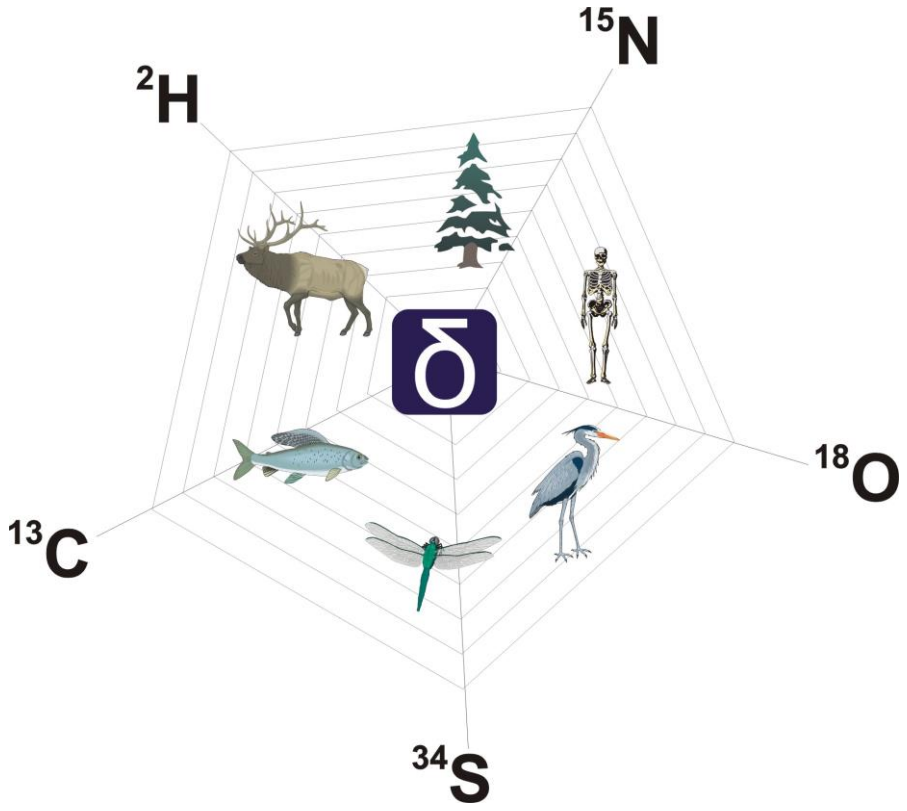
The 5th International Conference on Applications of Stable Isotope Techniques to Ecological Studies



Belfast, UK, August 13th to 18th 2006

Conference Organisers
Jason Newton & Stuart Bearhop

Organising Committee
Wolfram Meier-Augenstein
Rona McGill
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Event Organisers: *Happening*
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Welcome!

Dear Conference Participant

Welcome to Belfast and to the 5th International Conference on Applications of Stable Isotope Techniques to Ecological Studies. In this, the eighth year of the conference series, we are delighted once again to have that unique blend of researchers and students from universities, governmental institutions and industry that has made this series so successful.

We hope that Belfast will build on the success of the previous meetings in Wellington, Flagstaff, Braunschweig and Saskatoon, retaining the relaxed, friendly and collaborative atmosphere that sets *Isoecol* apart. The first conference in Saskatoon in 1998 had 115 delegates, this has grown steadily over the years and we will have close to 200 attending this meeting. As you can imagine with so many delegates, we had an extremely difficult task selecting presentations for oral slots (we could have probably run the conference twice!). However this has made for a very exciting, diverse and very full program. The timetable format at the Wellington conference (five days with a day off in the middle), proved to be a winning formula so we have retained this. We have also made sure that there are evening activities for you to attend (if you wish) from Sunday to Thursday.
This year we have gone for three plenary speakers, each an international leader in his field and these sessions will dovetail with seven themes which will run throughout the oral programme and poster evenings.

We hope that you enjoy your stay in Belfast and that you take some time out, either before after to travel further a-field in Northern Ireland and the Republic of Ireland, the north and west coasts are particularly beautiful at this time of year.

Most important of all we hope that you find this a rewarding and productive week.

Thank you for participating and helping to make this meeting a success.

Stuart Bearhop & Jason Newton

The Scientific Program

The program has 179 papers in total – 69 oral and 110 poster presentations. Many thanks to all the contributors.

We have three excellent plenary speakers, each a leading researcher in his field:

PLENARY SPEAKERS

On Monday morning Dr Carlos Martinez del Rio from the University of Wyoming will give a talk entitled: *Stable isotopes in animal ecology: what have we learned 8 years after a call for laboratory experiments?*

Carlos has a range of research interests relating to functional biodiversity. Carlos is the driving force behind a series of very important papers demonstrating both the utility and the limits of SIA applications in the understanding of ecological interactions and animal physiology.

On Tuesday we have Prof. Graham Farquhar, of the Australian National University, Canberra, and his presentation is entitled: *Carbon isotope discrimination by Rubisco and diffusion in leaves: applications to plant water-use efficiency and finding a gene.* Graham has been at the forefront of stable isotope work in plant physiology, both in terms of technical development and physiological experimentation.

On Thursday our final plenary will be given by Dr Tom Preston of SUERC, East Kilbride with a presentation entitled: *Tissue-diet spacing: what can we learn from experiments using enriched tracers?*

Tom was one of the architects of continuous-flow IRMS and has interests which involve many aspects of stable isotope biochemistry.

ORAL PRESENTATIONS

Oral presentations will be in the Peter Froggatt Centre (G06). All presenters should take their presentation files (PowerPoint) to Matt Lundy the day before they are due to talk (this will be on Tuesday for those presenting on Thursday). Matt will go through your presentation on a laptop in order to make sure there are no problems. Presentations should be15 minutes in length, with 5 minutes for questions.

POSTER PRESENTATIONS

Poster presentations will be held in the Whitla Hall on Monday and Tuesday evenings. The sessions will begin at 7:30pm and run until 9:30pm. Wine and soft drinks will be provided. Posters can be put up from Sunday (during registration) onwards and have to be removed by 4pm on Friday.

NAME BADGES

Please wear your name badge at all times during the meeting.

STUDENT AWARDS

We have over 70 students attending the conference and nearly all of them giving oral or poster presentations. Two awards will be given – best student oral paper and best student poster. Awards will consist of £250 and the presentations will be judged by four attending scientists. Good luck!

FINANCIAL SUPPORT FOR THE CONFERENCE.

We gratefully acknowledge the support of the following organisations and businesses for their financial support: Environment and Heritage Service, Belfast City Council, Scottish Universities Environmental Research Centre, The School of Biology & Biochemistry (QUB), Sercon, Thermo Electron/AGB, Pelican Scientific/Costech, CK Gas Products, GV Instruments, Elemental Microanalysis, Spectragases, IVA Analysentechnik

ACKNOWLEDGEMENTS

The organisers would like to thank: Kate Doherty and Karen Clarke from Happening, Robbie Mc Donald from Quercus (QUB) and Mathieu Lundy (QUB).
Very special thanks to Orea Anderson, Susie Brown, Kerry Crawford, Nicola Farmer, Isla Fraser, Gill Robb and David Tosh (all QUB) for their help with day to day running of the conference.

Social Events

PRE-CONFERENCE MIXER ***Sunday 13th August***

The School of Biological Sciences, Queen's University Belfast has sponsored this drinks reception and finger buffet, which will be held in the Medical Biology Centre on the Lisburn Road from 7pm to 9pm. A great chance to catch up with old friends and make some new ones. Registration packages will be available at the Whitla Hall for pick up from 5pm onwards.

CONFERENCE FIELD TRIPS ***Wednesday 14th August***

Trip 1: Giant's Causeway, Whiskey and the Antrim Coast

Trip 2: Rathlin Island

Trip 3: Dublin

Details on where and when to meet will be given on arrival at the conference. People travelling on trips 1 & 2 should bring walking boots and wet weather gear (just in case).

WEDNESDAY NIGHT PUB QUIZ ***Wednesday 14th August***

Venue and time to be confirmed at the meeting

CONFERENCE BANQUET ***Thursday 15th August***

The conference banquet will be held in the Belfast City Halls in the city centre. Belfast City Council has very generously sponsored a pre-dinner drinks reception which will begin at 7:15pm followed by dinner at 8:15pm. There will be a cash bar from 8:15pm onwards. You can either walk (20 - 25mins) or take a bus into the city centre (numbers 8A, 8B and 8C, pick up in front of the Lanyon Building every 10 - 15) all pass in front of the main University building on University road and stop outside the City Halls.

OVERVIEW OF EVENTS

Sunday 13th August

0930 - 1730	Short course in the *Medical Biology Centre, Lisburn Road*
1700 - 1845	Registration at the *Whitla Hall*
1900 - 2100	Pre-conference Icebreaker at the *Medical Biology Centre*

Monday 14th August

0815 - 0845	Registration at the *Peter Froggatt Centre (PFC)*
0845 - 0900	Welcome and introductions, *PFC G06*
0900 - 0940	Plenary: Carlos Martinez Del Rio, *PFC G06*
0940 - 1040	Session: From Individuals to Communities I, *PFC G06*
1040 - 1110	**Coffee break***, Whitla Hall*
1110 - 1250	Session: From Individuals to Communities I, *PFC G06*
1250 - 1420	**Lunch***, Whitla Hall*
1420 – 1540	Session: From Individuals to Communities I, *PFC G06*
1540 – 1610	**Coffee break***, Whitla Hall*
1610 – 1730	Session: Soil-Plant/Soil-Microbe Interactions, *PFC G06*
1930 – 2130	Poster Session*, Whitla Hall*

Tuesday 15th August

0830 - 0850	Registration at the *PFC*
0850 - 0900	Housekeeping, *PFC G06*
0900 - 0940	Plenary: Graham Farquhar, *PFC G06*

0940 - 1040	Session: Gas Exchange & Water Relations in Plants I, *PFC G06*
1040 - 1110	**Coffee break***, Whitla Hall*
1110 - 1250	Session: Riparian Ecology, *PFC G06*
1250 - 1420	**Lunch***, Whitla Hall*
1420 – 1540	Session: Isotopic Ecology of Salmonids, *PFC G06*
1540 – 1610	**Coffee break***, Whitla Hall*
1610 – 1730	Session: Pelagic Predators, *PFC G06*
1930 – 2130	Poster Session*, Whitla Hall*

Wednesday 16th August

Field trips and Wednesday night Pub Quiz

Thursday 17th August

0830 - 0850	Registration at the *PFC*
0850 - 0900	Housekeeping, *PFC G06*
0900 - 0940	Plenary: Tom Preston, *PFC G06*
0940 - 1040	Session: From Individuals to Communities II, *PFC G06*
1040 - 1110	**Coffee break***, Whitla Hall*
1110 - 1250	Session: From Individuals to Communities II, *PFC G06*
1250 - 1420	**Lunch***, Whitla Hall*
1420 – 1540	Session: Gas Exchange & Water Relations in Plants II, *PFC G06*
1540 – 1610	**Coffee break***, Whitla Hall*
1610 – 1730	Session: Paleoecology, *PFC G06*

1915	**Conference Banquet at Belfast City Halls**

Friday 18th August

0830 - 0850	Registration at the *PFC*
0850 - 0900	Housekeeping, *PFC G06*
0900 - 1040	Session: H & O Isotopes in Hair/Methods & Models, *PFC G06*
1040 - 1110	**Coffee break***, Whitla Hall*
1110 - 1250	Session: Methods & Models, *PFC G06*
1250 - 1420	**Lunch***, Whitla Hall*
1420 – 1540	Session: From Individuals to Communities III, *PFC G06*
1540 – 1610	**Coffee break***, Whitla Hall*
1610 – 1730	Session: From Individuals to Communities III, *PFC G06*
1730 – 1800	Student prizes and conference wind up

CONFERENCE PROGRAM

Monday 14th August

0815 – 0845 Registration

0845 – 0900 Welcome and introductions

0900 – 0940 Plenary: ***Dr Carlos Martinez del Rio***
Stable isotopes in animal ecology: what have we learned 8 years after a call for laboratory experiments?

SESSION 1: FROM INDIVIDUALS TO COMMUNITIES I (Individual based studies)
Chair: Stuart Bearhop

0940 – 1000 Unravelling how diet restriction extends lifespan: a stable isotope analysis of nutrient allocation in Drosophila.
O'Brien, D.M., *Min, K.J., Tatar, M.*

1000 – 1020 A novel method to study mating behaviour in mosquitoes: tracing ^{13}C labelled sperm in *Anopheles arabiensis* spermathecae.
Helinski, M.E.H.*, Hood-Nowotny, R., Mayr, L., Knols, B.G.J.'*

1020 – 1040 Using stable isotope analysis to identify dietary choices and trophic position of wireworms (Coleoptera: Elateridae) in Central European arable land. ***Traugott M.,*** *Schallhart K., Kaufmann R., Juen A.*

1040 – 1110 **Coffee Break**

SESSION 1: FROM INDIVIDUALS TO COMMUNITIES I (Individual based studies)
Chair: Howard Platt (Environment & Heritage Service)

1110 – 1130 Whitefish (*Coregonus laveratus*) intra-otolith stable isotope values of oxygen and carbon reveal spatial behaviour and variations in metabolic rate**.** ***Dufour, E.,*** *Gerdeaux, D., Wurster, C.M.*

1130 – 1150 Ontogenetic shifts in trophic position and habitat use by juvenile northern pike (*Esox lucius*) revealed by stable isotope and mark-recapture. ***Cucherousset, J.,*** *Paillisson, J.M., Roussel, J-M.*

1150 – 1210 Resource segregation and trophic specialisation in the *Coregonus lavaretus* species complex: stable isotopes, shape and stomach contents. ***Harrod, C.,*** *Kahilainen, K., Mallela, J.*

1210 – 1230 Microgeographic variation in isotopic composition of a rattlesnake prey base: implications for studies using stable isotopes as dietary indicators. ***Pilgrim, M.A.,*** *Farrell, T.M., Romanek, C.S.*

1230 – 1250 Daisy, what did you eat when we weren't looking? ***Zazzo, A.***, *Harrison, S., Bahar, B., Moloney, A. P., Monahan, F. J., Scrimgeour, C. M., Schmidt, O.*

1250 – 1420 **LUNCH**

SESSION 1: FROM INDIVIDUALS TO COMMUNITIES I (Individual based studies)
Chair: Blair Wolf

1420 – 1440 Foraging ecology of invasive American mink during an eradication campaign in the Outer Hebrides, Scotland. ***McDonald, R.A.,*** *Roy, S., Newton, J. Bearhop, S.*

1440 – 1500 Testing mechanisms for the evolution of dietary specialization: isotopic analysis of sea otters (*Enhydra lutris*). ***Newsome, S.D.,*** *Monson, D.H., Tinker, M.T., Oftedal, O., Ralls, K., Fogel, M.L., Estes, J.A.*

1500 – 1520 Stable isotope evidence of sex-specific differences in manatee Diets. ***Clementz, M.T.***

1520 – 1540 The stable isotope composition of the north Pacific and Arctic Ocean sea-scape: a closer look at the habitat of the migratory bowhead whale (*Balaena mysticetus*). ***DeHart, P.A.P.***, *Wooller, M.J.*

1540 – 1610 **Coffee Break**

SESSION 2: SOIL-PLANT/SOIL-MICROBE INTERACTIONS
Chair: Satoshi Tobita

1610 – 1630 The fate of proteinaceous material in soil. ***Knowles, T.D.J.***, *Mottram, H.R., Evershed, R.P., Bol, R., Chadwick, D.*

1630 – 1650 Modification of lipid distribution patterns and isotopic ($\delta^{13}C$) composition in plants and turnover of lipids in corresponding soils under enhanced (FACE) conditions. ***Wiesenberg, G.L.B.,*** *Schmidt, M.W.I., Schwark, L.*

1650 – 1710 Isotopomer studies of soil-derived nitrous oxide: evaluation of microbial origins and importance of nitrous oxide consumption. ***Ostrom, N.E.,*** *Sutka, R.L., Pitt, A., Jinuntuya, M., Ostrom, P.H.*

1710 – 1730 Methane emissions and microbial activity in a wetland grassland. ***Chamberlain, P.M.,*** *Chaplow, J., Parekh, N., Stott, A.W., McNamara, N.P.*

Tuesday 15th August

0830 – 0855 Registration

0850 – 0900 Housekeeping

0900 – 0940 Plenary: ***Professor Graham Farquhar***
Carbon isotope discrimination by Rubisco and diffusion in leaves: applications to plant water-use efficiency and finding a gene

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SESSION 3: Gas Exchange & Water Relations in Plants I
Chair: Leo Sternberg

0940 – 1000 Carbon and oxygen isotopes: a tool to analyze the fluxes of CO2 and H2O between plants and atmosphere. ***Ripullone, F****., Borghetti, M., Cernusak, L., Matsuo, N., Farquhar, G.*

1000 – 1020 The fate of carbon in a mature deciduous forest exposed to elevated CO2. ***Keel, S.G****., Siegwolf, R.T.W., Körner, C*.

1020 – 1040 Tracing C fluxes to the soil and atmosphere, through leaf litter decomposition in a poplar plantation by means of stable C isotopes. ***Rubino, M.,*** *Merola, A., Bertolini, T., Lagomarsino, A., De Angelis, P., Lubritto,C., D'Onofrio, A., Terrasi, F., Cotrufo, M.F.*

1040 – 1110 **Coffee Break**

ORAL SESSION: RIPARIAN ECOLOGY
Chair: Mat Wooller

1110 – 1130 Multiple stable isotopes reveal organic matter and mercury flow in a temperate river. ***Jardine, T.D****., Kidd, K.A., Doucett, R.R., Wassenaar, L.I., Cunjak, R.A.*

1130 – 1150 Identifying N inputs in river food webs: can δ^{15}N be used when streams are heavily impacted by agriculture? ***Roussel, J.M.,*** *Caquet, T., Cunjak, R.A., Haury, J., Jardine, T.D.*

1150 – 1210 Stable nitrogen isotope ratios of macrophytes and periphyton along a nitrate gradient in a subtropical coastal river. ***De Brabandere, L.****, Frazer, T.K., Montoya, J.P.*

1210 – 1230 Changes in carbon and nitrogen stable isotope ratios of periphyton exposed to landfill leachate. ***North, J.C.****, Cornelisen, C.D., Frew, R.D.*

1230 – 1250 The hidden information in the isotopic and stoichiometric composition of stream biofilms.
Evers, S.*, Waldron, S., Murphy, K., Penny, J.*

1250 – 1420 **LUNCH**

ORAL SESSION: ISOTOPIC ECOLOGY OF SALMONIDS

Chair: Rick Cunjak

1420 – 1440 Stable isotope studies on the use of marine-derived nutrients by coho salmon juveniles in the Oregon Coast Range. ***Church, M.R.***, *Ebersole, J.L., Wigington, P.J., Rensmeyer, K.M.*

1440 – 1500 Linking migratory patterns of females to ova traits in brown trout (*Salmo trutta*, L.) by means of stable isotope analysis. ***Acolas, M.L.***, *Roussel, J.M., Baglinière, J.L.*

1500 – 1520 Latitudinal clines in young-of-the-year Arctic charr habitat use in eastern North America. ***Storm-Suke, A.***, *Dempson, J. B., Reist, J. D., Power M.*

1520 – 1540 Linking ocean climate cycles to fish (Atlantic salmon) mortality using the stable carbon and nitrogen isotope composition of scale collagen. ***Trueman, C.N.***, *Moore, A*

1540 – 1610 **Coffee Break**

ORAL SESSION: PELAGIC PREDATORS

Chair: David Thompson

1610 – 1630 Employing chemical tags to determine trophic dynamics and movement patterns of migratory predators in the equatorial Pacific Ocean. ***Graham, B.***, *Popp, B., Olson, R., Allain, V., Galvan, F., Fry, B.*

1630 – 1650 Size and latitudinal effects on $\delta^{15}N$ reveal differential trophic ecology of two top predators in the western Indian Ocean. ***Lorrain, A.***, *Ménard, F., Potier, M., Marsac, F.*

1650 – 1710 Isotopic evidence for dietary shift in historical and modern white sharks off the coast of California. ***Kim, S.***, *Kerr, L.A., Suk, S., Koch, P.L.*

1710 – 1730 Evidence of niche partitioning between beaked whale species (Family Ziphiidae) in the North Atlantic from stable isotope analysis. ***MacLeod, C.D.***, *Herman, J., Sabin, R.C., Newton, J., Pierce, G.J.*

Thursday 17th August

0830 – 0855 Registration

0850 – 0900 Housekeeping

0900 – 0940 Plenary: ***Dr Tom Preston***
Tissue-diet spacing: what can we learn from experiments using enriched tracers?

ORAL SESSION: FROM INDIVIDUALS TO COMMUNITIES II (Population Studies)
Chair: Carlos Martinez del Rio

0940 – 1000 Does The Cost Of Living At Depth Force Dietary Switch In Chironomid Larvae? ***Grey, J.***, *Stott, A., Deines, P.*

1000 – 1020 Symbiosis of a Caribbean bivalve and shrimp: A field study using a stable isotope mixing model. ***Aucoin. S.***, *Himmelman, J.*

1020 – 1040 Migration dynamics of sand goby (Pomatoschistus minutus) between the North Sea and the Schelde estuary: a stable isotope approach. ***Guelinckx, J.***, *Maes, J., Dehairs, F., Ollevier F.*

1040 – 1110 **Coffee Break**

ORAL SESSION: FROM INDIVIDUALS TO COMMUNITIES II (Population Studies)
Chair: Manuela Forero

1110 – 1130 Interactive segregation among sympatric Arctic charr (Salvelinus alpinus) & brown trout (Salmo trutta) populations in Irish Loughs . ***Power, M.***, *Igoe, F.*

1130 – 1150 Isotope ecology of estuarine and freshwater crocodylians.
Wheatley, P. V., *Koch, P. L*

1150 – 1210 Detecting food web change in the Laurentian Great Lakes using stable isotope and fatty acid tracers.
Hebert, C.E., *Arts, M.T., Weseloh, D.V.C.*

1210 – 1230 Factors affecting prey choice in a despotic herbivore. ***Inger, R.***, *Ruxton, G., Newton, J., Colhoun, K., Robinson, J., Bearhop, S.*

1230 – 1250 Connecting breeding and wintering sites used by endangered southwestern willow flycatchers. ***Kelly, J. F.***, *Johnson, M.J., Langridge, S., Whitfield, M.*

1250 – 1420 **LUNCH**

ORAL SESSION: GAS EXCHANGE & WATER RELATIONS IN PLANTS II

Chair: Graham Farquhar

1420 – 1440 Stable oxygen isotopes of bulk leaf material reveal long-term chronic ozone effects in grassland species. ***Jäggi, M.****, Siegwolf, R., Fuhrer J.*

1440 – 1500 A novel stable isotopic approach to identify the fate of ozone in plants. ***Toet, S.****, Subke, J.-A., D'Haese, D., Barnes, J., Ineson, P., Emberson, L., Ashmore, M.*

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1500 – 1520 Elucidating the source of nitrous oxide in soils using stable isotope techniques**.** ***Baggs, E.M.,*** *Garbeva, P., Mair, L., Wrage, N., Shaw, L.J.*

1520 – 1540 Studying climate change: a novel tool using deuterium isotopomers quantification in tree ring cellulose.
Betson, T. R.*, Augusti, A., Schleucher, J.*

1540 – 1610 **Coffee Break**

ORAL SESSION: PALEOECOLOGY

Chair: Thure Cerling

1610 – 1630 Determining isotopic fractionations for carnivores: A case study at Isle Royale. ***Fox-Dobbs, K.****, Bump, J.K., Peterson, R.O., Koch, P.L.*

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1630 – 1650 Variation in herbivore bone collagen and tooth enamel $\delta^{13}C$ at a continental scale. ***Murphy, B.P.****, Bowman, D.M.J.S.*

1650 – 1710 Stable isotopic evidence of the effects of global change and sea-level rise on mammalian community ecology under glacial and interglacial conditions. ***Grawe DeSantis, L.R.***

1710 – 1730 Carbon isotopes, extinct megaherbivores, and supposed Amazonian refugia during the Pleistocene. ***MacFadden, B. J.***

Friday 18th August

0830 – 0855 Registration

0850 – 0900 Housekeeping

ORAL SESSION: H & O ISOTOPES IN HAIR/METHODS & MODELS
Chair: Wolfram Meier-Augenstein

0900 – 0920 Isotope turnover in animal tissues: the reaction progress variable. ***Cerling T.E.**, Ayliffe L.K., Bowen G.J., Elheringer J.R., Passey B.H., Podlesak D.*

0920 – 0940 Turnover of oxygen and hydrogen isotopes in the body water, CO2, hair and enamel of a small mammal after a change in drinking water. ***Podlesak, D.W.**, Bowen, G.J. , Cerling, T, Ehleringer, J. R. Passey, B. H.*

0940 – 1000 Hydrogen and oxygen isotope ratios in human hair are related to geography. **Ehleringer, J.R.**, Bowen, G.J., Chesson, L.A., West, A.G., Podlesak, D.,Cerling, T.E

1000 – 1020 Multi-isotope comparison of modern and pre-modern human hair and the homogenization of human diet. ***Bowen, G.**, Cerling, T., Podlesak, D., Chesson, L., Ehleringer, J.*

1020 – 1040 An isotope dilution approach to quantify the nutritional value of detritus. ***Vandewiele, S.**, van Oevelen, D., Kayal, E., Soetaert, K., Middelburg, J.J.*

1040 – 1110 **Coffee Break**

ORAL SESSION: METHODS & MODELS
Chair: Gabe Bowen

1110 – 1130 Error propagation and limits of resolution in inferring geographic origins from stable hydrogen isotopes. ***Wunder, M.**, Kester, C., Webb, C.*

1130 – 1150 When isotopes aren't enough: using additional information to constrain mixing problems. ***Phillips, D.L.**, Schuur, E.A.G., Brooks, J.R., Ben-David, M., Fry, B*.

1150 – 1210 Effects of temperature and ration size on carbon and nitrogen stable isotope trophic fractionation. ***Barnes, C.**, Sweeting, C.J., Jennings, S., Barry, J.T., Polunin, N.V.C.*

1210 – 1230 New developments in sulfur isotope analysis and applications to ecological research. ***Stricker, C.A.**, Rye, R.O., Guntenspergen, G.R.*

1230 – 1250 Coupled NCS isotope measurements. ***Fry, B.***

1250 – 1420 **LUNCH**

ORAL SESSION: FROM INDIVIDUALS TO COMMUNITES III (Community Studies)
Chair: Susan Waldron

1420 – 1440 Feeding strategies of Antarctic soil arthropods.
Bokhorst, S., *RonfortC., Huiskes, A.*

1440 – 1500 Zooplankton feeding selectivity on isotopically heterogeneous phytoplankton challenges classic stable isotope analyses of origins of zooplankton carbon. ***Perga, M.-E.,*** *Kainz, M., Mazumder, A.*

1500 – 1520 Effects of biomanipulation on feeding niches of perch (Perca fluviatilis) and roach (Rutilus rutilus) determined by stable isotopes.
Syväranta, J.*, Jones, R. I.*

1520 – 1540 Stable isotope analyses reveal aquatic food web complexity and conservation concerns at different spatial scales. ***Gaines, K.H.***

1540 – 1610 **Coffee Break**

ORAL SESSION: FROM INDIVIDUALS TO COMMUNITES III (Community Studies)
Chair: Mike Power

1610 – 1630 Community structure and food web based on stable isotopes (δ^{15}N and δ^{13}C) analysis of a North Eastern Atlantic maerl bed. ***Grall, J.****, Le Loc'h, F., Guyonnet, B.*

1630 – 1650 How fishing activities modify a benthic muddy-sand food web? A stable isotope approaches. ***Guyonnet, B****., Jacques, G.*

1650 – 1710 The role of stable carbon and nitrogen isotopes in determining a trophic cascade whereby invasive rats indirectly transform marine intertidal communities. ***Kurle, C.M.***

1710 – 1730 The importance of cacti to consumers in a desert food web.
Wolf, B. O.*, McKechnie, A. E., Warne, R., Mathiasen, C. C.*

1710 – 1730 **Student prizes and conference wind up**

Poster Sessions: Monday 14th & Tuesday 15th August, 1930-2130, Whitla Hall

FROM INDIVIDUALS TO COMMUNITIES

A1 Stable isotopes reveal alternate migration and foraging strategies in the parasitic phase of River Lamprey, *Lampetra fluvialis*, from the River Endrick, Scotland. *Adams, C.E.[1], Bissett, N.[1], **Newton, J.[2],** Maitland, P.S.[3]*

A2 Body size and stable isotope ($\delta^{15}N$ and $\delta^{13}C$) data to elucidate food web structure of trawl assemblage. ***Al-habsi, S.H.,*** *Polunin, N.V.C., Sweeting, C.J., Graham, N.A.J.*

A3 Foraging ecology and ecotoxicology in Southern Ocean seabird communities. ***Anderson, O.,*** *Phillips, R.A.,' Shore, R.,' McDonald, R.', McGill, R.A.R., Bearhop, S.*

A4 The influence of biodiversity on resource partitioning in intertidal gastropods. ***Andrew, G. M.,*** *Burrows, M. T., Hawkins, S. J., McGill, R. A. R.*

A5 Community structure and food web based on stable isotopes ($\delta^{15}N$ and $\delta^{13}C$) analysis of the North Bay of Biscay fishing ground (Northeast Atlantic). ***Le Loc'h, F.****, Hily, C., Grall, J.*

A6 Assessment of polychlorobiphenyl bioaccumulation in the spider crab food web using stable isotopes. *Bodin, N.**, Le Loc'h, F.,** Abarnou, A.*

A7 Influence of lipid extraction on stable carbon and nitrogen isotope analysis of crustacean tissues: potential consequences for marine food web studies. *Bodin, N., **Le Loc'h, F.** , Hily, C., Abarnou, A.*

A8 Carbon isotope ratios ($\delta^{13}C$) of macro-invertebrates in assessing lake trophic functioning. ***Borderelle, A-L.,*** *Verneaux, V., Gerdeaux, D.*

A9 Assessing the consequences of foraging strategy on cormorant productivity ***Brown, S.L.,*** *McDonald, R.A., Newton, J., Bearhop, S.*

A10 Seals as "pests": foraging strategies and potential for conflict ***Brown, S.L.,*** *McDonald, R.A., Newton, J., Bearhop, S.*

A11 A seasonal survey of the benthic food web of the Lapalme's Lagoon (Aude, France) assessed by carbon and nitrogen stable isotope analysis ***Carlier, A.,*** *Riera, P., Amouroux, J-M., Bodiou, J-Y., Escoubeyrou, K., Desmalades, M., Grémare, A.*

A12 Spatial and seasonal evolution of carbon cycling in the Scheldt estuary using stable isotopes. ***Chevalier, E. M.,*** *De Brabandere, L., Brion, N., Bouillon, S., Dehairs, F., Baeyens, W*

A13 Bat migration; a pilot study using stable isotope analyses. ***Crawford, K.,*** *McDonald, R., Newton, J., Bearhop, S.*

FROM INDIVIDUALS TO COMMUNITIES (cont)

A14 The Future of Madagascar's Lemurs: Coping with Change. ***Crowley, B.,*** *Koch, P., Godfrey, L.*

A15 Isotope trophic-step fractionation in marine suspension-feeding species
Dubois, S.*, Blin, J.L., Bouchaud, B., Lefebvre,S.*

A16 Macrobenthic assemblages associated with *Lanice conchilega* populations under oyster farming influences: trophic approach using natural stable isotopes
Dubois, S.*, Fuchs, S., Ropert, M., Marin-Leal, J., Lefebvre S.*

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ORAL SESSIONS

PLENARY

Stable isotopes in animal ecology: what have we learned 8 years after a call for laboratory experiments?

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Using stable isotope analysis (SIA) as a tool to study animal ecology is based on a paradox. Isotopically speaking, animals are what they eat; however, they are not exactly what they eat. The incorporation of the natural isotopic signatures of resources into animal tissues allows ecologists to investigate animal diets, animal movements, and the nutrient transfer across ecosystem boundaries. The difference between the isotopic composition of an animal's tissues and that of its diet creates novel isotopic signatures and allows diagnosing trophic position. Eight years ago we proposed that, given this paradox, SIA could only be used confidently as a tool in animal ecology if we developed laboratory research in three critical areas: 1) kinetics of isotopic incorporation, 2) isotopic routing, and 3) mechanisms of isotopic discrimination between animal tissues and diet. In the years since our call for laboratory experiments, progress is evident. In addition to gathering new data, isotopists are developing theoretical mixing and mass-balance models to guide data collection and aid in the interpretation of those data. I will use examples from a variety of animal species to illustrate how the potent combination of theory and experiments has led us to a clearer understanding of both the power and limitations of SIA in animal ecology.

FROM INDIVIDUALS TO COMMUNITIES I: Individual based studies

Unravelling how diet restriction extends lifespan: a stable isotope analysis of nutrient allocation in *Drosophila*

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Diet restriction extends lifespan in many animals. In *D. melanogaster*, reducing the quantity of dietary yeast (but not dietary sugar) available to adults dramatically increases survival and simultaneously reduces fecundity. While the mechanism of this control is unknown, it is a widely held tenet of life history theory that animals allocate limited nutrients away from reproduction and toward somatic maintenance or repair. Here we test this hypothesis explicitly by tracking the allocation of C and N deriving from dietary yeast to reproduction and to somatic maintenance in adults fed an *ad libitum* yeast diet (16%Y = "full diet") or a longevity-extending, yeast restricted diet (4%Y = "diet restricted"). We use two strains of isotopically labelled yeast as experimental diets; one labelled with beet sugar (δ^{13}C = -24‰) and unlabeled ammonium sulphate (δ^{15}N = -4‰), and one on cane sugar (δ^{13}C = -10‰) and labelled ammonium sulfate (δ^{15}N = 253‰). *Drosophila* have similar diets as larvae and adults, here comprising of yeast, sucrose (held constant), agar, and a vitamin mixture. By growing flies in which the isotopic composition of yeast diets contrast either in the larval stage or in the adult stage we can calculate investment of larval and adult yeast C and N to reproduction or somatic maintenance. As predicted, fecundity is greatly increased in full diet flies and investment of adult dietary C and N into eggs is many-fold higher than in diet restricted flies. However, lifespan is enhanced in diet restricted flies, as previous studies have demonstrated. Contrary to expectations, investment of adult dietary C and N from yeast into somatic maintenance is greater in shorter-lived, full diet flies than in longer-lived, diet restricted flies. We evaluate somatic turnover through two independent measures, and both indicate that somatic turnover (~ repair) is higher in the shorter-lived, full diet flies. However, if we express C and N investment into somatic maintenance relative to investment to reproduction [SM/R], relative somatic maintenance is higher in the longer-lived, diet restricted flies. These results demonstrate that longevity is not associated with *absolute* nutritional investment to somatic maintenance or repair, but that somatic investment *relative* to reproductive output may be more important in extending lifespan. This type of mechanism is consistent with a scenario in which reproduction induces somatic damage, and only somatic investment exceeding that required to counteract this damage increases lifespan. These data provide the first direct evidence to suggest that the relative allocation of resources plays a role in the longevity extension mechanism of diet restriction.

FROM INDIVIDUALS TO COMMUNITIES I: Individual based studies

A novel method to study mating behaviour in mosquitoes: tracing ^{13}C labelled sperm in *Anopheles arabiensis* spermathecae

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Mating studies in mosquitoes are notoriously difficult to conduct, in spite of their importance for future development of genetic control strategies (e.g. Sterile Insect Technique and transgenic approaches) to target disease vectors. The use of stable isotopes was proposed to study mosquito mating by following labelled sperm into spermathecae (female's sperm storage organ). Labelled-^{13}C glucose was incorporated into the larval and adult diet of the malaria mosquito *Anopheles arabiensis*. Treatments included the labelling of the larval water and adult sugar water only or a combination of both. Males were mated with unlabelled females and after mating females were immobilized and the spermatheca was dissected out. Insemination by microscopy was determined and subsequently spiked samples were analysed for isotope ratios using isotope mass spectrometry. Persistence of the label in females was studied by isolating females for a number of days.

Results demonstrated that spermathecae positive for sperm could successfully be distinguished from negatives and controls using the raw $\delta^{13}C$ values. A spermatheca was considered labelled when its raw $\delta^{13}C$ value was two standard deviations above the mean control value. Addition of ^{13}C label to the adult sugar diet alone was not sufficient to detect sperm transfer, but larval only labelling and larval and adult labelling resulted in detectable values. A slight loss of label was observed in females isolated for 3 days, however in most cases values were above the 2SD threshold. There were no detrimental effects of the addition of un-labelled glucose or labelled glucose on larval development and the males mating performance.

We have proven that is it possible to label male mosquitoes and detect the label in the females after sperm transfer. To our knowledge, this is the first study that has used stable isotopes to label sperm cells in insects. This method offers great potential to study a variety of issues related to mating including competitiveness studies of radio-sterilized males.

FROM INDIVIDUALS TO COMMUNITIES I: Individual based studies

Using stable isotope analysis to identify dietary choices and trophic position of wireworms (Coleoptera: Elateridae) in Central European arable land

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Wireworms are the soil-dwelling larvae of click beetles (Coleoptera: Elateridae). They can reach high population densities and thus play an important part in the soil food web of arable land and grasslands. Elaterid larvae are also known as pests: especially larvae within the genus *Agriotes* attack maize, potatoes, and other crops. Besides feeding on crops, previous laboratory feeding experiments suggest that wireworms are polyphagous; they may feed on the roots of a variety of plants (e.g. weeds) but also consume soil organic matter. Furthermore, the trophic position of most wireworm species within the soil food web is not exactly known. An analysis of their dietary choices under field conditions, however, has to precede any risk assessment of potential wireworm damage to agricultural crops, as well as any control strategy. Assessing wireworms' dietary choice under field conditions, however, is not a simple task. Elaterid larvae are fluid feeders, leaving no microscopically discernible food fragments to be found in gut dissection. Stable isotope analysis offers a new way to track wireworms' dietary choices under field conditions. Here, we present the first study investigating the dietary choices and the trophic position of wireworm species commonly found in arable land of Central Europe using stable isotope analysis. The interpretation of the field-derived data is based on the outcomes of extensive laboratory experiments investigating how wireworms' life history traits affect their isotopic signatures (see poster contribution by Traugott et al. "Evaluating ^{15}N and ^{13}C isotope ratio analysis to investigate diet choice in wireworms (Coleoptera: Elateridae)" this conference).
We sampled wireworms, plant roots, litter, and soil from 39 sites (representing 21 locations; grassland sites, potato- and maize fields) in Central Europe (Austria, Germany, and Italy) and analysed their ^{13}C and ^{15}N content. The analysis from an extensively sampled maize field at Rotholz (Tyrol, Austria) showed 15%, 58%, and 17% of *Agriotes obscurus* larvae (n=100) to have consumed exclusively weed roots, a mixture of weed and maize roots, and maize roots only, respectively. Interestingly, 10% of the larvae had to be classified as feeding on animal prey by their $\delta^{15}N$ signatures. This confirms previous speculations about the carnivorous nature of this "plant-feeding" species. Moreover, our findings reveal that *A. obscurus* represents a type B generalist where the dietary choice of the population as a whole is diverse, but the individuals obviously stick with a particular choice. These outcomes can be directly integrated into the design of new ways for controlling this pest species, such as the use of catch crops. *Hemicrepidius niger*, the other most abundant species at this site, turned out to be carnivorous questioning its status as a pest species attacking crops. This study highlights that wireworms have species-specific prey choices which determine their role and ecological function in the soil food web. Thus, for forecasting wireworm damage and wireworm control it is important not only to record the presence of wireworms *per se*, but also to consider elaterid larvae on a species level. An in-depth analysis of all sites and elaterid species will be presented at the conference in order to clarify the feeding ecology of wireworms in Central Europe.

FROM INDIVIDUALS TO COMMUNITIES I: Individual based studies

Whitefish (*Coregonus laveratus*) intra-otolith stable isotope values of oxygen and carbon reveal spatial behaviour and variations in metabolic rate

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Stable oxygen and carbon isotope values of otoliths ($\delta^{18}O_{oto}$ and $\delta^{13}C_{oto}$) enable a unique opportunity to gain insights in fish ecology and physiology. $\delta^{18}O_{oto}$ values are near physico-chemical equilibrium with the environment and provide a direct record of temperature and $\delta^{18}O$ values of the ambient water ($\delta^{18}O_w$). Although, otolith carbon is derived from dissolved inorganic carbon (DIC) and diet, the inability to constrain the contributions of each source have restricted the application of this tracer. Previous studies have separately concluded diet, DIC, metabolic rate, and activity to drive variation in $\delta^{13}C$ values of otoliths. We investigated 1) vertical summer depth positioning and 2) sources of ontogenetic variations in $\delta^{13}C_{oto}$ values for whitefish (*Coregonus lavaretus*) in Lake Annecy (France).

Whitefish is a commercially valuable zooplanktivorous species common in many European lakes such as Lake Annecy. We used micromilling techniques to recover high-resolution carbonate and reconstructed 10 individual intra-otolith $\delta^{18}O_{oto}$ and $\delta^{13}C_{oto}$ profiles at bi-weekly to monthly increments over the first three seasons of otolith growth. Contemporaneous water temperature, and $\delta^{18}O_w$, zooplankton $\delta^{13}C$ ($\delta^{13}C_{zooplankton}$), and dissolved inorganic carbon $\delta^{13}C$ ($\delta^{13}C_{DIC}$) values were also measured. To determine vertical summer depth positioning, we first reconstructed fish individual thermal history using $\delta^{18}O_{oto}$ thermometry via a freshwater fish otolith-specific temperature fractionation equation. Comparison of reconstructed and environmental temperatures indicate that adults generally inhabited areas close to the thermocline during summer, while juveniles often occupied areas offshore. We then investigated sources of intra-otolith variations in $\delta^{13}C_{oto}$ values. When we compared ontogenetic $\delta^{13}C_{oto}$ profiles with variations of $\delta^{13}C_{zooplankton}$ or $\delta^{13}C_{DIC}$ values in a common temporal frame, we failed to demonstrate any strong relationships. However, when we used a generalized *Corregonus* spp. bioenergetic model to estimate specific respiration rate (SRR), we found a strong and significant linear relationship (r^2=0.68, $F_{1,14}$=30.2) between SRR and $\delta^{13}C_{oto}$ value for age 1+ and 2+. We conclude that ontogenetic profiles of whitefish $\delta^{13}C_{oto}$ in Lake Annecy are governed by metabolic rate, which is in turn driven primarily by temperature. Finally, we estimated the contribution of metabolic carbon over the fish's life.

This study demonstrates that when environmental parameters are known the two tracers can be used for investigating behaviour of freshwater fishes, which is often difficult to determine by classical methods.

FROM INDIVIDUALS TO COMMUNITIES I: Individual based studies

Ontogenetic shifts in trophic position and habitat use by juvenile northern pike (*Esox lucius*) revealed by stable isotope and mark-recapture analyses

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Recent technological advancements in Passive Integrated Transponder (PIT) technology have proven successful in designing mark-recapture experiments to study the behaviour of large numbers of individually tagged, small-bodied animals, including early life stages of fishes. Similarly, the use of Stable Isotope Analysis (SIA) to trace animal migration and foraging behaviour is increasingly well documented in literature, but the potential benefits of repeated SIA on the same individually tagged organisms remain unexplored. This approach was developed to study early life history shifts in foraging behaviour and habitat use by individually tagged northern pike (*Esox lucius*) in areas exposed to natural fluctuations of water level.

The study was conducted in the Brière marsh (Northwest France), from May to August 2005. Larvae and juvenile pike were artificially fed on zooplankton in hatchery tanks, then PIT-tagged (fork length 51.0 ± 5.3 mm [mean ± S.D.], n = 192) before release in a flooded grassland (FGL). FGL progressively dried out in spring and the only connection with the adjacent temporary water pond (TWP) was equipped with a fyke net to capture migrating fish. Similarly, water level in TWP dropped off in summer and the fish were trapped while moving to a permanent ditch; additional recaptures were also achieved by electrofishing in the TWP. Any time individuals were handled (*i.e.* at tagging and subsequent recaptures), they were fin clipped, and stable nitrogen and carbon isotope analysis was run on fin tissue collection. The same tissue sampling protocol was performed on few wild juveniles that were naturally born in FGL. SIA were also run on potential preys in FGL and TWP, *i.e.* zooplankton, invertebrate, juvenile crayfish, and other fish taxa.

Survival rate of PIT-tagged fish through the experiment was 34% and 10% in FGL and TWP, respectively. δ^{15}N and δ^{13}C values for fish reared at hatchery and their food (zooplankton) revealed the existence of two distinct groups identified as zooplanktivorous and piscivorous (*i.e.* cannibalistic) fish (fractionation factor +2.01‰ δ^{15}N and +0.82‰ δ^{13}C). Initial fish length at tagging was positively correlated with δ^{13}C and δ^{15}N. After release, time spent in FGL was negatively correlated with δ^{15}N and fish size, indicating that early migrants were cannibalistic individuals. The dilution of initial hatchery signatures clearly suggested that juvenile pike preyed on primary consumers in FGL (zooplankton, invertebrate and juvenile crayfish). Conversely, individuals were significantly ^{15}N-enriched (+2.83‰) after their stay in TWP where they likely preyed on fish. A similar pattern was observed for wild juvenile pike, although sample size was small. Taken together, the results indicate that ontogenetic shifts in habitat use by juvenile pike and their trophic position in the food web were tightly associated, and appeared to be size dependant. The study shows that multiple SIA on same individually tagged organisms in concert with mark-recapture experiments can provide a mechanistic approach to understand life history variants at the population level and may therefore open a rich area of research.

FROM INDIVIDUALS TO COMMUNITIES I: Individual based studies

Resource segregation and trophic specialisation in the *Coregonus lavaretus* species complex: stable isotopes, shape and stomach contents

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Evolutionary ecologists often rely on short-term indicators of diet *e.g.* stomach content analysis (SCA) to infer long-term niche segregation between closely-related taxa thought to be undergoing speciation[1]. We are interested in the potential of stable isotope analysis (SIA) to provide a measure of long-term resource use for workers in this fast-moving field, and are applying this approach to our work on the evolution of trophic polymorphism in European whitefish. In northern Scandinavia, whitefish populations originated from a single evolutionary lineage[2], but following post-glacial colonisation, rapidly diverged (over *ca.* 10 000 years) into different forms or morphs with distinct morphology (*e.g.* gill raker counts) and ecology[2, 3]. Lake fish communities in this region are typically dominated by a single whitefish morph (LSR) - in some lakes the LSR morph is found in sympatry with a small-bodied planktivorous morph (DR), and some large/deep lakes include an additional, profundal morph (SSR).

Previous studies using SCA have demonstrated that whitefish morphs segregate food resources in sympatry[3]. However, as SCA only provides a dietary snapshot, we were interested in the potential of SIA to reveal long-term foraging patterns, resource segregation between morphs, and whether morphological traits could be related to trophic ecology. Using a comprehensive sampling scheme, we collected whitefish from subarctic lakes in the north of Finland: 2 lakes supported 3 sympatric morphs (LSR, DR & SSR); 1 supported 2 sympatric morphs (DR & LSR) and in 2 lakes a single allopatric morph (LSR) was found. We used a combination of SIA ($\delta^{13}C$ & $\delta^{15}N$), SCA and geometric morphometrics (GM) to examine how sympatric morphs partioned resources (SIA & SCA) and differed in shape (GM), and how these patterns contrasted with monomorphic populations. GM demonstrated that morphs differed in shape, whilst short- (GCA) and long-term (SIA) measures of diet revealed that morphological differences were reliably associated with specialisation on food resources from different habitats (LSR = littoral, DR = pelagic & SSR = profundal). We then compared SIA, GM and SCA data across lakes for morphs found only in sympatry, or in sympatry and allopatry. Shape and diet overlapped in those morphs found only in sympatry, suggesting common relationships between morphology and diet specialisation. Allopatric morphs were generally similar in mono and polymorphic lakes, but SIA data suggested evidence of ecological release in one lake.

This study highlights the utility of SIA derived data to studies examining the role of ecological specialisation in the evolution of closely related taxa. We suggest that evolutionary ecologists should join the diverse set of scientists who routinely utilise stable isotope analyses in their research.

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FROM INDIVIDUALS TO COMMUNITIES I: Individual based studies

Microgeographic variation in isotopic composition of a rattlesnake prey base: implications for studies using stable isotopes as dietary indicators

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Variation in stable isotope ratios has been used to quantify trophic relationships in many communities. My long term research goal is to test the validity of the stable isotope approach for determining differences in pigmy rattlesnake (*Sistrurus miliarius*) diet composition among three Florida populations located less than 4km apart. To use stable isotopes as dietary indicators, prey species must have distinct isotope profiles. In order to evaluate isotopic variation in the rattlesnake prey base, I used drift fence arrays to collect a representative sample of prey items from each population. I collected 992 prey items, representing 10 amphibian, 8 reptile and 7 mammal species. For each species, I combined captures from all three populations and generated site-wide average $\delta^{13}C$ and $\delta^{15}N$ values. Site-wide averages were variable ($\delta^{13}C$ values ranged from -31.1 to -14.5 and $\delta^{15}N$ values ranged from 0.3 to 7.0) and showed considerable overlap. Taxonomy was not the best predictor of isotopic similarity among prey species. *Hyla squirrella* (Squirrel treefrogs) had the most depleted $\delta^{13}C$ values, while its congener *Hyla cinerea* (Green treefrogs) had among the most enriched $\delta^{13}C$ values. Conversely, congeners *Anolis carolinensis* and *Anolis sagrei* showed two of the most similar isotopic compositions. Generating site-wide averages was not the appropriate scale for my investigation; however, it illustrated pitfalls associated with the traditional isotope approach to studying diet. For example, there was no a priori reason to expect prey species that occurred in all three populations to look isotopically similar. *Rana utricularia* (leopard frogs) were a major prey item available in each population. Leopard frog $\delta^{13}C$ values showed significant variation among populations. In addition, ontogenetic shifts in leopard frog isotopic composition varied by isotope and population. Assessment of rattlesnake diets using stable isotopes requires generating population-specific and size-specific prey profile plots that incorporate ontogenetic shifts in isotope ratios.

FROM INDIVIDUALS TO COMMUNITIES I: Individual based studies

Daisy, what did you eat when we weren't looking?

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Over the last ten years or so, there has been increasing interest in high-resolution, time-resolved stable isotope records in animal tissues such as teeth, hair or hooves. Controlled feeding experiments are instrumental in improving our interpretation of such records in domestic and wild animals as well as fossils. Diet-switch studies provide information on the mechanisms and timing of isotope incorporation into tissues, allowing us to reconstruct the individual dietary history of modern and fossil animals. However, complex experiments can sometimes have unexpected outcomes.

Here we report on a large-scale experiment designed to investigate the dynamics of C and N isotope changes recorded in bovine hair and hooves resulting from a diet-switch over a 24 week period. Post-mortem high-resolution isotopic profiles in hair and hooves of nine individual cattle revealed that at least two unplanned dietary shifts in C and N occurred several weeks after the cattle had been switched to an experimental, isotopically distinct diet.

Using our isotopic data, we calculated the growth rates of hair and hooves from each individual and determined the timing of the two unexpected diet switches. Applying a modelling approach similar to that developed for horses (Ayliffe et al. 2004; Cerling et al. 2004), we were able to calculate the isotope value of the questionable feed and reconstruct precisely the individual dietary history of the nine cattle. This study demonstrates very convincingly the power of stable isotope time series in hair and hooves as forensic tracers of dietary history. Even though we were not watching Daisy all the time, we now know what she was up to.

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FROM INDIVIDUALS TO COMMUNITIES I: Individual based studies

Foraging ecology of invasive American mink during an eradication campaign in the Outer Hebrides, Scotland

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The American mink *Mustela vison* is a small mammalian carnivore. The species is a widespread and damaging invasive alien species in western Europe, and has had a major ecological impact as a predator of native wildlife. Populations of mink became established in the Outer Hebrides (Scotland) after escapes from fur farms. On these remote islands, mink have had a grave impact on a variety of island wildlife, particularly internationally important populations of breeding shore birds. Mink living on several major islands of the Outer Hebrides have been subject to a concerted eradication campaign since 2001. The mink live in a variety of habitats from small coastal islets, to extensive bog and marginal farmland. Conventional dietary studies offer some insight into foraging, based on instantaneous sampling of diet from gut contents. Using stable isotope approaches, we characterised the foraging ecology of the species in its non-native range and identified patterns of coastal and inland foraging, that varied according to sex, season and a range of other ecological parameters. Our results offer an insight into the evolutionary ecology and adaptation of an established invasive mammal. From an applied perspective, we describe the effect of culling on foraging ecology and provide advice with respect to the management of problem species.

FROM INDIVIDUALS TO COMMUNITIES I: Individual based studies

Testing Mechanisms for the Evolution of Dietary Specialization: Isotopic Analysis of Sea Otters (*Enhydra lutris*)

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Individual dietary specialization has been documented for nearly 100 species distributed across a broad range of taxonomic groups. The large majority of these studies, however, have not been able to accurately quantify the degree to which individuals are specialized relative to their population. Furthermore, while theoretical approaches have identified potential mechanisms responsible for dietary specialization, few studies have examined this topic across the spatial and temporal scales necessary to identify the ecological conditions that favour the evolution and maintenance of individual specialists.

We are using stable isotope analysis to explore the degree of dietary specialization within and among sea otter (*Enhydra lutris*) populations in the North Pacific Ocean. Sea otters offer a unique opportunity for testing specialization mechanisms because recent fluxes in population sizes and the recolonization of previously occupied habitat mean that otters and their prey coexist at different densities in various areas. In addition, sea otters are known to consume a wide variety of prey items that range across multiple trophic levels and habitats. Not surprisingly, $\delta^{13}C$ values of common prey items range from –17‰ to –10‰ and $\delta^{15}N$ values range from 7‰ to 15%, with significant separation among ecologically distinct prey types.

Our approach utilizes carbon and nitrogen isotope values from two sea otter tissues, bulk bone collagen and keratin (i.e., serial sampling of whiskers), to characterize inter- and intra-individual diet variability between populations on a sub-annual to multi-seasonal basis. This strategy allows us to test the degree of individual dietary specialization over a range of temporal scales and provide a substrate for comparison of modern populations to individuals from historic and/or archaeological contexts. Lastly, an examination of isotopic data from individuals whose dietary preferences have been characterized through extensive observational studies provides an important comparison of isotopic proxies to more traditional techniques used to assess foraging behaviour.

FROM INDIVIDUALS TO COMMUNITIES I: Individual based studies

Stable Isotope Evidence of Sex-specific Differences in Manatee Diets

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Florida manatees (*Trichechus manatus latirostris*) are large aquatic herbivores that are generally considered to be indiscriminate foragers of aquatic plants. Extensive field observations and examination of stomach contents has found no evidence of sex-specific differences in foraging habits for this species (Ledder 1986), but recent telemetry data has documented differences in habitat selection and foraging distances traveled by male and female manatees during the year (Deutsch et al. 2003). Males typically travel extensively during the spring and summer, while females restrict their movements to small areas during these seasons before migrating to warm water refugia in the fall. By traveling over larger areas than females during the warmer months, male manatees may encounter and forage on a wider variety of aquatic vegetation than females. We tested this hypothesis by examining the carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope composition of two tissue types from male and female manatees: metabolically active tissues with different turnover rates (liver, muscle, collagen); and metabolically inactive tissues that are continuously accreted without turnover (vibrissae). Examination of isotope values from these two tissue types allowed us to compare dietary differences between male and female manatees at both long term (months to years) and short term (weeks) time scales.

We collected tissue samples from thirteen individuals (6 males, 7 females) from the Indian River Lagoon (IRL) and seven individuals (6 males, 1 female) from freshwater rivers and lakes in eastern and central Florida. Mean isotope values in metabolically active tissues were significantly different between male and female manatees from the IRL with males typically yielding lower $\delta^{13}C$ and higher $\delta^{15}N$ values than females. This pattern was also observed within the weekly isotope records of the sub-sampled vibrissae. Again, we found the same difference in mean isotope values between the sexes, but also discovered that the variability within a single vibrissa was typically greater for males than for females; the range in $\delta^{13}C$ and $\delta^{15}N$ values for most females was less than 1.0‰, but was up to 4.0‰ for male manatees collected in the summer and fall. Manatees collected from freshwater habitats were found to have significantly lower $\delta^{13}C$ values and higher $\delta^{15}N$ values than either sex from the IRL. Since male manatees from the IRL have isotope values that approach those of freshwater individuals, tissue isotope values indicate that these males are likely incorporating more freshwater plants into their diets than are females. Our results suggest significant sex-specific differences in manatee diets, but that most of this difference can be accounted for by changes in the diets of male manatees during the summer and fall seasons. These findings show that the foraging strategies of manatees may be more complicated than previously thought and illustrate how the stable isotope analysis of multiple tissues can offer a more detailed record of manatee diets.

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FROM INDIVIDUALS TO COMMUNITIES I: Individual based studies

The stable isotope composition of the north Pacific and Arctic Ocean sea-scape: a closer look at the habitat of the migratory bowhead whale (*Balaena mysticetus*)

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Stable isotope analyses have been used in many different ecological applications to trace nutrient transfer, source location, and migratory behaviors of consumers. Much of this original research has focused on the use of the elements carbon, nitrogen, and sulphur. Given the more negative oxygen ($\delta^{18}O$) and hydrogen (δD) values for freshwater sources (e.g. Mackenzie River = ~ -19‰, ~ -150‰, respectively) versus the fairly constant $\delta^{18}O$ and δD values for ocean water (Standard Mean Ocean Water = ~ 0‰ for both $\delta^{18}O$ and δD), we examined whether stable oxygen and hydrogen isotope analysis may further enhance studies of marine food web dynamics and whale migratory behavior. To investigate these possibilities, we measured the $\delta^{18}O$ and δD in water and multiple zooplankton species along the migratory track (e.g. Bering Sea to Mackenzie River outflow basin) of the western arctic bowhead whale (*Balaena mysticetus*) to examine the isotopic variation in typical prey items. To complete the trophic perspective, baleen from six Alaskan *B. mysticetus* were sampled at 2 - 5cm intervals and analyzed for their stable oxygen and hydrogen isotope composition. Over all zooplankton samples, there was a wide range in the values observed ($\delta^{18}O$ = -13‰ to 56‰; δD = - 220‰ to -75‰) but species-specific separation yielded closely paired patterns with regional water values, and there was a clear regional separation in the zooplankton between the winter (Bering Sea) and summer (eastern Beaufort Sea region) habitat of the whales. Baleen samples not only confirmed the seasonal annual migration of the bowhead, but appeared to reflect the diet consumed in these two isotopically distinct regions. The results of this study confirm that oxygen and hydrogen isotope analyses promises to be an effective tool for ecological studies of marine systems at all trophic levels, from establishing fractionation factors for marine plankton to providing accurate resolution for tracking shifts in long-term whale migration patterns due to environmental change.

SOIL-PLANT/SOIL-MICROBE INTERACTIONS

The Fate of Proteinaceous Material in Soil

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Nitrogen is vital to all life and is often a limiting factor in the rate of primary biomass production. The availability of biologically accessible forms of nitrogen can control the diversity, dynamics and functioning of many ecosystems including soils [1]. Although the cycling and inter-conversions between inorganic forms of nitrogen in soils are relatively well understood, much less is known about the fate of organic nitrogen containing compounds, especially at a compound-specific level. Every year in the UK, 45 million tonnes of manure are deposited directly onto fields by livestock in addition to 67 million tonnes collected from farm buildings and yards to be spread on fields. A considerable proportion of the organic nitrogen applied to soil in this way is proteinaceous, hence it is important to develop a molecular understanding of the fate of both the nitrogen from proteins and amino acids and the associated carbon once in the soil. We wish to measure the relative rates of mineralization or assimilation of amino acid nitrogen by soil microorganisms, as well as characterising the products of these processes and the organisms responsible for carrying out these transformations. This study aims to answer these questions by following the fate of stable isotopically labelled amino acids in soil.

Uniformly ^{15}N- and ^{13}C-labelled amino acids were added to grassland soils held in small lysimeters, which were incubated for time periods of between 1 and 64 days. Gas chromatography/combustion/isotope ratio mass spectrometry (GC/C/IRMS) was used to follow the loss of ^{13}C and ^{15}N from these labelled amino acids and incorporation into other amino acids. These data provide an insight into the rates at which the nitrogen (and carbon) from amino acids is assimilated by microorganisms and used in the biosynthesis of new amino acids. Similarly, compound specific δ^{13}C and d^{15}N values for amino sugars enable microbial or fungal action to be monitored, whilst the incorporation of ^{13}C into phospholipid fatty acids will allow characterisation of the soil microorganisms using amino acids as a carbon source.

This work forms part of a wider study which will ultimately lead to incubation experiments in which the fate of organic nitrogen containing compounds from uniformly dual (^{13}C and ^{15}N) labelled animal wastes will be investigated in intact soil cores.

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SOIL-PLANT/SOIL-MICROBE INTERACTIONS

Modification of lipid distribution patterns and isotopic ($\delta^{13}C$) composition in plants and turnover of lipids in corresponding soils under enhanced CO_2 (FACE) conditions

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Lipids are assumed to represent a relatively stable fraction of soil organic matter (SOM) [1]. In contrast to macromolecular SOM-fractions these compounds are suitable for detailed structural and compound-specific $\delta^{13}C$-isotope characterization. Alternatively to the standard approach for assessment of SOM turnover rates using natural isotopic labelling after monoculture crop switching, carbon enrichment experiments (FACE) facilitate turnover estimations of bulk SOC and organic substances as a result of labelling with ^{13}C-depleted CO_2. In this study, we applied isotopic and biomarker analysis to obtain information on the development of lipids in plants and turnover into SOM.

Surface soil and plant samples derive from the Eschikon FACE experiment after 10 years of parallel treatment with i) natural CO_2 concentrations, and ii) an enhanced CO_2 concentration of 600ppm. Samples were taken of both experiments with *Lolium perenne* and *Trifolium repens*. Lipids were recovered by accelerated solvent extraction and separated into eight fractions by automated liquid chromatography [2]. Fractions of aliphatic hydrocarbons and carboxylic acids were analysed by GC-MS and GC-irmMS.

Turnover of bulk carbon seems virtually identical for both experiments with an replacement of 30% by new carbon as estimated from bulk organic carbon isotopic ($\delta^{13}C$) differences between ambient and elevated CO_2 plots. This turnover is in good agreement with literature data from standard natural isotopic labelling experiments.

In contrast to bulk carbon, lipid turnover shows large varieties between both, different lipid fractions (alkanes and carboxylic acids), and different plantations. Long-chain plant-derived carboxylic acids turn over significantly faster than bulk organic carbon, which is in perfect agreement with literature data [3]. Contrastingly, long-chain plant-derived *n*-alkanes reveal significant lower new carbon proportions and hence slower turnover times than the bulk soil organic carbon of the FACE experiment and alkanes of agricultural trials with natural ^{13}C-labelling [3]. This might be due to a combination of three effects: i) the plant-internal translocation rate of alkanes from aboveground biomass to SOC is lower for the plants of the FACE experiment than for typical agricultural crops with larger biomass production rates, ii) alkanes in the soils can be derived from other sources like e.g. incorporation of fossil carbon, and iii) degradation processes in the no-till soil are slower than in well aerated agricultural soils.

The FACE experiments gave new insights into the behaviour of plant lipids under elevated atmospheric CO_2 conditions, which are to be expected within the next decades.

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SOIL-PLANT/SOIL-MICROBE INTERACTIONS

Isotopomer Studies of Soil-Derived Nitrous Oxide: Evaluation of Microbial Origins and Importance of Nitrous Oxide Consumption

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Nitrous oxide contributes to 6% of the warming effect on global climate and agricultural activities are the primary anthropogenic source of this trace gas. We demonstrate that Site Preference (SP), the difference in $\delta^{15}N$ between the central (α) and outer (β) nitrogen atoms in N_2O, is a powerful approach for apportioning fluxes of N_2O from soils to nitrification and denitrification sources. Within a never-tilled soil, experiencing cultivation for 3 consecutive years, we observed markedly higher N_2O fluxes than have previously been reported for agricultural and early successional soils. This observation substantiates early findings of large fluxes of N_2O from soils following initial cultivation. Values of SP for soil-derived N_2O varied between -0.1 and 12.9 ‰ indicated that the majority of N_2O produced was from denitrification (60.9 to 100%). As this sampling occurred during the period of the highest flux over a three year period we conclude that the release of N_2O upon the initial cultivation of native soil is predominantly from denitrification. Our determinations of the isotopic composition of soil-derived N_2O upon initial cultivation may provide a basis to recognize the importance of the initial clearing and tilling of soils to historical records; notably ice core records.

A critical aspect of the use of isotopomer data to apportion sources of N_2O to nitrification and denitrification is the need to correct data for isotope shifts that may have occurred during consumption of N_2O in soils prior to its escape to the atmosphere. The challenge of such a correction is that isotopic enrichment factors for biogeochemical processes tend to be quite variable and our results for N_2O consumption in soil mesocosm and pure culture experiments substantiate this finding. However, we have found that N_2O consumption produces consistent relationships between $\delta^{18}O$ and $\delta^{15}N$ and $\delta^{18}O$ and $\delta^{15}N^{\alpha}$ of 2.7 and 1.6, respectively, which are clearly diagnostic of this process. Based on the values of production rates and isotopomer values of soil-derived N_2O we model the isotope effects of simultaneous production and consumption and find increasingly curvilinear relationships result with increased consumption. Consequently, a deviation from the linear mixing relationship between soil-derived and atmospheric N_2O is an indication of extensive consumption. We find, however, that the ^{15}N and ^{18}O depleted values of soil-derived N_2O in our study and others and the linear mixing relationships between isotopomer data are strong indications that the impacts of consumption on field isotope data are minor. Furthermore, at a level of consumption 10% of that of production, we find only minor isotope shifts that would impact the SP estimate of N_2O from denitrification by only a few percent. Consequently, we conclude that while N_2O consumption is undoubtedly an important process in soils it is not likely an important process during periods of high flux and we now have a definitive means for identifying and potentially correcting for isotope shifts resulting from this process.

SOIL-PLANT/SOIL-MICROBE INTERACTIONS

Methane emissions and microbial activity in a wetland grassland

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Tadham Moor is a managed wetland in the Somerset Levels, UK, which is flooded for the period Nov-March every year. Our project aimed to examine greenhouse gas emissions from Tadham under contrasting hydrological regimes (dry, flooded, post flooded), to identify drivers of soil methane (CH_4) fluxes, and to examine responses of microbial populations and activity to hydrology. Additionally, a nitrogen fertiliser addition experiment carried out at Tadham 8 years previously gave us the opportunity to examine the effects of N additions on greenhouse gas fluxes and microbial populations.

A mobile laboratory consisting of instruments to measure CH_4 fluxes was used in 3 field campaigns to establish greenhouse gas fluxes at the site. Soils were a net source of CH_4 in the summer, but net sinks of CH_4 during- and post- flooding. To examine the effect of hydrology on the soil microbial community, microbial phospholipid fatty acids (PLFAs) were extracted from soils taken from Tadham. Whilst water table had no effect on PLFA abundance and composition, historic additions of N fertiliser caused increases in Gram negative bacteria that persisted 8 years after the end of the N additions.

Soil cores from Tadham were subjected to a long-term water table hydrology manipulation experiment in which cores were either waterlogged (water table 0 cm), or had a water table at 10 or 20 cm below the surface. After 3 months, waterlogged cores were a strong source of CH_4 (mean flux 35 mg CH_4-C m^{-2} hr^{-1}), whilst cores with water tables at 10 or 20 cm were a weak source (2 mg CH_4-C m^{-2} hr^{-1}) or sink (-0.5 mg CH_4-C m^{-2} hr^{-1}) respectively.

Soils from the manipulation experiment were incubated with $^{13}CH_4$ at two concentrations (40 & 400 ppm) for 7 days, after which microbial PLFAs were extracted and analysed for composition and ^{13}C content in four different soil depths: 0-5, 5-10, 10-15 & 15-20 cm. Whilst there was no difference in PLFA abundance and compositions between the water table treatments, PLFA $\delta^{13}C$ values demonstrated differing incorporation of ^{13}C-CH_4 down the soil profile and between water table levels, with significantly greater CH_4 oxidising activity in oxic zones compared to waterlogged anoxic layers.

Methanotrophic bacteria are categorised as high or low affinity based on their ability to consume low and high concentrations of CH_4, respectively, and Type I and Type II based on their physiology. Type I and II methanotrophs contain C_{16} and C_{18} chain PLFAs, respectively. Determinations of the PLFAs into which the ^{13}C-CH_4 label was incorporated showed that both high and low affinity methanotrophs were active in Tadham soils, although low affinity methanotrophs were more active in the upper soil layers relative to the lower layers. Additionally, since C_{18} PLFAs were labelled with ^{13}C at low concentrations of CH_4, and greater proportions of ^{13}C label were identified in C_{16} PLFAs at high concentrations of CH_4, we identify Type II organisms with high affinity methanotrophs, and Type I with low affinity methanotrophs.

Our results are consistent with Tadham soils being a net source of CH_4 when the water table is in the upper 10 cm of the soil profile. The ability of methanotrophic bacteria to consume CH_4 is significantly affected by water table depth, and when the water table is below 10 cm all methane which is produced in the lower waterlogged soils is consumed by active methanotrophic bacteria in the soil above.

PLENARY

Carbon isotope discrimination by Rubisco and diffusion in leaves: applications to plant water-use efficiency and finding a gene

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The carbon isotope ratio ($^{13}C/^{12}C$) of organic material is less than that of carbon dioxide in the atmosphere. That is because of fractionation by the primary carboxylating enzyme, Rubisco[1], an exciting area of research revealing evolutionary pressures at the molecular level[2]. The Rubisco effects are modified by diffusion limitations in the transport of CO_2 from the atmosphere to that enzyme[3]. The latter principles have been understood for a long time[4] and have been exploited to examine genetic variation in the ratio of carbon gain and water loss (water-use efficiency)[5]. A practical outcome has been the release of wheat varieties with improved water-use efficiency[6]. Carbon isotope discrimination has been used to phenotype recombinant inbred lines of Arabidopsis and to identify a gene modifying water-use efficiency [7].

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GAS EXCHANGE AND WATER RELATIONS IN PLANTS I

Carbon and oxygen isotopes: a tool to analyze the fluxes of CO_2 and H_2O between plants and atmosphere

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In recent years, the use of oxygen isotopes has became an useful tool for obtaining information on the global carbon cycle, thanks to alteration of the oxygen isotope composition ($\delta^{18}O$) of atmospheric CO_2, due to exchanges of oxygen atoms between air CO_2 and water in leaves. When predicting atmospheric $\delta^{18}O$ with existing models, complications arise due to uncertainties associated with:

i) leaf water heterogeneity
ii) calculation of the CO_2 concentrations at the site of CO_2-H_2O equilibrium
iii) rate of catalytic activity of enzyme carbonic anhydrase (CA)

This research aimed to investigate the applicability of the Péclet model (ΔL = ^{18}O enrichment of leaf water compared to source water) and the extent to which the evaporative enrichment of oxygen isotopes in leaf water is reflected in the oxygen isotope ratio of CO_2 passing over the leaf in a wide range of environmental conditions, while leaf gas exchange is at steady-state.

The experiment was carried out on cotton plants grown from seeds in a temperature and humidity controlled glasshouse (ANU, Canberra, Australia). Environmental conditions (vapour pressure deficit, leaf temperature, light intensity and oxygen composition) were altered to induce large variation in photosynthetic activity (cc/ca = ratio of chloroplast and ambient CO_2) and evaporative conditions (ea/ei = ratio of ambient and intercellular vapour pressure) and therefore different values of Δe ($\Delta^{18}O$ of H_2O at the evaporating site) and Δc ($\Delta^{18}O$ of chloroplast CO_2) respectively.

The results indicate that Péclet model can predict ΔL more accurately than the Craig-Gordon model. We obtained the reasonable L value, an important parameter in the Péclet model, compared to those of the previous reports when we removed the unenriched vein water. The L was not influenced by the change in the environmental conditions. The results showed that δc was highly significantly correlated with δe. The proportional oxygen isotope equilibrium between CO_2 and chloroplast water was calculated to be very near unity at leaf temperatures of 29°C, and approximately 0.8 at leaf temperatures of 20°C. This large discrepancy confirmed that leaf temperature has a large impact on CA activity and as a consequence on the equilibrium of CO_2 with water inside the leaf. Such differences should be taken into consideration in carbon balance model at the ecosystem level.

GAS EXCHANGE AND WATER RELATIONS IN PLANTS I

The fate of carbon in a mature deciduous forest exposed to elevated CO_2

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A crane and a specially designed free air CO_2-enrichment system permitted to label the canopy of a mature deciduous forest with ^{13}C depleted CO_2. Thus, the carbon flow was traced continuously for four years through the forest without disturbance.

Potted C_4 grasses in the canopy ('isometers') served as a reference for the stable carbon isotope label applied. After four growing seasons, leaves were completely labelled, while newly formed wood (tree rings) still contained approximately 10% unlabelled carbon. Distinct new carbon labels were found in fine-roots (39%) and sporocarps of mycorrhizal fungi (62%). Soil particles attached to fine-roots contained 8% new carbon, whereas no measurable signal was detected in bulk soil. Soil-air CO_2 consisted of 35% new carbon indicating that considerable amounts of assimilates were rapidly respired to the atmosphere.

These data illustrate a pronounced allocation of very recent assimilates to carbon pools of short residence times.

GAS EXCHANGE AND WATER RELATIONS IN PLANTS I

Tracing C fluxes to the soil and atmosphere, through leaf litter decomposition in a poplar plantation by means of stable C isotopes

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Litter decomposition is one of the major processes determining C fluxes between terrestrial biosphere and atmosphere and C stabilization into SOM. Nonetheless, factors influencing C fractions lost by decomposing litter are still not well known. Positive mutual feedbacks were found between leaf litter and rhizosphere respiration (Subke, Hahn et al. 2004). In order to test the hypothesis that an enhanced rhizosphere activity can also influence the fractions of C lost by decomposing litter, a field experiment was performed at the experimental POPFACE site of Tuscania (Viterbo, Italy). In this site the rhizosphere activity was found to be stimulated by the enrichment in atmospheric CO_2 concentration (Lukac, Calfapietra et al. 2003) as well as litter decomposition was higher in CO_2 enriched plots (Cotrufo, De Angelis et al. 2005). Our experiment is based on the substitution of the original leaf litter of *P. nigra* with strongly ^{13}C enriched ($\delta^{13}C$ ~ +160 ‰) leaf litter of the same species. During the one year of field incubation soil CO_2 efflux and its isotopic composition was measured at monthly intervals. A two source mixing model was applied to quantify the litter contribution to total soil respiration, as well as, at harvest, to quantify the litter-derived C input to SOM. The variation in the isotopic composition of different soil layers (0-2, 2-5, 5-10, 10-20 cm) and the fractions of litter derived C for each soil layer will be shown. The effects of N fertilization and postFACE on the above processes will be discussed.

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RIPARIAN ECOLOGY

Multiple stable isotopes reveal organic matter and mercury flow in a temperate river

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Little is known about the relative importance of exogenous versus endogenous carbon in riverine food webs and its effects on the trophic transfer of contaminants. Changes in the type of organic matter used by consumers along longitudinal gradients in rivers may influence their concentrations of contaminants such as mercury that are known to accumulate up the food chain. The presumed dominance of terrestrial inputs in small headwater streams and shift to aquatic production in downstream reaches may have consequences for mercury exposure if concentrations of this contaminant differ between the two food sources. To assess this, we measured $\delta^{13}C$, $\delta^{15}N$, δD and mercury concentrations in biofilm, leaf litter, predatory invertebrates and a minnow species (blacknose dace) in the Renous River, New Brunswick, Canada in 2005. This 5th order river is in a relatively undisturbed and forested catchment and is far removed from mercury point sources in the province.

The $\delta^{13}C$ signatures of aquatic and terrestrial primary producers showed considerable overlap at upstream sites but were distinct at downstream sites; mixing models for these downstream sites showed a wide range in % aquatic carbon in the diet of invertebrates and fishes (2-100%). We found a significant positive relationship between mercury concentrations and the % aquatic carbon in the diet for predatory aquatic invertebrates (water striders, stoneflies, dragonflies), suggesting that in-stream primary production may be a higher source of mercury than terrestrial carbon for these organisms. Blacknose dace, with a high proportion of aquatic carbon in the diet (91-100%), had elevated mercury concentrations (1.0 to 2.5 ppm dry weight) that were related to their higher trophic position as measured by $\delta^{15}N$. Analysis of δD will be used to better discriminate between organic matter sources at upstream sites where carbon signatures overlapped.

These measurements allow us to assess mercury and organic matter flow in pristine systems. Our goal is to later compare with systems that are influenced by local mercury deposition or land-use activities. Better information on mercury in both pristine and human-altered systems will help guide policy makers in developing proper fish consumption advisories.

RIPARIAN ECOLOGY

Identifying N inputs in river food webs: can δ15N be used when streams are heavily impacted by agriculture?

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There is a large body of evidence that N-inputs from agricultural practices result in profound changes to freshwater ecosystem structure and function including eutrophication, hypoxia and biodiversity loss. Stable nitrogen isotope ratios (δ15N) have been used to trace agriculture-derived N in river food webs, since δ15N values of inorganic N from animal waste and synthetic fertilizers are respectively high (+8‰ to + 26‰) and low (-4‰ to +4‰) compared with natural N sources. However, recent advances in our understanding of allochthonous N uptake into riverine food webs are mostly based on studies of streams where N concentration is low to moderate (i.e. < 5 mg.l-1).

Streams in Brittany (France) most frequently drain agricultural watersheds where livestock production (pigs, cattle, poultry) is intensive and animal wastes are spread on the land. We initiated a program in 2002-2003 designed to determine if δ15N can be used to trace allochthonous sources of N in the food web of the Scorff River, where dissolved NO3 concentrations varied monthly from 20 to 35 mg.l-1. Macrophytes, invertebrates (arthropod primary consumers from different functional feeding groups and predatory invertebrates) and fish (non lethal fin-clips of insectivorous taxa) were collected from 5 sites in headwaters including a tributary exposed to manure spreading, 6 sites in the lower reach including the vicinity of the effluent from a fish hatchery, and 1 site in a small forested tributary.

Within-site δ15N values (Δδ15N) showed high variability among macrophyte species (2.05 to 7.8‰). The high variations in δ15N were measured among functional groups of primary consumers (0.17 to 4.0‰) in the manure-influenced and wastewater-exposed sites below the hatchery, suggesting between-group differences in the incorporation of either organic or inorganic allochthonous N. Mean δ15N values of primary consumers (all functional groups pooled) differed among sites and were significantly correlated with δ15N of predatory invertebrates (average fractionation factor +2‰). In contrast, δ15N of insectivorous fish was weakly correlated to δ15N of primary consumers, but the highest δ15N values in fish samples (up to 18.4‰) were observed in the most heavily impacted sites. It is suggested that δ15N in fin tissues of non-migratory insectivorous fish is a good tracer of anthropogenic N contributions to riverine food webs highly influenced by agricultural activities.

RIPARIAN ECOLOGY

Stable nitrogen isotope ratios of macrophytes and periphyton along a nitrate gradient in a subtropical coastal river

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An increase in human population and associated changes in land use have affected an increase in groundwater nitrate concentrations throughout central Florida. Within the region, this nitrate-laden groundwater returns to the surface via numerous large springs that serve as the origin of flow for many coastal streams and rivers. These rivers can exhibit strong nitrate gradients due to the high nutrient uptake potential by dense stands of macrophytes. We hypothesized that downstream declines in nitrate concentrations would be manifested spatially as increases in the $\delta^{15}N$ of the residual pool of nitrate, submersed aquatic vegetation and periphyton as a consequence of isotopic fractionation associated with preferential uptake and assimilation of $^{14}NO_3^-$. This hypothesis was tested in two spring-fed river systems, i.e. the Chassahowitzka and Homosassa rivers, along Florida's central Gulf of Mexico coast. In general, $\delta^{15}N$ values of nitrate, submersed aquatic vegetation and periphyton increased with decreasing fraction of nitrate remaining in each of the two study systems. The fractionation associated with nitrate uptake by macrophytes and associated periphyton was determined from the relationship between $\delta^{15}N$ of both constituents of the submersed aquatic vegetation community and the fraction of nitrate removed from the system. Values for macrophytes and periphyton ranged from 0.4‰ to 2.4‰ and from 1.3‰ to 3.3‰, respectively. These are the first such values reported for photoautotrophs in flowing waters.

RIPARIAN ECOLOGY

Changes in carbon and nitrogen stable isotope ratios of periphyton exposed to landfill leachate

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Periphyton was allowed to grow on glass tiles placed in the water column of a small, landfill-associated stream to investigate the influence of landfill leachate contamination on carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope signatures of periphyton. Samples of periphyton were collected from upstream and downstream of a closed landfill known to be leaking leachate into the stream. Isotope signatures of dissolved inorganic species ($\delta^{15}N\text{-}NO_3^-$, $\delta^{15}N\text{-}NH_4^+$ and $\delta^{13}C$-DIC) within samples of stream water and leachate were also measured.

Upstream periphyton had enriched $\delta^{13}C$ and $\delta^{15}N$ values (average $\delta^{13}C$ = -29.5 ± 2.0 ‰; average $\delta^{15}N$ = 7.2 ± 0.9 ‰) relative to downstream periphyton (average $\delta^{13}C$ = -33.0 ± 1.5 ‰; average $\delta^{15}N$ = -7.0 ± 0.6 ‰). Downstream $\delta^{13}C$-DIC (3.7 ± 0.3 ‰) indicates a mixture of background DIC (upstream $\delta^{13}C$-DIC = -11.6 ± 0.01 ‰) and more enriched leachate DIC ($\delta^{13}C$-DIC = 4.58 ± 0.04 ‰). Alkalinity data for stream and leachate samples further support the likelihood of a leachate contribution, with an upstream value of 52 mg/L, a downstream value of 119 mg/L, and leachate alkalinity of 790 mg/L. The $\delta^{13}C$ value of downstream periphyton is indicative of uptake of dissolved CO_2, whereas uptake of HCO_3^- would typically result in a more enriched $\delta^{13}C$ signature. Therefore, although leachate may be contributing to the downstream DIC pool, isotope results suggest that periphyton is not using HCO_3^- derived from leachate as a carbon source.

The highly significant difference between upstream and downstream $\delta^{15}N$ values (14.2 ‰; $p<0.001$) suggests changes in the nitrogen source pool between upstream and downstream locations and/or significant fractionation during uptake and assimilation by periphyton located downstream. Indeed, stream water levels of ammonium and nitrate upstream of the landfill (0.02 mg $N\text{-}NH_4^+$/L and 1.39 mg $N\text{-}NO_3^-$/L) are considerably different from downstream levels (1.9 mg $N\text{-}NH_4^+$/L and 1.05 mg $N\text{-}NO_3^-$/L). The downstream increase in ammonium results from the input of leachate, which is characterised by high ammonium concentrations (22 mg $N\text{-}NH_4^+$/L). Stream $\delta^{15}N\text{-}NO_3^-$ values are comparable between upstream and downstream water samples (5.2 ± 0.8 ‰ and 4.4 ± 1.4 ‰ respectively). However, downstream $\delta^{15}N\text{-}NH_4^+$ values (7.9 ± 0.9 ‰) were enriched relative to $\delta^{15}N\text{-}NH_4^+$ values of leachate (5.9 ± 1.2 ‰), which suggests leachate-derived ammonium may be the primary nitrogen source for the periphyton and that significant discrimination against the heavier isotope occurs during NH_4^+ uptake and assimilation. These results are consistent with previous studies that have demonstrated $\delta^{15}N$ depletion in algae exposed to high concentrations of ammonium (e.g. Cornelisen *et al*., in revision). The results of the current study suggest that the landfill leaks sufficient quantities of ammonium into the stream to alter the nitrogen source pool and in turn the $\delta^{15}N$ signature of periphyton.

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RIPARIAN ECOLOGY

The hidden information in the isotopic and stoichiometric composition of stream biofilms

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Biofilms comprise algal species, decomposing particulate organic matter, bacteria and fungi within a polysaccharide matrix, and characteristically grow on solid substrates. Within this matrix, autotrophic algae excrete photosynthetically-derived nutrients, and the bacteria and fungi are essential to the breakdown of allochthonous detritus[1]. This consortium may contribute significantly to lotic carbon cycling. In addition, biofilms contribute to water quality remediation and purification. The adsorption properties of the polysaccharide matrix promote suspended sediment removal, and retention of nutrients and heavy metals[2]. Biofilms provide food for aquatic organisms, either through indiscriminate ingestion, or selectively ingestion of certain preferential components[3].

For studies on biofilm consumer assemblage-structures and additional information on in-stream microbial functioning capacity (in terms of detrital breakdown and water purification), determining the functional composition of the biofilm may be worthwhile. Clearly, there are multiple levels of complexity. Here we explore a combined isotopic and stiochiometric approach to determine the relative contribution of biofilm allochthonous biomass, important as allochthhonous organic matter provides substantial contributions to nutrient availability and thus increases biofilm bacterial activity[4]. Our approach over-comes microscopic misidentification of algal cells from detritus of varying sources in the matrix[5].

Biofilm biomass and species composition are influenced by a suite of factors including temperature, invertebrate grazing, and flow[6], with the impact likely to vary temporally and spatially. We have some knowledge of how isotopic and stoichiometric composition are similarly influenced[7] but our knowledge is quite limited. Thus, in addition to determining the relative contribution of allochthonous material, we chose study of two different catchments to consider whether there was consistentency is isotopic composition despite differing habitats. Study sites were in NE Scotland on a moorland tributary of the Dee (samples summer ’03 and ’04), and in SW Scotland in a conifer-forested sub-tributary of the River Cree (sampled Nov. ’03 – Oct. ’05). Dee sites varied both in size (from 1 – 10m) and also overhanging tree cover. In SW Scotland all sites were located on the same stream. In this talk we will present detail of the following: **1)**That in both sites the carbon isotopic composition of the biofilm was very similar to allochthonous carbon, thus this parameter was not powerful in determining allochthonous contributions. **2)** A consistent negative linear relationship prevailed with molar C:N and $\delta^{15}N$ at both sites throughout the sampling periods, allowing the use of a two-source mixing model (e.g.[8]), to apportion allochthonous and autochthonous organic material within the biofilms. **3)** The monthly sampling reveals temporal variation in isotopic signature. Despite the number of aquatic food-web studies, there is little information in variation of baseline resource composition. Temporal variation can reflect in stream-processes or catchment-based processes and we illustrate the latter through consideration of the impact of a forest clear-felling event on the biofilm composition.

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ISOTOPIC ECOLOGY OF SALMONIDS

Stable Isotope Studies on the Use of Marine-Derived Nutrients by Coho Salmon Juveniles in the Oregon Coast Range

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Greatly reduced spawning runs of anadromous salmon in streams of the Pacific Northwest (USA) have led to concerns about the effects of reduced marine derived nutrients (MDN's) on sustaining over-wintering juvenile salmon in those streams. In response to these concerns, state and volunteer organizations have placed many thousands of hatchery-supplied salmon carcasses in streams to supplement nutrient sources. Almost no research has accompanied these management programs.

We are using stable isotopes (^{13}C, ^{15}N, ^{34}S) to study the potential use of naturally-occurring salmon carcasses, eggs and resulting fry by over-wintering coho salmon juveniles in two streams of the Oregon Coast Range. Our work is paired with detailed data gathering on stream habitat condition, temperature, chemistry and PIT-tagging studies to monitor movement and growth. We have sampled two cohorts of coho over their stream residencies from eggs to smolts, with particular emphasis on critical life stages (e.g., eggs, emergence from gravel, initial growth, late summer feeding stress, winter feeding, and smolting).

The transition from egg to late-summer parr serves as an uncontrolled diet-switch. During this period isotopic signatures decrease from equilibrium at an enriched state due to higher trophic oceanic feeding (e.g., white muscle $\delta^{15}N=17$), to equilibrium (white muscle $\delta^{15}N=6$) with in-stream diets. One year with high winter spawner returns (and thus higher numbers of carcasses, eggs and fry) was associated with pre-smolts and smolts that showed not only $\delta^{15}N$ elevated above late summer equilibrium but also wide scatter in $\delta^{15}N$ (from 4 to 8), approximating a shift within the cohort equivalent to one trophic level. These results hint that some fish are consuming significant amounts of MDN's while others concurrently shift to lower signature diets. The year with much lower spawner returns did not show such patterns.

To help resolve questions of potential rapid and short term diet switching from lower signature in-stream or terrestrial food sources to higher signature MDN's during the winter period, we are experimenting with analyzing a very rapid turnover (hours/days) "tissue" fraction of the fish – mucus. To our knowledge, we are the first to perform stable isotope analyses of fish mucus. Sampling procedures (including non-lethal) have been developed and isotopic analyses are ongoing. We will compare this sampling with concurrent non-lethal sampling of caudal fins of the same fish to provide contrasting and complementary information on both longer-term slower turnover pools (fins) and much faster turnover fractions (mucus) to discern abrupt and fine time-scale diet switching to marine derived sources of nutrition.

ISOTOPIC ECOLOGY OF SALMONIDS

Linking migratory patterns of females to ova traits in brown trout (*Salmo trutta*, L.) by means of stable isotope analysis.

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Brown trout (*Salmo trutta*) populations in coastal rivers often split into two components; freshwater resident and anadromous individuals. However mechanisms that govern this migratory behaviour *i.e.* stay in the river or migrate to sea, are still misunderstood. Recent investigations in the Oir River (Normandy, France), where anadromous and freshwater resident trout use the same spawning grounds, have led to conclude that there is no genetic differentiation between the two groups. An individual-based analysis of migratory patterns has recently suggested the existence of several life history tactics among the freshwater trout, including residency in headwater tributaries and downstream migration along the river. Because fish have different body sizes when returning to spawn in tributaries, the hypothesis that migratory fish encounter higher feeding opportunities than non-migratory fish is broadly admitted. Shift in feeding behaviour, for instance from insectivory to piscivory is also expected. In this study, we particularly focused on female brown trout that invest large amounts of energy into gonads before their return to spawning tributaries. The aim was to investigate all possible correlations between size, migratory and feeding behaviour of females and their ova traits.
A total of 55 resident females (fish length ranged from 153 to 371 mm) and 26 anadromous females (from 294 to 660 mm) were sampled on the Oir River at the time of reproduction in 2003, 2004 and 2005. Manual stripping was performed to assess fecundity and sub-samples were taken to measure ova traits (size and weight) and run stable isotopes analysis (C and N). Fish age was also assessed by means of scale reading. Among freshwater trout, positive correlations were found between female length, age, fecundity and ova weight. Moreover, the biggest ova were ^{15}N and ^{13}C enriched and a positive correlation between female length and ova $\delta^{15}N$ was observed. This could be explained by a difference in feeding behaviour, with small females feeding on invertebrates and large migratory females being more piscivorous, but this result has to be confirmed by further SIA on potential preys (invertebrate and fish) collected on the Oir River basin. Anadromous females were found to produce smaller ova compared with freshwater females of similar size. Anadromous female length was positively related to age and fecundity but ova weight and $\delta^{15}N$ were steady whatever female length. Moreover, $\delta^{13}C$ suggests differences in migratory patterns at sea among anadromous trout according to fish age, but no correlation between ova $\delta^{13}C$ and ova size was found.
Ova size may correlate not only with female status, but also with size, growth and survival of the progeny. Therefore major differences in ova traits according to maternal migratory patterns and food sources may be a significant cause of variation in early life history traits of brown trout and could play a role in maintaining different migratory components in the population.

ISOTOPIC ECOLOGY OF SALMONIDS

Latitudinal clines in young-of-the-year Arctic charr habitat use in eastern North America

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Concerns over the potential impacts of climate warming on northern fish communities have increased as a result of recent changes in seasonal phenologies and increases in mean annual temperature. Arctic latitudes are likely to be significantly affected by climate change, yet they are one of the least studied areas and have few climate-related biological studies available on which to base impact predictions. For cold adapted species such as Arctic charr (*Salvelinus alpinus*), climate-warming impacts will be variable throughout the range and particularly negative at the southern extremes of the range. Accordingly, there is an urgent need to improve understanding of climate-related variability in the biological performance of Arctic charr stocks to develop appropriate management actions to mitigate possible negative climate change effects. Otolith analysis overcomes existing information deficiencies by facilitating reconstruction of the environmental history of individual fish, particularly with respect to habitat use. Here, otoliths collected from young-of-the-year Arctic charr from across the latitudinal range in the eastern Canadian Arctic and sub-Arctic are analysed for $\delta^{18}O$. Isotope results were then used to estimate average experienced growth temperatures using a genus-specific fractionation equation. Where possible, estimated temperature values were compared to monitored water temperature data. In general the two temperature measures differed. Differences likely reflect the effect of behavioural thermoregulation motivating juveniles to seek the warmest available water temperatures to maximize growth. Maximization of experienced water temperatures will hold direct consequences for survival and fitness and suggests caution in the interpretation of paleo-climatic data that do not account specifically for knowledge of species thermal preferences. Across the latitudinal range obtained isotope results were negatively correlated with latitude and positively correlated with the $\delta^{18}O$ of surface waters. The difference between the two isotope measures was similar thus indicating constant $\delta^{18}O$ fractionation throughout the distributional range. Growing temperatures inferred from $\delta^{18}O$ analysis, however, were non-linearly related to latitude as a result of either thermal limitations (high latitudes) or a high incidence of temperatures above the thermal optimum for growth at lower latitudes.

ISOTOPIC ECOLOGY OF SALMONIDS

Linking ocean climate cycles to fish (Atlantic salmon) mortality using the stable carbon and nitrogen isotope composition of scale collagen

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Global stocks of Atlantic salmon (*Salmo salar*) have declined consistently for at least 30 years, and much of the observed increase in mortality is believed to have an origin during the marine phase of life. Several largely theoretical or statistical studies suggest that *S. salar* mortaliy may be influenced by the strength and timing of phytoplankton blooms in the North Atlantic, thus climatic factors such as the North Atlantic Oscillation (NAO) and ocean warming may impact salmon dietary behaviour in predictable ways, Unfortunately monitoring of open marine pelagic fish species is extremely difficult and expensive and consequently relatively little is known of the marine dietary behaviour of S. salar.

Fish scales are routinely collected from many commercial fish species as they are incrementally grown structures and are used in age determination. Fish scales are composed of collagen and apatite, and several experimental studies have determined the relationship between the isotopic composition of carbon and nitrogen in scale collagen and diet. In many fisheries laboratories, multi-decadal archives of fish scales exist potentially providing a record of behavioural response to climate variation.

We measured the isotopic composition of C and N in archived *S. salar* scales sampled in the North Sea during the return homeward migration from 1986-1996. We show clear dietary separation between age classes of fish in most years, with larger, older fish occupying higher mean trophic levels. Most interestingly, the isotopic composition of carbon recorded in *S. salar* collagen shows large (>2‰) fluctuations over the decade of sampling. Furthermore, the isotopic composition of carbon in scale collagen correlates negatively with estimated population size, suggesting that the mechanism controlling variations in the isotopic composition of scale collagen also significantly influences marine mortality. We suggest that carbon isotope compositions in marine consumer tissues reflect the relative size and duration of the spring phytoplankton bloom, with large or early blooms resulting in more positive $\delta^{13}C$ values throughout the supported ecosystem. If this is true, then our stable isotope data suggest that marine mortality is reduced during years with high levels of phytoplankton productivity. As plankton abundance is linked to ocean climate variability, the stable isotope composition of archived fish scales can be used to study mechanisms linking ocean climate variables and population size.

PELAGIC PREDATORS

Employing chemical tags to determine trophic dynamics and movement patterns of migratory predators in the equatorial Pacific Ocean.

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Previously, small and large-scale movements of pelagic predators in the equatorial Pacific Ocean (eqPac) have been examined using catch statistics, conventional tag and release studies, and electronic tag tracking studies. Isotopic compositions of fish tissues can serve as internal chemical tags that provide information on both their movements and foraging habitat. Our isotope dataset from the eqPac, now consisting of over 3000 samples of top predators and their prey, shows spatially-explicit patterns of trophic dynamics. Species-specific geographical δ^{15}N maps are a powerful tool to examine differences in the trophic ecology among predators. For example, δ^{15}N spatial patterns of bigeye (*Thunnus obesus*) and yellowfin (*Thunnus albacares*) tuna suggest that in the central and eastern eqPac, bigeye tuna forage more exclusively near the equator than do yellowfin tuna. If a predator migrated extensively throughout the eqPac, then little geographical isotopic variation would be expected because regional δ^{15}N differences would be integrated over space and time. δ^{15}N spatial variability is high (>12‰) for tropical tunas, and thus, these species exhibit a large degree of regional residency. In this isotope cartography context, geographically anomalous δ^{15}N values are an excellent indicator of recent basin-wide movements. Furthermore, by coupling anomalous δ^{15}N values with our experimentally-determined tissue turnover rates of yellowfin tuna we are able to define the recent foraging area of migrating individuals.

Pelagic predators collected from around French Polynesia and Micronesia have remarkably high δ^{15}N values (≥ 20‰) relative to all other regions of the eqPac and thus, these isotope 'tags' can discriminate regional movements. To address whether changes in δ^{15}N values in these regions are a function of either a change in food web structure or δ^{15}N of nutrient inputs, we will present compound-specific isotope analysis (CSIA) of individual amino acids in predator tissues. Our work in the eastern tropical Pacific (ETP) on yellowfin tuna indicate minimal isotope fractionation in essential amino acids (EAA), whereas, a trophic enrichment occurs in non-essential amino acids (NAA) (Popp et al. *Submitted*). By comparing the differences in the δ^{15}N of EAA and NAA from the same tissue sample we can determine if shifts in bulk tissue δ^{15}N are a function of changes in trophic dynamics or nutrient dynamics. Furthermore, we have found that isotope baseline shifts incorporated into tuna have a remarkable degree of overlap with the δ^{15}N patterns of organic matter analyzed from ETP sediments, which is dependent upon nutrient utilization in the surface waters. Thus, the δ^{15}N overlap between tuna and sediments in the ETP further suggests that although tuna are capable of basin-wide movements, there is a large degree of regional residency. By coupling isotope cartography, CSIA amino acid research, and existing isotope baseline datasets we can now examine regional and basin-wide movement patterns of migratory predators in the open ocean.

PELAGIC PREDATORS

Size and latitudinal effects on $\delta^{15}N$ reveal differential trophic ecology of two top predators in the western Indian Ocean

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Ecologists primarily use $\delta^{15}N$ values to estimate the trophic level of organisms. However, it is increasingly being acknowledged that many factors can influence the stable nitrogen isotopic composition of consumers, e.g. age, starvation, food quality or isotopic signature of primary producers (isotopic baseline). Such sources of variability can complicate trophic position estimations from $\delta^{15}N$ values. However, information on the ecology of the species may be revealed by the analysis of these variations (e.g., degree of residence, habitat preference, foraging strategies).

Muscle tissues of yellowfin tuna (N = 264, FL = 40-170cm) and swordfish (N = 136, MFL = 68-225cm) were sampled between 2001 and 2004 in the western Indian Ocean during different seasons and along a latitudinal gradient (23°S to 5°N). Size and latitude effects on $\delta^{15}N$ were examined using linear mixed-effects models, and different grouping factors tested (year, season, cruise, fishing gear).

The latitudinal effect was significant for yellowfin only. This is discussed with respect to the global environmental parameters extracted from the Levitus World Ocean Atlas (e.g., oxygen levels, nitrate concentrations). These environmental gradients control the isotopic signature of the baseline and so may be propagated through the food web. The observed latitudinal effect in the yellowfin data suggests that these populations exhibit a relatively resident behaviour compared to swordfish.

The size effect was significant for both species, but was much more pronounced in the swordfish and seasonally dependant for yellowfin tuna. During the three months of the reproduction period, juveniles and adults of yellowfin tuna form mixed schools. Stomach samples indicate they fed on the same surface prey species, explaining the lack of size effect. Between species, the differing size relationships might be explained by their foraging strategies, which are related to their respective physiological abilities. Swordfish adults are able to reach very deep layers (900m) and can feed on mesopelagic organisms during the day leading to a distinct isotopic signature. Swordfish juveniles cannot reach such deep layers. In non-reproductive periods yellowfin juveniles and adults have a similar but much reduced vertical segregation within the surface layers (200m).

Thus, stable isotope analyses allow the investigation of complex vertical and spatial segregation, both within and between species, even in the case of highly opportunistic feeding behaviours.

PELAGIC PREDATORS

Isotopic evidence for dietary shift in historical and modern white sharks off the coast of California

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White sharks (*Carcharodon carcharias*) are top-level opportunistic predators that have been observed preying on marine mammals off the coast of California. However, California's pinniped populations were dwindling until 1970s. Populations began to rebound after enactment of the Marine Mammal Protection Act in 1972. We used stable isotope analysis to determine feeding patterns of white sharks from 1955 through present as marine mammal populations fluctuated off California. Stable isotope ratios of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) can be utilized as tracers for ecological studies. Carbon isotopes vary at the base of the food web with primary productivity, onshore versus offshore location, and latitude. Nitrogen isotopes are strongly sorted by trophic level, with greater ^{15}N-enrichment at higher trophic levels. The life history of a white shark may be recorded in its concentrically accreted vertebrae, assuming subsequent turnover does not overprint earlier events. To track the diet of a shark through its lifetime, we determined the $\delta^{13}C$ and $\delta^{15}N$ values of organic matter extracted from individual vertebral growth rings. Our preliminary results indicate that white sharks fed at a high trophic level in the 1950 and 1960s, when pinniped populations off California were not high. Isotopic data suggest that white shark diets consisted of pinnipeds from higher latitude or migratory populations and perhaps baleen whales. In addition, ontogenic dietary shifts are discernable within vertebral centra. A controlled feeding study is needed to verify diet-to-tissue fractionation factors and remodeling dynamics of vertebral centra. Additional samples from historic and archaeological sites will also help define the trends of white shark feeding habits through time.

PELAGIC PREDATORS

Evidence of Niche Partitioning Between Beaked Whale Species (Family Ziphiidae) In The North Atlantic From Stable Isotope Analysis.

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As a group, the beaked whales (Ziphidae) remain the least known family of large mammals. Strandings data and stomach contents analysis suggest a number of possible niche differences between species, particularly related to water temperature and prey size preferences. However, such data are subject to a number of biases and limitations.

Stable isotope analysis can be used investigate various aspects of a species' niche and will not be subject to the same biases and limitations as these other data types. As a result, stable isotope analysis can provide an independent indication of niche differences between species. Stable isotope ratios of carbon and nitrogen were measured in samples of bone collagen from 144 individual beaked whales belonging to six species from the North Atlantic. Two species, northern bottlenose (*Hyperoodon ampullatus*) and Sowerby's beaked whale (*Mesoplodon bidens*), had significantly lower $\delta^{13}C$ values than the other species. This may indicate that these two species occur at higher latitudes than other species. In terms of these two species, northern bottlenose whales had significantly higher $\delta^{15}N$ values than Sowerby's beaked whales. Of the four species with ^{13}C-rich collagen, these could also be separated into two groups based on nitrogen isotope ratios, with True's and Cuvier's beaked whales (*Mesoplodon mirus* and *Ziphius cavirostris* respectively) having the higher $\delta^{15}N$ values.

It is possible that differences in carbon and nitrogen isotope ratios between species could be related to other confounding ecological correlates, such as variation in body size, latitude of occurrence, sex and when the samples collected and not differences between animals *per se*. However, generalised additive modelling (GAM) demonstrated that species was still a significant factor when these possible confounding variables were taken into account, suggesting that the interspecific differences are real. These interspecific differences are consistent with the hypothesised niche partitioning in terms of prey size and water temperature based on analyses of other types of data and provide independent support for niche partitiong between these beaked whales in the North Atlantic.

PLENARY

Tissue-diet spacing: what can we learn from experiments using enriched tracers?

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A generation before DeNiro and Epstein[1] wrote their seminal papers on the ^{15}N and ^{13}C abundance in animal tissues, Schoenheimer[2] and co-workers were conducting the first studies using enriched stable isotope amino acids. Using amino acids labelled with ^{15}N and with ^{2}H, they were the first to show that body protein is constantly being remodelled Contemporary with DeNiro and Epstein, Waterlow[3] and co-workers pioneered procedures using enriched tracers to measure mammalian protein metabolism in health and disease. This field has continued to develop with studies of intermediary metabolism and on the synthetic rate of individual proteins. Stable isotope tracer work continues to enter new fields such as functional proteomics.
This presentation considers the large body of work using enriched stable (and radioisotope) tracers to study protein metabolism, and attempts to use findings from nutritional physiology and medicine to inform ecological studies. It is also recognized that recent advances from ecology, especially with respect to the relationship of ^{15}N tissue-diet spacing with nitrogen stress[4], can inform human medicine. New insights into the natural world often come hand in hand with technological developments, as is the case with compound specific isotope analysis. The need for further innovation in our tools to measure stable isotope abundance is also discussed.

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FROM INDIVIDUALS TO COMMUNITIES II: POPULATION STUDIES

Does The Cost Of Living At Depth Force Dietary Switch In Chironomid Larvae?

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Evidence for fuelling of food webs by methane-derived carbon is accumulating but we still do not have a definitive picture of the linkages. For example, bulk stable isotope studies of lake-dwelling chironomid larvae have inferred increasing reliance upon methanotrophic biomass with increasing lake depth and potential methane production.
We used fatty acid analyses and compound-specific stable carbon isotope analyses of those fatty acids to seek diagnostic biomarkers characteristic of methanotrophic bacteria within individual larval chironomid tissues collected from different depth zones in a lake. The aim was to provide direct evidence of trophic linkage from field-derived samples, and qualify whether isotopic-depletion occurred in fatty acids that can only be synthesized de novo, indicating use of acetate units from methane-derived sources as an alternative explanation for the light $\delta^{13}C$ values recorded. Depth-specific differences in fatty acid profiles are discussed with reference to current knowledge of feeding mode plasticity, and in relation to local environmental variables that may influence feeding mode.

FROM INDIVIDUALS TO COMMUNITIES II: POPULATION STUDIES

Symbiosis of a Caribbean bivalve and shrimp: A field study using a stable isotope mixing model

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We describe the relationship between a shrimp *Pontonia* sp. living in the mantle cavity of the bivalve *Pinna carnea* in a seagrass community in southwestern Dominican Republic. The majority of shrimp were found in breeding pairs and living in bivalves measuring >140 mm in length. Observed size relationships indicated that bivalves >140 mm in length provided the shrimp with more space because these larger shells, in contrast to smaller ones, were more rigid and did not bend with valve closure to reduce the mantle cavity. Moreover, predation experiments indicated bivalves measuring >140 mm in length provided the shrimp with a safer refuge because the bivalve itself was less vulnerable to predators.
We applied a multiple-source mixing model which considered values of stable ^{13}C and ^{15}N isotopes of consumer tissues and potential food items to estimate the relative contribution of different foods to the diets of both the bivalve and shrimp (i.e., quantified the relationship between animal tissues and assimilated foods). The model, which used $\delta^{13}C$ and $\delta^{15}N$ values and conserved for isotopic mass balance, calculated the range of feasible solutions that could explain the isotopic value of the consumer. It indicated that particulate organic matter (POM) filtered from water made up a major proportion of the diet of both the bivalve (34-36%) and the shrimp (46-53%). The model also showed that the bivalve assimilated more epibionts shed from seagrass (59-66%) than POM from bottom sediments (0-5%), whereas the shrimp assimilated more POM from bottom sediments (26-38%) than materials from epibionts (0-28%). The removal of bottom sediment POM by the shrimp should benefit the bivalve since silt can reduce growth and survival in bivalves.
This is the first study making use of isotopic mass balance in a mixing model to describe the nature of a symbiotic relationship.

FROM INDIVIDUALS TO COMMUNITIES II: POPULATION STUDIES

Migration Dynamics of sand goby (*Pomatoschistus minutus*) between the North Sea and the Schelde estuary: a stable isotope approach

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The seasonal species composition in the brackish water area of Holarctic estuaries is consistent and predictable. Changes in temporal fish distribution are caused by seasonal migrations of marine fish species, which enter the estuary during their juvenile stage for a relatively short period of time. For this reason estuaries are regarded as nursery areas for marine fish species. However the underlying ecological principles for these fish migrations are still subject of debate. In order to understand the factors that cause this shift in habitat use we aim to clarify the migration dynamics of the marine sand goby (*Pomatoschistus minutus*) between the North Sea and the Schelde estuary. Stable isotopes of carbon and nitrogen can serve to study these movements in detail provided that the marine and estuarine environments differ isotopically for carbon and/or nitrogen and that isotopic turnover rates in sand goby tissues are known.

A laboratory diet switch experiment was conducted to estimate $\delta^{13}C$ and $\delta^{15}N$ turnover rates, defined as the change in isotopic composition due to growth and metabolic tissue replacement, in dorsal muscle, liver and heart tissue. The rate of change differed among tissues and among elements. For both $\delta^{13}C$ and $\delta^{15}N$ muscle tissue had the slowest turnover rate with half-lives of 24.7 and 27.8 days respectively. $\delta^{15}N$ had the fastest turnover in liver, while $\delta^{13}C$ changed most rapidly in heart tissue. Differences in turnover rates could be attributed to differences in metabolic activity.

From May 2003 to April 2004 gobies were collected monthly in the upper and lower Schelde estuary. Stable isotope analysis on monthly samples of their stomach contents was applied to establish the existence of a consistent $\delta^{13}C$ and $\delta^{15}N$ gradient between the upper and lower estuary. Only $\delta^{13}C$ showed to be a reliable tracer in the Schelde estuary as the $\delta^{15}N$ gradient alternated throughout the year. Finally, to infer fish movement dorsal muscle samples of 15 fish from each month caught in the upper estuary were analyzed. Based on these $\delta^{13}C$ values individual estuarine residence times were assessed. It was concluded that the summer abundance peak probably consisted of fish, which recruited to the estuary in distinct migration pulses. This suggests sequential, obligate migrations that occur independently of estuarine environmental conditions. No immigration was detected during July, but from August until March there was continuous immigration, although the abundance started to decrease from November onwards. The temporal overlap in immigration and emigration indicates a more complex series of movements between offshore spawning grounds and estuarine nurseries, supporting the hypothesis of an individual optimal habitat choice based on environmental and physiological conditions.

FROM INDIVIDUALS TO COMMUNITIES II: POPULATION STUDIES

Interactive segregation among sympatric Arctic charr (*Salvelinus alpinus*) and brown trout (*Salmo trutta*) populations in Irish Loughs

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Arctic charr are a cold water adapted fish and were among the first species to re-occupy Irish freshwater habitats following the retreat of the last ice-age. In Ireland, all known Arctic charr populations are lake resident and many co-exist with brown trout. In Scandinavian lakes, co-existing Arctic charr and brown trout are known to display "interactive segregation", a mechanism by which co-existing ecologically similar species segregate as a result of small behavioural differences, with dietary differences between allopatric and sympatric populations typically providing the best evidence of the mechanism. Stable isotope analyses are ideally suited for the study of persistent dietary separation within and among populations and, as a consequence, are ideal for the study of interactive segregation. Here we use stable isotope analyses to examine dietary separation between sympatric Arctic charr (*Salvelinus alpinus*) and brown trout (*Salmo trutta*) along a productivity and latitudinal gradient of Irish Loughs and test the applicability of the interactive segregation hypothesis to observed trophic relationships among co-existing populations. Stable isotope analyses are supplemented with gut content analysis as a means of contrasting short- and long-term differences in resource use. Gut contents showed both species fed seasonally on similar prey in many, but not all loughs. Stable isotopes indicated the long-term nature of the similarity or separation in feeding relationships inferred with gut contents. In southern loughs the two species showed significant overlap in diet. In northern or higher altitude loughs the two species showed varying degrees of dietary separation. Results, therefore, provided equivocal support for the interactive segregation hypothesis as used to explain dietary differences between the species in Scandinavian lakes. Differences in lough productivity are suggested as a proximate cause of the differences in dietary separation. In Lough Muckross, where dietary overlap was substantial, feeding and capture patterns indicative of differences in habitat use were consistent with the niche compression hypothesis, with differences in temperature preferences providing a plausible mechanism for persistent habitat separation.

FROM INDIVIDUALS TO COMMUNITIES II: POPULATION STUDIES

Isotope Ecology of Estuarine and Freshwater Crocodylians

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Aquatic and marine taxa are difficult to observe in the wild. As a result, food and habitat preferences of many aquatic animals are unknown. This is especially true for animals, such as many crocodylians, that utilize estuaries. Crocodylians, as top-level carnivorous taxa, are especially poorly understood because they can potentially feed from three different food webs - marine, riverine, and terrestrial. Similarly, their drinking water could come from either the ocean or a freshwater source, and there are currently no analytical techniques to estimate the amount of ocean water that contributes to a crocodylian's drinking water. Stable carbon and oxygen isotopes provide an empirical way to study aquatic and marine animals to learn more about diet and habitat. For estuarine animals, stable isotopes provide a unique opportunity to estimate proportions of diet and drinking water provided by oceanic versus terrestrial sources. Marine and estuarine mammal ecology has been previously studied using a stable isotope approach, but systematic data for marine and estuarine reptiles does not exist. Our carbon and oxygen data from tooth enamel carbonate indicate that *Alligator mississippiensis* has isotope values consistent with a freshwater habitat, whereas some *Crocodylus acutus* individuals are utilizing ocean water and oceanic food sources to a greater extent that *A. mississippiensis*. Habitats and diets may be differentiated based on $\delta^{13}C$ and $\delta^{18}O$ values from tooth enamel carbonate as well as the $\delta^{18}O$ value variability when multiple individuals of the same species are measured.

FROM INDIVIDUALS TO COMMUNITIES II: POPULATION STUDIES

Detecting food web change in the Laurentian Great Lakes using stable isotope and fatty acid tracers

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In the Laurentian Great Lakes, significant changes in food web structure have occurred through time. Invertebrate and fish communities have changed and these alterations have been linked to anthropogenic activities, e.g. the introduction of exotic species. However; it is difficult to predict how changes in community composition at lower trophic levels will affect apex predators. Developing ecological tracers as tools to define food web interactions and detect change is an important part of filling this gap. Here, we discuss the use of stable isotopes and fatty acids as tracers of food web interactions. Through retrospective analysis of archived herring gull egg samples we have identified temporal changes in the diets of gulls breeding on the Great Lakes, particularly on Lake Erie. Stable nitrogen isotopes have indicated that birds are feeding at lower trophic levels now than in the past. These changes reflect reductions in prey fish availability. Metabolically-stable fatty acid markers have provided corroborative evidence of dietary change. Dietary change is an important factor affecting the interpretation of data from environmental monitoring programs, e.g. contaminant temporal trends. The simultaneous application of stable isotopes and fatty acids, particularly through retrospective studies, will lead to a better understanding of food web dynamics and environmental change.

FROM INDIVIDUALS TO COMMUNITIES II: POPULATION STUDIES

Factors Affecting Prey Choice in a Despotic Herbivore.

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Ideal free models predict that animals should move to food patches where their intake rate is highest. However such models do not consider the implications of group living, including competition, dominance and social rank. For despotic species these effects can be particularly strong, with dominant individuals and groups expected to monopolise the best resources. To test this, we must accurately quantify resource utilisation of individual animals, which is problematical in natural situations. However recent advances in the use of stable isotope analysis have removed many previous constraints.
Light-bellied Brent Geese are particularly suited to this type of study, being despotic in nature and foraging in habitats (marine and terrestrial) with particularly robust isotopic gradients. Using stable isotopes ratio of Carbon and Nitrogen (expressed as $\delta^{13}C$ and $\delta^{15}N$) from the tissues of geese, we take advantage of these gradients to determine dietary choice of individuals. This is coupled with field observations to determine both social rank of individuals and groups, and mechanisms driving the maintenance of dominance. This approach allows us to describe resource partitioning amongst social groups with some surprising results, highlighting the costs of living in a group and raising a family.

FROM INDIVIDUALS TO COMMUNITIES II: POPULATION STUDIES

Connecting Breeding and Wintering Sites Used By Endangered Southwestern Willow Flycatchers

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A primary constraint on effective conservation of migratory animals is our inability to track individuals through their annual cycle. One such animal is the endangered southwestern subspecies of the willow flycatcher (*Empidonax traillii extimus*), which is difficult to distinguish from other subspecies in the field. In particular, conventional efforts to identify members of the endangered subspecies on the winter grounds have met with limited success. Our objective was to use stable isotope ratios as a means of identifying wintering sites of southwestern willow flycatchers. Between 1998 and 2005, we collected feathers in Peru, Central America, Mexico and the US. We analysed stable isotope ratios of carbon, nitrogen, and hydrogen from feathers of breeding and winter willow flycatchers. We document a positive trend in hydrogen stable isotope ratios across latitude and negative trends between latitude and carbon and nitrogen stable isotope ratios. The geographic pattern in hydrogen stable isotope ratios was similar to that reported elsewhere in the literature for other passerines. The negative trend in carbon stable isotope ratios with increasing latitude reflected an absence of C_4 contributions to the diet north of 12° north latitude. The mechanism driving the negative relationship between nitrogen stable isotope ratios and latitude is unknown. We used these stable isotope ratios in a discriminant function analysis optimised for correctly assigning wintering birds to the region where they were captured. These functions were only useful (> 50% correct) for assigning individuals to their winter regions if the regions used were large and the threshold probability for assignment was relatively high (e.g., 0.33 0.50). When using these same discriminant functions to assign breeding samples of southwestern willow flycatchers, most birds were assigned to two regions, central Mexico and Costa Rica/Panama. We suggest these regions are more likely to harbor wintering southwestern willow flycatchers than other winter regions. Targeted research and management in these areas would be a wise use of limited conservation funding

GAS EXCHANGE AND WATER RELATIONS IN PLANTS II

Stable oxygen isotopes of bulk leaf material reveal long-term chronic ozone effects in grassland species

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A shift in the stable oxygen isotopic signature ($\delta^{18}O$) of bulk leaf material directly reflects changes in stomatal conductance (g_s). Because g_s can be affected by reduced soil moisture or by elevated ozone (O_3) levels, the aim of this study was to test whether $\delta^{18}O$ values of bulk leaf material responds to irrigation and O_3 stress, both singly and in combination. In a free-air fumigation experiment, we determined in 2002 and 2003 $\delta^{18}O$ values of *Holcus lanatus* L., *Plantago lanceolata* L., *Ranunculus friesianus* (Jord.), and *Trifolium pratense* L. kept under ambient and elevated O_3 levels with and without irrigation, and we measured g_s. All species generally reacted with an increase in $\delta^{18}O$ under elevated O_3, in both irrigated and non-irrigated plots, which during both growth periods (GP1 and GP2) in 2003 was associated with a decrease in g_s, except for *T. pratense* during GP1. At higher O_3 levels during GP2 in 2002 and 2003, the increase in $\delta^{18}O$ was less in irrigated than in non-irrigated plots, and the associated decrease in g_s was smaller. Both, the reduced soil moisture in the absence of irrigation and the elevated O_3 caused an increase in $\delta^{18}O$ and thus these effects could not be separated during GP2 in 2003.
A dual stable isotope approach indicated that during GP2 (2002 and 2003) increased $\delta^{13}C$ and $\delta^{18}O$ in all species but *R. friesianus* were related to stomatal limitation due to elevated O_3. In *R. friesianus* $\delta^{18}O$ was increased during all GPs without a change in $\delta^{13}C$, thus indicating that elevated O_3 caused a reduction in both g_s and maximum photosynthetic capacity (A_{max}).
Our data suggest that $\delta^{18}O$ can directly reveal long-term chronic ozone impacts on g_s, except under extremely dry weather conditions.

GAS EXCHANGE AND WATER RELATIONS IN PLANTS II

A novel stable isotopic approach to identify the fate of ozone in plants

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Ozone is a secondary air pollutant that causes damage to sensitive crops, trees and semi-natural vegetation in Europe and other parts of the world at current levels. Risk assessment of ozone impacts on vegetation is essential, since background ozone concentrations are expected to increase further during the next decades. A model (DO_3SE) has been developed to quantify and predict ozone deposition and stomatal flux, but some of its components are severely limited by current knowledge. A new approach with isotopically labelled ozone (^{18}O-ozone) was applied to trace ozone and its derivatives directly in plants and soil.

Ozone-sensitive white clover (*Trifolium repens*, NC-S) plants grown in pots were exposed to ^{18}O-ozone at two ozone concentrations during the photoperiod under summer conditions in a climate-controlled chamber. The aims of the study were to assess the temporal change in accumulation of ozone-derived ^{18}O in different plant parts and soil, and to determine the effects of ozone concentration and pre-exposure with ozone on rates of ^{18}O accumulation. Results on the fate of ^{18}O-ozone in the dry fraction of plant parts and the soil surface are presented.

Fumigation of plants with ^{18}O-ozone during the photoperiod resulted in a continuous increase in δ^{18}O of dry leaf biomass, indicating accumulation of ozone-derived ^{18}O in the dry constituents of leaves. Dry biomass of petioles was a very small sink for ^{18}O originating from ozone, while no ^{18}O accumulation was detected in the dry fraction of root biomass and soil. ^{18}O-enrichment of dry leaf biomass was 3.5 times smaller at 50 ppb than at 100 ppb of ^{18}O-ozone, which partly resulted from the difference in ozone concentration. δ^{18}O of dry leaf biomass was significantly higher in plants which experienced a 1-month ozone pre-exposure than in plants grown in filtered air prior to the 100 ppb ^{18}O-ozone fumigation. However, stomatal conductance was significantly lower in plants which experienced the ozone pre-exposure. This may imply that defence mechanisms were stimulated in plants that have been pre-exposed to ozone. Initial results also suggest that the ozone-derived ^{18}O associated with dry leaf biomass was for a large part located in the cell walls and for a much smaller part in the apoplast.

The potential of the new experimental approach for improving our knowledge of ozone deposition to plants at both the individual plant and ecosystem level, and the implications for ozone models will be discussed.

GAS EXCHANGE AND WATER RELATIONS IN PLANTS II

Elucidating the source of nitrous oxide in soils using stable isotope techniques

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Nitrous oxide (N_2O) is produced in soils during denitrification, nitrification, nitrifier denitrification and nitrate ammonification. These microbial processes may occur simultaneously in different microsites of the same soil but there is often uncertainty associated with which process is predominantly contributing to measured emissions. Recent advances in stable isotope techniques facilitating direct measurement of ^{15}N-N_2O-^{18}O allows determination of the source of N_2O and an accurate quantification (^{15}N enrichment) or estimation in natural systems (natural abundance, isotopomer) of emissions from each source. Here we will introduce the techniques we have developed, present selected results from studies where they have been applied, and present ideas for the way forward.

Our ^{15}N-enrichment technique for differentiating between nitrification and denitrification, whereby $^{14}NH_4{}^{15}NO_3$ and $^{15}NH_4{}^{15}NO_3$ are applied to different replicates (Baggs et al. 2003), has been successfully employed in several soil microcosm and field studies. By enabling quantification of the contribution of nitrification for the first time, this technique has unequivocally shown the significance of this process in N_2O production in soils, for example at a wider range of soil water contents than previously thought (Bateman & Baggs 2005). We have built upon this method to also quantify N_2O production during nitrifier denitrification by adding a treatment with ^{18}O-labelled water (Wrage et al. 2005) and have shown that this process is much more important in soil N cycling than originally thought, accounting for up to 50% of N_2O production, depending on soil water content, oxygen availability, pH and nitrogen availability. This supports indications from our physiology experiments that the ability to undertake nitrifier denitrification may be a universal trait in the betaproteobacterial ammonia oxidising bacteria (Shaw et al. 2006).

We are now coupling the above techniques with isotopomer (intramolecular distribution of ^{15}N) analysis in soils for quantification of N_2O production during nitrate ammonification, and for differentiation of all processes in unfertilised/natural systems. We are also linking emissions from each source to diversity and activity of relevant microbial functional groups, for example through the development and application of a specific *nirK* primer for the nitrite reductase in ammonia oxidizing bacteria involved in nitrifier denitrification.

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GAS EXCHANGE AND WATER RELATIONS IN PLANTS II

Studying climate change: a novel tool using Deuterium isotopomers quantification in tree ring cellulose

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Deuterium (D), the heavy stable isotope of hydrogen, is used as tracer in hydrology and plant sciences. D measurements by isotope ratio mass spectrometry (IRMS) yield only the whole-molecule D/H ratio (δD). This disregards that biological processes incorporate D selectively into non-equivalent intramolecular positions, giving rise to molecules deuterated at specific positions called D isotopomers. The D isotopomer distribution (DID) in a compound is non-random and reflects the biochemical history of that compound.

In tree rings, the DID of cellulose results from a series of mechanisms. If the D discrimination of each mechanism is known, a signal related to the underlying process can be reconstructed: **First**, precipitation water enters the tree. The δD of that water depends on ambient temperature. **Second**, leaf transpiration, depending on air humidity, enriches leaf water in D. Thus, δD of the water entering photosynthesis contains a combined temperature and humidity signal. Enzyme isotope effects during photosynthesis constitute the **third** D discrimination mechanism. Enzymes act on a specific C-H bond, affecting specific D isotopomers in photosynthates. For example, we measured the DID of leaf photosynthates of annual plants grown under different CO_2 concentrations and discovered that the abundance ratio of the two C(6)-D isotopomers reflects photorespiration levels. Enzyme isotope effects create physiological signals, but they blur the climate signal contained in leaf water δD. The **fourth** mechanism operates after photosynthates have been exported to the trunk. During cellulose formation in the trunk, hydrogen exchange occurs between C-H groups of translocated leaf photosynthates and xylem sap water. Because xylem sap water has the same δD as precipitation water, this exchange re-introduces the temperature signal into tree ring cellulose. All four mechanisms influence the final δD of tree ring cellulose, but because the signals of the mechanisms overlap, they can not be reconstructed from δD. In contrast, the DID of tree ring cellulose corresponds to seven D/H ratios, which offers a means of accessing multiple signals simultaneously. To measure DIDs, we use Nuclear Magnetic Resonance spectroscopy (NMR). We developed a technique to obtain a glucose derivative from wood material which is suitable for NMR and conserves the original DID of tree ring cellulose. We showed experimentally that the hydrogen exchange at trunk level takes place mainly at position C(2)-H, with the two C(6)-Hs exchanging the least. Therefore, the C(2)-D isotopomer abundance should adopt the temperature signal from precipitation (mechanisms 1+4), while the two C(6)-D isotopomer abundances should retain information about leaf-level processes (mechanisms 2+3). In agreement, we found from a CO_2 enrichment experiment on adult trees that the mentioned photorespiration signal was transferred to tree rings. This confirms that physiological information is retained in tree ring cellulose and can be retrieved by measuring DID.

Applied to tree ring series, DID measurements offer the unique possibility to study plant-atmosphere interactions on century time scales. This allows investigating if there has been a globally coherent response of the vegetation to increasing CO_2 (CO_2 fertilization), and if it is lasting or transient. We also expect to retrieve information about temperature, humidity and past climate variability from DID measurements. Such information is of prime interest to refine climate models and forecast climate change.

PALEOECOLOGY

Determining isotopic fractionations for carnivores: A case study at Isle Royale

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The application of stable isotope biogeochemistry to paleoecologic questions, such as the reconstruction of past predator-prey interactions, requires assumptions about how isotopes are sorted by trophic level. For example, previous isotopic reconstructions of late Pleistocene megafauna from La Brea found that dire wolves and sabertooth cats fed primarily on grazers (bison, camel). A larger than generally assumed N isotope enrichment factor would change the dietary interpretation for both carnivores to include other herbivores (sloth, horse, mastodon). Unfortunately, the isotopic enrichments of ^{13}C and ^{15}N that occur between mammalian carnivores (predators) and their prey have not been well defined in modern populations. We use bone collagen from the Isle Royale National Park wolf and moose populations to determine enrichment factors for $\delta^{13}C$ and $\delta^{15}N$ for a wild population of predators and their prey. Isle Royale is a closed system with low food web complexity, where wolves are the top predator and moose are the only ungulate and dominant prey species. This system allows us to measure a fractionation under natural conditions, while minimizing other inputs (i.e. prey selection) that often complicate this calculation for wild carnivore populations. We also examine how the isotopic record of wolves and moose reflect known climatic and ecologic events that have occurred since the 1960's.

PALEOECOLOGY

Variation in herbivore bone collagen and tooth enamel $\delta^{13}C$ at a continental scale

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Carbon isotope analysis of the skeletal remains of grazing animals is a powerful and increasingly popular tool for reconstructing changes in the relative abundance of C_4 *versus* C_3 grass biomass (C_4 relative abundance) in both the recent and distant past. Despite the extensive use of this approach, there has been few attempts to quantify the strength of the relationship between C_4 relative abundance and $\delta^{13}C$ of grazing herbivores in extant ecosystems. We used the Australian continental as a natural laboratory and examined variation in the bone collagen and tooth enamel $\delta^{13}C$ of kangaroos, a group of closely related, predominantly grazing herbivores, in relation to C_4 relative abundance.

We measured C_4 relative abundance at 168 locations throughout the tropical and temperate zones of Australia, in ecosystems ranging from desert to humid forest. Statistical modelling showed that C_4 relative abundance was closely related to the relative abundance of rainfall in the C_4 and C_3 growing seasons (seasonal water availability) (76% of deviance explained). This relationship was used to predict C_4 relative abundance at 793 locations where kangaroo specimens were collected. Modelling showed that C_4 relative abundance, predicted from seasonal water availability, explained a large proportion (66%) of the variation in both bone collagen and tooth enamel $\delta^{13}C$. This figure increased to 73% when interspecific differences were accounted for; $\delta^{13}C$ of species with diets dominated by grass had a steeper relationship with C_4 relative abundance than of those with diets containing less grass. That the bone collagen and tooth enamel $\delta^{13}C$ of a predominantly grazing herbivore is highly correlated with C_4 relative abundance throughout a diverse range of ecosystems strongly supports the use of carbon isotopes to reconstruct past environments.

PALEOECOLOGY

Stable isotopic evidence of the effects of global change and sea-level rise on mammalian community ecology under glacial and interglacial conditions

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Sea-levels are rising and are predicted to continue increasing with global warming, tripling rates of rise by the end of the 21st century. Understanding the effects of climate change and sea-level rise on past environments is important for elucidating the potential consequences of current global warming. Glacial and interglacial cycles have influenced floral and faunal diversity in the past. Typically, warmer interglacial sites have higher species richness due to the ability of diverse floral communities to persist and subsequently support assorted faunal consumers. Artic and high-latitude temperate sites clearly demonstrate this pattern as ice sheets prevent floral and faunal communities from establishing themselves during glacial periods. Interglacial periods, however, may not always be synonymous with high species richness. Currently, Florida's coastal forest communities demonstrate a decline in floral species richness over time, in response to rising sea-levels and the increased frequency of tidal flooding. Due to Florida's flat topography, increased warming and subsequent sea-level rise reduces the land area available for flora and fauna communities.

A series of interglacial and glacial fossil sites spanning approximately 1.5 million years across the Pliocene-Pleistocene boundary were examined. Stable carbon and oxygen isotopes of biogenic apatite were used to: 1) compare the dietary niches of species present at multiple sites, 2) quantify the dietary niches of taxa unique to the glacial and interglacial sites, and 3) examine mammalian microfaunal niche partitioning through time and between interglacial and glacial sites. High-crowned horse teeth can also allow serial samples to quantify environmental variation at each of the sites, as inferred from the variation of carbon and oxygen isotope ratios over the course of the tooth's development. In order to quantify taxon-specific dietary shifts, I compare the inferred diets of the herbivorous megafauna present at the majority of the sites, including: the tapir *Tapirus* sp., the armadillo *Holmesina floridanus,* the ground sloths *Megalonyx leptostomus* and *Eremotherium eomigrans, the deer Odocoileus virginianus*, and the horse *Equus* spp. Tooth enamel, bone apatite, or orthodentine is compared among taxa. I also compared the occupied niche space of mammalian taxa unique to glacial or interglacial sites in order to identify potential differences in the ecology of mammalian communities. Lastly, the isotopic analysis of the mammalian microfauna can clarify how small mammals partition their niches with varying levels of species richness and presence of sympatric species. Quantifying ecological differences between glacial and interglacial sites in Florida provides a test of the effects of climate change and sea-level rise on past mammalian community dynamics. This has immediate relevance to clarifying possible biotic responses to current global warming.

PALEOECOLOGY

Carbon Isotopes, Extinct Megaherbivores, and Supposed Amazonian Refugia during the Pleistocene

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Much speculation has centered on the supposed presence of refugia in what is now dense tropical rainforest in the Amazon of northern South America. Refugia are “habitat islands” thought to promote speciation, e.g., as evidenced by the extraordinarily high Neotropical faunal and floral biodiversity today. With regard to historical biogeography and paleoecology, during the late Pleistocene it has been argued that the Amazon region consisted of patches of rainforest habitats (“refugia”) surrounded by tropical savanna grasslands.

This study presents carbon isotope data analyzed from teeth of extinct toxodont megaherbivores (Order †Notoungulata; Family Toxodontidae) that were widespread in Central and South America during the Pleistocene. Toxodonts have high-crowned teeth and thus traditionally have been interpreted as grazers. Toxodont fossils have been collected from Pleistocene localities in what is dense tropical rainforest of the Amazon today. If savanna grasslands surrounding forested refugia had existed during the Pleistocene, then it is likely that toxodonts would have fed on C4 tropical and temperate grasses (as can be documented elsewhere in drier regions of the ancient Neotropics during this time).

The mean carbon isotope values for toxodonts from what is now tropical Amazon rainforest (in Bolivia, Peru, and Brazil, latitude 8 to 14^{o} S) is –13.4 per mil (range –15.1 to –11.0 per mil), which translates into a plant diet with a mean value of –27.5 per mil. The latter value indicates a diet of predominantly C3 plants, not C4 grasses. As such, it is hypothesized that toxodonts lived in C3 forests, the precursors to those found in the Amazon today. There is no isotopic evidence for C4 savanna grasslands surrounding forested refugia in the Amazon during the Pleistocene.

H & O ISOTOPES IN HAIR

Isotope turnover in animal tissues: the reaction progress variable

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The reaction progress variable is applied to stable isotope turnover of biological tissues. We use the implicit assumption used in biological isotope turnover experiments that the system can be described by first-order rate kinetics. This approach has the advantage of readily determining whether more than one isotope turnover pool is present. In addition, the normalization process inherent to the model means that multiple experiments can be considered together although the initial and final isotope compositions are different. Consideration of multiple isotope turnover pools allows calculation of diet histories of animals using a time sequence of isotope measurements along with isotope turnover pools. The transit time or delayed release of a material, such as blood cells from bone marrow during a diet turnover experiment, can be quantified using this approach. Turnover pools can also be corrected for increasing mass during an experiment, such as when the animals are actively growing.

H & O ISOTOPES IN HAIR

Turnover of oxygen and hydrogen isotopes in the body water, CO_2, hair and enamel of a small mammal after a change in drinking water.

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Stable isotopes of oxygen and hydrogen in animal tissues are increasingly used by ecologists to study migration, to study resource utilization and as a method to reconstruct past climate. In general, the oxygen and hydrogen isotopic signatures of animal tissues are strongly correlated with the isotopic signature of local drinking water, and as a result, tissues such as hair, feathers and teeth may be used identify the location of origin for a selected animal, or as proxy for past climate. Despite the widespread utilization of this relationship, there has been limited research on the relative importance of various sources of oxygen and hydrogen to tissue signature. Likewise, there has been limited research on the rate of change in the oxygen and hydrogen isotopic signatures of various tissues after a change in location or a change in resource use by animals. We quantified the relationship between drinking water and tissue signature, and we determined the rate of change in oxygen and hydrogen of the body water, teeth and hair for a group of captive woodrats (*Neotoma cinerea*). Woodrats were supplied with isotopically depleted drinking water ($\delta^{18}O$ = -15‰; $\delta^{2}H$ = -120‰) or isotopically enriched drinking water ($\delta^{18}O$ = 15‰; $\delta^{2}H$ = 338‰). All woodrats were fed the same food throughout the experiment ($\delta^{18}O$ = 24‰; $\delta^{2}H$ = -109‰). After the woodrats had equilibrated with their drinking water, selected woodrats were switched to the opposite drinking water. We collected blood, breath and hair samples during the water switch, and we collected incisors at the end of the experiment. We used the reaction progress method to calculate the half-lives of oxygen and hydrogen in the body water. The half-lives of oxygen and hydrogen in water extracted from the blood samples were 3.6 and 5.8 days, respectively, and the half-life of oxygen in CO_2 was 3.1 days. Oxygen in the body water and CO_2 were in equilibrium (α = 1.038), and thus, breath samples were a reliable and non-invasive method of measuring $\delta^{18}O$ of body water. Using the reaction progress model to calculate the half-lives of oxygen in hair allowed us to calculate the half life for two pools of oxygen used in hair synthesis; a slow turnover pool (half-life = 144 days) and a long turnover pool (half-life = 13 days). Lastly, we used laser ablation to sequentially sample $\delta^{18}O$ of tooth enamel in the continually growing incisors. The drinking water switch was distinctive in the tooth enamel and using forward and inverse modelling techniques, we were able to model the input signal (body water $\delta^{18}O$) as measured by CO_2 samples. As a result of this experiment, we can reliably model the $\delta^{18}O$ of body water, hair and tooth enamel for a small mammal based on estimated $\delta^{18}O$ values of drinking water. Experiments that quantify and explain the relationships between sources of oxygen and hydrogen and tissue signature will increase the ability of ecologists to use tissues such as hair, feathers and teeth to study migration, resource utilization and climate.

H & O ISOTOPES IN HAIR

Hydrogen and oxygen isotope ratios in human hair are related to geography

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The hydrogen and oxygen isotope ratios that are recorded in human hair should reflect dietary food sources, drinking water sources, and atmospheric oxygen in some proportions. Here we provide evidence from randomly-sampled modern humans that geographic information related to the region-of-origin of individuals is recorded in their scalp hair. The hydrogen and oxygen isotope ratio values of organic matter in scalp hair (primarily keratin) were significantly and linearly related to the isotope ratios of tap waters in 65 cities across the USA. Based on linear regression analyses, 27% of the hydrogen and 35% of the oxygen atoms in human hair were attributable to tap water, with each linear regression explaining 86% of the observed variation. Based on the geographical distributions of the isotope ratios of tap waters across the USA, we constructed geographic information system (GIS) maps of the mean expected organic hydrogen and oxygen isotope ratios in human hair across the across the contiguous 48 states of the USA assuming equilibrium conditions. These spatial maps revealed discernable regions across which the hydrogen and oxygen isotope ratios of human hair were isotopically distinct. For instance, the hydrogen and oxygen isotope ratios of individuals from Texas, a state in the southwestern USA, were isotopically distinct from the states of Colorado and Wyoming to the north. Possible applications of these observations are extensive and do include reconstructing providing region-of-origin information for unidentified human remains.

H & O ISOTOPES IN HAIR

Multi-isotope comparison of modern and pre-modern human hair and the homogenization of human diet.

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Stable isotope ratio analysis of animal body tissues is now widely applied to elucidate the diet and feeding behaviour of modern, sub-fossil, and fossil animals. The stable isotopes of carbon and nitrogen have found the widest application, but an increasing number of studies reporting data on the hydrogen and/or oxygen isotope ratios are providing improved context for the interpretation of these data. Humans, like any other animal, are amenable to study using isotopic methods. Although several studies have previously presented C & N or H & O isotope ratio data from archaeological or modern human remains, to this point there has been no survey of sufficient scope to clearly document the characteristic isotopic domains of modern and pre-modern humans and compare these in the context of recent global changes in human behaviour and demographics.

We have analyzed the H-, C-, N- and O-isotope ratios of 157 hair samples collected during the 1930's to 1950's from individuals living in culturally isolated native populations on 5 continents and Polynesia. We compare the data for these samples of "pre-modern" human hair to a large, global database of stable isotope ratios of modern human hair, and find that the pre-modern dataset is distinctive in several ways. Within 3 of the 4 individual isotope systems, the pre-modern data span a larger range of values, despite the much larger sample number for the modern data set. For all 4 isotope systems, the pre-modern data exhibit a flatter (lower kurtosis) distribution than the modern samples, which in most cases approximate a normal distribution. The pre-modern data also commonly deviate from covariate trends among multiple isotope systems that are strongly preserved in the modern data, such as the characteristic co-variation between hair H- and O-isotope ratios. These patterns document a level of dietary diversity in the pre-modern populations that is not present or has been obscured in the data for modern, globalized humans.

Each of the distinctive characteristics of the pre-modern data set can be attributed largely to samples from a subset of the populations studied. In each case, consideration of the case study within the dietary context of the individual population indicates potential sources of the unusual isotopic data. Although the dominant cause can not be clearly identified in all examples, the probable mechanisms each relate to the distinctive and highly specialized diets of the pre-modern populations. This implies that stable isotope ratios of historically collected hair (or other human body tissues) provide a tool for reconstructing the loss of specialized diets by human populations responding to a range of external influences (recent globalization pressures being one among these). Closer inspection of the data for modern humans provides evidence for the partial preservation of specialized diets among several local populations. These represent cases where the process of dietary homogenization is ongoing and it, as well as its isotopic expression, can be studied in action.

METHODS AND MODELS

An isotope dilution approach to quantify the nutritional value of detritus

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Detritus represents an important, though heterogeneous, food source for benthic organisms. Bulk measurements such as C/N, lipids or amino acids are often used as food quality indicators. But what is taken up specifically from the detritus pool still stays largely unknown. Labelling food sources provides a powerful approach to study transfer in food webs, but due to its heterogeneity and unknown composition, it is difficult to produce labelled detritus.
We present an inverse approach: the deposit-feeding polychaete *Capitella capitata* was isotopically labelled by feeding them enriched ^{13}C and ^{15}N diatoms. After the enrichment period, the organisms were subjected to different treatments. When organisms take up carbon and nitrogen from unlabelled detritus, their isotope enrichment will decrease in proportion to the carbon and nitrogen availability of the food source to the deposit feeder.
In a preliminary experiment bulk $\delta^{13}C$ and $\delta^{15}N$ measurements were performed to investigate if we see the expected isotope dilution. Three treatments were applied: the labelled polychaetes were placed in either burnt sediment, which represents starvation, in high quality natural sediment, which represents a rather good food source, or in burnt sediment with diatoms, which represents an excellent food source. The expectations were that the amount of label would stay constant in the starvation treatment and decrease in the high quality sediment and diatom treatment, with the latter treatment showing the highest degree of isotope dilution. The method does work, but there were differences between the dilution of $\delta^{13}C$ and $\delta^{15}N$.
In a second experiment not only bulk $\delta^{13}C$ and $\delta^{15}N$ measurements, but also isotope measurements of fatty acids and amino acids were performed to study which specific compounds are taken up from detritus. Four treatments were applied: burnt sediment (starvation), low and high quality natural sediments (resp. a rather poor and a rather good food source) and burnt sediment with diatoms (excellent food source). The compound specific analyses of the labelled polychaetes should enable us to discern which compounds are preferably taken up from the detritus by *Capitella capitata* and should give us an idea about the carbon and nitrogen availability of these compounds in the different treatments.

METHODS AND MODELS

Error propagation and limits of resolution in inferring geographic origins from stable hydrogen isotopes

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Although there has been a recent and rapid proliferation of case-studies applying stable hydrogen isotope measurements to studies that infer geographic origin of tissues, there has been much less work done to define the inferential limits of the approach (e.g. geographic resolutions). Migration problems using hydrogen isotopes are generally approached deterministically and virtually none of the associated assumptions have been rigorously tested. While the work of testing assumptions and elucidating mechanisms progresses, we offer a stochastic approach to study the limits of deterministic models that have been used thus far.
We characterized and modelled two fundamental sources of variation universal to all hydrogen isotope values observed in the field. Because it is not possible to directly measure the number of isotopes in a sample gas, observed data are calibrated against a standard gas measured at the same time. These calibrated estimates include error that is never formally carried forward into subsequent ecological models. The second source of variation is metabolically derived in response to environmental stress. There are estimates of the amount of background fluctuation in hydrogen delta values expected from birds under various temperature conditions within the range of values expected in the field.
These sources of variation cannot be avoided or controlled for in natural studies. Therefore, by modelling the effects of compounding these sources of variance, we approximated the finest scaling realistically expected from natural datasets. We used nested probability distributions coupled with commonly applied deterministic models to simulate datasets of varying sample sizes. The statistical distribution of the analyses of simulated data describes the lower limit of the precision (the “grain”) that we can expect from studies using hydrogen to make inferences of geographic origin. This varies with geographic setting and choice of deterministic model approach.

METHODS AND MODELS

When isotopes aren't enough: using additional information to constrain mixing problems

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Stable isotopes are often used as chemical tracers to determine the relative contributions of sources to a mixture. Ecological examples include partitioning pollution sources to air or water bodies, trophic links in food webs, plant water use from different soil horizons, sources of respired C from ecosystems, and others. Mixing models based on isotopic mass balance can be used to solve for the source contributions as long as the number of sources does not exceed the number of isotopic tracers plus one. If the number of sources is greater than this, there is no unique solution but mixing models can still be used to place bounds on the source contributions (Phillips & Gregg, 2003). In many cases these bounds are wide, limiting the degree of ecological insight based solely on the isotopic evidence. However, researchers often have other ecological knowledge that can be used to further constrain these broad limits of source contributions. For example, in an animal dietary study, data on gut contents, prey abundance, and energetic or nutritional constraints may be applied to filter out sets of source proportions that are not ecologically feasible even if they satisfy isotopic mass balance.

Combining various isotopic and non-isotopic constraints on possible source contributions requires an inverse modeling approach. This may be implemented in several different ways; we used the IsoSource model (Phillips & Gregg, 2003), which is a convenient tool for providing the full range of solutions that satisfy isotopic mass balance. This model output can then be further processed to trim the range of solutions by imposing other non-isotopic constraints. Here we outline the specific procedure for this approach, and demonstrate it on three varied ecological studies that utilized stable isotope analysis to determine the relative contributions of numerous sources to a mixture. The first example analyzes the contributions of various organic C pools to CO_2 release in a forest fire, reflecting C pool sizes and logical rankings of the completeness of combustion (e.g., bark > wood). The second example examines plant water use from different soil horizons, constrained by soil water potential, root distribution, and horizon thickness. The third example analyzes food sources for a carnivore, with constraints on prey availability.

As demonstrated by these examples, other types of information can be fruitfully combined with stable isotope data in order to provide improved resolution of source contributions in mixing problems. Judiciously applied, additional non-isotopic constraints can lead to more realistic and interpretable results.

Reference:

Phillips DL, Gregg JW (2003) Source partitioning using stable isotopes: coping with too many sources. Oecologia 136: 261-269.

METHODS AND MODELS

Effects of temperature and ration size on carbon and nitrogen stable isotope trophic fractionation

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Stable isotope data are widely used to track the origins and transformations of materials in food webs. Reliable interpretation of these data requires knowledge of the factors influencing isotopic fractionation between diet and consumer. For practical reasons, isotopic fractionation is often assumed to be constant but, in reality, a range of factors may affect fractionation.

To investigate effects of temperature and feeding rate on fractionation of carbon and nitrogen stable isotopes in a marine predator, we reared European sea bass (*Dicentrarchus labrax*) on identical diets at 11 and 16°C at 3 ration levels for over 600 days. Growth rate was greatest in the warm tanks and within temperature growth was correlated with ration size. Nitrogen trophic fractionation ($\Delta\delta^{15}N$) was affected by temperature. Bass $\Delta\delta^{15}N$ was 4.41‰ at 11°C and 3.78‰ at 16.1°C. Carbon fractionation ($\Delta\delta^{13}C$) was also affected by temperature. Bass $\Delta\delta^{13}C$ was 1.18‰ at 11°C and 1.64‰ at 16.1°C. The higher lipid content in the tissues of bass reared at cooler temperatures accounted for the temperature effect on $\Delta\delta^{13}C$. When $\Delta\delta^{13}C$ was determined in the absence of lipid, there was a direct effect of ration size and $\Delta\delta^{13}C$ was 2.51, 2.39 and 2.31‰ for high, medium and low rations respectively. Reported $\Delta\delta^{15}N$ for all treatments exceeded the mean of 3.4‰ widely used in ecological studies of fish populations and communities. This would confound the interpretation of $\delta^{15}N$ as an indicator of trophic level when comparing populations that are exposed to different temperatures. Biases introduced by assuming constant trophic fractionation should always be quantified if it is impractical to undertake studies of the complex array of factors that might influence fractionation in the field. The $\Delta\delta^{13}C$ of 0-1‰ commonly applied in food web studies did not hold under any of the temperature or feeding regimes considered and a value of 2‰ would be more appropriate.

METHODS AND MODELS

New Developments in Sulfur Isotope Analysis and Applications to Ecological Research

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Sulfur (S) isotopes are well known to be extremely useful in deducing geologic and geochemical sources and processes, yet continue to receive only limited attention in ecological and biogeochemical research. Recent advances in the analytical aspects of S isotope analysis have been made and include improved accuracy, precision, and sample throughput. The development of novel analytical approaches has added greater flexibility and eased constraints imposed by small sample size and/or low S concentrations in organic matter and eliminated time consuming off-line extraction procedures. These developments have made it possible to assemble large datasets that can be combined with C and N isotope data. The broad range in δ^{34}S values that characterizes many ecosystems offers great promise in resolving sources and partitioning ecosystem processes, with applications to animal ecology, nutrient cycling, mass balance studies, and past climate reconstruction. For example, in collaboration with colleagues we were able to exploit a 55‰ range in δ^{34}S in a ten-year study of brown bear ecology. The range in δ^{34}S of potential diet items was more than double the range in δ^{15}N, which allowed for enhanced resolution of assimilated diet estimates and provided isotopic separation among ungulate species that were indistinguishable in δ^{15}N. Incorporation of marine-derived nutrients (salmon) into resident freshwater fish (potential bear diet item) was best traced with δ^{34}S, whereas δ^{15}N values appeared to be influenced by trophic position and/or by the relative protein content of fish diets. Studies of prairie wetland biogeochemistry provided an opportunity to use S isotopes to constrain S sources and fluxes to pothole wetlands and determine the mechanisms leading to ^{34}S depleted food web signatures. This application exploited the fact that S has multiple oxidation states and S isotopes are therefore sensitive to redox conditions. We demonstrated that S cycling is highly sensitive to wetland hydroperiod and that oxidation of soil sulfides to secondary sulfate was the dominant pathway coupling S geochemistry and prairie wetland food webs. These studies and others that we have pursued, illustrate the potential power in the addition of S isotopes to traditional C and N isotope investigations. In some situations, S isotopes may be the measurement of choice to address specific questions.

METHODS AND MODELS

Coupled NCS Isotope Measurements

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Ecologists have benefited greatly from technological advances that allow coupled isotope measurements of N and C isotopes from the same sample. This talk discusses a new, routine way to add S isotope measurements to the coupled N+C isotope measurements. The N+C measurements are made with using an elemental analyzer (EA) coupled to an isotope ratio mass spectrometer (IRMS), with N_2 and CO_2 gases produced by EA combustion separated on a gas chromatography (GC) column. The S measurements become possible when a second gas chromatography column is employed, with SO_2 gas slowly transiting a 1m column held at 50^{o}C while N_2 and CO_2 are separated in a second downstream GC column (a 2m column held at room temperature). After the C+N measurements, the second GC column is switched out of the gas stream, allowing a doubling of flow rates and SO_2 elution to the mass spectrometer. The combustion system for the coupled NCS system is simple, quartz + copper, and analysis times are 20-25 minutes per sample, yielding nine parameters for each sample: δ^{13}C, δ^{15}N, δ^{34}S, %C, %N, %S, N/C, S/C and S/N.

δ^{18}O contributions to the S analyses potentially confound the δ^{34}S measurements, but have been evaluated with new calculations based on a combination of SO + SO_2 isotope measurements. The overall sample prep system has been optimized successfully to buffer and minimize δ^{18}O variations, allowing accurate and precise δ^{34}S measurements from the EA system.

The next step in this technological evolution with elemental analysis will be to use the water of combustion for δD measurements. A likely future end-point for the EA-IRMS systems will be coupled HCNS 4-isotope measurements, with 4 dimensional data challenging us all to produce better science with the multiple tracers.

At the moment, the three-tracer NCS analyses allow construction of 3-D mixing models with four sources, tetrahedron mixing models that are useful in many contexts. An example application for an estuarine food web concerns the Rowley River estuary north of Boston, MA. A combination of elemental ratios and δ^{15}N in *Fucus* macroalgae shows that stream inputs are supplying pollutant N to this estuary. The elemental composition of animals is much more constant than that of plants, even for marsh mussels that in the past have been suspected of incorporating sulfidic sulphur from marsh sediments. The NCS tracers also show strong niche separation in benthic-feeding mummichogs (*Fundulus*) vs. plankton-feeing silversides (*Menidia*), with the niche separation preserved all along a 10km marine-to-freshwater salinity transect.

FROM INDIVIDUALS TO COMMUNITIES III: COMMUNITY STUDIES

Feeding strategies of Antarctic soil arthropods

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Antarctic soil ecosystems are relatively simple as compared to temperate soil systems. There are fewer species and there is less functional redundancy. Because there are no large grazers in the Antarctic terrestrial biome all the carbon and nitrogen from the primary producers appear to be directly entering the decomposition cycle. Due to the lack of large grazers the role of the soil arthropods is probably of larger importance than at more benign systems. The exact role of soil arthropods in Antarctic terrestrial ecosystems is however not completely clear.
Do these springtails and mites graze on the primary producers or do they only take part in the decomposition process? The potential food sources such as, alga, mosses and lichens show clear differences in ^{13}C and ^{15}N values. Field and lab studies on the stable isotope composition of the primary producers and soil arthropods have given us a better understanding of the role of these species in the flow of C and N.
To find the feeding preference of one of the dominant springtails (*Cryptopygus antarcticus*) we have conducted cafeteria, feeding experiments and analysed field specimens on ^{13}C and ^{15}N. By combining this information, we have gotten a clearer picture on the role of this species in the food web. Other species such as the oribatid mite *Alaskozetes antarcticus* and the mesostagmatid *Gamasellus racovitsai* have also been identified in their role of the food web.

FROM INDIVIDUALS TO COMMUNITIES III: COMMUNITY STUDIES

Zooplankton feeding selectivity on isotopically heterogeneous phytoplankton challenges classic stable isotope analyses of origins of zooplankton carbon

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Stable isotope analyses have been widely used to estimate terrestrial carbon contribution to zooplankton secondary production. In such studies, the $\delta^{13}C$ values of the two potential C sources for zooplankton, i.e. bulk phytoplankton and terrestrial carbon, are assessed and subsequently introduced in two-source mixing models. Subsequently, two assumptions are implicitly made: (1) zooplankton do not feed selectively on particular algae-classes within bulk phytoplankton, or (2) if zooplankton do, the variability of the $\delta^{13}C$ values amongst the different algae classes is small compared to the variability between the $\delta^{13}C$ values of bulk phytoplankton and terrestrial end-members.

In a field study of six coastal lakes of British Columbia, we investigated these assumptions, at two different seasons (July and October), performing stable isotope and carotenoids pigment analyses on particulate organic matter and zooplankton. Analyses of carotenoid pigments revealed that herbivorous cladocerans, in these lakes, consistently avoided diatoms at these seasons and positively selected cryptophytes in July, thus rejecting the first assumption. In addition, cryptophytes were shown to be substantially ^{13}C-enriched compared to bulk phytoplankton and even to the terrestrial end-member, thus refuting the second assumption. In July, the actual phytoplanktonic food source for herbivorous cladocerans was significantly ^{13}C-enriched compared to bulk phytoplankton. We conclude that applying bulk phytoplankton $\delta^{13}C$ as an end-point in a two-source mixing model in these lakes would overestimate the contribution of terrestrial carbon to zooplankton secondary production.

FROM INDIVIDUALS TO COMMUNITIES III: COMMUNITY STUDIES

Effects of biomanipulation on feeding niches of perch (*Perca fluviatilis*) and roach (*Rutilus rutilus*) determined by stable isotopes

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We are using natural abundance stable isotope analysis of carbon and nitrogen to characterise trophic linkages and carbon flow patterns in a freshwater lake recovering from severe eutrophication and undergoing biomanipulation. Here we present preliminary results on how the mass removal of fish can affect the food niches of two key fish species, perch (*Perca fluviatilis*) and roach (*Rutilus rutilus*). Sampling of perch and roach was started in 2003 (a year before the biomanipulation was started) and sampling will continue until the end of 2006. A significant change has already been observed in both perch and roach population mean $\delta^{13}C$ and $\delta^{15}N$ signatures after the biomanipulation, accompanied by wider statistical deviations around these mean isotopic signatures, indicating increased feeding niche breadth. In addition to adult fish, similar changes in isotopic signatures have also been observed in juvenile perch and roach.

FROM INDIVIDUALS TO COMMUNITIES III: COMMUNITY STUDIES

Stable isotope analyses reveal aquatic food web complexity and conservation concerns at different spatial scales

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The Bitter Lake National Wildlife Refuge in the southwestern United States is an unusual ecological complex encompassing wetlands, desert grassland-scrub, and dozens of saline sinkholes. Some of these water-filled sinkholes contain the only known populations of several endemic fish and macroinvertebrate species, and an understanding of the food web interactions in this system may allow refuge personnel to more effectively manage these sensitive species. In order to characterize the trophic structure of the sinkhole complex, samples of fish, aquatic insects, snails, amphipods, algae, and macrophytes were collected from eight sinkholes for stable carbon and nitrogen isotope analysis.

Contrary to expectations, distinct site-specific differences in 15N signatures, inferred trophic levels, and carbon sources were observed in conspecific organisms. For instance, delta 15N values for consumer and producer biota in one sinkhole are frequently about 3-5‰ higher than are the values from their conspecifics in the seven other sinkholes in this study. Because even macrophyte values are elevated, unique geographic effects may be the cause of the unusually high ^{15}N signatures observed at this site. In another case, a comparison of ^{15}N signatures for biota sampled in two sinkholes only twenty meters apart suggests that while fish act as top predators in one sinkhole (with the introduced red shiner as apex predator), dragonfly and damselfly larvae appear to occupy the upper trophic positions in the relatively species-poor sinkhole nearby.

These and the many other differences observed between the study sinkholes suggest that superficially similar aquatic habitat patches in close proximity can support communities with significantly different food web structures. As a result, the scale at which food webs are investigated may determine the degree of trophic complexity perceived in a patchy landscape, and this complexity must be taken into account when planning conservation activities.

FROM INDIVIDUALS TO COMMUNITIES III: COMMUNITY STUDIES

Community structure and food web based on stable isotopes (δ^{15}N and δ^{13}C) analysis of a North Eastern Atlantic maerl bed

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Maerl beds are highly biodiverse biogenic substrata that have been receiving increasing attention in the last decade. Although maerl beds represent important nursery areas for commercial fishes and molluscs, little is known on the trophic web of their communities. Community structure parameters of one maerl bed of the southern basin of the Bay of Brest (species richness, abundance, biomass and dominating species) were studied in parallel with the carbon and nitrogen isotopic composition of their main benthic species (macrofaunal, and megafaunal organisms) in order to assess the trophic levels and differences in the potential food sources of maerl inhabitants.
On both maerl grounds, the major potential sources of energy were identified to originate either from epiphytic macroalgae and microphytobenthos both growing on maerl thalli, together with sedimenting (sedimentary) particulate organic matter (POM) originating from the water column. The majority of the macro- and megafaunal organisms investigated were filter feeders, selective-deposit feeders and predators/scavengers. Filter feeders fall into three different groups representing different trophic pathways (i) sponges feeding directly on POM (water column filter feeders I), (ii) ascidians and holothurians feeding on POM and probably captured pelagic preys (water column filter feeders II), and (iii) filter feeding molluscs and crustaceans were hypothesised to feed on microphytobenthos or on decaying sedimented POM (Interface filter feeders). Selective deposit feeders were also divided into two subgroups. Carnivores were distinguished between those with scavenging habits and true predators.
Coupling of the trophic levels observed with the community biomass structure revealed that most of the benthic biomass derives its food from detritic sedimented POM and/or microphytobenthos, with interface filter feeders (15% of the biomass), selective deposit feeders (16%). Carnivores made up to 14% of the total biomass. Generally stable isotopes ratio mean values overlap and cover a large range within feeding types, indicating a strong overlap in food sources and a high degree of complexity of the food web presumably due to the diversity of the potential food sources.

FROM INDIVIDUALS TO COMMUNITIES III: COMMUNITY STUDIES

How fishing activities modify a benthic muddy-sand food web? A stable isotope approaches.

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The trophic structure of a muddy sand ecosystem of the Atlantic North East is studied in order to better understand biotic interactions between species but also to evaluate the impact of fishing activities on the benthic food web. This approach was lead through dual stable carbon (13C/12C) and nitrogen (15N/14N) isotope analysis of the organisms' trophic levels and potential food sources, coupled with community structure analysis of the fishing impact.

Demersal fishing gears currently used, in particular all over the North Atlantic, have become a global environment concern because of the damage inflicted to numerous non-target species. In addition to causing the loss of habitats or damage to their integrity and the death of non-target species, the physical perturbations induced by such devices significantly affect the structure of benthic communities and functioning of ecosystems.

The aims of this study were to better understand the food web of a characteristic muddy sand bay and to identify the possible interaction of fishing impact with the trophic chain. Direct impacts on benthic organisms were observed on by-catch as well as on non-captured organisms during an in situ experimental trawling of a previously unfished ground. Many isotopic studies have focused on complex benthic shallow waters ecosystem such as seagrass beds or mangrove but this study investigates how the food web can changed with the fishing impact. So, our main goals were therefore i) to try to identify the different food sources supporting the functioning of the muddy sand bay community, ii) to estimate trophic levels of its dominant species, iii) to delineate trophic relationships between these species and iv) to corroborated this food web with short term impact study and asses the potential long term impact on this trophic structure and its consequences at the ecosystem level.

Our results show that benthic trawling leads to modifications of trophic interactions (predation and competition) through species removal (target and bycatch species), direct and indirect mortality (stimulation of scavenging and opportunistic predation), mechanical disturbance of benthic habitats and finally changes the marine food web. Benthic food chains of heavily fished ecosystems are affected through the predominance of predators and scavengers which induces consequences on the ecosystem functioning and in turn for the resilience of the ecosystem.

FROM INDIVIDUALS TO COMMUNITIES III: COMMUNITY STUDIES

The role of stable carbon and nitrogen isotopes in determining a trophic cascade whereby invasive rats indirectly transform marine intertidal communities

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The vast majority of studies documenting the impacts of invasive species concentrate on the direct effects of predation or herbivory by an invader within a native ecosystem. Here I present data supporting the hypothesis that introduced rats in the Aleutian Islands indirectly affect invertebrate and marine algal abundance in the rocky intertidal via a cross-community trophic cascade. Specifically, I investigated how rat presence or absence indirectly affected the structure of the marine rocky intertidal through direct predation on birds such as Glaucous-winged Gulls and Black Oystercatchers that forage in the intertidal. Rat predation has significantly reduced marine bird abundances on rat-infested (n=8) vs. rat-free (n=15) islands which translates into significant differences in intertidal invertebrate abundance and algal cover on the two island types. Gull and oystercatcher densities were an order of magnitude lower on islands with rats (t = 2.6, df = 99.9, P = 0.01 and t = 4.7, df = 105.9, P < 0.01, respectively). Densities of herbivorous snails and limpets were over four times greater on islands with rats than without (Nested ANOVAs, $F_{1,21}$ = 7.89, P = 0.011 and $F_{1,21}$ = 5.19, P = 0.033, respectively). The differences in grazing invertebrate abundance resulted in a reduction by half in the percentage of area covered by fleshy algae on islands with rats ($F_{1,21}$ = 9.78, P < 0.01). Non-grazing invertebrates eaten by marine birds also demonstrated differences in abundance between island types. Barnacles covered over four and a half times as much area in the rocky intertidal on islands with rats as islands without ($F_{1,21}$ = 6.31, P = 0.020), while mussels and sea stars demonstrated a similar but non-significant trend ($F_{1,21}$ = 1.75, P = 0.201 and $F_{1,21}$ = 1.99, P = 0.173, respectively). Finally, less algal cover translated into increased settling space in the intertidal for other invertebrates not eaten by marine birds. Densities of sea anemones were four and a half times greater on islands with rats ($F_{1,21}$ = 4.42, P = 0.0.048) and tunicates and sponges exhibited a similar but non-significant trend ($F_{1,21}$ = 1.71, P = 0.205 and $F_{1,21}$ = 2.85, P = 0.106, respectively). I estimated the foraging ecology of coastal dwelling rats through behavioural observations and analyses of $\delta^{13}C$ and $\delta^{15}N$ values from rat (n = 41) liver, kidney, muscle, and fur and all possible prey items. The $\delta^{13}C$ and $\delta^{15}N$ data indicate that rats forage on a continuum that encompasses contributions primarily from coastal terrestrial plants and marine invertebrates. The analysis of several tissues allowed me to estimate their diets over time. Individuals appear to specialize on plants, invertebrates or a combination of both with little variation throughout the time periods represented by the various rat tissues. Both the stable isotope data and the behavioural observations indicate that the most common marine source for rats was amphipods. Two foraging behaviours were observed during scan sampling and rats spent six times longer foraging on amphipods than they did foraging on unknown invertebrates in the intertidal (t = 7.495, df = 4, P = 0.002). Isotopes indicated that birds contribute very little to the rats' diet. This was expected as rats decimated bird populations soon after rats were introduced up to 100+ years ago and therefore birds are currently a rare food source. My results illustrate an unexpected consequence of invasive animals, their potential to initiate trophic cascades that can lead to large-scale ecological impacts on plant abundance and community structure, and the role of stable isotopes in clarifying the ecological function of invasive species.

FROM INDIVIDUALS TO COMMUNITIES III: COMMUNITY STUDIES

The importance of cacti to consumers in a desert food web

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Columnar cacti are prominent features of arid and semi-arid ecosystems in the Neotropics. Cacti are unusual among arid zone plants in that they offer an abundance of succulent, energy rich fruit to vertebrates in an environment where water and nutrient abundance may constrain animal function. Quantifying the importance of these resources to consumers provides important insight into the role that cacti play in structuring animal communities. In the Sonoran Desert of Arizona, saguaro, *Carnegiea gigantea* and organ pipe cacti, *Stenocereus Thurberi*, provide extensive water and energy resources to the consumer community during the hottest and driest periods of the annual cycle. Between May and August, columnar cacti release a huge pulse of nutrients into the ecosystem in the form of floral nectar and fruit pulp.

This nutrient pulse can be tracked into consumers by means of its stable isotope signal, which differentiates cacti from other plant resources in the environment. Plants such as saguaro use CAM photosynthesis and have tissue carbon isotope ratios that differ strongly from the isotopic values of the majority of desert plant species, which use C3 photosynthesis (saguaro/organ pipe $\delta^{13}C$ = -13.0 ‰ VPDB versus $\delta^{13}C$ = -25.8 ‰ VPDB average for Sonoran Desert C3 plants).

During June, the peak period of fruit production, stable isotope analysis of avian plasma indicates that saguaro fruit represents approximately 43% of the bird community's carbon intake. These data show that the saguaro resource penetrates deeply into both insectivorous and granivorous avian guilds where it provides water, energy and nutrients.

In contrast, these resources have very limited penetration into other consumer groups. In small mammals, for example, cacti resources comprise less than 10% of the carbon intake of the rodent community during the same period. Carbon isotope measurements of arthropods, such as ants and bees (Hymenoptera), flies (Diptera) and beetles (Coleoptera), demonstrates that these groups apparently make only limited use of cacti resources as well. At higher trophic levels, in both arthropods and reptiles, there is little penetrance of cacti resources into consumers. This study is the first to provide insight into the potentially important role that this plant functional group plays as a consumer resource to an animal community in a desert ecosystem.

POSTER SESSIONS

Stable isotopes reveal alternate migration and foraging strategies in the parasitic phase of River Lamprey, *Lampetra fluvialis*, from the River Endrick, Scotland.

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It has long been known that the adult river lamprey population in the River Endrick, an inflowing tributary of Loch Lomond, shows a bimodal size distribution with the more numerous "small" individuals (range 166 to 257 mm length) migrating into spawning areas in addition to the less numerous "large" individuals (range 269 to 323 mm length), this being the typical size for river lamprey in other catchments in Scotland.
Here we test the hypothesis that this body size pattern is a consequence of differential migration and foraging strategies, specifically that:
a) the "large" (and typical) adults follow a migration and foraging pattern usual for the species i.e. migrating to sea to forage parasitically on sea fish before returning (after 1-2 years) to spawn and
b) that the "small" adults only migrate within the freshwater Loch Lomond system to parasitize freshwater fish before returning to spawn.
Individuals of the "large" and "small" body forms were collected by trapping as they migrated onto the spawning areas of the River Endrick between 2004 and 2006. The muscle tissue of 9 "small" and 6 "large" form river lampreys was analysed for stable isotopes of C and N.
The muscle tissue of all "large" form river lampreys showed a very clear and distinct marine isotope signature confirming that this form had been feeding at sea over a period of at least months prior to the return migration. All except one "small" river lamprey had isotopic signatures consistent with a period of feeding in fresh water over a period of months prior to the return migration. Thus we conclude that the two body size forms of river lamprey represent differing migration and foraging strategies with the large form migrating to sea and the small form only migrating within freshwater and foraging on other fresh water fish species.
One "small" form lamprey had a stable isotope signature that indicated marine feeding, however it is more likely that this individual remained in freshwater but parasitized one of the 3 marine feeding fish species which enter Loch Lomond, as lamprey wounds have been reported on these species in their freshwater phase.

Body size and stable isotope ($\delta^{15}N$ and $\delta^{13}C$) data to elucidate food web structure of trawl assemblage

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Modelling food webs based on body size spectra or body mass relies on the assumption that energy is transferred from the smallest to the largest animal through predation. Several studies have employed body size and stable isotope data to elucidate food web structure. The North Sea trawl assemblage shows a strong correlation between body size and $\delta^{15}N$ at community but not at species level (Jennings et al. 2001), however, fish body size and $\delta^{15}N$ are not related in coastal lagoon (Persic et al. 2004). Here it is shown that the Western Arabian Sea fish assemblage exhibits significant positive correlation between both $\delta^{15}N$ and $\delta^{13}C$ and $\log_2$ body mass classes. This suggests that for this assemblage, trophic level is determined substantially by fish body size at community levels as in the North Sea. Of three targeted sparid fish species studied in detail, *Cheimerius nufar* and *Pagellus affinis* showed no variation in $\delta^{15}N$ and $\delta^{13}C$ with body mass, while *Argyrops spinifer* did. Community and species level analyses were similar overall, $\delta^{15}N$ data indicating a difference of just one trophic level between the smallest and the largest size classes. In the Western Arabian Sea spatial and temporal sources of indirect variation appear to be important factors, feeding plasticity implying that the strong relationship seen in North Sea data is weaker here.

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Foraging ecology and ecotoxicology in Southern Ocean seabird communities

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Although there has been some work describing pollutant burdens of seabirds in the Southern Ocean, the factors that drive both intra- and inter-specific variation in these burdens remain poorly understood. While it seems likely that differences in prey preferences and foraging locations among individuals/species are likely to play a role, the relative importance of each is unknown. We will combine stable isotope and conventional approaches to assess the importance of variables such as trophic position, foraging location and prey preferences in describing variation in tissue pollutant levels both within and between species. Pilot data from the blood of adult petrels demonstrate that functional groups, (as defined by morphology and conventional dietary analysis), within the Procellariiform community are separable using stable isotope analysis. Moreover stable carbon isotopes also give an indication of where individuals are foraging. The intention is to understand how changes in trophic position and community structure, associated with crashes in krill population; may affect subsequent pollutant burdens of individuals both within and between species. The project will focus on heavy metals burdens of a number of Procellariiform species, mainly located at Bird Island, South Georgia and New Island in the Falklands. Further work examining the relationship between trophic positioning and Polychlorinated Biphenyls (PCBs) and Organochlorine (OCs) pesticides is also intended.

The influence of biodiversity on resource partitioning in intertidal gastropods.

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Niche differentiation and trophic partitioning are common mechanisms by which organisms found in the same habitat are able to co-exist whilst avoiding excessive competition for food resources. On rocky shores grazing gastropod molluscs compete for the epilithic biofilms and macroalgae which make up their diets. The biodiversity of these grazers varies naturally with latitude in the UK and it is likely that differences in feeding strategies and specialisations occur where the number of sympatric species differs. This work aims to determine whether this is the case by using stable isotopes to reveal dietary preferences within the guild of grazing gastropods found on British rocky shores at different levels of diversity. Eight shores were chosen and matched according to wave exposure and geology, four close to Oban in Scotland, an area of naturally low grazer biodiversity, and four near Plymouth on the southwest coast of England where diversity is higher.

Stable carbon and nitrogen isotope values were obtained for foot tissue from grazers in the two regions and compared with values for epilithic biofilm, live macroalgae and macroalgal detritus from the relevant shores which provide baseline signatures for the foodweb. Initial results from two of the eight study sites indicate that grazer isotope ratios are closely related to those of macroalgal food resources and that epilithic biofilms contribute surprisingly little to their diets: the $\delta^{13}C$ values of biofilms were substantially lower than those of grazers for both the northern and southern shores (mean differences are -3.94‰ and -5.42‰ respectively). In addition to this, trends indicating resource specialisation were shown in both regions. At the northern site $\delta^{13}C$ values of tissue from *Littorina obtusata* show that this species occupies a different trophic niche to co-occurring grazers (mean $\delta^{13}C_{L.\ obtusata}$ = -15.53 ± 0.25‰, 1.44‰ higher than the sample mean of other species present). At the southern site the $\delta^{15}N$ value of tissue from the top shell *Gibbula umbilicalis* was elevated relative to that of other grazers; indicating that this species may be deriving some of its nutrition from higher trophic levels. No evidence of such a strategy was found for *G. umbilicalis* at the northern site suggesting it may be more specialised in its feeding preferences at higher levels of diversity.

Further analyses will establish how these trends apply across a number of sites and clarify the interactions between grazers and their food resources. Additional isotopic examination of the grazer-biofilm relationship is also required to confirm the potentially contentious findings that intertidal grazers apparently derive little nutrition from biofilms.

Community structure and food web based on stable isotopes ($\delta^{15}N$ and $\delta^{13}C$) analysis of the North Bay of Biscay fishing ground (Northeast Atlantic)

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The North Bay of Biscay continental shelf is an important French benthic and demersal fishery, but little is known on the trophic food web of its benthic communities. In order to assess the benthic trophic web in relation with the different potential food sources, the purpose of this study is to describe the macro and megafaunal benthic communities structure, in parallel with the carbon and nitrogen stable isotopic composition of the main benthic and demersal species.
Two distinct benthic communities were sampled: the muddy sands *Brissopsis lyrifera*, *Dasybranchus gajolae, Callianassa subterranea* community of the Grande Vasière central part and outer Bay of Biscay *Ditru*pa sands community of higher species richness, abundance and biomass than the muddy sand community.
Both communities trophic structure are dominated by deposit, suspension feeders and predators, distributed in three main trophic levels. Large differences in stable carbon ratios values within the primary consumers attest of two different food sources components: i) a pelagic component made up of fresh sedimenting particulate organic matter on which zooplankton and suprabenthos feed and ii) a benthic component supplying deposit feeders. Isotopic differences were also observed within the upper trophic levels which allowed estimation of the contribution of each component to their diet.
Finally, the use of stable isotopic compositions together with species feeding strategy allowed discrimination between the trophic functioning of the two benthic communities.

Assessment of polychlorobiphenyl bioaccumulation in the spider crab food web using stable isotopes.

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Maja brachydactyla is a decapod crustacean of great commercial interest which is largely distributed along the Northeast Atlantic coast. The main objective of this work was to assess the bioaccumulation processes of polychlorobiphenyls (PCBs) which are lipophilic organic contaminants, in this species.

Carbon and nitrogen stable isotope compositions and PCB contamination were investigated in the spider crab food web from the Iroise Sea (Western Brittany) and the Seine Bay (Eastern English Channel). The biota examined included sediment, macroalgae and phanerogames, as well as various benthic species of polychaetes, molluscs, echinoderms and crustaceans. From primary consumers to upper predators, the benthic food web of the spider crab from the two sampling areas covered almost three trophic levels. PCB concentrations were all significantly higher in organisms from the Seine Bay than those from the Iroise Sea. The examination of the PCB patterns showed increased influence of higher chlorinated congeners with the isotopically derived trophic level (TL) of the organisms. Moreover, PCB concentrations were significantly related to TL in the spider crab food web from the two sampling areas. The highest food web magnification factors (FWMFs) were calculated for the congeners with 2,4,5-substitution, and were lower in the spider crab food web from the Seine Bay, compared to the Iroise Sea.

Influence of lipid extraction on stable carbon and nitrogen isotope analysis of crustacean tissues: potential consequences for marine food web studies.

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The analysis of naturally occurring stable carbon and nitrogen isotope ratios is an important tool to work out trophic relationships, especially in marine ecosystems. However, the interpretation of $\delta^{13}C$ and $\delta^{15}N$ results is not always straightforward because of the influence of multiple factors such as the tissue-specific lipid content.

The aim of this work was to evaluate the effects of lipid extraction on $\delta^{13}C$ and $\delta^{15}N$ values in muscle, hepatopancreas and gonads of a marine crustacean, the spider crab *Maja brachydactyla*. For that, samples were analyzed for stable isotopes before and after lipid removal, using a derived Soxhlet extraction method.

Differences in $\delta^{13}C$ and $\delta^{15}N$ were observed among tissues before and after pre-treatment. Lipid extraction from muscle did not lead to any significant effect on either $\delta^{13}C$ or $\delta^{15}N$. By contrast, ecologically significant shifts for both carbon and nitrogen stable isotopes were observed in the spider crab hepatopancreas. As regards gonads, lipid extraction led to a shift only for $\delta^{13}C$.

Finally, the derived Soxhlet extraction method removed the lipid influence for stable isotopic analysis. We recommend this pre-treatment especially in the case of lipid-rich tissue but also for stable isotope studies on whole organisms.

Carbon isotope ratios ($\delta^{13}C$) of macro-invertebrates in assessing lake trophic functioning

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The European Water Framework Directive (WFD) (2000/60 EC) requires, for all members, an assessment of the ecological status of aquatic ecosystems. This status has been defined as a result of the structure and the functioning of aquatic systems. Currently, most of the biological methods for lake monitoring are based on their trophic level and not on their functioning. Therefore, the aim of this study is to contribute to a better knowledge of lake trophic functioning i.e. the lake's ability to transfer the organic matter up to consumers.
Two approaches based on the macro-invertebrate communities have been applied on 12 French lakes:

- ✓ The Lake Biotic Index (LBI) (Verneaux & *al*., 2004), a new lake biological quality assessment method, comprising two indices, each of them giving rise to a peculiar interpretation: the Bl index reflecting a trophic potential and the Df index, interpreted as the trophic functioning result i.e. the efficiency of the organic matter transfer.
- ✓ The carbon isotope ratios ($\delta^{13}C$) of macro-invertebrates sampled according to the LBI method. These ratios have been analysed to test relationships of the LBI interpretations (in term of trophic potential and organic matter transfer) with carbon sources and organic matter recycling.

The LBI obtained, from 12 French lakes, showed that systems greatly differed through both their trophic potential and their organic matter transfer. The results, concerning the LBI, showed also that all combinations between high/low trophic potential and efficient/no efficient organic matter transfer exist. The results of carbon isotope ratios, from 7 lakes, revealed great differences between mean $\delta^{13}C$ values, standard deviations and the depth related $\delta^{13}C$ variations.
Based on the macro-invertebrate $\delta^{13}C$ results obtained from 7 lakes, preliminary interpretations could be proposed:

- ✓ The littoral $\delta^{13}C$ standard deviations would be related to the lake trophic potential; despite none correlation between Bl index and $\sigma\delta^{13}C$ have been obtained, two groups of lakes appeared: lakes with weak trophic potential and standard deviation of $\delta^{13}C$ values less than or equal to 1 and lakes with high trophic potential and standard deviation of $\delta^{13}C$ values superior to 1.
- ✓ The variations in $\delta^{13}C$ values between littoral and deep zones, correlated to the Df index, could reflect the trophic functioning. Through the 7 lakes, two types of trophic functioning have been defined: one type based on a high heterotrophic organic matter recycling activity and another based on direct organic matter consumption without recycling.

The $\delta^{13}C$ values could be used as descriptors of lake trophic functioning.

FROM INDIVIDUALS TO COMMUNITIES A9

Assessing the consequences of foraging strategy on cormorant productivity

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Cormorant numbers in Britain and Ireland have increased in recent years resulting in concerns regarding the possible impacts on commercial fishing interests. Initially conflicts focused on cormorants overwintering at inland freshwater habitats, but increasingly colonies of cormorants have begun to feed inland during the breeding season and consequently there is now the potential for conflict with fisheries throughout the year. In Northern Ireland, the contribution of freshwater prey items to the diet of breeding cormorants has been shown to exceed that of non breeding cormorants, with breeding birds traveling up to 60km per day to feed at inland freshwater sites [1]. Since the strategy of inland freshwater feeding requires a greater energy investment than a marine diet because of the increased distance traveled during foraging trips, it seems likely that there must be some benefit associated with feeding on freshwater fish. This may take the form of increased nutritional value of prey items or greater predictability of prey. Further, the fact that breeders appear to employ this strategy while non-breeders do not suggest that freshwater foraging may be crucial in terms of productivity and fitness. However, these hypotheses have yet to be tested.

We are using stable isotope analyses to assess the amount of marine prey in cormorant diets at one of Northern Ireland's largest coastal breeding colonies. For the first time we will investigate the potential consequences (and determinants) of variability in dietary preferences among cormorants, by relating foraging specialisation to a suite of reproductive, demographic and condition related parameters. We will also fit GPS loggers to a sub-sample of birds and thus be able to link specific foraging locations to fine scale foraging strategies (i.e. within marine or freshwater habitats as opposed to between).

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Seals as "pests": foraging strategies and potential for conflict

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It is often the perception of those who depend on the fishing industry that piscivores have a substantial impact on fish stocks which affects their livelihoods. This can lead to conflict between statutory obligations to protect biodiversity and the need to ensure that fishing industry remains sustainable. In extreme circumstances this conflict can result in the illegal culling of predators, which fishermen consider pests, such as seals. If legal obligations to protect seals are to be met then the impact of seals on fishing interests must be understood.

It has been suggested that seals feeding at salmon nets and fish farms are specialist foragers or "rogue" seals [1] exploiting that particular resource, therefore the occurrence of generalist and specialist feeders within a seal population is of relevance when considering the conflict between seals and fisheries. This project aims to investigate the foraging ecology of the common seal and to assess the prevalence of generalist and specialist foragers in the seal population.

Specialist and generalist foragers can be identified by comparing the isotopic signatures of tissues which integrate information over short time scales (e.g. blood) with those which integrate information over longer time scales (e.g. whiskers). In specialist foragers there will be little or no variation in isotopic signature between short term integrators and long term integrators. Conversely in generalist foragers there will be greater variation in isotopic signatures of short term integrators than long term integrators [2]. Variation in isotopic ratios in blood and whisker samples will be used to identify specialist and generalist common seals.

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A seasonal survey of the benthic food web of the Lapalme's Lagoon (Aude, France) assessed by carbon and nitrogen stable isotope analysis

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Northwestern Mediterranean brackish Lagoons constitute privileged ecological area for the growth of many migrant species (marine and freshwater fishes, migratory birds, etc…), as well as for a few resident species, which can support frequent and important fluctuations of abiotic factors. Receiving run-off from upland, Mediterranean Lagoons are very productive ecosystems and generally feature multiple sources of organic matter (freshwater inputs, salt-marsh plants, phytoplankton, seagrass and macroalgae,…), all potentially sustaining the aquatic food web. The identification of the origin of organic matter fuelling this food web in such complex ecosystems is thus a challenging topic.

We used carbon and nitrogen stable isotope analysis to describe the structure of the food web of the Lapalme's Lagoon (Aude), one of the best preserved brackish Lagoons of the French Mediterranean coast. δ^{15}N and δ^{13}C of the main potential sources of organic matter and consumers (macro invertebrates and fishes) were measured during three seasons (June 2004, September 2004 and February 2005). The aims of our work were 1) to investigate the major organic matter sources sustaining the food web of the Lagoon and possible heterogeneity in the basis of this food web along a salinity/confinment gradient, 2) to study the structural complexity of this food web through the determination of trophic levels, and 3) to assess whether the major trophic pathways of this food web varied seasonally and to identify which species are responsible for such temporal changes.

The food web of the Lagoon appeared to be mainly based on both sedimentary organic matter (SOM) and suspended particulate organic matter (POM). In view of its δ^{13}C, SOM pool seemed to be mostly a detritic mixture of ^{13}C-depleted salt-marsh plants and macroalgae and ^{13}C-enriched seagrass. Low C/N ratio of SOM suggested however that phytoplankton contributed substantially to this pool of organic matter. On the ground of δ^{15}N values, the food web was found to be distributed on 4 trophic levels (TL), bivalves corresponding to TL2 and fishes occupying the top of the food web. Although the overall structure of the food web did not change significantly throughout the year, detailed investigation of the main species of the Lagoon (especially *Cerastoderma glaucum*) showed some marked temporal and spatial isotopic variations. Most of the spatial variation originated from high δ^{15}N and low δ^{13}C values obtained in a very confined basin, suggesting possible intense denitrification processes in the sediment and a strong contribution of salt-marsh plant as food source via the detritic pathway. Slight but significant spatial variability in the main species of the rest of the Lagoon may be interpreted as a higher influence of salt-marsh plants and macroalgae against seagrass with increasing distance from the open sea connection.

Spatial and seasonal evolution of carbon cycling in the Scheldt estuary using stable isotopes

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Since several decades, human activities strongly impact the functioning of the Scheldt river and estuary, located in one of the most densely populated regions of Western Europe (Belgium – The Netherlands). Since 1996, a monthly monitoring follow-up was performed over a longitudinal transect in the freshwater to mesohaline reaches of the Scheldt (20 sites), as well as in four major tributaries. This time series was initiated in the framework of the OMES project and aims to assess the health condition evolution of the Scheldt ecosystem resulting from the implementation of waste water treatment measures and possibly also from natural long term changes. In addition to parameters such as total alkalinity, pH and nutrients, a stable isotope approach was conducted on dissolved inorganic carbon (DIC), particulate organic carbon (POC), particulate nitrogen (PN). Moreover, for the years 2005 and 2006, a recently developed technique allowed us to determine the C isotopic signature of the dissolved organic carbon (DOC).

All these measurements provide valuable insight in temporal changes in the biogeochemical cycling of carbon in the Scheldt system. We will discuss the seasonal and longer term evolution of parameters such as $\delta^{13}C_{DIC}$, $\delta^{13}C_{POC}$□□, $\delta^{13}C_{DOC}$, $\delta^{15}N_{PN}$ in terms of chemical and biological processing, and as useful tools for improving our understanding of source and sink terms for carbon.

Bat migration; a pilot study using stable isotope analyses

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Many European bat species migrate, but there has been no direct evidence to date, that suggests that species actively migrate to the UK. However some authorities have argued that the Daubenton's bat and the Serotine bat do migrate to the UK and that Pipistrellus species may show signs of a partial migration, as shown within continental Europe. However, assessing migration in a cryptic and nocturnal group, such as bats, is problematic. Conventional approaches such as ringing have provided some information on long distance movements, but this technique requires recapture of the animal, which tends to occur only infrequently.

Here we use δ^2H to investigate seasonal movements of bats to and from the UK. Although latitudinal gradients of δ^2H in precipitation are not as pronounced in Western Europe as they are in North America, previous work has indicated that they offer considerable potential in this respect. We use museum specimens collected within the UK during spring and autumn, between the years 1996-2004, comprising 22 *Myotis daubentonii*, 13 *Eptesicus serotinus* and *48 Pipistrellus* species. We will present the preliminary results from this work.

The Future of Madagascar's Lemurs: Coping with Change

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Lemurs have been evolutionarily isolated on the island of Madagascar since their arrival roughly 60 million years ago. Most extant and all extinct lemur species are or were forest-dependent. Even *Lemur catta*, the least arboreal extant lemur, relies on forest species for food. Unfortunately, survival of the island's diverse forest communities and their resident lemurs is uncertain. Today, what little remains of the island's natural habitat is highly fragmented. Despite continuing field research, surprisingly little is known about most lemur taxa. It is unclear if lemurs that comprise modern communities are taxa that have acclimated to human change, or if these modern species are just as vulnerable to habitat loss as their extinct counterparts. The majority of our understanding of lemur diet and habitat use comes from observational data and tooth-wear analysis. New isotopic evidence will augment these data sets. Using stable carbon, nitrogen and oxygen isotopes from bone, our research will identify ecological niches of multiple sympatric modern lemur taxa at several localities and to compare these results with subfossil material. By evaluating isotopic signatures from subfossil and modern data, our project will address: (1) how vegetation may have changed at specific localities over time, and (2) if lemurs that persist at a site have changed their foraging strategies or ecological niche space to survive. Results from this research have the potential to help identify those lemur species that are resilient to environmental change and those that are not.

Preliminary $\delta^{13}C$ and $\delta^{15}N$ collagen data suggest that modern *Lemur catta* are a full trophic level higher than *Propithecus verreauxi*. Subfossil and modern $\delta^{13}C$ and $\delta^{15}N$ values for *L. catta* and *Lepilemur leucopus* differ by one to several permil. Part of this difference might be related to geographical variation between modern and subfossil sites. Among the extinct taxa, *Pachylemur insignis* has $\delta^{15}N$ and $\delta^{13}C$ values similar to subfossil *P. verreauxi,* and *Archaeolemur sp*. has values similar to subfossil *L. leucopus*. All lemurs have lighter N and C isotopic values than the sympatric, carnivorous *Fossa fossana*.

Isotope trophic-step fractionation in marine suspension-feeding species

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Multiple stable isotope analyses are becoming a useful tool to study trophic webs and interest in this technique largely spread in terrestrial and marine ecology to trace both the fate of organic matter sources and investigate trophic relationships in ecosystems. Typically, the principle is to compare isotopic signatures of primary producers with consumers, keeping in mind that there's an enrichment of the isotopic tracer – heavy isotope ^{13}C or ^{15}N – from prey to predator.

In most of the studies dealing with marine food webs, the value of this enrichment (called fractionation) is considered to be unique whatever the species or the food source considered. Common values of ^{13}C and ^{15}N fractionation (hereafter $\Delta\delta^{13}C$ and $\Delta\delta^{15}N$) are 1‰ and 3.5‰ respectively. Nevertheless, in order to accurately estimate trophic interactions and to avoid misinterpretation in the results, fractionation must be valued precisely for each studied species. The aim of this study was to assess experimentally $\Delta\delta^{13}C$ and $\Delta\delta^{15}N$ values in some marine invertebrates of ecological importance in trophic food webs of coastal zones.

Six suspension feeding species – *i.e.* bivalves *Crassostrea gigas*, *Mytilus edulis*, *Cerastoderma edule*, gastropod *Crepidula fornicata*, and polychaetes *Sabellaria alveolata* and *Lanice conchilega* – have been fed during 3 months under highly controlled conditions with a single food source of microalgae. $\delta^{13}C$ and $\delta^{15}N$ values have been assessed at 0, 4, 8, 15, 30, 45, 60 and 90 days in consumers and every 3 days in primary producers.

Overall, a new equilibrium has been reached with a shorter interval for $\delta^{13}C$ than for $\delta^{15}N$: respectively by c.a. 30 and 40 days after the dietary change for cockles and by 100 and 110 days for slipper limpets. For 3 of 6 species, model indicated that the new equilibrium has not been reached after 90 days. Fractionation values are not as consistent as suggested in literature. Variations are larger for δ15N than for δ13C. Trophic steps in oysters, mussels and sand mason are closed to assumed values (1‰ for d13C and 3.5‰ for d15N) but cockles, honeycomb worms and slipper limpets exhibited much higher values. Because of strong differences in bivalve species, we suggested that feeding process and metabolic rates are involved in fractionation.

Macrobenthic assemblages associated with Lanice conchilega populations under oyster farming influences: trophic approach using natural stable isotopes

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The terebellids tubiculous polychaete *Lanice conchilega* colonise coastal areas and is often associated with shellfish farming. Polychaete tubes are known to enhance local biodiversity as engineer species by modifying surface heterogeneity and creating habitat for numerous invertebrates. This species has strong colonisation capacities and is considered as invasive species by oyster farmers. In this study, we examined infauna associated with *Lanice conchilega* tubes according tube density and/or presence of cultivated oysters. In France, oyster are reared off-bottom in culture bags on iron tables at a distance of ca. 50 cm from the sediment allowing benthic communities to colonize the substrate under them. We hypothesized that these two factors may influence trophic food web functioning – i.e. trophic competition, prey-predator interactions, diversity of trophic guilds etc. – and we used $\delta^{13}C$ and $\delta^{15}N$ natural stable isotopes compositions of organisms to analyse trophic food web within *L. conchilega* patches, under or at a distance of cultivated oysters.

We showed that *L. conchilega* individuals had not the same stable isotope signature when they were under oyster bags meaning that they probably had different feeding behaviour and diet in that case. We suggested that oysters feces and pseudofeces could be considered as organic matter source for suspension feeders. We also noted that trophic structure is much more equilibrated and richer in terms of trophic guild away from oyster bags: while all trophic guilds – i.e. suspension-feeders, surface deposit-feeders, sub-surface deposit-feeders, predators – are represented out of the tables, infauna associated with soft sediments under oyster bags are strongly dominated by predators. Nevertheless, we noted that isotope signature of predators – i.e. carnivorous – are widely dispersed in $\delta^{15}N$ range, indicating various feeding sources. One of our main conclusions is that analysis of feeding guilds using species abundances and diversity is not enough to understand trophic interactions and food web complexity. We clearly show here the useful nature of stable isotope analysis in the investigation of food webs.

Using stable isotopes to evaluate the impact of nesting seabirds on island vegetation

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Double-crested cormorant (*Phalacrocorax auritus*) populations breeding on the Great Lakes have increased tremendously over the last 30 years. Seabird nesting activities have long been recognized to modify vegetation at nesting and roosting sites. Previous work has demonstrated a decline in forest health at a stand level however; the physiological impacts at a tree level and specific mechanisms of seabird impact have not been explored. Here, we examine stable carbon and nitrogen isotopes in tree rings and foliage for potential signs of stress (carbon) and altered nutrient uptake (nitrogen). Our results suggest that trees in cormorant colonized areas are experiencing increased levels of stress manifested as a shift in $\delta^{13}C$ values detected in both foliage and tree ring samples. Foliage $\delta^{13}C$ were significantly greater from trees in colonized areas which suggests less isotopic discrimination was occurring than in leaves collected from a non-colonized area. In bird colonies, studies have shown that foliar $\delta^{15}N$ is enriched, approaching the signal evident in seabird guano. Our results corroborate this; significantly higher $\delta^{15}N$ values were found in tree rings from islands with nesting cormorants and these values were similar to those measured in cormorant guano. Our study demonstrated how analyzing stable isotopes in tree rings can enhance traditional dendrochronological research. In our study, stable isotopes in tree rings reflected the impact of a subtle environmental stressor that caused a sub-lethal response in affected trees. This response would not have been detected using growth data alone.

Stable carbon and nitrogen isotopes in faeces and body of locusts, *Schistocerca gregaria,* fed mixtures of isotopically distinct diets

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For decades, stable isotopes have been used by ecologists to derive the food sources of animals by analysing their whole body or samples of body tissues. More recently, this interest has spread to animal scientists and food chemists looking for ways to trace the origin or production system of animal products used as human food. In spite of this expanded interest, the number of well controlled studies in which animals were fed different mixtures of isotopically distinct diets is still limited.

In the experiments described here, locusts (*Schistocerca gregaria*) were fed different mixtures of wheat and corn leaves. Corn leaves were collected in 2003 and 2004 from commercially managed fields at the research station "Meiereihof" of the University of Hohenheim, Germany, when plants were flowering. Wheat for the first experiment was cultivated in a green house and fertilised with guano in order to induce an enrichment of ^{15}N in the wheat compared to the corn fertilised with synthetic urea. For the second experiment, wheat was collected from the research station for organic farming "Klein-Hohenheim". All leave material was freeze-dried and milled to a fine powder; in the case of corn, leave veins were removed before milling. For each experiment, 5 diets were prepared containing 100% corn leave meal, 25% wheat/75% corn leave meal, …, 100% wheat leave meal (w/w). Egg pouches were collected from a laboratory culture of *Schistocerca gregaria* and incubated at 30°C. Immediately after hatching, nymphs were transferred to five glass containers in an incubator set to a 12h day at 35°C and 12h night at 25°C cycle, relative humidity was kept <40%. The five diets were randomly assigned to the five containers. Nymphs were fed *ad libitum*, but in order to ensure that the nymphs did not preferentially ingest one ingredient of the mixtures, new feed was only offered after the previous ration had been completely ingested. During the last nymph stage, faecal material was collected for analysis. After moulting to adult stage, locusts were collected, frozen and freeze-dried. Feeds were analysed for proximate composition. Total feeds, faeces, lipids and lipid-free matter of feed, faeces and total locusts and chitin of locusts were analysed for $\delta^{13}C$, feeds, faeces, total locusts and chitin for $\delta^{15}N$.

While the composition of corn leaves was rather constant between the two batches, (I: 24.3% crude protein, $\delta^{13}C$ = -12.4‰; $\delta^{15}N$ = 4.2‰; II: 21.7% crude protein, $\delta^{13}C$ = -12.4‰; $\delta^{15}N$ = 4.2‰), composition of wheat leaves differed strongly (green house: 47.2% crude protein, $\delta^{13}C$ = -27.2‰; $\delta^{15}N$ = 11.9‰; organic farm: 5.9% crude protein, $\delta^{13}C$ = -29.2‰; $\delta^{15}N$ = 5.3‰). In the 2nd experiment, it was not possible to rear locusts on the 100% wheat diet to adult stage, probably due to the low protein content. Isotopic composition of locusts followed generally that of their diets, however, not strictly parallel. Trophic shift for carbon in lipids and lipid-free matter differed between wheat and corn based diets. In both experiments, faeces of locusts fed corn leave rich diets were depleted in ^{15}N compared to the diet, while those of animals fed entirely wheat leaves were enriched.

The results of these two experiments stress the importance of considering proximate composition and substrate-specific values of trophic shift when applying stable isotopes in studies on nutritional ecology of animals.

Effect of dietary protein level and feeding rate on trophic shifts of C and N isotopes and on the activity of enzymes involved in the amino acid metabolism of Nile tilapia, *Oreochromis niloticus*

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Dietary back-calculation using incorrect estimates for the trophic shift may lead to huge mistakes in the calculated contributions of possible food sources to the growth of animals. The influence of dietary protein content and feeding level on the trophic shifts of C and N isotopes ($\Delta\delta^{13}C$, $\Delta\delta^{15}N$) has been described in different species and therefore has to receive attention in dietary back-calculations. In order to test whether the measurement of the activities of enzymes involved in the amino acid metabolism could be used to estimate $\Delta\delta^{13}C$ and $\Delta\delta^{15}N$ more precisely, Nile tilapia (*Oreochromis niloticus*) were fed three isolipidic and isoenergetic diets differing in their protein contents (20, 29 and 39%) at three levels (2, 4 and 8 g per kg metabolic body mass per day). All diets were made of casein, wheat starch**,** and corn germ oil and were supplemented with vitamins, minerals and L-arginine. The experiment lasted eight weeks. Body composition, gross energy and $\delta^{13}C$ and $\delta^{15}N$ values were determined in diets and fish bodies. The livers of fish were assayed for the activity of aspartate aminotransferase (ASAT) and glutamate dehydrogenase (GDH).

The feeding level affected the activities of ASAT and GDH as well as $\Delta\delta^{13}C$ and $\Delta\delta^{15}N$ significantly, but the dietary protein content had no significant effect except on the specific activity of ASAT. The activities of ASAT and GDH in the whole liver were significantly correlated with the protein gain of the individual fish. $\Delta\delta^{13}C$ and $\Delta\delta^{15}N$ were significantly lower in fish with a higher protein utilisation. The correlations between activities of both enzymes with $\Delta\delta^{13}C$ and $\Delta\delta^{15}N$ were not satisfactory to predict the trophic shifts from enzyme activities.

Further studies should examine whether the measurement of activities from enzymes involved in the amino acid metabolism combined with enzymes involved in the lipogenesis, the glycolysis and the gluconeogenesis could help to obtain reliable estimates for the trophic shift in situations where the diet quality as well as the amount of diet consumed are not known.

Stable isotopic comparison in otoliths of Atlantic cod *Gadus morhua* and Pacific cod *Gadus macrocephalus*

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Atlantic cod *Gadus morhua* and Pacific cod *Gadus macrocephalus* are commercially important fish species with similar life history and behaviour. We report a comparison study on stable isotopic compositions of their otoliths. In general, the mean $\delta^{18}O$ values of otoliths of Atlantic cod were about 1.0‰ more positive than those of Pacific cod, with a range of -0.49‰ to +3.58‰ representing the progression of spawning to adult stages. These differences appear to show that spawning conditions and adult habitat of the two species were markedly different. In contrast, $\delta^{13}C$ mean values in otoliths of Atlantic cod were as high as 1.95‰ more negative than those of Pacific cod ranging from -4.43‰ to +0.66‰, indicating the diet of Atlantic cod was broader but the age of maturity of Pacific cod was most likely younger. Previous genetic studies showed that no clear differentiations were found for Pacific cod coast-wide, and the results for stock structure of Atlantic cod seemed controversial. Nevertheless, stable isotopic and trace elemental variations from otoliths of Pacific cod did clearly identify two or more spawning stocks between the Washington western coast and Puget Sound. Examination of a large number of otoliths (218 Atlantic cod and 150 Pacific cod) suggests that the age of 1-2 years is a critical period for survival, as this may be the time for the fish to migrate or move to deeper ocean waters. We conclude from this study that the isotopic comparison and $\delta^{18}O$ and $\delta^{13}C$ variations of otoliths can be used as a natural tag in investigations on cod's life history, stock structure, and environmental changes that an individual fish encountered.

Using isotopes to examine the links between terrestrial and marine systems along the Central California coast.

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Rivers are important sources of dissolved and particulate fluxes to the world's oceans and directly influence many regional processes including habitat functioning, carbon export, and nutrient export. However, the effects of these allochthonous inputs on community composition and functioning and the conditions in which they are important are not well understood.

Big Sur is an isolated coastal region in central California that is characterized by a steep landscape and numerous streams that drain from nearly pristine catchments. These streams are highly ephemeral and deliver large pulses of nutrients and particulates during rain events throughout the winter and spring seasons. Supplemental nutrients are supplied during spring and early summer via wind-induced upwelling that brings nutrient-rich oceanic waters to the surface.

The resulting highly productive and diverse nearshore giant kelp forests are an ideal system for examining the extent of the connections between terrestrial and marine systems. *Macrocystis pyrifera*, the largest of the kelps, is a foundation species for nearshore ecosystems providing a three-dimensional structure utilized for habitat and protection by a diverse group of species. Moreover, the life history of *Macrocystis* has been shown to be affected by variation in nutrients, trace elements, and sediment, which may come from terrestrial and/or marine sources that may impact the health and longevity of these kelp forests.

At sites near-to and far-from river inputs, I am using δ13C and δ15N to investigate the composition of particulate organic matter in the nearshore system and to determine which sources are incorporated by consumers in nearshore kelp communities. Samples are also collected to determine the major nutrient sources for primary producers in this system and how those sources vary seasonally during periods of high and low stream flows.

Comparisons of $\delta^{15}C$ and $\delta^{13}N$ isotopes in Australian sea lion (*Neophoca cinerea*) teeth.

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To study historic diets and variation over time, samples for isotopic analysis can be acquired from the teeth of museum specimens[1,2].
To minimize possibilities of individual variation it may be preferable to analyse samples taken from teeth located in the same position in the tooth rows of specimens. However, this not always possible when using museum specimens as some teeth may be missing or stored loose so the original position of the tooth cannot be determined with certainty. To maximize possibilities in sample size it is therefore important to understand isotopic heterogeneity between teeth in different positions of the tooth row. Then comparisons between individuals, using teeth from different positions may be made.
The present preliminary study uses carbon and nitrogen isotopic signatures from dentine of museum specimens of Australian sea lions (*Neophoca cinerea*) collected from the South Australian coast between 1922 and 2000. Isotopic heterogeneity between five post-canine teeth from an individual tooth row was examined. Post-canine teeth were chosen for analysis as these are most numerous in the sea lion dentition and therefore the most likely to be available. Results showed that teeth from all positions in the tooth row had similar $\delta^{15}C$ and $\delta^{13}N$ isotopic values (varying less than $\pm 0.4\ ^{o}/_{oo}$). Another preliminary study to indicate variation over time was also undertaken. Teeth from five individuals collected over a seventy-eight year time span were analysed. Results of this preliminary study did not suggest change in feeding regime over this time period, however, additional sampling is required for clarity and confirmation.
This preliminary research suggests that isotopic ratios gained from analyses of specimens of post canine teeth taken from different positions in an Australian sea lion tooth row of may be comparable between individuals.

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Stable carbon and nitrogen isotope analysis of vibrissae: long-term indicators of dietary change

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Vibrissae may be a useful tissue for isotopic analysis and the study of long-term and seasonal dietary changes. Vibrissae lengths can be transformed into measurements of time, which can then be related to isotopic ratios.
Growth rates have been determined for a number of pinniped species using dated vibrissae. Harbour seal (*Phoca vitulina*) vibrissae growth rates were irregular or variable, while grey seal growth rates (*Halichoerus grypus*) varied according to the length and age of the vibrissae with asynchronous shedding and discontinuous replacement. Conversely Steller sea lions (*Eumetobias jubatus*) displayed more consistent growth and annual retention of their vibrissae. It has been suggested that vibrissae growth follows a von Bertalanffy growth curve.
This study transformed leopard seal (*Hydrurga leptonyx*) vibrissae lengths into time-lines using both a simple linear and von Bertalanffy growth model. With information gained through stable carbon and nitrogen isotope analysis of sequentially segmented vibrissae their potential as indicators of seasonal change in the leopard seal diet was confirmed. Temporal variations in stable isotope ratios consistent with changes in source of feeding (inshore versus offshore) and prey types were identified.
Further studies of captive seals are required to identify vibrissae growth, loss and replacement rates.

Combined use of stable isotope and fatty acid analyses reveal almost total segregation by salinity habitats in a coastal population of European eel.

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Using discriminant function analysis (DFA) of $\delta^{13}C$, $\delta^{15}N$ and C:N values from European eels (*Anguilla anguilla*) inhabiting a coastal catchment, we recently demonstrated strong evidence for population sub-structuring along a short fresh-brackish water gradient[1] (salinity range = 0-25). In the current study, we expanded our focus to include fully marine habitats, extending the salinity range from 0 to 34. Yellow phase eels were collected from four areas along a 5km salinity gradient, representing freshwater (FW, salinity <1, n=30); brackish (BW, ~12, n=16); marine-dominated (MW, ~25, n=31), and full seawater habitats (SW, ≥34, n = 29).
Discriminant function analysis following Harrod *et al.*[1] demonstrated that isotope ratios differed greatly in eels collected along the salinity gradient (Wilk's lambda = 0.075, P < 0.0001). However, the ability to classify eels according to salinity habitat declined relative to Harrod *et al*. (classification success 70% v 85% in Harrod *et al.*[1]), largely due to overlap in $\delta^{13}C$ values between eels captured from fresh and brackish water habitats. As other data (mark-recapture and telemetry) indicated that eels typically remained in their respective salinity zones, we followed a method used by ecologists studying marine mammals[2] and combined stable isotope analysis (SIA) with fatty acid analysis (FAA). The utility of this technique is that consumer fatty acid compositions, like stable isotope ratios, reflect that of their diet, and that different food webs (e.g. marine and freshwater) are characterised by contributions of certain fatty acids, (e.g. n-3:n-6)[3].
Using secondary samples from eels previously analysed for SIA, we extracted, esterified and analysed 32 fatty acids via gas chromatography. Individual fatty acid compositions were quantified using standards of odd-chained fatty acid methyl esters. DFA demonstrated clear differences in the fatty acid composition of eels from each of the four salinity habitats (Wilk's lambda = 0.023, P < 0.0001). Discrimination of eels from FW and BW habitats was improved relative to SIA data, but classification success was similar (75%), due to some overlap in the fatty acid composition of eels from BW and MW habitats. Finally, we combined SIA and FAA data in a single discriminant function analysis. The results were striking, with almost total separation of eels according to salinity habitat (Wilk's lambda = 0.007, P < 0.00001; classification success = 93%). This study demonstrates the potential for combined use of SIA and FAA techniques to identify variation within animal populations, and provides further evidence for population sub-structuring in coastal eel populations.

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Use of ^{13}C and ^{15}N as population markers for the malaria mosquito *Anopheles arabiensis* in a Sterile Insect Technique (SIT) context

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Monitoring of sterile to wild insect ratios in field populations can be useful to follow the progress in genetic control programmes such as the Sterile Insect Technique (SIT).

The feasibility of using the stable isotope of carbon, ^{13}C and ^{15}N as potential chemical markers for the malaria mosquito Anopheles arabiensis Patton was evaluated in the laboratory. Either ^{15}N labelled glycine or labelled ^{13}C glucose was incorporated into the larval diets in powder or liquid form. The contribution of adult sugar feeding to the total mosquito carbon pool and the metabolically active carbon pool was determined by tracing the decline of the enrichment of the adult male mosquito as it switched from a labelled larval to an unlabelled adult diet. This decline in the adult was monitored by destructive sampling of the whole mosquito and analysed using isotope ration mass spectrometry. In the ^{15}N labelling experiments only persistence of the marker was monitored.

A two-pool model was used to describe the decline of the ^{13}C enrichment of adult mosquitoes. The proportion of the total adult carbon pool derived from the adult sugar diet over the life span of mosquitoes was determined and the ratio of structural to non-structural carbon assessed (~50%). The uptake and turnover of sugar in the metabolically active fraction suggests that after 3 days >70% of the active fraction is derived from sugar feeding (increasing to >90% by day 7).

It was possible to “fix” the ^{13}C isotopic label in adult An. arabiensis and to detect the label at an appropriate concentration up to 21 days post-emergence. The optimum labelling treatment using ^{13}C would cost around 250 US$ per million mosquitoes. Stable isotope marking may thus aid research on the fate of released insects besides other population-based ecological studies.

Using daily ration models and stable isotope analysis to predict biomass depletion by herbivores.

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Food availability is one of the major factors governing the distribution of animals. Since animal numbers can in turn regulate the availability of food, the relationship between the two can be described in terms of resource depletion (which can be difficult to quantify). We present a novel method utilising multi-source stable isotope mixing models and a daily ration approach to predict the depletion of seagrass (*Zostera* spp.) by grazing light-bellied Brent Geese (*Branta bernicla hrota)* at Strangford Lough, Northern Ireland. The model successfully predicts the depletion of *Zostera* spp over the whole Lough when compared to empirically derived estimates of biomass. Further, the model was able to predict biomass depletion over two years with different population sizes. The predicted quantities of *Zostera* consumed were not in agreement with previous studies, being considerably higher, but this may be explained in terms of the lower energetic value of *Zostera* at Strangford Lough, compared to other sites. When re-parameterised with energetic values from previous studies, the model predictions for *Zostera* consumption were comparable to estimates derived by standard techniques.

This approach offers a novel, efficient and widely applicable method to investigate the relationship between consumers and their food sources. It may have application in deriving site carrying capacities and in quantifying agricultural damage by consumers.

Determining the importance of decayed macroalgae in an intertidal ecosystem, using $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ stable isotope analysis.

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In coastal ecosystems a significant fraction of the macroalgal biomass is deposited ashore after being dislodged by currents and waves. Decomposition of the wrack by microbes and abiotic fragmentation returns nutrients to the sediment and into the interstitial trophic chains of these environments. Thus the wrack becomes an important energy source for invertebrates. Large amounts (6.73kg av. wwt m^{-1}, May 2005, Elmer, West Sussex, UK)) of drift algae accumulate around the coastal defence structures (CDS) at Elmer, on a regular basis and remain for several weeks, sometimes several months at a time and must contribute significantly to the diet of detritivores. The significant role of seaweed-derived detritus as a source of organic carbon and nitrogen, for subtidal and rocky-intertidal consumers, has been well documented in marine and freshwater habitats (Mann 1988). But its importance to consumers in and around CDS remains uncertain. Using $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ stable isotope analysis of fresh and decayed macroalgae we aim to clarify the uptake of detrital nutrients derived from drift algae in foodwebs based around CDS.

12 species of living and decayed macroalgae species were sampled along with a range of faunal species from soft sediments and from CDS's on Elmer beach. In order to gain further understanding of the mechanisms involved within in this complex system, seaweed decay experiments in both the lab and field were undertaken over 30 days in March 2005. Six seaweed species were decayed in the field using litterbags (25x25cm, 5mm mesh size) and 14 species in 1L glass beakers for the lab experiment. Samples were initially taken every 2 days for both lab and field studies. Preliminary results from the decay experiments have shown a considerable range of $\delta^{13}C$ values for the living algal species studied; they ranged from -11‰ to -34.5‰. For the chlorophyta, the $\delta^{13}C$ values ranged from -16.41‰ to -19.40‰, phaeophyta from -16.56‰ to -21.99‰ and for the rhodophyta, from -20.20‰ to 34.50‰. On average the $\delta^{13}C$ values for the phaeophyta and chlorophyta were more terrigeneous than the rhodophyta. The degree of change in isotopic signatures as the algae decayed varied depending on the algal species, for example, *Ulva lactuca* showed a $\delta^{15}N$ depletion of 3.56‰ over 4 days in the lab but only 1.36‰ in the field and *Griffithsia sp.* a depletion in $\delta^{15}N$ of 2.56‰ in the lab over 7 days. *Osmundea pinnatifida* became $\delta^{13}C$ depleted by 4.16‰ from -16.56‰ to -20.72‰ over 2 weeks in the lab experiment. Furthermore, *Fucus vesiculosus*, *Mastocarpus stellatus* and *Sargassum muticum* were amongst many that showed an initial enrichment of $\delta^{15}N$ (0.61‰/0.21‰, 1.59‰/0.50‰ and 0.35‰/0.36‰ respectively (lab/field results)) in the first couple of days before becoming depleted, a trend attributed to microbial growth (Tenore, *et al* 1982).

Based upon these findings, the decay process of macroalgae has been shown to be a complex process with considerable isotopic variations both between macroalgal species and during decay, due to both the biological makeup of each and the microbial activity on the macroalgae themselves. The implications of these findings suggest that care needs to be taken when interpreting macroalgae isotopic signatures in particular when using them in food web studies.

References:

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Across ecosystem boundaries: from lacustral benthic methane to aerial vertebrates

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It has long been established that emergence of aquatic insects can subsidise terrestrial consumers. Recent stable isotope studies have revealed the importance of chemosynthetic carbon to chironomid larval nutrition, and thus there is great potential for the wider dispersal of such recycled carbon when these insects emerge en masse in spring and summer. In a eutrophic Cumbrian lake, UK, chironomid larvae exhibit highly depleted 13C signatures and using this natural isotopic marker it is possible to track the carbon through the food chain. Localised insect abundance is very attractive and important to breeding barn swallows (Hirundo rustica). By systematically trapping insects and sampling barn swallows at their nests around the lake, we tracked the progress of lacustral chemosynthetic carbon to vertebrate predators in terrestrial food webs.

Hair Segment Analysis of Female Alaskan Brown Bears: Resolving Seasonal Salmon Consumption with Sulfur Isotopes

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Understanding the nutritional ecology of large animals has traditionally relied on labor intensive field observations, analysis of fecal residues, or body weight indices. During the last 10-15 years, stable isotopes have provided a robust tool for quantifying assimilated diet, with the approach representing perhaps the most important break through in animal nutritional ecology in recent times. Hair has been a preferred sample matrix, mainly because it provides an opportunity for non-invasive sampling and records assimilated diet as a function of time. In a 10-year radio collar study (1993 – 2003) of female brown bears (*Ursus arctos*) in southwest Alaska, we were able to use stable isotopes (C, N, S) to demonstrate a strong dependency on Pacific salmon as a protein source, despite the fact that this population occurred at the fringes of salmon availability. It was also shown that not all female bears utilized this protein source and alternative forage items were exploited following a decline in salmon escapement between 1998 and 2001. However, that analysis was based on whole hairs which were thought to have an integration period spanning late spring (den emergence) through fall (den entry) and therefore may have weighted assimilated diet estimates toward salmon. To further resolve diet assimilation on a seasonal scale, we analyzed segments of female brown bear hair from a subset of individuals that were captured multiple times over the course of the study. Guard hairs were sectioned into thirds (tip, mid, base) and assumed to grow at a constant rate, resulting in integration periods of approximately 1.7 months per hair segment. We characterized $\delta^{34}S$ of hair segments and used it as an indirect measure of the mean proportion of salmon in the diet. We found that most female bears consumed less salmon early in the hair growing period (tip) compared to the middle (mid) and later (base) periods. When the availability of salmon declined, our results demonstrated a corresponding reduction in salmon consumption which was also more constrained to the middle hair growth period. Further, despite a rebound in salmon availability in 2002, we observed a similar pattern in salmon consumption to continue. This study demonstrates the potential power of stable isotope analysis of hair segments for increasing the temporal resolution of assimilated diet estimates in salmon eating bears.

Turnover rates and stoichiometry couplings in Collembola

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In this study mass balances, C and N turnover rates, C:N:P stoichiometry, and direct measurements of ammonium excretions were conducted on two Collembola species, *Proisotoma minuta* (Tullberg, 1871) and *Protaphorura armata* (Tullberg, 1869). The purpose was to investigate life history strategies and physiological characteristics of the two physiologically distinct species. The animals were fed unlabelled or dual labelled (^{13}C and ^{15}N) yeast enriched to about twice the natural abundance and investigated over 28 days after entering the reproductive stage. The half lives of both C and N were between 6-7 days for *P. minuta* and 8-11 days for *P. armata*, and increased with age. *P. minuta* allocated substantial resources for oviposition (5-6% of dry mass day^{-1}) opposed to *P. armata* (1-1.5%). Conversely, *P. armata* allocated more resources for growth (2.3 % of dry mass day^{-1}) than *P. minuta* (0.7 %). In total the productivity was higher for *P. minuta* than *P. armata*. *P. minuta* also had higher excretion rates of ammonium than *P. armata*. The percentage of C and N allocated directly from the yeast pool to reproduction was calculated from $\delta^{13}C$ and $\delta^{15}N$ values after diet switch. While more than 50% of C and N was allocated directly from yeast in *P. minuta* the equivalent number for *P. armata* was around 10%. That a much larger part of yeast was incorporated into the eggs in *P. minuta* without prior metabolism is supported by the isotopic signatures of ^{15}N in eggs at natural abundance equilibrium. The $\delta^{15}N$ values were similar for eggs and yeast in *P. minuta* but higher (3.0) for *P. armata* eggs. The adults of the two species had relatively similar stoichiometric compositions but the C:P ratios of eggs were twice as high for *P. armata* than *P. minuta*. The eggs had similar C:N ratios. The higher P content of *P. minuta* eggs could be a result of different life history strategies of the two species with *P. minuta* being more productive and metabolically active.

Structure of the Dublin Bay food web: a dual C and N stable isotopes analysis

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Dublin bay is an open bay with has significant estuarine influence from the river Liffey. The bay receives substantial organic matter inputs from allochthonous sources, mainly in the form of particulate matter. Around 90% of the total carbon is due to these external inputs, with sewage contributing 156.6 $gC.m^{-2}.y^{-1}$ and the rivers 30.1 $gC.m^{-2}.y^{-1}$. Similarly, 97% of the nitrogen is from allochthonous sources. When tidal exchange is considered, both budgets are heavily influenced, such that the N input doubles, and the C input increases by over an order of magnitude.
Carbon and nitrogen stable isotopes have been used to characterise the C and N transfer pathways within the Dublin bay food web and to identify the major sources constituting the basis of the food chains. Benthic invertebrates, fishes, and all the primary producers and organic matter sources have been sampled in spring 2005 in the sublittoral and the main intertidal sub-environments of Dublin bay (sandflats and mudflats).
Riverine inputs of particulate matter could clearly be excluded from the food chains as its isotopic signature was strongly ^{13}C-depleted (mean -31.93‰) compared to the sampled animals (-21.45 < mean $\delta^{13}C$ < -13.57‰, over all sites). In addition, the sewage effluent organic matter did not seem to contribute to the food chains, considering its relatively low $\delta^{13}C$ and $\delta^{15}N$ values (-25.77‰ and 3.41‰ respectively). In intertidal areas, primary consumers appeared to rely in general on a pool of sources composed of marine particulate and sedimented organic matter, microphytobenthos and detrital macrophytes. Inter-specific patterns have also been shown, such that the diet of the bivalve *Macoma balthica* was highly influenced by microphytobenthos, while the cockle *Cerastoderma edule* relied more on marine particulate matter. Conversely, the sublittoral food chain, for which different trophic levels have been defined from the animals isotopic signatures, was shown to be based on marine planktonic carbon, either in suspended or sedimented forms.

Combining molecular genetic and stable isotope technology for plant biosecurity: geographic origins of exotic insect pests.

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Stable isotope technology is being considered as a novel tool for plant biosecurity in New Zealand to provide accurate information on the geographic origins of invasive exotic insect pests. Molecular genetic methods provide some resolution, but require significant sampling of all potential source populations making it impractical to develop for each new species risk. Stable isotopes on the other hand vary spatially based on locality-specific biogeochemical processes. They therefore offer a potentially more sensitive and feasible means to map geographic origins.
The isotopes $d^{13}C$ and $d^{2}H$ have been demonstrated as useful for tracing the natal origin of a migratory, host specific insect, the monarch butterfly[1]. They have also been used in conjunction with population genetic markers (DNA barcode sequence of the mitochondrial cytochtome oxidase gene), to determine if recent incursions of painted apple moth (Lepidoptera; Lymantriidae) represent specimens new to New Zealand or an as yet undetected population that has escaped erradication. Painted apple moth is a potentially devastating pest of New Zealand's horticultural and forestry industry and geographic origin information is essential prior to launching the appropriate quarantine response.
Difficulty in interpreting this data has highlighted the need for research relevant to polyphagous organisms of unknown geographic path, i.e., different to the more predictable status of the monarch butterfly[1]. Research is planned using a model insect system to understand aspects such as the influence of varying dietary and climatic histories, the spatial resolution based on existing global isotopic maps, which isotopes are most appropriate to use, and how signatures may be influenced during transit to New Zealand through respiration and continued growth on a sea voyage or by subsequent feeding on plants in New Zealand.

Reference:
[1]Hobson K.A., Wassenaar L.I., Taylor O.R. (1999) Stable isotopes (δD and $\delta^{13}C$) are geographic indicators of natal origins of monarch butterflies in eastern North America. *Oecologia* 120:397-404

Feeding in estuaries by three commercially important marine fishes

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Stable isotopes of carbon, nitrogen and sulphur have been employed to quantify feeding in estuaries by three commercially important marine fishes: common sole (*Solea solea*), whiting (*Merlangius merlangus*) and European sea bass (*Dicentrarchus labrax*). Sampling was concentrated in the Thames estuary (UK) and adjacent offshore waters, the Thames acting as a proxy for other estuaries, allowing generalisations about the connectivity and energetic contributions of North Sea estuaries to offshore adult fish stocks.

The stable isotope signatures of invertebrate species along the estuarine-marine gradient demonstrate distinctive spatial patterns, and allow invertebrates to be classified to their collection location with a high degree of accuracy. We have implemented the invertebrate data as a proxy for basal isotopic signatures and assessed stable isotope data from fishes both graphically and using mixing models.

We infer the estuarine feeding strategies of young-of-the-year fishes of the three species, with the results demonstrating marked differences between species. We also analyse the relative importance of estuarine feeding and persistence of estuarine signals in older fishes, highlighting further differences between species and age groups.

^{15}N enrichment in marine organisms (*Aplysina aerophoba, Balanus perforatus, Anemonia sulcata, Posidonia oceanica*) as a tracer of aquaculture-derived effluents in the Eastern Central Adriatic (Croatia)

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Information on aquaculture-related environmental impacts along the Eastern Adriatic coast is very scarce. The effect of fish farm-derived particulate organic nitrogen on the benthic organisms the wider area around the fish farm at Vrgada Island (with annual production of 450 t sea bass and 1000 t tuna) in Central Adriatic (Croatia) was investigated was investigated in the period from May to September 2005. Stable nitrogen isotope compositions (δ^{15}N) of particulate fish farm effluents (fish food and faeces), particulate organic matter (POM) and benthic sessile biota in the area impacted by the aquaculture activity were compared to those of the POM and same species of organisms at an unpolluted reference site. Geochemical maps showing the spreading of particulate N effluents derived from aquaculture were drawn.

The pelleted fish feed used at the fish farm had a mean δ^{15}N value of +9.3±1.0‰. Faeces stripped from the fish were depleted in ^{15}N in average by approx. 1.7‰. POM collected under the fish cages had δ^{15}N values between 4.5±0.2 and 5.3±0.4‰, depending on the season, and was consistently enriched in ^{15}N compared to the POM collected at the reference and some other off-shore locations (with δ^{15}N between 2.6±0.2 and 3.6±0.2‰). The mucilage (which occurred in July) collected under the cages had a very high δ^{15}N value of +11±0.8‰.

Variability of δ^{15}N values between different tissues of the same individual was determined; the differences were not significant at *P. oceanica*, while they reached up to 1.2‰ in *A. aerophoba.* To assure that the δ^{15}N values of biota collected at different locations were comparable, only the results of isotope analyses of the same tissue of each species collected from individuals of the same size (age) were used. The δ^{15}N values in the selected organisms were significantly higher around the fish cages, but also in the coastal part of the Murter Sea, exposed to the anthropogenic pollution (sewage). The ^{15}N enrichment in selected organisms reached different levels, from up to 4.7% in *P. oceanica* to up to 6.8‰ in *A. aerophoba*, which was significantly higher than the natural variability of δ^{15}N values of the same species at each location.

Different organisms exhibit different degree of ^{15}N uptake from the environment. While δ^{15}N values of *A. sulcata* and *B. perforatus* showed an excellent linear correlation with those of POM, a weaker correlation was found between δ^{15}N of *A. aerophoba* and POM, although its δ^{15}N values consistently increased with increasing δ^{15}N values of POM. This is attributed to the presence of different bacterial populations hosting in the sponge, thus making sponge a less reliable indicator of aquaculture impacts than previously assumed. Sponge and other organisms, although known as non-selective suspension feeders, but hosting extended bacterial populations, may have δ^{15}N values lower than POM, although still reflecting the overall enrichment of the environment in ^{15}N due to the presence of ^{15}N enriched effluents. Geochemical mapping of effluent abundance in an area based on different indicator organisms may thus differ from each other.

Impact of sewage discharge on the benthic food web in a mangrove ecosystem: evidences from carbon and nitrogen stable isotopes ratios

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In order to assess sewage discharge and its impact on mangrove ecosystems, carbon and nitrogen stable isotope ratios (δ13C, δ15N) were determined in suspended particulate organic matter (SPOM), sediment, primary producers and benthic invertebrates at different point along a mangrove creek, in two intertidal mangrove forests. Mtoni mangrove is located in the vicinity of Dar es Salaam (Tanzania) and is thus likely to receive extensive amounts of sewage whereas Ras Dege is a pristine site located in a rural area, 20 km away from Dar es Salaam and was used as a reference site.

Nitrogen stable isotope signatures of different biological compartments in the peri-urban site all showed higher values compared to those from the pristine site (average increase of +3‰ to +7‰), providing evidence of sewage contamination of Mtoni mangrove. However, due to the diffuse nature of sewage inputs within the system, no spatial variations of $\delta^{15}N$, in relation to sewage discharges, were detected. Nevertheless, the high $\delta^{15}N$ signal observed along all the successive trophic levels of the foodweb showed that sewage did become incorporated into the benthic foodweb and suggested the great importance of sewage-derived organic matter in sustaining the foodweb in polluted mangrove systems. Furthermore, the $\delta^{13}C$ signatures of invertebrates indicated some shifts in the trophic behaviour of some species were assessed in the polluted site. $\delta^{13}C$ values of some widespread species indeed showed significant differences between both sites with more depleted values in the pristine site (decrease of -2.4‰ to -7.7‰, depending on the species, in the pristine site compared to the polluted site). Those results revealed that invertebrates shifted on a diet composed of less mangrove detritus in Mtoni mangrove. This could be correlated with the important proliferation of the algae *ulvae* sp. in this system, probably induced by higher nutrients loads coming from sewage discharges, and which could then be preferred as carbon source in this system.

Food sources of cultivated oysters in two contrasted ecosystems of Normandy (France), as analysed by stable isotope natural compositions ($\delta^{13}C$, $\delta^{15}N$)

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Food sources for marine cultivated bivalves are generally not well identified although they are essential for the sustainability of such activities together with a better understanding of coastal ecosystems. Besides the well-known phytoplankton, other sources of organic matter as microphytobenthos (MPB) and detritus (continental or marine origins) can contribute significantly to the growth of marine bivalves. The aim of this study was to identify the potential food sources and to estimate their contribution to the growth of the Pacific oyster in two shellfish ecosystems: the Baie des Veys and the Lingreville area. Each of these coastal zones are characterised by different biological performances of the cultivated species. The consistent methodology used was based on the analyses of the food sources simultaneously to the animal tissues using stable natural isotope composition ($\delta^{13}C$, $\delta^{15}N$). A field study was undertaken during 1 year with sampling every two months. The sampled food sources were suspended organic matter from marine and continental origins, MPB, detritical organic matter from the sediment and macroalgae. Results showed that isotopic composition of the animal tissue varied larger than the potential food sources over the year within one ecosystem. Besides, differences between the two systems were also observed. For instance, the $\delta^{13}C$ and $\delta^{15}N$ values of the oysters ranged from –21.97 to –21.11‰ and 3.37 to 7.32‰ in the Baie des Veys and from –23.79 to –21.38‰ and 2.63 to 4.81‰ for in Lingreville area, respectively. Therefore it is assumed that contribution of these different sources to the animal growth differed depending on the ecosystem and the period of the year. From all evidence, nitrogen stable isotope should be more discriminating than carbon. Models - both ecophysiological and mathematical ones - were used to estimate the contribution of food sources: marine particulate organic matter (mostly phytoplankton) was assumed to be a major food source but MPB could contribute significantly. Finally, results will be discussed in the context of oyster farming sustainability.

Comparing trophic interactions of brown trout (*Salmo trutta* L.) and roach (*Rutilus rutilus* L.) in eutrophic and non-eutrophic Irish lakes using stable isotopes analysis

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Ireland's freshwater fish fauna was historically dominated by salmonids. Among the native species the wild brown trout (*Salmo trutta* L.) represents an economically important resource with high socioeconomic value making a significant contribution to the local tourism industry. Brown trout population density often fluctuates markedly because it is regulated by a complexity of environmental and biological factors. The sensitive natural balance is potentially threatened by introductions of non indigenous fish species and water pollution.
One introduced species roach (*Rutilus rutilus* L.) has become widely distributed in freshwaters systems due to their high adaptable ecology. Roach has been regarded as a possible threat to salmonid species because of competition for food and space due to their high recruitment rate and high population density. To characterize the trophic interactions of brown trout and roach, populations of both species were sampled in a non-polluted lakes, Lough Corrib and stable isotopes analysis was performed on samples of different age classes and the trophic level data will be compared afterwards with samples from an another non polluted lake, Lough Ennell
The other main threat to brown trout populations is cultural eutrophication. Field evidence suggests that eutrophication enhances competitiveness where roach and salmonids co-exist and induces a shift in the food web trophic levels. To assess the consequences of eutrophication on fish communities the trophic interactions among different age classes of brown trout and roach populations were compared in a group of eutrophic lake, Lough Conn and Lough Cullin and a non-eutrophic lake, Lough Corrib. Dataset from another non polluted lake, Lough Ennell, will be added to the study at a letr date.

A model to predict foraging behavior of big brown bats using stable isotope signatures $\delta^{13}C$ and $\delta^{15}N$ of skin tissue

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Stable isotopes are commonly used to evaluate trophic relationships, nutrient state, and temporal and spatial variation in diet, food webs, and behavior both within and between species. Here we describe the development and application of models to predict the foraging behavior of big brown bats, *Eptesicus fuscus*, based upon $\delta^{13}C$ and $\delta^{15}N$ of skin tissue. We utilized a 42 specimen sample collected from three well-characterized ecogeographies disparate both in photosynthetic mechanism and fertilizer use to generate the models. Significant differences between these three sites in terms of $\delta^{13}C$ ($F_{2, 39}$ = 112.92***), $\delta^{15}N$ ($F_{2, 39}$ = 97.06***), and the ratio of these two tissue types (*Wilk's* Λ = 0.032, $F_{4, 76}$ = 87.02***) allowed for the following three models to be developed using Fisher's linear discriminant functions: 1) A model predicting if bats forage in C_3 or C_4 sites, 2) A model predicting if bats forage in agricultural areas which utilize artificial fertilizers, and 3) A synthesis model using both variables to predict specific foraging ecogeography. We present the results of model application to a dataset of 310 bats sampled from 9 states which included a broad range of $\delta^{13}C$ (-31.71‰ < $\delta^{13}C$< -17.20‰) and $\delta^{15}N$ (6.36‰ < $\delta^{15}N$ < 15.60‰) signatures. Model predictions are discussed in terms of implications for pest management and bat conservation. We validated the use of skin tissue samples in the model by comparing each site across five tissue types. We found significant correlations (*p*-values range from 0.0184 to <0.0001 for each of the twenty comparisons conducted) between each of the following tissue types: skin biopsies, hair, whole blood, plasma, and feces and selected skin samples in the analysis due to low variance within this tissue type.

Isotopic analysis of trophic relations in Ground beetles (Carabidae)

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Ground beetles are common in many ecosystems. Since this family encompasses a range of feeding specializations, including seed feeding, predation and omnivory, knowledge of the feeding habits of individual species is required to assess their roles in food webs. Most research into trophic relations of carabids has relied on direct observation or gut content analysis. Unlike these direct, observational methods which only yield a snap-shot of current food sources, stable isotope analysis of different tissues may provide retrospective information on the feeding history of individuals belonging to different species.

The objective of this study was to analyse trophic relations in a species-rich carabid community (26 species) using the isotopic composition of two different tissues. During June to August 2005, 26 sympatric species of carabid beetles were trapped alive in two cropping systems in Ireland. Beetles were frozen, identified to species level and dissected to obtain two tissue samples: muscle and elytra (wing cases). Between 5 and 10 pooled samples of each tissue were analyzed per species for $\delta^{13}C$ and $\delta^{15}N$. Results from a related laboratory diet-switching experiment suggest that muscle reflects adult diets, while the elytra primarily reflect larval diets. Thus, we hypothesize that the isotopic data in this survey will reveal firstly, the relative trophic positions of adult beetles of these sympatric species during an active season and secondly, possible differences in this positioning for larval developmental stages.

$^{15}N/^{14}N$ fractionation of different herbivorous fish on a coral reef

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The value applied to $^{15}N/^{14}N$ fractionation between diet and consumer can have profound affects on the determination of food web structure. We compare empirical calculations with outputs from a new fractionation model to demonstrate that diet type and food processing mechanism can lead to fractionation greater than the currently accepted value of 3.4‰. Small differences in isotopic signature currently being lost in the assumption of a single level of fractionation may carry important information regarding the feeding and metabolic rate of individuals. The model incorporates consumer feeding and excretion rates and diet quality to determine the $\delta^{15}N$ of the diet mix and hence a value for trophic fractionation. We use three herbivorous reef fish as field study organisms as their diet can be well described and easily sampled. Model parameters were derived from feeding observations and literature data. The trophic fractionation of *Acanthurus sohal*, *Zebrasoma xanthurum* and *Pomacentrus arabicus* was 4.20, 4.24 and 3.57‰ by model and 4.69, 4.47 and 5.25‰ by empirical measurement. The model is sensitive to the daily feeding rate of the fish, which may be lower for *P. arabicus*. The model is the first to determine isotope signatures of a consumer's diet mixture without applying a fractionation value.

Stable Isotopes (C and N) record past changes in mangrove ecosystems: Spanish Lookout Cay, Belize

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Mangrove ecosystems exist in a dynamic coastal environment and must adapt to changes in sea-level. Mangroves produce peat which accumulates over time and preserves evidence of the past ecosystem history. In this regard, the peat serves as a chronicle of ecosystem changes, which can be compared with records of past sea-level variations. Evidence preserved in peat cores from mangrove islands, such as Spanish Lookout Cay, Belize, allows examination of how past mangrove ecosystems behaved through the Holocene (~10,000 years). Spanish Lookout Cay is presently dominated by Red mangroves (*Rhizophora mangle* L.). A Russian peat corer was used to obtain two mangrove peat cores (SC1 and BT-79) to depths of 7.5m and 9.0m respectively; SC1 provides a 5,900 ± 55 ^{14}C year record of the past mangrove ecosystem. Fossilized Red mangrove leaves are preserved within the peat and are used for stable isotopic analyses to understand past stand structure (dwarf vs. tall trees) and nutrient status. The stable carbon and nitrogen isotope compositions (δ^{13}C and δ^{15}N) for modern Red mangroves have been observed to range from -29 to -22‰ and from -11 to 2‰ respectively. Dwarf Red mangrove stands (< 1m) are typically located in the interior of the island, are generally phosphorous limited, and have a more negative δ^{15}N (< -3‰). Tall Red mangrove stands (> 3m) are generally located on the fringe of the island and demonstrate a more negative δ^{13}C (≤ -27‰). Preliminary data show the location of core SC1 had a predominantly tall Red mangrove stand (mean δ^{13}C = -27.0 ± 0.5‰) over the past ~5,900 ^{14}C years while the modern location is dominated by dwarf Red mangroves. Comparing bulk stable isotope data with additional lines of evidence (e.g. compound specific stable isotope data, sub-fossil pollen, and peat stratigraphic characteristics) provides a holistic picture of past mangrove ecosystems. Stable isotopic analyses of the dominant mangrove species from peat cores are providing a unique perspective into past mangrove ecosystem dynamics during Holocene sea-level changes.

Changes in δ13C and δ15N in breast feathers and relationships with post-release survival of the endangered Attwater's prairie chicken (*Tympanicus cupido attwateri*) and greater prairie chicken (*T. c. pinnatus*) in the USA

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The Atwater's prairie chicken (*Tympanicus cupido atwateri*) is an endangered species with a narrow restricted distribution primarily along coastal grasslands and prairies in Texas, USA. Breast feathers from 125 Atwater's Prairie Chickens (APCs) and 104 greater prairie chickens (GPCs) (*T. c. pinnatus*) were collected from several locations in Texas and in various Midwestern states in the U.S.A. to measure stable isotopes of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) and to determine if there was a correlation with nutritional deficiencies or with survival in the wild. One hypothesis was that different C and N intake in the wild could be related with nutrient deficiencies that affected post-release survival of APCs. A few of the APC samples were from captive individuals, however, the majority were from APCs that had been released in the wild and had survived for one or more molting periods. $\delta^{13}C$ decreased slightly with the number of days in the wild (r^2=0.42) while $\delta^{15}N$ tended to increase (r^2=0.33). Similar results were obtained with birds recaptured during two consecutive years. The captive APCs held at two different locations in Texas had similar $\delta^{13}C$ values and were greater than those observed in APCs that had been in the wild for at least one molting period. $\delta^{13}C$ values in feathers of released APCs were greater than those observed in GPCs collected at most locations in Midwestern states. $\delta^{15}N$ values of wild APC and GPCs were more similar suggesting that both species are feeding at the same trophic level regardless of differences in latitude or habitat use. Our results indicate that wild APCs prefer or are restricted to feeding on plants and insects that are lower in carbon than that in the diet while raised in captivity. Whether the minor observed changes in the diet of APCs are related with changes in survival remains to be determined.

Use of stable isotopes to estimate trophic ecology in Common Octopus (Octopus vulgaris) from three sites off the Atlantic coast of Galicia (NW Spain)

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During winter of 2005, 41 common octopus *(Octopus vulgaris)*, 26 females and 15 males, were sampled at three fishing localities (Meira, Finisterre and Cedeira) off the Atlantic coast off Galicia (NW Spain). We explored the potential use of stable isotope analysis in octopus tissues to approach its trophic ecology, given the limitations found to the study of feeding habits in this species, which is fished using bait. Some of their life-history characteristics, predator habits and territorial behaviour make the octopus a good candidate to explore its usefulness when looking for geographical differences in local trophic webs. In the present study, stables isotopes of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) were analysed in samples of lipid-free beak and mantle musculature. On the other hand, we collected biometric data and determined reproductive status of these individuals. Signatures of nitrogen were significantly lower in beaks than in mantle tissue, whereas differences in carbon signatures between these tissues were much smaller. A positive relationship between $\delta^{15}N$ and $\delta^{13}C$ was found in the muscle mantle as well as in the beak; isotopes signatures between both tissues were also found to be significantly correlated. Differences were found in both isotopes amongst sampling localities. The interpretation of these differences is discussed in relation to the oceanographic characteristics of Galician coast and in relations to the biological parameters of octopuses.

Contribution of stable isotopes and mercury to the study of the trophic ecology of the yellow-legged gull in Galicia (NW Spain)

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Half of the Atlantic breeding population of yellow-legged gull *(Larus michahellis*) nests in the Galician islands. Yellow-legged gulls are opportunistic feeders predating on a variety of marine and terrestrial food resources. Previous studies based on the examination of pellets have shown spatial variation in the diet of adults; however, little information is available on the feeding ecology of chicks and adults during the winter. In the present work, we used analysis of stable isotopes and mercury in mantle feathers of adults (moulted during the winter) as a complementary tool to characterize their trophic ecology during that time of the year. The same approach was used for chicks, given the scarcity of data on their diet. During the summer of 2004, we collected mantle feathers of both, chicks and nesting adults breeding in four insular colonies of Galicia (Cíes, Ons, Ansarón and Vionta) and nitrogen and carbon stable isotopes and mercury levels were determined. In chicks, values of δN were similar for all colonies and showed low CV values, indicating that they were feeding at a similar trophic level; otherwise, differences in δC values may reflect differences related to the local food webs exploited. Mercury concentrations in chicks feathers were significantly different amongst colonies, and were influenced by both, nitrogen signature and locality. In adults, δN showed significant inter-colony differences, while no differences were found in δC values. Nevertheless, the interpretation of results in adults is further complicated by the fact that we do not know the foraging area of these populations during the winter.

Characterising open ocean ecosystem structure using stable isotopes: an example from the Chatham Rise SW Pacific Ocean

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The Chatham Rise and Subtropical Front (STF) to the East of New Zealand support one of New Zealand's most productive deep-water fisheries. Current rates of extraction of many fish species however, are proving unsustainable and recently a reduced total allowable catch (TAC) fishing quota has been imposed on both the orange roughy and hoki fisheries. Distributions of pelagic and benthic communities, along with information of feeding strategies of key Chatham Rise fish species, are reasonably well documented; however fisheries management is currently hampered by the lack of detailed information on the trophodynamic structure of the ecosystem. We attempted to redress this by using carbon and nitrogen stable isotopes to characterise the trophic interactions within the open ocean ecosystem of the Chatham Rise, focussing not only on the STF, but also on the ecosystems north of the STF, in sub-tropical waters and south of the front, in sub-antarctic waters. The Chatham Rise ecosystem appears to have four trophic levels between primary producers and fish and, despite the seasonally productive nature of the STF, isotopic signatures indicate that overall the system may be food-limited. The trophodynamic structure of the ecosystem and implications to fisheries management are discussed in light of the C and N isotopic data.

Using stable isotope to determine if there is trophic competition between an invasive species, *Crepidula fornicata*, and a commercial species, *Pecten maximus*.

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The study addressed in the trophic links between two benthic suspensivores: the invasive species, *Crepidula fornicata*, and a commercial species, *Pecten maximus*. Carbon and nitrogen isotopic ratios were used to determine whether there is trophic competition between these two species. The study was carried out over one year with weekly sampling. For each species, the muscle and the digestive gland were investigated, two organs with different turnover. In parallel, the water in the water-sediment interface was analysed. This survey raised other questions than the trophic competition linked to the stable isotope theory: which is the lipid effect on the carbon stable isotope ratio? Which is the impact of the organ turnover on the change speed of the isotope composition? What are the differences of the fractionation between organs?
Concerning the lipid effect, results underline the importance to define a correction factor depending of the quantity of lipid of the organ to compare different species and also different organs. The higher metabolic activity of the digestive gland compared to the muscle can be shown for both species. In that respect, the study of two organs with different turnover allowed us to work on two time scales within the scope the incorporation of different food sources. Two potential food sources were identified depending on the time of the year. Phytoplankton was a food source during the spring time, second food source more depleted in ^{13}C was incorporated during the other seasons. Potentially, this second food source could be the microphytobenthos. An other important point evidenced by this survey was particularly high variation coefficient of the isotopic composition during the spring period for the two species and the two organs.

Detecting changing landuse at Porirua Harbour-Pauahatanui Estuary using stable carbon and nitrogen isotopes and heavy metals

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Urbanisation, farming, industry, forestry, agriculture and native wetlands are contributing to changing the estuarine environment around two estuaries located at Porirua-Pauahatanui Inlet, Wellington, New Zealand. The fragile estuarine environment is sandwiched between city and rural life, and the effects of storm water run-off into the estuary is having a noticeable effects in most areas.
A baseline study has been conducted in 2004 using stable carbon and nitrogen isotopes, and heavy metals, to determine the level of impact from various inputs to the system on the resident flora and fauna. Changing biodiversity of species around both estuaries is also noted, with relevance to site specific inputs.
Stable isotopes of sediments, shell fish and algae show delineation between landuse changes around the estuaries. Heavy metals have been studied in cockles taken from both estuaries, and levels reported with respect to World Health Organisation (WHO) standards. The study is part of a monitoring project undertaken to ascertain long term effects on the estuarine environment, and help to define acceptable and high-risk areas around the estuary as change occurs.

Linking relative trophic position and contaminants in Asian carps, invasive species in the Mississippi and Illinois Rivers

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Populations of nuisance invasive fishes quickly reach extremely high biomass. Before all possible control methods can be applied, however, an understanding of the contaminant levels these invaders carry is needed. We investigated differences in contaminant levels of two invasive carp species as a function of sampling site, fish species, length and trophic differences using stable isotopes ($\delta^{15}N$, $\delta^{13}C$). Fish were collected from three different sites, the Illinois River near Havana, Illinois, and two sites in the Mississippi River, upstream and downstream of the Illinois River confluence. Five bighead carp (*Hypophthalmichthys nobilis*) and five silver carp (*Hypophthalmichthys molitrix*) from each site were collected for muscle tissue analyses. Total fish length explained more variation in muscle contaminants than species, or sample location; however, dietary and trace metal differences did exist between the two species. Total fish length was positively correlated with mercury and negatively correlated with selenium in both species. This result was not unexpected because larger fish have likely been in the system longer giving them a higher probability of exposure and accumulation of contaminants. Bighead carp had higher isotope ratios ($\delta^{15}N$, $\delta^{13}C$) and lower levels of arsenic and selenium, suggesting that phytoplankton constitutes a smaller percentage of their diet compared to silver carp. Stable isotope ratios of nitrogen in Asian carp were at levels that are more commonly associated with higher-level predators, or from organisms in areas containing high loads of wastewater effluent.

The effect of macrodecomposers and litter type on plant growth on abandoned alpine pastureland: a mesocosm experiment

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After the abandonment of alpine meadows and pastures litter quantity increases and the litter quality changes remarkably due to the lacking management activities and the immigration of dwarf shrubs. The decomposer community has to adapt to these new conditions. The primary decomposer *Lumbricus rubellus* (Lumbricidae) and the secondary decomposer *Enantiulus nanus* (Diplopoda) are known to be important members of the detritivore community on abandoned alpine pastureland in the Central Alps (Kaserstattalm, Tyrol) (Seeber et al., 2005). To investigate their contribution to the cycling of nutrients on these sites, their effect on the growth of *Dactylis glomerata* (Poaceae), a grass species which is abundant on abandoned areas, was evaluated.
Litter material of different qualities (*Luzula sylvatica*, a grass species, *Vaccinium gaultheroides*, a deciduos dwarf shrub, and *Calluna vulgaris*, a hardy dwarf shrub) was ^{15}N-labelled and offered as food sources to the decomposers in a mesocosm experiment in a plant growth chamber. *L. rubellus* and *E. nanus* had the choice to either feed on one, two or three litter types to find out which litter material they preferentially consume. To determine their contribution to the cycling of nutrients the ^{15}N-labelled material was used to trace the path of nitrogen from the litter material to shoots and roots of *Dactylis glomerata*. The results of this experiment will contribute to a better understanding of decomposition processes in abandoned alpine ecosystems.

Reference:

Seeber, J., Seeber, G.U.H., Kössler, W., Langel, R., Scheu, S. & Meyer, E., 2005. Abundance and trophic structure of macrofauna decomposers on alpine pastureland (Central Alps, Tyrol): effects of abandonment of pasturing. Pedobiologia 49: 221 – 228.

Food sources of macrodecomposers on abandoned alpine pastureland

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On managed alpine meadows and pastures, decomposition is similar to that in meadows and pastures at lower altitudes. Abandonment of alpine meadows and pastures, however, leads to a change in litter composition, its quality, and the quantity of litter due to the invasion of dwarf shrubs. The decomposer fauna present in managed sites is adapted to process the litter which is produced by the vegetation found under traditional land-use practices. These decomposers, however, might be unable to process and translocate the different litter material, such as dwarf shrub litter, which is produced within abandoned pastures (Seeber et al., 2005). As a consequence, more differentiated, irregularly distributed humus layers develop (Seeber and Seeber, 2005).
In laboratory mesocosm experiments we previously have shown that earthworms and millipedes are able to feed on dwarf shrub litter (Seeber et al., 2006). It is not known, however, if and to what extent this is true under field conditions. Here, we report on the outcomes of a field experiment designed to determine to what extent earthworms and millipedes feed on dwarf shrub litter under natural conditions. Besides unlabelled grass litter, ^{15}N-labelled litter of the dwarf shrub *Vaccinium gaultheroides* was offered to the decomposers in an abandoned alpine meadow. Decomposers, plant- and soil material were sampled at several time points after applying the labelled substrate. This will allow us to determine by stable isotope analysis if and to what extent dwarf shrub litter is actually consumed by the two decomposer groups under field conditions. The outcomes of this field experiment will provide new insights into the feeding habits of key decomposer species found in alpine pastures. Moreover, these findings will contribute to a better understanding of decomposition processes and soil formation in alpine grassland ecosystems after abandonment.

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Functioning of food webs across ecosystems of different biodiversity level (*FOODWEBIO*, the *MarBEF* RMP project)

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Processes ongoing in the ecosystem and mediated by organisms are determined by interactions among organisms and between organisms and the ambient environment, thus by biodiversity. Diversity of habitats and species vary geographically across environmental and ecological gradients, structuring ecosystems and their functioning at local and global scales. Ecosystem metabolism is intimately linked to carbon and nitrogen fluxes from primary producers to consumers of higher trophic levels. This trophic transfer determines the productivity of ecosystems depending on the efficiency of the food webs. Systems with high diversity and complex trophic interrelations such as the Mediterranean Sea or Atlantic are considered to be stable and productive, while in systems such as the Baltic Sea average diversity is low and food web structure relatively simple. Despite such striking differences in their structures, the productivity of the food web in the Baltic is reported to be similar to that of the Atlantic. This would indicate that biodiversity might not be an essential prerequisite for ecosystem (food web) functioning. However, the number of interactions between species increases with their number and even so does the number of material cycles and pathways within a food web. Therefore the question arises, to what extent food web efficiency of an ecosystem is related to the entire diversity as well as to the species pool of the higher ranking systems.
To address this question, the *FOODWEBIO* project investigates the relations between diversity of habitats and species, and the functioning food webs in European coastal waters that differ in biodiversity. The objectives of the project are:

1) to define the structure and functioning of the food webs and inter-relations between various trophic levels in ecosystems of different biodiversity level based on key taxa
2) to compare the structure of the food webs among systems based on the selected BIOMARE flagship sites across a range of geographical and environmental gradients
3) to assess the effect of changes in biodiversity on the efficiency of food web at a pan-European scale.

Two methodological approaches are integrated: (1) $\delta^{13}C$ and $\delta^{15}N$ ratios to identify the origin of organic matter in various components of the systems studied and define trophic relations and (2) Network Analysis to unravel the interactions between the living and non-living components of ecosystems and energy flow within them.
The project was commenced last year for a period of nearly four years (until 2008) within the activity of EU Network of Excellence Marine Biodiversity and Ecosystem Functioning (*MarBEF*). The project gathers nine partners representing six European countries bordering the Baltic Sea, North Sea, Bay of Biscay and Mediterranean Sea.

Benthic food web structure in brackish waters of the southern Baltic Sea (the Gulf of Gdansk) as determined by dual stable isotope analysis

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Data on population dynamics were combined with $\delta^{13}C$ and $\delta^{15}N$ ratios of the key benthic species and their potential food source (organic matter from surficial sediments 0-1cm, suspended total particulate matter) to investigate trophic relationships across variety of habitats in the southern Baltic Sea (the Gulf of Gdansk). The organisms analysed for stable isotopes included a range of macrobenthic plants and animals such sea grass, green and red algae as well as polychaetes, crustaceans, molluscs, priapulid worms and demersal fish of different food preferences, that play a predominant role in benthic biocenoses.
Biodiversity of benthic communities was low but showed variability at a local scale. The total number of taxa ranged from 5 to 19, their abundance from 90 ind./m^2 to 1307 ind./m^2 and biomass from 1.3 g dry weight/m^2 to 30.9 g dry weight/m^2 at six locations, demonstrating different community structure and various environmental conditions in the Gulf.
Regardless spatial variations in the composition and density of benthic communities, food chains were usually limited to three or maximum of four trophic levels, exception was deep-water muddy and anoxic habitat where only facultative deposit feeding Baltic clams can permanently occur. Trophic network linkage density increased with the number of species, from deeper locations towards shallow and semi-shelter locations situated close to the coast. The same species or group of species represented the same trophic level across habitats but varied in $\delta^{13}C$ and $\delta^{15}N$, indicating various origin of organic matter. Adjacent to the mouth of the Vistula river and in semi-sheltered areas the impoverishment of seston in $\delta^{13}C$ (down to -26.4‰) and enrichment in $\delta^{15}N$ (up to 8.1‰) suggested terrestrial input of allochthonous materials to the Gulf while in more open-sea areas more enriched $\delta^{13}C$ and impoverished $\delta^{15}N$ organic pools were found. Dissimilarities in carbon and nitrogen isotopic signatures of suspended matter between locations were reflected in the $\delta^{13}C$ of consumers, specifically infaunal and epifaunal bivalves. For the dominant mussels *Mytilus trossulus* and the Baltic clams *Macoma balthica,* $\delta^{13}C$ varied from 20.8‰ to 23.6‰ and from 19.5 to 22.1‰, respectively and $\delta^{15}N$ was between 6.4‰ and 8.6‰ and 6.2‰ and 9.5‰, respectively. In contrast, the $\delta^{13}C$ and $\delta^{15}N$ ratios in surficial sediments showed rather low variations, indicating homogenous and mixed terrestrial-marine origin of sedimented materials.

Monitoring Ecosystem Health and Environmental Change Using Seabird Guano Chemistry

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Scottish Natural Heritage

Stable-isotope analysis has become an increasingly popular tool in ecological research. In particular, stable-isotopes have been used to elucidate trophic relationships and to identify and quantify sources of dietary material within food chains. Seabirds form an integral part of the marine ecosystem and are therefore sensitive indicators of ecosystem function, change and health. This study will present initial results obtained from the analysis of guano from the main seabird species from the Isle of May and the St Andrews coastline, Scotland. Through analysis of the stable isotope composition and inorganic pollutant levels in the guano, it is possible to monitor contaminant levels, trace dietary shifts and assign trophic levels to individual seabird species.

Bulk guano is composed of a variety of components from a variety of sources and of these components uric acid a single compound derived from both nitrogen and carbon that has been metabolized by the bird. Isolation and analysis of uric acid from the guano therefore eliminates uncertainty introduced by analysing the bulk guano. Analysis of guano also has the advantage that it non-invasive and causes less disturbance to birds than sampling of other tissues. This study will present initial results which show that (i) uric acid can be quantitatively isolated from guano for analysis of carbon- and nitrogen-isotope composition by EA-IRM-MS (ii) the stable-isotope composition of uric acid can be directly related to diet (with fractionation of <1-2‰) and (iii) responds rapidly (days) to changes in diet.

Algal-bacterial coupling and microzooplankton grazing in the Scheldt estuary, using stable isotope labelling experiments.

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Quantifying carbon and nutrient flows through pelagic food webs is still a challenge. Especially the microbial food web in highly eutrophied, estuarine environments has its specific difficulties, as autotrophic processes as well as inflow of allochtonous organic matter provide potential carbon sources for bacteria.
Carbon flows in a natural phytoplankton assemblage from the Scheldt estuary (Netherlands) were followed by measuring changes in concentrations and stable isotope ratios in a 10-day period after addition of ^{13}C to a batch experiment. Two experiments were performed, one in the euhaline reaches of the estuary, and one in the brackish, turbid part.
Coupling of algal and bacterial production was inferred from ^{13}C-uptake in algal- and bacterial-specific polar lipid fatty acids (PLFA) with aid of a dynamic model and using Bayesian techniques for parameter estimation. As expected, algal exudates turned out to be the dominant source for bacterial carbon production in the euhaline estuary, showing a strong microbial loop. In the brackish zone, although allochtonous DOM could be identified as the major carbon source, algal products still showed to be important for bacterial production.
Combined experiments where ^{13}C bicarbonate and ^{13}C glucose were added to different batches, allowed also estimates of carbon flows due to microzooplankton grazing on bacteria. Microzooplankton grazing turned out to be a dominant process in the turbid zone of the estuary, exceeding primary production.

Habitat segregation and divergence in the Lesser Whitethroat (*Sylvia curruca*) complex: a combined molecular and isotopic approach

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The Lesser Whitethroat (*Sylvia curruca*) is one of the most widely distributed and morphologically diverse Old World warblers (family Sylviidae). Within the Lesser Whitethroat superspecies there are a number of morphologically distinct allospecies/sub-species described, based on a combination of morphological traits and habitat selection, however the true taxonomy is unclear.

Here we combine molecular approaches, with stable isotope analysis of tail feathers (grown in the breeding areas) to study the relationship between genetic variability and ecological variability in this diverse species complex. Initial results using C & N isotopes show that there are several distinct ecological groupings and that haplotypes are not shared between these. The addition of H isotopes to this data set are likely to provide increased resolution. This combined approach is likely to provide a much more robust taxonomy for the complex

Stable isotopes reveal different diet patterns between age groups and across villages in Yup'ik Southwest Alaska.

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Naturally occurring stable isotope variation has only recently been used for diet assessment in medical applications. We are developing a stable isotope approach to assess diet pattern, particularly subsistence and non-subsistence intake, in Yup'ik Eskimo communities in Southwest Alaska. This research is part of a larger study of the genetic and dietary factors underlying obesity and diabetes risk in this population. Seasonal diet assessment is challenging in these study, where people travel to hunting or fishing sites in the summer and access can be difficult in the winter months. We are evaluating whether isotopic signatures could provide a quick, non-invasive index of usual diet that can be extrapolated back through time via hair analysis. Although diets vary among villages, diets can include significant marine resources (salmon, seal, whitefish, walrus), terrestrial subsistence (moose, reindeer, muskox, caribou, berries), birds, and market foods grown outside Alaska. Because these food types are expected to vary widely in C,N,O/H and S isotope signatures, this population is an ideal test case for evaluating the information that isotope analyses can provide on individual-level dietary variation. Diet survey data from one coastal community indicates large age-related disparities in diet pattern, with marine subsistence foods contributing more than half of total calories to participants over 60 yrs and less than 10% of total calories of participants age 14-20 yrs. Refined sugar and high fructose corn syrup (soda) contribute nearly 20% of total calories in the 14-20 yrs age bracket. We expected that these differences would cause highly significant age-related differences in red blood cell (RBC) δ^{15}N and δ^{13}C, and tested this relationship with samples from 6 villages. RBC δ^{15}N varied from 7 to 13‰, with older participants significantly enriched relative to younger participants. Variation in RBC δ^{13}C (-21.5 to -19‰) was also age-related, with younger participants enriched relative to older participants. However, these differences varied widely among villages, with coastal villages exhibiting the greatest age-related differences in δ^{15}N and riverine villages showing the greatest age-related differences in δ^{13}C. RBC δ^{15}N correlates with RBC phospholipid DHA and EPA, two polyunsaturated fatty acids abundant in marine foods, which supports the assertion that δ^{15}N reflects consumption of marine foods. Despite greater intake of marine foods by elders, carbon signatures were comparatively enriched in teens, suggesting that C4-based food consumption (chicken, beef, refined sugar, and/or high fructose corn syrup) is higher in this age group. Data from 3 day food records for these same participants support these interpretations.

Tracing *in situ* amino acid uptake in plants and microbes with $^{15}N^{13}C$ labelled compounds

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Attention on ecosystem cycling of amino acids is increasing due to the potentially high flux rates in nitrogen limited ecosystems. Amino acids serve as substrate for fungi and bacteria in the soil, and plants are able to take up small amino acids as intact compounds. Amino acids in soil water origin as root exudates, lyzed microbes and decomposing plants. We investigated uptake of amino acids and ammonium in plants and soil microorganisms by injecting ^{15}N labelled ammonium or fully ^{15}N and ^{13}C labelled amino acids into the soil. As in previous studies, the obtained enrichment in plant shoots showed species specific preference for the different nitrogen sources (Andresen & Michelsen 2005). There was a significant plant uptake of carbon from free amino acids in the soil solution.
Though measurements of amino acids (the 20 essential; HPLC) yielded low concentrations in the soil pore water (~ 1% of dissolved organic nitrogen) and showed seasonal variations, the $^{15}N^{13}C$ tracer experiment revealed high plant and microbial enrichment, suggesting a significant cycling of amino acids. Furthermore, the influence of colonisation and type of mycorrhizal association (ericoid and arbuscular) of the roots on amino acid uptake was investigated. Mineralization (decarboxylation) of the ^{13}C labelled amino acids at uptake (through mycorrhizae or directly through root) and after xylem transport decreased the ^{13}C enrichment of plant shoots, which therefore may not necessarily reflect root acquisition of intact amino acids. Furthermore, tannin addition tended to reduce plant uptake of label.
By combining data on ^{15}N recovery after 1 day in shoots and roots (fine and coarse) of the dominant heathland plants: the evergreen dwarf shrub *Calluna vulgaris* and the graminoid *Deschampsia flexuosa,* in soil microorganisms (chloroform fumigation extraction) and in soil water, we discuss the relative importance of free amino acids and ammonium as plant nutrients and microbial substrates in natural N-limited ecosystems with a high proportion of soil N held in tannin-N complexes.

Reference:
Andresen and Michelsen 2005 'Off-season uptake of nitrogen in temperate heath vegetation' *Oecologia* (2005) 144 Stable Isotopes Issue: 585 - 597.

Unravelling the mystery of tree water uptake along a Namibian ephemeral river – which tree gets what and from where?

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Ephemeral rivers, in which surface flow occurs for less than 10% of the year, are found in arid regions. Due to the rare surface flow events these rivers do not have a permanently established aquatic fauna and flora associated with them. Instead terrestrial life forms, like trees and shrubs, thrive along their banks. There is concern that extensive water abstractions from such river systems will affect the health and distribution of riparian trees, especially the outer riparian fringe vegetation that is not regularly inundated by floods.
In this paper we investigate the water use strategies of three riparian tree species (*Acacia erioloba, Faidherbia albida and Tamarix usneoides*), growing mid-river and on the riparian fringes of the ephemeral Kuiseb River in the Namib Desert. We used stable hydrogen and oxygen isotopes (δD and $\delta^{18}O$) to determine the dependency of these trees on groundwater, fog, soil water and floodwater. In addition, we determine water stress (Xylem Pressure Potentials) and transpiration rates for all the species both mid-river and on the riparian fringe. The isotope results suggest that two of the species at both locations depend primarily on a mixture of ground - and soil water. None of the *T. usneoides* specimen (at either location) had a signature that corresponded directly to groundwater. Instead, they lined up with the signatures of either floodwater, or fog water, or that of shallow soil layers. Pre-dawn xylem pressure potential, determined with a Scholander Pressure Chamber, was used as an indicator for water stress. The results suggest that the fringe and mid-river stands are equally water stressed, supporting the isotopic findings of equal access. However, transpiration rates suggest that the fringe vegetation transpires less than lush mid-river stands. Dendrometer measurements also indicate that the fringe vegetation has slower growth rates than the mid-river vegetation. Hence we argue that both mid river and riparian fringes have access to the same water sources, but mid-river stands access greater water quantities, as reflected in transpiration and growth rates. We suggest that slower growth rates coupled with lower transpiration rates for the riparian fringe trees suggest that increased abstraction will affect the distribution of these trees. Trees growing mid-river may take on the characteristics of the fringe trees, fundamentally changing the demography of the river system.

Quantifying Methanotroph Biomass and Methane Oxidation Capacity in Agricultural Soils using ^{13}C Labelling Techniques

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Methane (CH_4) is an important greenhouse gas that currently contributes *c*. 15% to global warming. Concentrations of CH_4 have been increasing such that the present day atmospheric CH_4 concentration is the highest in over 160,000 y. This increase can be attributed to an increase in anthropogenic CH_4 emissions since the industrial revolution, and a reduction in the oxidation capacity of the CH_4 sinks. Soils are recognized as an important terrestrial sink, estimated at between 15-45 Tg CH_4/yr, due to the presence of methanotrophic bacteria which utilise CH_4 diffusing through the soil. As developing countries continue to clear native land to grow crops to sustain a growing economy, it is becoming increasingly important to understand the impact agricultural practices have on the global CH_4 sink. Many farming practices alter the structure and chemistry of the soil. On-going research in this laboratory is aimed at improving our understanding of how the active methanotrophic community responds to these changes.
A wide range of agricultural soils from the UK (Rothamsted Experiment, Harpenden) and Brazil (Vilhena) have been incubated with $^{13}CH_4$ labelled methane at atmospheric concentrations for varying lengths of time. The stable isotopically labelled methane was metabolised into the active methanotrophic biomass. Phospholipid fatty acid (PLFA) analysis in conjunction with compound-specific isotope analysis (Bull et al. 2000) was used to identify and quantify the bacteria responsible for the atmospheric oxidation of CH_4. Analyses were based on PLFA profiles obtained by GC-FID, GC-mass spectrometry (GC-MS) and GC-combustion-isotope ratio-MS (GC-C-IRMS). The ^{13}C-labelled PLFAs were used to assess the diversity and size of the methanotrophic bacterial community using the Frostegard and Baath (1996) approximation. As a refinement of this approach the PLFAs of a selection of enumerated low affinity methanotroph cultures have been quantified by GC-FID and the concentration of PLFA per cell calculated. The biomass of the incubated soils will be estimated using this new value. Applying stable isotopes in this way, to quantify the biomass in the incubated soils, will help answer important questions concerning the impact various agricultural practices have on this important bacterial community.

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Nitrogen cycling in an unpolluted forest ecosystem: integrating novel concepts in a comprehensive methodology

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Since the 50's, the classical ^{15}N pool dilution theory (Kirkham and Bartholomew, 1954) provides a powerful tool to study N cycling. In these days, the mineralization - immobilization theory (MIT) combined with the nitrification process was assumed to be dominant in ecosystem N functioning.

This methodology is up till now commonly used in most N transformation studies. However, since the 50's, the view on N cycling has continuously changed (Schimel and Bennett, 2004). Nowadays, the MIT theory is often undermined, especially in "unexplored" ecosystems, such as boreal and unpolluted forests. The functionality of the dissolved organic nitrogen (DON) pool (Neff et al., 2003) and the importance of abiotic processes (up to 90% of the total N flux; Johnson et al., 2000) serve as examples of the novel N cycle in a broad range of ecosystems.

In our opinion, the changing view on N cycling requires an upgrade in some methodological aspects. Complex ecosystem N cycling suggests a comprehensive approach, integrating different complementary methodologies. Improvements in mathematical modelling, and isotope ratio mass spectrometry techniques, combined with the correct use of ^{15}N pool dilution techniques, might help to explore N cycling beyond the MIT borders.

As an example, we present a step by step study on N retention mechanisms in a South Chilean forest soil. In a laboratory experiment, ^{15}N material was applied to the mineral soil layer, and a ^{15}N tracing model was set-up (Huygens et al., in prep). Results indicated the importance of NH_4^+ as an important substrate for DON forming processes. Consequently, natural abundance measurements of the DON pool (Huygens et al., 2005; Huygens et al., in prep.), combined with a DON fractionation method showed the importance of adsorption to the soil matrix before decomposition can potentially take place. The water-affinity of the DON pool is indicated to be an important property with respect to N losses from the ecosystem. In a ^{15}N pulse chase field experiment, the fate of labelled of N material was studied in different aqueous and terrestrial ecosystem compartments. In this way, the results obtained in laboratory experiments can be validated in the field.

Overall, the combination of different novel methodologies provides a more complete picture of ecosystem N functioning, emphasizing the advantage of integrating complementary approaches. In our case, this step by step methodology appeared to be an excellent means to study N retention mechanisms and the role of DON in ecosystem functioning.

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Molecular and compound-specific stable C isotope investigation of the fate of dung C in a temperate grassland soil

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Establishing the potential of soils to sequester C and elucidating the mechanisms whereby this is achieved is vital in order to understand the capacity of terrestrial systems to act as a sink for anthropogenic C. Significant quantities of cattle dung are applied to temperate grassland annually and traditional long-term use of manures as soil improvers has been proven to increase SOM (Haynes and Naidu, 1998). This project aimed to utilise molecular and compound-specific C isotope methods to investigate the fate of cow dung in a temperate grassland soil, utilising the $\Delta^{13}C_{treated - control}$ values of compounds from natural abundance ^{13}C-labelled dung ($\delta^{13}C$ = -12.6‰; C_4) and C_3 ($\delta^{13}C$ = -30.3‰) soil. Compound-specific stable C isotopic determinations of 0 - 1 and 1 - 5 cm soil horizons sampled from beneath C_4 cow pats at seven occasions over 372 d showed differences between degradation rates for major organic components of C_4 dung. C_4 dung dry matter comprised 80% carbohydrate, 5% lignin and <1% lipid (Dungait *et al*., 2005). Carbohydrates were analysed as the alditol acetates of xylose, glucose, arabinose, galactose and mannose; percentage incorporation of xylose was considerably greater than any other dung carbohydrate accounting for over 10% of dung C at peak bulk dung C incorporation compared with 3% for glucose. Mannose concentrations were negligible in the applied dung but were determined in the soil suggesting that the soil microbial biomass had used dung components as a substrate. This was confirmed by microbial PLFA analysis that revealed that soil bacteria and fungi used C_4 dung components for membrane biosynthesis for the duration of the experiment. The unexpected re-emergence of dung-derived carbohydrates to the surface soil horizons at the end of the experiment suggested short-term sequestration of dung C in soil horizons below 5 cm depth with reintroduction by bioturbation. 4-hydroxypropanoid products of the off-line pyrolysis of dung lignin displayed a range of degradation rates in the soil, but appeared to be more labile than expected and did not contribute significantly to bulk $\delta^{13}C$ values in the latter part of the experiment. Determinations of the trimethylsilyl ethers of 5β-stanols and methyl esters of *n*-monocarboxylic acids (C_{14} – C_{32}) suggested that concepts of recalcitrance might rely not so much on individual chemistry, but on the potential of certain compound classes to escape degradation or leaching, perhaps by forming associations with basic minerals or humic substances; very long chain *n*-monocarboxylic acids ($\geq C_{22}$) appeared to be the most resistant to decomposition in the soil.

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Differentiated respiration of an external carbon source at contrasting soil depths and temperature in a Beech forest soil.

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Heterotrophic respiration contributes typically 40 – 60 % to the total belowground respiration in forest soils. However, this ratio may to a large extend be affected by the seasonality of carbon cycling as influenced by abiotic (temperature, moisture) and biotic (litter fall; root exudation) parameters. In this study we have examined the response in soil heterotrophic respiration to increased carbon substrate availability at different temperatures and different soil depths.
Respiration of external and ambient soil carbon was differentiated based on the carbon-13 content of respired CO_2. Respiration rates in the mineral soil below 10 cm depth was generally not changed by addition of an external carbon source (sucrose). In contrast, respiration in the litter layer and surface organic soil horizons increased 1.5 - 3 times when sucrose was added, independent of incubation temperature. The $\delta^{13}C$ character of CO_2 from ambient respiration was significantly stratified with soil depth increasing from -24.5 ‰ in the litter layer to -23 ‰ in the deep mineral soil. The isotopic signature of respired CO_2 decreased significantly upon application of sucrose derived from C3 sugar beets ($\delta^{13}C$ = -26‰), however, differences in $\delta^{13}C$ between ambient and sucrose induced respiration were narrow in most samples and the ratio of sucrose derived respiration intractable. Application of C4 sugar cane derived sucrose ($\delta^{13}C$ = -11‰), however, facilitated separation of ambient and sucrose induced respiration. These results indicate that 33 – 78 % of the soil respiration upon sucrose application was due to respiration of the sucrose-C, the ratio increasing with increasing soil depth. This emphasizes two important findings. The increase in soil respiration upon addition of external sucrose was due not only to respiration of the external sucrose, but combined with increased respiration of ambient carbon (priming effect). Secondly, the microbiota in the deep mineral soil apparently changed metabolic pathway in response to the sucrose, rather than increasing respiratory activity.

Dual-labelled ($^{13}C/^{15}N$) green manure to differentiate between plant uptake of organic and inorganic N

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Experimental data is still lacking for determining whether plant uptake of organic nitrogen in soils contributes substantially to the total N uptake. Pulse-injection studies with dual-labelled amino acids have confirmed that crops possess the capacity to take up organic N but failed to quantify the uptake relative to total N uptake. In this study, homogeneously, dual-labelled (^{13}C and ^{15}N) green manure was added to soil with wheat plants. An advantage of using $^{13}C/^{15}N$-labelled green manure as opposed to pulse injection of dual-labelled amino acids is that the amino acids in the green manure are released gradually and naturally, and that N dynamics and assimilation can be monitored. The plants were harvested after 27, 56, and 84 days after sowing and analyzed for ^{13}C and ^{15}N. From the soil-plant system there was 11-13 % loss of green manure N and 51 % loss of green manure C within the initial 27 days after which there was little or no losses. Uptake of N derived from the green manure was for all three harvest dates 83.3% (±0.9) and 88.6% (±0.7) for roots and shoots, respectively. This significant difference might be explained by a differential assimilation of N form containing different fraction of N from the green manure. The study gave also a clear indication of uptake of organic N using the ^{13}C values. A small, but significantly higher ^{13}C value was measured in roots from the labelled treatments at 27 days. From this value, the uptake of organic N was estimated to be minor and constitute between 2.1 % and 6.3 % of total root N. This supports the hypothesis that organic N uptake does not contribute substantially to N acquisition in plants. Nonetheless, the use of using homogeneously $^{13}C/^{15}N$-labelled green manure in this study gave evidence of some organic N uptake in wheat and future studies with higher ^{13}C enriched green manures may reveal this more clearly.

Isotope ratio analysis for the assessment of N use efficiency in Irish pasture systems

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The objective of this study was to investigate the usefulness of N isotope ratio analysis of soil, plants and an invertebrate predator as an integrated assessment tool of management intensity in Irish pasture systems. We hypothesize that $\delta^{15}N$ reflects the system-wide N use efficiency of such soil-plant-animal pasture systems because high external N inputs (as fertilizers or livestock excreta) are associated with higher N (preferentially ^{14}N) losses, which in turn will lead to system-wide ^{15}N-enrichments.
Samples of bulk soil, a grass and a legume plant species as well as one spider species were collected in June–August 2005 from 50 commercial pasture fields in south-eastern Ireland. Samples were dried and powdered; isotopic analysis is awaited. Detailed data on farm management (including N fertilizer use, stocking density) is available and will be related to isotopic data.

The long term influence of manure and inorganic fertiliser applications on the $\delta^{15}N$ value in plants – preliminary results

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The Broadbalk agricultural field experiment was established in 1843 and provides a unique opportunity to examine long term effect of animal manure and mineral fertiliser on natural abundance soil and plant $\delta^{15}N$ trends. Many previous agricultural studies have observed that a long term application of manures generally lead to higher plant and soil $\delta^{15}N$ values compared to (no N) control plots. However, such $\delta^{15}N$ changes are less clear when inorganic N fertiliser have been applied. Indeed, previous studies have shown that long term applications of mineral fertiliser can decrease, increase or to have no significant effect on the plant and soil $\delta^{15}N$ values in fertilised plots when compared to control (no added N) plots.

To improve the understanding of possible reasons behind these sometimes contradictory plant and soil $\delta^{15}N$ values after fertiliser application, we examined the annual plant $\delta^{15}N$ values over two 10 year periods with respect to: i) manure and mineral fertiliser application, ii) mineral fertiliser rates ranging from 0 to 192 kg N ha^{-1} yr^{-1} and iii) two mineral fertilisers, i.e. calcium ammonium nitrate (applied 1969-1979) and ammonium nitrate (applied 1996-2005).

Estimation of the contribution of natural fallow plant species to the nitrogen budget of agro-ecosystems in the Sahel

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As well as uncertain rainfall and locust outbreak, poor nutrient (carbon, nitrogen and phosphorus) status of the sandy soils is one of the causes for low and unstable productivity in agro-ecosystems in the Sahel. Improvement of the soil fertility through organic matter management is proposed with high possibility and practicality for smallholder farmers and coexisting nomads in this region. As concerned with nitrogen, a most limiting factor in the soil, biological nitrogen fixation (BNF) by plant-microbe interactions can be efficiently utilized in croplands and also in fallow lands (Sanginga, 2003). In this study, we have measured $\delta^{15}N$ values of fallow plant species for quantitative estimation of BNF by means of the natural abundance method, to evaluate the contribution of plants to the maintenance and improvement of fertility of the sandy soils in the Sahel.

Plants of higher dominance in fallow lands of different ages (1, 2, and 10 years as well as millet fields) were photographed and collected in dry season (November 2003 and December 2004) and rainy season (August 2004 and 2005) at the Fakara region, 80 km east of Niamey, Niger. Each plant species was identified as a scientific name from local nomenclature (Zarma and/or Peul) with use of the "*Lexique de noms vernacularies des plantes du Niger*," published by INRAN, and verified by ordinary ways of plant taxonomy. The plant samples were dried and finely powdered and an adequate quantity of the samples was then introduced to an element analyzer (Carlo Erba, Flash EA-1112, Milan, Italy) connected to an isotope ratio mass spectrometer (ThermoFinnigan, Delta XP^{plus}, Hamburg, Germany) to determine $\delta^{15}N$ (‰).

Among annual leguminous herb species, *Cassia mimosoides* (Caesalpiniaceae), had the highest frequency in fallow lands of one or two years old, followed by *Zornia glochidiata*, *Alysicarpus ovalifolius* and *Indigofera strobilifera* (all Papilionaceae). The $\delta^{15}N$ value of these leguminous species was around -1.0 to +1.5‰, showing higher dependency on BNF. Young twigs of *Acacia albida* (Mimosaceae), a leguminous tree often reserved in farmer's field, had its $\delta^{15}N$ value of +1.0‰.

Fallow lands, especially in dry season, were highly dominated by annual grass species (Gramineae), such as, *Ctenium elegans*, *Eragrostis tremula*, and *Schizachyrium exile*, and perennial *Andropogpn gyanus*. The $\delta^{15}N$ values of these annual grasses were low in samples of dry season (+2.0 to +2.5‰), which may suggest associative nitrogen fixation in non-leguminous plants. However, the values were much higher in samples of rainy season (+8.0 to +12.0‰). Therefore, this discrepancy should be necessarily elucidated by further studies, with an experimental cultivation of these species in homogenous soil as to have a uniform N profile in content and isotopic ratio.

For the improvement of soil N fertility in the Sahel, it would be a rational technical option to efficiently utilize the natural fallow plants, i.e., legumes with higher ability of fixing air nitrogen, such as *C. mimosoides* and *Z. glochidiata*, and some grasses with higher biomass production.

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Insight into the N dynamics of beech forests using highly ^{15}N labelled beech litter

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In forests, leaf and root litter is the major annual input of organic matter to the soil. During the process of litter decomposition, carried out by a complex interaction among soil fauna, fungi and bacteria, the litter is broken down into particles of smaller size and soluble compounds. The degree and depth of incorporation of leaf litter products depend both on the activity and the composition of the soil fauna and on the leaching intensity of soluble organic compounds (DOC, DON). Newly formed organic matter may follow different pathways (mineralisation vs stabilisation) depending on soil and climatic conditions.

The fate of litter N has been studied in a large number of beech forests (n = 17) in Europe using ^{15}N-labelled beech litter. The selected forests represent the ecological conditions where this tree species is dominant, i.e. acid and calcareous soils with mull or moder as humus type. At each site, the litter was applied on the soil in amounts close to the annual litterfall and covered a surface of 1 – 15 m^2 around selected trees. This approach allows us to monitor the N dynamics in the decomposing litter, the underlying soil and the uptake of litter-released N by the trees over one or more decades. Additionally, the mineralisation of litter N was studied, at selected sites, after incorporation of litter particles into the topsoil three and eight years after the start of the experiments.

Litter decomposition was fastest at sites with a high abundance of earthworms (mull humus) and lowest at high altitude sites (moder humus). Nevertheless, at all other sites there was no clear difference in litter mass loss according to the humus type. In forests with moder humus litter-released N was incorporated mainly in the organic layers and the upper mineral soil. In forests with mull humus, incorporation of litter N into the soil was deeper compared to forests with moder humus. After three years of litter decomposition, between 65 and 100 % of the litter N had been recovered in the decomposing litter, the organic and mineral soil and the trees. Recovery of litter N after eight years was similar to after three years, except for a higher percentage in the trees. In contrast to classical observations, the N cycle in moder humus on sandy soils appears to be short and efficient, mineral N being mainly provided by the mineralization of litter derived POM. These sites are often productive forests where the uptake of litter N into the trees is also high. Nevertheless, with ongoing time, mineralisation of litter N decreases to values close to the overall N mineralisation. In conclusion, there is a positive correlation between tree growth and use of recent litter N on soils with low N reserves.

A detailed examination of hydrogen and oxygen isotopes to increase the precision of leaf water modelling

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There is much interest in understanding the fractionation processes that determine the oxygen/hydrogen isotopic relationship in organic matter and leaf water. While the composition of plant organic matter is of interest for environmental reconstruction, precise data on leaf water composition is an essential input to biosphere/atmosphere modelling. A number of factors contribute to the isotopic composition of the water at any point in the leaf. Some of the major ones are: the composition of the water entering the roots, the amount of evaporation, the composition of the vapour around the leaf and the Péclet effect. Previous models, incorporating the Péclet effect have accounted for the 'gross' variation in isotopic enrichment of the leaf water between the petiole and the sites of evaporation, calculated as though all the processes affecting the evaporation and condensation of water, both hydrogen and oxygen isotope fractionation, change smoothly along transects of the leaf.

Our objective is to examine whether transpiration is uniform over the leaf, a one dimensional response to O and H enrichment, or whether the model needs to be modified to take into account other 'feedback' mechanisms. In this study, we are producing detailed maps of leaf transpiration to improve the modelling of the isotopic composition and reflect the spatial variation found within a leaf. By examining these affects on a micro scale we hope to very much improve the reality of our models. Variables that will be measured will include atmospheric vapour composition, ambient temperature, leaf surface temperature, and wind velocity, the isotopic composition of growth water and transpiration rate.

New methods have been developed to analyse the oxygen and hydrogen isotopic composition of leaf water. The aim is to measure sub-microlitre amounts of leaf mesophyll water in a steady state environment using freshly cut leaves. We plan to remove about 1 mm^3 of leaf material from growing leaves and immediately seal it into a glass tube with CO_2. The leaf water will then equilibrate with the CO^2 which will subsequently be measured by injection into a continuous-flow stable isotope ratio mass spectrometer (using a loop valve) and the $\delta^{18}O$ of the water determined. A similar method will be employed for hydrogen, except that the water will be cryo-distilled from the leaf in a deep-freeze, then injected onto a chromium column and reduced to produce pure H_2. For more information on these techniques see the poster of Hilary Stuart-Williams.

Data from our studies will be presented on the poster.

Water source utilization and nutrient status of hammock and pineland plants in the Everglades, USA.

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Tropical hardwood hammocks and pinelands are two emergent ecosystems in the predominantly wetland Everglades in south Florida, USA. While hammocks have a significantly greater biomass per unit area and an organic soil horizon built up over time, pinelands are devoid of soil except in small pockets in sinkholes in the pitted exposed limestone surface. This lack of soil is attributed to burn off by periodic natural fires in pinelands; these fires usually do not encroach inside hammocks owing to the high ambient humidity inside.

The organic soil horizon in hammocks is thought to provide a greater nutrient availability to hammock plants by virtue of higher nutrient concentrations as well as entrapment of rainwater. In comparison, adjacent pineland plants are expected to rely upon groundwater which has a lower nutrient concentration. Hammocks are thus viewed as localized nutrient concentrations on the oligotrophic everglades landscape. This yearlong study attempts to (i) characterize and contrast the various water sources of hammock and pineland plants and (ii) examine whether hammock plants have higher foliar nutrient contents.

The first part examines the isotopic (^{2}H and ^{18}O) composition of stem waters in plants from hammocks and pinelands in Everglades National Park (ENP) and compares that with potential source water compositions. The various sourcewater pools (rain, GW and soil litter water) were found to be isotopically distinct from each other thereby allowing determination of water source usage in plants. Rainwater was lighter in the wet season (-8 ‰) compared to the dry season (-1‰). This was reflected in groundwater (wet season, dry season) which also exhibited capacitance, damping the amplitude of variation in isotopic composition of rain water, owing to the regional extent of groundwater. Soil water was enriched evaporatively in the dry season and was lighter in the wet season owing to precipitation inputs. Both hammock and pineland plant stemwaters followed a similar isotopic dilution in the wet season, with hammock stemwaters being more enriched than pineland plants, implying an uptake of both isotopically lighter groundwater and enriched soil water by hammock plants. Pineland plant stemwater ^{18}O composition was more closely correlated to groundwater.

In the second part of the study, leaf tissue was analyzed for total C, N and P, with the majority of hammock species exhibiting higher N and P % per leaf tissue weight than pineland plants. However, *Lysiloma, Myrica* and *Quercus* were three species present in the abrupt hammock-pineland interface that occasionally exhibited higher nutrient levels in pinelands. Reasons might include phenology (lysiloma had new leaves in the pineland) and nitrogen fixation (myrica in the pineland). Further sampling and tissue analysis is in progress.

Partitioning of Autotrophic and Heterotrophic contributions to Soil Respiration in Maize Based Agroecosystem using stable carbon isotope ratio methodology

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In this study, we partitioned soil respiration into its autotrophic and heterotrophic components by combining root exclusion with stable carbon isotope ratio methodology in production scale (~65 ha) maize-based agroecosystems. Autotrophic soil respiration (Ra) is defined as combined root respiration and the respiration of soil microorganisms residing in the rhizosphere and using root-derived carbohydrates as an energy source, while heterotrophic respiration (Rh) is defined as the respiration of soil microorganisms and macroorganisms not directly under the influence of the live root system and using SOM as an energy source. Throughout the 2005 growing season, $\delta^{13}C$ measurements of soil respiration were made in maize fields representing three agroecosystems: irrigated continuous maize, irrigated maize-soybean rotation, and rainfed maize soybean rotation. After soil surface CO_2 flux was measured using a Li-Cor LI-6200 Portable Photosynthesis System, small chambers were placed on collars in both root excluded shields and in non-root excluded soil for collection of soil respiration samples. Ambient headspace CO_2 was first removed using a soda lime trap, and soil-respired C was allowed to collect in the chambers. Soil respiration samples were then collected in 12mL evacuated exetainers and analyzed for $\delta^{13}C$ by means of a Finnigan Delta-S isotope ratio mass spectrometer interfaced with a Thermo Finnigan GasBench II using a cryogenic trap to increase CO_2 concentration. Proper timing of this trapping method allowed for maximization of this signal as well as adequate separation of peaks associated with nitrogen and oxygen.
In all three sites, within row $\delta^{13}C$ of soil respiration was generally less negative than between row due to the greater influence of Ra in that region (root material was determined to have a $\delta^{13}C$ of -12.6 ‰). Root excluded soil had a generally more negative $\delta^{13}C$ signature in comparison to non-root excluded soil in the maize-soybean rotations during midseason, reflecting the greater influence of the more negative soybean residue from the previous season as compared to the greater contribution to soil respiration from living maize roots in the non-root excluded soil. In continuous maize, there was little difference between $\delta^{13}C$ of soil respiration from root excluded and non-root excluded soil, since the previous crop was also maize in this case.

Using stable isotopes to trace ozone deposition to soil – a novel approach

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Ozone is widely acknowledged as a major air pollutant affecting forests, crops and wild plants. New techniques to model ozone flux to vegetation in order to assess potential damage and make predictions for future impacts now rely on a flux based approach, but there is still a lack of understanding regarding some aspects of ozone flux to stomata, plant external surfaces, and soil that are needed to parameterise such models. We describe a new approach in which ozone is generated from the stable isotope ^{18}O, to allow tracing of ozone deposition in plant-soil systems, and present first results for the O_3 deposition to mineral soil.

Mineral soil samples were exposed to different ozone concentrations and over increasing lengths of time to measure the accumulation of ozone derived ^{18}O. Soil parameters that were varied within these treatments were either soil water content, or microbial activity (through sucrose additions before fumigation). Based on analysis of dry soil samples, the results show a linear increase of ^{18}O content with time. Soils with no or low water content (0 or 30% of water-holding capacity, WHC) showed similar ^{18}O accumulation, while a higher soil water content (60% WHC) resulted in a reduction to about 30% of dry soil ^{18}O accumulation. Variation in soil microbial activity resulted in no measurable variation in soil ^{18}O accumulation. ^{18}O accumulation in soil under 50ppb was approximately 45% of the accumulation under 100ppb over the same length of time, indicating an approximately linear relationship between ozone concentration and ozone deposition to soil.

We discuss these results with respect to their significance for the understanding of deposition processes and implications for ozone deposition modelling. The successful proof of concept for labelling ozone with ^{18}O and tracing deposition into different compartments opens new possibilities for applying this technique in larger fumigation set-ups, such as open-top chambers or free air concentration enrichment studies.

New insights into plant lipid formation and translocation from plant to soil organic matter obtained from compound-specific isotope ($\delta^{13}C$) analyses

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Seasonal and plant-internal variations of lipid composition and compound-specific isotopic signatures were previously described for various plant groups [1,2]. Only a few observations were documented concerning lipid variations of annual and perennial crops [3]. So far, the translocation and stabilization mechanisms of lipids within plants are still unknown. Pulse-labelling of plants with $^{13}CO_2$ gives the possibility to determine lipid incorporation, translocation, and fixation mechanisms by using compound-specific isotope analysis of plant and soil lipids collected at different times after labelling. In this study, we simultaneously applied biomarker and isotopic analysis to obtain information on compartmentalization of plant lipid distributions.

In addition to several crops collected from different agricultural trials during the growing season, selected crops were cultivated under controlled conditions and received a pulse-labelling in a ^{13}C enriched atmosphere. Plant samples were divided into leaf, stem and root biomass for each growth stage and analyzed separately. Extractable lipids of plant and soil samples were recovered by accelerated solvent extraction and separated into fractions of different polarity by automated liquid chromatography [4]. Fractions of aliphatic hydrocarbons and carboxylic acids were analyzed by GC-MS and GC-irmMS.

Maize plants from agricultural trials show significant differences in the isotopic composition of belowground biomass and aboveground biomass. $\delta^{13}C$ values of belowground carboxylic acids remained fairly constant during the growing season. Root *n*-alkanes, however, became isotopically depleted in comparison to aboveground *n*-alkanes. The difference in isotopic signature of roots versus aboveground biomass argues against an origin of alkanes and carboxylic acids in roots. Root lipids do not derive from photosynthates of aboveground biomass, which were then plant-internally translocated towards the roots. These results are supported by results of the labeling experiment, where the isotopic signature of carboxylic acids even in maize roots is modified due to the labeling, while alkanes show identical isotopic signatures for labeled and unlabeled roots, similar to soil alkane isotopic signatures. These lipids must be biosynthesized *in situ* by either (a) direct assimilation of soil organic carbon by root tissues, or (b) interaction with soil microbes growing on or within the roots as proposed by [5]. Compound-specific $\delta^{13}C$-signatures of long-chain carboxylic acids and alkanes are thus suitable for source apportionment of lipid production in different plant compartments.

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A temporal and spatial survey of dissolved inorganic carbon (δ^{13}C-DIC) and dissolved oxygen (δ^{18}O-DO) in Loch Lomond, Scotland.

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δ^{13}C-DIC and δ^{18}O-DO have individually been used to consider photosynthesis and respiration in aquatic systems[1,2]. Dual isotope approaches are still rare, yet are powerful tools linking nutrient pools that are naturally associated in ecology. Here we examined the spatial and temporal patterns of DIC and DO in a large freshwater body, to consider if they offer any insight into dissolved inorganic cycling. Our study site, Loch Lomond is the largest lake in mainland UK, covering 71km^2 and draining a catchment of ~700km^2. A geological fault line bisects the Loch, leading to two distinct basins. The north basin is deep (max 204m), narrow and oligotrophic, the south basin is broad, shallow (max ~25m) and mesotrophic. A middle basin can also be defined and is intermediary in physio-chemical characteristics. Mean residence time for the loch is 1.9 years but this can vary with location and prevailing wind direction[3].

Samples were collected four times over a ten-month period (November '04, March '05, June '05 and September '05), at the surface, middle and bottom of 21 profiles. The nine major inflows were also sampled. At each site δ^{13}C-DIC, δ^{18}O-DO, [DIC], [DO] and temperature were measured. The range observed in [DIC] was 0.14mM to 0.19mM, with maximum in June '05. δ^{13}C-DIC ranged from –10.5‰ to –7.8‰ with maximum in June 05. δ^{18}O-DO ranged from 23.2‰ to 25.3‰ with maximum in Sep. '05.

Our sampling strategy revealed surface spatial variation in [DIC], δ^{13}C-TIC and δ^{18}O-DO, which seems to be related to lake structure. For example, [DIC] was highest in the southern basin where the largest inflow enters the lake. This inflow may enhance [DIC] by direct import, or alternatively respiration could be higher due to increased nutrient availability. δ^{18}O-DO increased ~1‰ from southern basin to northern basin for all sampling seasons, suggesting respiration may be proportionally more significant in the north. δ^{13}C-TIC decreased from –7‰ in the southern basin to –14‰ in the northern basin in March 05. No significant change in δ^{13}C-TIC was observed in the other sampling periods.

However, spatial variation was also observed in δ^{13}C-DIC and δ^{18}O-DO as a function of depth, (less markedly with [DIC]), particularly in the northern and middle basins when the lake is stratified due to development of a thermocline. The southern basin only forms a temporary thermocline during the summer in stable conditions. δ^{13}C-DIC and δ^{18}O-DO showed an antipathetic relationship with depth. δ^{13}C-DIC decreased by as much as 10‰ from the surface to lake bed in the northern and middle basins during June and Sep., with δ^{18}O-DO increasing by ~3‰ in the same conditions. Enrichment in both isotopic signatures with depth may suggest the relative increase in importance of heterotrophic production.

By considering each isotope individually and in relation to the other, elucidating patterns in overall lake production may be achievable. Here we have observed significant changes in δ^{13}C-DIC and δ^{18}O-DO, which allows inference of spatial and temporal differences in the manner in which DIC/DO is utilised and thus, gross lake productivity and nutrient cycling.

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δ^{15}N dynamics of ammonium and particulate nitrogen in a temperate eutrophic estuary

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We monitored the stable nitrogen isotopic composition (δ^{15}N) of suspended matter and ammonium in the freshwater stretch of the Scheldt estuary (Belgium) over a full year to investigate for seasonal evolution and possible co-variation between isotopic signatures. The δ^{15}N value of ammonium remained rather constant during winter (average = +11.4‰) but increased significantly with the spring and summer bloom, reaching values as high as +70‰. This enrichment of the ammonium pool in ^{15}N coincided with significant ammonium depletion during summer period, suggesting a close causal relationship. Based on a semi-closed system approach we deduced an apparent fractionation factor associated with NH_4^+ utilization (i.e. combining effects of uptake and nitrification) of 18.4‰ (SE=2.0‰), which is similar to values reported in literature. Observed variations of ammonium δ^{15}N could account for about 69% of δ^{15}N variation in suspended matter.

An overview of the uses of stable carbon, nitrogen and sulfur isotopes in the oil sands region of Alberta, Canada.

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Naphthenic acids (NAs) and polycyclic aromatic hydrocarbons (PAHs) are naturally occurring organic compounds associated with bitumen from the Athabasca oil sands region in Alberta (Canada). The process of extracting bitumen from sand generates large volumes of process-affected water containing elevated levels of NAs and PAHs. These groups of complex mixtures are of environmental concern since both groups cause chronic toxicity to aquatic organisms. Aquatic organisms may be exposed to these compounds in naturally eroded environments and areas of anthropogenic activities along the Athabasca River and its tributaries or in reclaimed aquatic environments constructed with oil sands process-affected material. To some extent, both NAs and PAHs are biodegradable which is important in terms of potentially reducing toxicity. In oil sands reclamation, biodegradation of these compounds may also be important in carbon cycling.

There are many ways in which stable isotopes have been applied to issues related to the oil sands region. Stable carbon isotopes of fish were used as a tracer of exposure to oil sands constituents in the tributaries of the Athabasca River and at sites downstream of a confluence along the Athabasca River. Since oil sands constituents are not uniformly distributed in natural or reclaimed aquatic environments, mobile species such as fish may be exposed to varying levels of oil sands constituents. Defining the level to which organisms are exposed to oil sands constituents, using tools such as stable isotope analyses, is important to developing exposure and effects relationships for the assessment of cumulative impacts of oil sands development.

At oil sands reclamation sites, trends of ^{13}C depletion and ^{15}N enrichment in benthic invertebrate groups were correlated to increased levels of process-affected material used as construction material. However, these trends varied among invertebrates which lead to the need for further understanding of the cycling of carbon and nitrogen at the base of the benthic foodweb in oil sands aquatic reclamation environments. Field studies showed elevated dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC) concentrations at oil sands processed-material (OSPM) sites. DOC and/or DIC $\delta^{13}C$ values differed between low and high organic (peat) reference and OSPM sites which may be due to enhanced microbial activity at sites with elevated DOC concentrations. Laboratory degradation studies using a commercial NA mixture also showed shifts in $\delta^{13}C$ values for DIC which were consistent with the ^{13}C enrichment of DIC at OSPM sites containing elevated levels of NAs suggesting that DIC $\delta^{13}C$ values may be useful for examining carbon dynamics at reclamation sites. Future research includes assessing factors that influence microbial degradation and isotope values, in terms of NA concentration, and nitrogen and sulphur sources.

$^{15}N/^{14}N + {}^{18}O/^{16}O$ tracing of nitrate in UK upland waters

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Although problems relating to nitrate in water are mainly associated with modern farming methods in more populated parts of the UK, nitrate also constitutes a potential problem in more remote, upland areas. Here it is nitrate in atmospheric deposition which has a particular impact due to a combination of factors: 1) these are high rainfall areas; 2) low concentrations of base cations in upland soils makes them particularly prone to acidification, and nitrate is soon likely to become the main acidifying agent in 'acid' rain; and 3) upland ecosystems are adapted to low nutrient levels and are often N-limited.

Current trends suggest critical loads for acidity and N deposition will be exceeded in many upland areas by 2010, factors which threaten future compliance with the EU Waters Directive. Predicting the response of upland areas to nitrate deposition, however, depends on knowledge of the extent to which their soil/plant ecosystems are already N-saturated. In saturated systems, excess soil N is likely to undergo bacterial nitrification, and be released as nitrate into surface waters. To this end, being able distinguish between nitrate derived from atmospheric deposition, and nitrate formed by soil nitrification is particularly important.

In the first combined $^{15}N/^{14}N$ and $^{18}O/^{16}O$ study of nitrate in rainfall and surface waters in the UK, we analysed samples from four upland sites of the Acid Waters Monitoring Network: Afon Gwy (Wales), Lochnagar (Cairngorms), River Etherow (Peak District), and Scoat Tarn (Lake District). For the data available to date several very clear results emerge:

- $\delta^{15}N$ values for rainfall nitrate were typically in the range –2 to +3‰, and therefore not distinguishable from the values expected for nitrate in upland soils.
- $\delta^{18}O$ values for rainfall nitrate were typically in the range +60 to +80‰, and therefore very different, and distinguishable from the values of +2 to +4‰ which would be theoretically expected for bacterially-produced soil nitrate (based on one third of the oxygen being derived from atmospheric O_2 = +23‰, and two thirds being derived from water = -8 to –6‰).
- Nitrate in most of the streams had $\delta^{18}O$ values corresponding very closely to the theoretical values for bacterial soil nitrate; implying that, at least during the sampled period, very little atmospheric nitrate passes directly through the soils into the streams.
- Only the outflows of Lochnagar and Scoat Tarn showed significant atmospheric nitrate (c. 17%), and this may represent rainfall which has fallen directly onto the surface of these water bodies.

Seasonal variability of oxygen stable isotopes across the northern 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 for the development of hypoxia are linked to freshwater and nutrient inputs from the Mississippi River, which enhance shelf primary productivity and lead to increased algal biomass that eventually sinks to bottom waters. Ultimately, decomposition of this material in the lower stratified water column and in bottom sediments decreases oxygen concentrations and leads to hypoxia. To better understand oxygen sources and sinks in this area, we have analyzed oxygen stable isotopes in combination with oxygen concentrations.

Atmospheric oxygen has a $\delta^{18}O$ value of 23.5‰, and 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 can significantly change oxygen isotopes. Oxygen derived from primary production has an isotopic value of the ambient water (approximately -2‰ in this area), and will lower the $\delta^{18}O$ of dissolved oxygen. On the other hand, fractionation during respiration can significantly increase $\delta^{18}O$, to values 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 - 30m deep) at 5m 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 October2002, due to wintertime conditions, which favour 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. In July 2002, summertime development of surface phytoplankton blooms and accompanying bottom water hypoxia resulted in very different oxygen stable isotope patterns across the study area. Since photosynthesis produces low $\delta^{18}O$ oxygen (near -2‰), surface waters showed reduced $\delta^{18}O$ values of 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. On the other hand, in June and July of 2003 two tropical storms disrupted the stratification and bottom hypoxia was much less severe during this time, despite comparably high surface productivity.

During calm periods of summer stratification, oxygen dynamics were predominantly controlled by biological processes. Nevertheless, physical mixing was more important during the fall and winter months, but also in the summer of 2003. 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 estimate productivity and respiration on a large scale without time-consuming incubations.

What can we tell about source of sinking lacustrine particulate organic matter (seston) from isotopic and stoichiometric composition?

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Seston (sinking particulate organic material) plays an important role in lacustrine biogeochemistry through:

i) providing nutrients that can be microbially recycled or grazed by higher trophic level species either whilst sinking in the water column or post-deposition on the sediment.

ii) burial in the sediment, which generally reduces the rate at which carbon and nitrogen pass through atmospheric component of their biogeochemical cycle and thus reduces the contribution to radiative forcing from immediate respiration of this material.

In higher latitude lakes, allochthonous DOM (introduced by rivers) is considered an important energy source for lacustrine foodwebs. We wondered whether allochthonous material is equally important to lake seston budgets?

Our field site is Loch Lomond in Scotland. This is an interesting lake in which to consider seston dynamics as it has the largest (surface area) in mainland United Kingdom, and is bisected by a geological fault line, leading to within a narrow latitude, three distinct basins with identical or similar environmental controls (e.g. daylength, external temperature). Further, the north and south basin receive almost identical volumes of inflowing water, but the north basin is considered oligotrophic and the south basin mesotrophic due to differences in the amount of nutrient received.

Thus from May 2005, we have collected, approximately monthly, seston from traps deployed in mid- and bottom-water at the deepest point in each basin (a location considered to integrate lake processes). Carbon and nitrogen isotopic and stoichiometric analyses have been used to consider spatial and temporal changes in seston quality, in addition to construct budgets of carbon and nitrogen delivery to lake sediments.

A habitat-scale field survey of the stable oxygen and hydrogen isotope composition of aquatic insects (Chironomideae: Diptera) in a subarctic lake ecosystem with paleoenvironmental implications

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The stable oxygen isotope composition ($\delta^{18}O$) of chironomid headcapsules preserved in lake sediments can be used to reconstruct past environmental changes in the Arctic and subarctic region. Application of this technique is based on the assumption that the $\delta^{18}O$ of chironomid head capsule chitin faithfully records the $\delta^{18}O$ of the lakewaters in which they live. However, the details associated with this assumption and the magnitude of $\delta^{18}O$ variations in aquatic ecosystems (from both organisms and water) have rarely been examined. Field results from Smith Lake, a subarctic lake in interior Alaska, show no significant difference in $\delta^{18}O$ of whole body organic matter between two chironomid species. Difference in $\delta^{18}O$ between soft tissues and chironomid headcapsules (primarily composed of chitin) suggests that soft tissue may more strongly reflect seasonal lake water $\delta^{18}O$ values, whereas the $\delta^{18}O$ of chitin from chironomid headcapsules seems to more strongly reflect mean annual lake water $\delta^{18}O$ values, possibly influenced by the reabsorption of old chitin during larval ecdysis. Significant differences detected between the δD of the two examined chironomid species from Smith Lake may reflect different feeding behaviors and a trophic level increase in δD. Stable isotope data of the chironomids from Smith Lake are presented in the context of data derived from plants and other organisms in the same lake ecosystem. These field observations are also currently being supported by a laboratory-based study involving chironomids grown under known conditions (i.e. controlled $\delta^{18}O$ of habitat water and diet). Our research aims to lay the groundwork for a more rigorous application of $\delta^{18}O$ and δD data derived from the analysis of chironomid headcapsules preserved in arctic and subarctic lake sediments to infer past environmental conditions.

Combining Stable Isotope Analysis and mark-recapture experiments to study juvenile brown trout (*Salmo trutta*, L.) life history variants.

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Recent improvements in Passive Integrated Transponder (PIT) technology have proven successful in tracking small fish in shallow streams. A mark-recapture project was initiated at large scale in La Roche Brook, a second order tributary of the Oir River (Normandy, France) where more than 550 juvenile brown trout were PIT-tagged in 2005. The aim of this study is to assess movement, habitat selection and life history variants within the population, with particular emphasis on individual growth trajectories since growth has been identified as a key factor for juvenile survival and dispersion. Thanks to non-destructive tissue sampling, repeated Stable Isotope Analysis (SIA) is used to assess changes in food source and trophic position on individually tagged fish. Young-of-the-year (YOY, fork length 50-71 mm) and older juveniles (one or two years old, fork length 100-277 mm) were captured in La Roche brook, a 2.2 Km long site, in June and October 2005. Each fish was PIT-tagged, measured (length and weight), and fin clipped before release. From June to October, juveniles were tracked every two weeks using a portable PIT detector; habitat features were mapped and a GIS data base was used to analyse fish movements and habitat selection. As a first examination, stable carbon and nitrogen isotope analysis was run on a sub-sample of fin tissue from 50 fish that displayed singular growth patterns, including fish that migrate to the Oir River during summer.

No major differences in $\delta^{15}N$ and $\delta^{13}C$ values were found in fin tissue samples according to fish location in the brook. Older juveniles were significantly ^{15}N-enriched in June and October compared with YOY, whereas no difference was observed for $\delta^{13}C$ values. $\delta^{15}N$ values tended to increase from June to October for YOY (up to 2‰). Within age-class variations in $\delta^{15}N$ and $\delta^{13}C$ values were different between YOY and older juvenile only in June, with a higher dispersion for YOY $\delta^{13}C$ values and for older juveniles $\delta^{15}N$ values. Individual growth rate varied from 0.03 to 0.55 mmday^{-1}; YOY grew significantly more than older juveniles during summer, and higher fish growth was observed in the lower reach of the brook. Growth rate was not correlated to $\delta^{15}N$ and $\delta^{13}C$ values or to fish mobility. No difference in isotopic signature was found between the most mobile individuals (more than 100 m distance covered between consecutive tracks) and those with higher site fidelity. However, a significant decrease in $\delta^{13}C$ values of mobile fish was observed from June to October.

In spite of significant differences in growth performances and mobility among individuals, preliminary SIA on fin tissue suggest no major shift in food source and prey selection by juvenile during summer. Complementary investigations, especially on fine scale frequency of movement, habitat selection and prey availability, are now needed to understand spatial variations in fish growth observed in La Roche Brook.

Marine nutrient inputs and uptake in food webs of Atlantic coast rivers: bottleneck to freshwater productivity?

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Anadromous fishes can deliver considerable marine derived biomass to freshwater systems leaving detectable “marine signatures” as these nutrients are incorporated into riverine, and riparian, food webs. Much of this research has focused on the Pacific salmon rivers of western North America. There is little evidence of the importance of marine nutrients in food webs of Atlantic river ecosystems. We used stable isotope analysis in an attempt to detect marine subsidies to freshwater benthos delivered by anadromous fishes such as Atlantic salmon (*Salmo salar*), sea lamprey (*Petromyzon marinus*) and blueback herring (*Alosa aestivalis*).
Benthic macroinvertebrates sampled from egg incubation baskets set in Atlantic salmon redds had elevated $\delta^{13}C$ and $\delta^{15}N$ values suggesting consumption of salmon eggs. Similarly, macroinvertebrates in the West River, Nova Scotia, Canada, also had elevated $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ values in a downstream reach that suggested consumption of marine-derived organic matter from spawning blueback herring; invertebrates at an upstream reference site with no spawning had similar $\delta^{13}C$ but lower $\delta^{15}N$. However, sculpin (*Cottus* sp.), a common benthic fish species and known egg predator, showed no evidence of having consumed Atlantic salmon eggs in Catamaran Brook, New Brunswick, Canada or the Scorff River, Brittany, France. These analyses suggest that marine organic matter subsidies may be important in Atlantic rivers with concentrated spawning such as by alosid species wheras carbon and nitrogen contributions from Atlantic salmon may be minimal, or localized. These results will be discussed in the context of historically large numbers of anadromous fishes in Atlantic coast rivers, and the potential limitation in freshwater productivity today as a consequence of reduced marine nutrient inputs.

Diet and movement of the Atlantic bluefin tuna (*Thunnus thynnus*) through carbon and nitrogen stable isotope analysis

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The Atlantic bluefin tuna (*Thunnus thynnus*) is a highly migratory, pelagic apex predator. Bluefin are unusual among fish species in their capacity for endothermy and their ability to reach large body masses exceeding 600 kg. These same features allow bluefin to migrate widely to exploit the most productive forage grounds throughout the north Atlantic. Bluefin are important apex predators that may exert top down control on pelagic food webs. Bluefin diet is therefore an important component of pelagic food web dynamics that can be applied to an ecosystem-based approach to fisheries management in these regions.

We used stable isotope analysis to better determine bluefin diet and trophic position throughout its range and to test the feasibility of tracing large-scale movements. Bluefin diet and movement patterns have mainly been assessed through stomach content analysis (SCA) and tagging studies, respectively. Both approaches have provided valuable information about bluefin ecology, but are logistically challenging. SCA is further limited by inherent biases associated with this method, while the cost of tagging limits this approach to smaller sample sizes. We analyzed $\delta^{13}C$ and $\delta^{15}N$ from bulk tissue samples of bluefin white muscle (presumed moderate turnover) and liver (presumed fast turnover) as a complementary approach to these established methods. Adult bluefin samples were collected from forage grounds off North Carolina, New England, and Canada in the western Atlantic, and from offshore forage grounds in the eastern Atlantic. Juvenile samples were collected from forage grounds off Virginia and New England in the western Atlantic and the Bay of Biscay in the eastern Atlantic. Bluefin stable isotope values were compared with regional prey isotope data to quantify bluefin diet and trophic position. Bluefin isotope values were generally found to be depleted or only moderately enriched relative to local prey values, suggesting possible contributions of lower trophic level prey items, minimal bluefin diet-tissue trophic level discrimination, or contributions of forage from previous migrations. For New England bluefin, we also analyzed stomach contents and compared individual liver and muscle values to estimate duration of feeding at local forage grounds. SCA depicted a diet primarily of herring (*Clupea harengus*) for adults and sand lance (*Ammodytes americanus*) and euphausiids for juveniles in this region. Greater scatter in muscle isotope values relative to liver values suggests equilibration over a short time scale but incomplete equilibration over the duration represented by muscle tissues.

We extracted lipids from all tissue samples prior to isotopic analysis to correct for variable $\delta^{13}C$ depletion associated with differing lipid content between and within tissue types. Four different solvents were used to test their efficiency of lipid removal in bluefin liver, bluefin white muscle, and whole homogenized Atlantic herring, an important bluefin prey item: 2:1 chloroform-methanol, chloroform, ether, and hexane. Preliminary data suggest chloroform-methanol provides the most exhaustive lipid extraction. Nitrogen isotope values for each extraction method were compared to non-treated samples to test for extraction-induced $\delta^{15}N$ alteration. Chloroform-methanol and ether induced $\delta^{15}N$ enrichment for liver and muscle while hexane and chloroform caused depletion. Whole herring experienced minimal $\delta^{15}N$ alteration.

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Teeth reveal sperm whale ontogenetic movements and trophic ecology through the profiling of $\delta^{13}C$ and $\delta^{15}N$

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Teeth from male sperm whales stranded in the Northeast Atlantic were used to determine whether chronological profiles of stable isotope ratios of carbon and nitrogen across dentine growth layers could be used to detect known ontogenetic benchmarks in movements and foraging ecology. Teeth profiles show a general depletion of $\delta^{13}C$ (median=1.91‰) and an enrichment of $\delta^{15}N$ (median=2.42‰) with age. Marked isotopic changes occur for most teeth around 9/10 years, 13 years and again at 21/22 years. These results are consistent with male segregating from natal groups in low latitudes at around the age of 9, with the onset of puberty, and gradually, during the following 3/4 years, dispersing pole ward into $\delta^{13}C$-depleted temperate waters of the north Atlantic. Penetration into further depleted and productive high latitudes occurs at around 21/22 years, which might facilitate the spurt of accelerated growth rate observed at this age. Breeding migrations, back to lower latitudes, are not reflected in the $\delta^{13}C$ profiles possibly due to being short compared to the time spent feeding in high latitudes. The observed enrichment in $\delta^{15}N$ with age is likely to be caused by a trophic level increase as males age and grow in size, probably feeding on larger prey. Additional explanations could be that in the higher latitudes of the north Atlantic the main prey source is the high trophic level squid *Gonatus sp*, as well as the fact that lower latitudes from where males disperse are depleted in basal $\delta^{15}N$. This study confirms the potential of using $\delta^{13}C$ and $\delta^{15}N$ in teeth to investigate movements and dietary history of individual sperm whales. Such information gathered from different regions, sexes, and periods in time, could provide a unique way to understand the ecology of this species across oceans.

Feeding ecology and trophic status of pelagic sharks from the eastern north Pacific inferred from $\delta^{13}C$ and $\delta^{15}N$

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Like many elasmobranch fish, the common thresher shark (*Alopias vulpinus*) and the shortfin mako shark (*Isurus oxyrinchus*) have slow intrinsic growth rates and relatively low fecundities, making them vulnerable to overexploitation. Both species are fished recreationally and commercially along the west coast of the United States. Despite their vulnerabilities to exploitation, in-depth trophic studies on these species are lacking from the eastern north Pacific. In addition, there are anecdotal evidence of trophic shifts in both species with ontogeny but infrequent access to large individuals and the intrinsic limitations of stomach contents (snapshot view, often empty) have constrained such interpretations.

To complement and validate stomach content-based food habits studies and to look for evidence of trophic shifts, we utilized stable isotopes of C and N to infer the feeding ecology of these two species of pelagic sharks. We sampled liver and dorsal white muscle from a wide range of sizes of both species caught by commercial and sport-fishermen along the coast of California in 2004-2005. From the liver and muscle tissues, we measured $\delta^{13}C$ and $\delta^{15}N$ to reflect short-term versus long term feeding differences within an individual since different tissues have different isotopic turnover rates.

We also sampled vertebrae from large shortfin mako from sport-fishing tournaments in 2005 and utilized archived large common thresher vertebrae from fish caught in 1995–2002. Using micro-sampling techniques, we were able to utilize $\delta^{13}C$ and $\delta^{15}N$ of distinct vertebral centrum growth rings of the corpus calcareum as a temporal record of the feeding history of individual sharks.

The common thresher soft tissues showed a linear increase in $\delta^{15}N$ with increasing size suggesting a gradual gape limited trophic increase with ontogeny. The common thresher $\delta^{13}C$ suggests limited individual variability in their diet. The muscle tissue $\delta^{15}N$ was always enriched relative to the liver tissue $\delta^{15}N$ from the same individual suggesting tissue related isotopic differences versus seasonal differences.

The shortfin mako soft tissues did not show a clear increase in $\delta^{15}N$ with increasing size. The shortfin mako $\delta^{13}C$ suggests a more opportunistic diet with more individual variability. The muscle tissue $\delta^{15}N$ was enriched relative to the liver tissue $\delta^{15}N$ from the same individual in smaller sharks but all females greater than 250 cm fork length, showed an inverse relationship. This could possibly be due to seasonal diet differences and could also reflect differences in the physiology of these female sharks as they reach sexual maturity.

We also present the preliminary results of the stable isotope analysis of individual vertebral growth bands.

Dietary and geographical fingerprint of ancient British Columbian glacier body through molecular and isotope characterisation of bone and skin lipids and amino acids.

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Molecular fingerprinting and compound-specific carbon isotope analysis was performed on individual lipids and collagen amino acids extracted from bone and skin sampled from a glacier body exhibiting outstanding preservation due to an exceptional glacial depositional environment. The remains of Kwaday Dän Ts'inchí, the finest preserved ice body unearthed in North America, were recovered from a retreating glacier within the Tatshenshini–Alsek Park in British Columbia on August 14, 1999. The aim of this molecular investigation was to elucidate where this individual originated and how much time he endured such inhospitable surroundings prior to his death. High temperature gas chromatographic (HTGC) analysis of both tissues revealed a considerable abundance of endogenous straight-chain *n*-alkanoic acids ($C_{12:0}$, $C_{14:0}$, $C_{16:0}$, $C_{16:1}$, $C_{18:0}$, $C_{18:1}$ fatty acids) and cholesterol, in addition to hydroxy acids, comprising 10-hydroxyhexadecanoic acid, 10- and 12-hydroxyoctadecanoic acid. Two further unusual long-chain hydroxy acids, 10- and 12-hydroxyeicosanoic acid and 10- and 12-hydroxydocosanoic acid, were also detected in considerable abundances in the bone sample. The origins of these hydroxy acids was assigned to microbially-activated hydration of the double bonds in the $C_{20:1}$ and $C_{22:1}$ fatty acids present in the glacier body's bone at the time of death, possibly originating from a substantial dietary intake of marine food by the individual. The marine biomarker trilogy of isoprenoidal compounds, phytanic acid, pristanic acid and trimethyltetradecanoic acid, were also detected in the bone sample. In contrast, the skin sample lipid composition was dominated by $C_{16:0}$ with only trace abundances of the long chain hydroxy acids and isoprenoidal compounds. Although little disparity was observed between bone and skin fatty acid $\delta^{13}C$ values, cholesterol values were higher in the latter, possibly owing to a deviation to include terrestrial dietary sources in the last months of life, observable due to the more rapid turnover rate of skin. This result was highly congruent with the results of amino acid $\delta^{13}C$ analysis, in particular $\Delta^{13}C_{Glycine\text{-}Phenylalanine}$ values, where the lower values observed for skin (12.7 ± 1.7‰) than bone (15.6 ± 1.7‰) is likely to indicate a divergence away from an exclusively marine diet in the last months of life. Hence, both the lipid and amino acid composition and carbon isotope composition are consistent with the consumption of a high marine protein diet throughout life (bone signature), followed by a reliance on more C_3 terrestrial foods in the months prior to death (skin signature). This individual evidently spent the majority of his life in a coastal environment followed by either a seasonal or single journey inland in the final months of life.

Are isotopes the key to understanding ancient ecosystems?

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Animals fractionate nitrogen and organic carbon isotopes during food digestion and preferentially excrete the lighter isotopes. Consequently their body tissues become enriched in $\delta^{15}N$ and $\delta^{13}C_{org}$. This simple relationship between consumer and food means that the trophic level of organisms within the same ecosystem can be distinguished on the basis of their isotopic signature. Various authors have successfully applied this technique to modelling trophic structure in present day and Neogene vertebrate ecosystems. However, in this current study nitrogen and organic carbon isotopic ratios are being used to investigate the community structure and palaeoenvironmental changes within a much older fossil ecosystem.
The Early Eocene fish of Fossil Lake in the Green River Formation of SW Wyoming are part of an exceptionally well-preserved diverse aquatic community. Specimens have been collected from throughout the succession, particularly from mass mortality beds. Preliminary analyses of fossil fish from these beds indicate that isotopic signatures can be used to define the trophic structure of extinct communities. The results generated are directly comparable to those from modern fish. In addition to this data relating to trophic structure, nitrogen isotopes are providing information regarding nutrient flux in Fossil Lake through time.

Prehistoric Evidence for Flexibility in Northern Fur Seal (*Callorhinus ursinus*) Maternal Strategies in the Northeast Pacific Ocean

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Archaeological sites along the northeast Pacific margin, including temperate latitude sites (35-50°N), contain a high abundance of northern fur seal (NFS) remains relative to other pinnipeds. The modern pattern of offshore foraging and largely high-latitude breeding, coupled with the relatively short time period NFS congregate onshore (~4 months), would have made NFS less available to prehistoric human populations in comparison to other pinnipeds. Furthermore, harvest (mortality) profiles from archaeological assemblages provide two firm conclusions. First, all sites contain young pups 0-4 months of age, confirming that NFS had rookeries in close proximity to these sites. Second, strong representation of 5-12 month old individuals is odd in light of modern NFS nursing and attendance behavior; young-of-the-year were available to human hunters year-round, not just during the ~4 month breeding season.

To explore whether the modern maternal strategy (abrupt weaning at ~4 months) is a result of their high-latitude breeding distribution, we measured δ^{15}N values of fossil NFS between 2 and 20 months of age. Isotopic results suggest that prehistoric NFS breeding south of the Bering Sea used a maternal strategy similar to other otariids who breed at temperate latitudes, who typically wean their pups at ~10-14 months of age. In the absence of strong selective pressure for early weaning imposed by the onset of severe winter conditions in the Bering Sea, weaning at an older age may have been adaptive for NFS populations breeding in seasonally ice-free environments at temperate latitudes. Overall, our study confirms there were more temperate latitude NFS rookeries in the past and that these rookeries were large enough to allow NFS to be a dominant species in areas where, today, it represents a small fraction of the marine mammal community. Our results have implications for the conservation of NFS, confirming that a wider range of sites are viable rookery locations, and that certain aspects of their reproductive behaviour may shift as they adapt to life at temperate latitudes.

Parts Underground: Corms, Conundrums, and the Diet of Mole-Rats

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Plant underground Storage Organs (USO's), including tubers, grass rhizomes, corms, and bulbs, are a source of nutrition for some animals in xeric regions. Plants living in these arid environments store nutrients and water in USOs for use during long dry seasons. Modern African mole-rats (family Bathyergidae) are known to utilize this resource nearly exclusively. Faunal analyses show that mole-rats co-occur with ancient hominins in Late Pliocene sub-Saharan Africa, possibly indicating similar dietary reliance on USO's. Isotopic studies of fossil hominins have been interpreted by some authors to indicate USO utilization. We measured the isotopic composition of enamel and bone apatite from five species of bathyergids distributed across a broad range of habitats; each species relies on USO's to different extents. The different species of mole-rats have distinct $\delta^{13}C$ values, with a total range among all specimens of -15 to -3‰. Our data suggest a broad range of dietary specialization along the C_3-C_4 vegetation axis. Some species have $\delta^{13}C$ values suggesting narrow dietary preferences; values for others indicate broader diets. Some of the $\delta^{13}C$ values from modern mole-rats overlap isotope data for hominin species. An analysis of Plio-Pleistocene mole-rats, found co-occurring with early hominins, show further isotopic similarities between these taxa. Plio-Pleistocene mole-rat $\delta^{13}C$ values are more constrained than modern data, and range from –9 to –8‰. Results of this study confirm that C_4 USO's were available on the landscape for utilization in Pliocene southern Africa, and indicate possible dietary similarities between African mole-rats and early hominins. These data are consistent with the hypothesis that USO's were consumed by hominins.

Variations in tap water isotope ratios

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Precipitation exhibits seasonal variation in isotopic composition in well-modelled patterns across the United States. Most precipitation values – either rain or snow – fall along the Global Meteoric Water Line (GMWL). Temporal variations in stable isotope values of precipitation within a single location can be significant, especially across seasons of the year. Variations in precipitation isotopic composition would inherently affect the isotopic composition of drinking (tap) water, but to different degrees, based on the specific source of tap water: ground water, reservoirs and/or transported water.

The stable isotopic composition of tap water could be useful for understanding relationships between precipitation source and human water use, especially in high-impact (urban) areas. Tap water sources are intimately linked to precipitation and may show a similar pattern of seasonal variation. The amalgamation of multiple precipitation events into one mixed body of water may act as buffer, dampening the magnitude of any potential temporal variation. On the other hand, if that water is stored in reservoirs, the isotope ratios of tap water may be enriched relative to precipitation.

We initiated a monthly sampling effort to describe monthly variation in tap water isotopes in different cities within the United States through the year. Sampling sites across the contiguous USA (with one site in Canada) were chosen to represent different geographical regions. Results indicated that variation in tap water isotopic composition through the year was insignificant in some regions and significant in other regions of the USA. Of the 49 locations sampled, 53% of the waters sampled showed an annual range in δ^2H values of ≤8‰, whereas 18% of the waters sampled varied by 20‰ or more in δ^2H values. At one extreme, the temporal range of tap water isotope ratios was 39‰ for δ^2H in Minneapolis, MN; at the other extreme, temporal variation was 3‰ for δ^2H in Las Vegas, NV.

Tap water variations in isotope ratios were less than variations in precipitation. For example, precipitation data from Chicago, IL (GNIP online database, 2005: http://isohis.iaea.org) showed a range of 170‰ for δ^2H and 25.1‰ for δ^{18}O (19 year average). Yet the range of tap water isotopic composition we observed was much smaller: 6‰ for δ^2H and 0.8‰ for δ^{18}O.

Tap water from several cities showed seasonal shifts in isotopic composition between one sample and the next, indicative of a switch in tap water source. This switch was evident in highly urbanized areas, such as Southern California. In Buena Park, a suburb of Los Angeles, tap water samples between April and May changed -21‰ for δ^2H and -2.1‰ for δ^{18}O as the water source switched from local ground water to water imported from the Sierra Nevada Mountains 400 km distant.

Sulphur isotopes ratio in pollutants migration investigations.

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The stable isotopes sulphur composition in compounds of industrial origin, present in atmosphere, biosphere, hydrosphere, groundwater, soil, etc., may differ from those for natural sources. Sulphur isotope ratio can be treated as an environmental tracer, and may be applied to study the sulphur pollution distribution from coal combustion process, in the environment.
Literature review shows that there are a few data on sulphur isotope ratio in Polish coals and on fractionation of sulphur isotopes in process of coal combustion. The first step of the investigation was characteristics of coals from Polish coalmines. This study seems to be the preliminary and can be used for further geological and hydrological investigations.
The next studies concerned possibility of application of sulphur isotope ratio to investigate the pollution of environment by sulphur compounds, coming from coal combustion process. The two paths of sulphur compounds separation can be distinguished. $\delta^{34}S$ values in slag and ash from Turów Power Station are enriched in the heavier isotope ^{34}S in the coal combustion process. The same effect has been observed for the Belchatow Power Station. The results regard in $\delta^{34}S$ value for Patnow Power Station and Kaweczyn Power Station are opposite. Sulphur in ash and slag is depleted in heavy isotope ^{34}S in the coal combustion process. It is not very clear why this difference between these results occurs but probably it arises due to different combustion process condition.
The sulphur dioxide from outlet gas was absorbed to determine sulphur isotope fractionation in desulphurization process. Sulphur dioxides from outlet gases are enriched in light isotope ^{32}S in comparison to coal, which is used in power plants. Fractionation of sulphur isotopes between inlet and outlet gases has been observed. However, products from this process are different (gypsum and ammonium sulphate), $\delta^{34}S$ are enriched in both desulphurization processes.
This method may be applied to investigate of different air pollution control technologies as well, for example method can be used to establish further fate of by-products e.g. elution of gypsum from landfilled waste, monitoring of water contamination and investigation of landfill deposited ashes leaching. However, introduction of desulphurization units has changed isotopic ratio of sulphur in the outlet gas streams. Normally, SO_2 remaining in this outlet flue gas is depleted in the heavy isotope 34. These phenomena should be taken into account during the elaboration of sulphur balance for the country and the region.

Stable Isotope Analysis of Wood

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The potential applications of isotope ratio mass spectrometry (IRMS) to forensic science are currently under investigation by many individuals. The technique has been utilised in a wide range of disciplines with success, including the monitoring of wine, natural oils and honey for illegal food adulteration; detection of illegal drug doping in sport and monitoring remediation of contaminated land.

The suitability of the technique for the analysis of wood was assessed, with the aim of identifying the geo-location of wood, for use in cases of illegal logging. A number of studies were carried out during method development including assessment of the potential of the principle of identical treatment and a hydrogen exchange experiment, to determine the validity of inter-laboratory data comparison. In addition the natural variation within wood samples was assessed.

The work was used with success following a criminal case in which wooden safety matches were collected from a crime scene and compared to those collected from a suspect. Wooden safety matches, produced for sale in the UK are manufactured from *populus tremula*, sourced from a variety of locations worldwide, although a majority are produced from managed forests in Sweden. The case illustrated here, shows that using stable isotope profiling, a match found at a scene of crime could be distinguished from that collected from a suspect.

The Role of Stable Isotopes in Human Identification: Variables Affecting the Interpretation of Data.

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Recent natural catastrophes with large scale loss of life have demonstrated the need for a new technique to provide information for disaster victim identification where traditional methods fail to yield the identification of an individual, or in other situations where authorities need to determine the recent geographical life history of people. One proposed solution is the use of Stable Isotope Profiling (SIP) using Isotope Ratio Mass Spectrometry (IRMS).

Links exist between the isotopic signal of dietary components and the isotopic composition of body tissue, which can be exploited to provide information on a person's life history. Generally, the ^{13}C and ^{15}N found in human material reflects the ^{13}C (and ^{15}N) isotopic composition of the food consumed by an individual whereas the ^{2}H (and to a degree ^{18}O) content is a reflection of a person's direct and indirect water intake, and, hence geographic origin.

In order to utilise Isotope Ratio Mass Spectrometry in forensic investigations involving the identification of human remains, factors that can affect the isotopic signature of human tissues needs to be explored. These factors include the effect of burning and soot/smoke exposure, the effect of weathering on exposed/buried remains and the interaction of insects and bacteria, as well as the effect of different storage containers on the samples themselves.

Here we present the preliminary results of a series of experiments investigating these variables. Samples were analysed using EA IRMS and isotope ratios determine for $^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{2}H/^{1}H$ and $^{18}O/^{17}O$.

Evaluating the contribution of seagrass and mangroves to prawn food webs across northern and eastern Australia using stable isotope analysis.

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Multiple stable isotope analysis was used to examine the relative importance of mangroves, seagrass or epiphytes to coastal food webs supporting juvenile penaeid prawns. Sampling of prawns and potential food sources was conducted at mangrove and seagrass sites in each of two areas of the Gulf of Carpentaria, northern Australia, and in two regions on the east coast of Australia. The carbon, nitrogen and sulfur isotope signatures of prawns from most sites confirmed that mangroves provided very little contribution to the diet. Prawns sampled from within small mangrove-lined creeks had $\delta^{13}C$ values midway between those of mangroves and seagrass, suggesting that mangrove carbon could contribute at most 50% of the dietary source of prawns in these habitats; however, the $\delta^{34}S$ values indicate that this is unlikely. The diet of most juvenile prawns was clearly derived from within seagrass beds in each region. Previous studies have questioned the relative contribution of seagrass versus their epiphytes to coastal food webs, because the two often have similar isotope signatures. We found that much of the observed spatial and temporal variation in the $\delta^{13}C$ values of prawns could be explained by epiphyte signatures; more so than by the variation in seagrass values. These data confirm that seagrass beds are important habitats in coastal mangrove systems, providing structure for epiphytic algae, which forms the primary base of the food web supporting juvenile prawns.

Hooves: a new tissue for high-resolution reconstruction of bovine dietary histories

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Stable isotope analysis of incremental tissues such as hair and teeth are powerful tools used to track dietary changes and movement in animals. Spatially separated samples record the isotopic composition of the tissue at the time it was deposited. Our objective was to establish whether sequential analysis of hooves can be used to reconstruct the dietary history of cattle.

A controlled, on-farm experiment was conducted in which six cattle were switched from a barley-based diet to an isotopically distinct diet incorporating maize and urea (the isotopic spacing between diets was 15‰ for $\delta^{13}C$ and 11‰ for $\delta^{15}N$) and maintained on that diet for 168 days. Postmortem sampling of the cleaned wall of the outer, left front toe was carried out using a micro-drilling technique. A 15 mm thick slice of the toe was cut with a band saw, 15 mm away from the inner wall. The soft tissues were removed with a blade and the horn defatted and dried. Bands less than 1 mm deep were drilled into the hoof wall using a diamond drill bit attached to a Dremel® 400 drill. The average width of sampled bands was 1.2 mm and the spacing between them was less than 1 mm. Therefore, at least 25 samples with a mean C and N content of 393±46 µg and 116±19 µg (n=198, ±SD), respectively were collected from the top 60 mm of each toe.

The isotopic composition of hooves responded very quickly to the new diet, suggesting that at least one of its pools has a rapid turnover with a half-life of less than 20 days. However, the N response was delayed somewhat compared to that of C. The calculated mean growth rate of cattle hooves was 6.85±0.79 mm per month (n=6, ±SD), a value that is considerably higher than previous estimates. The temporal resolution of sampling used here was about 5 days.

In conclusion, these experimental results demonstrate for the first time that hooves are a suitable incremental tissue for high-resolution isotopic reconstruction of the dietary and life history of bovine animals.

Lipid extraction in stable isotope ecology: a call for consensus

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It has been long known that lipids have the potential to confound interpretation of food webs constructed using stable carbon isotopes. One of the key steps in endogenous lipid synthesis exhibits strong discrimination against carbon-13, leading to more negative $\delta^{13}C$ in lipid rich tissues that is independent of diet. Despite this knowledge, there is little consensus on proper procedures for dealing with the lipid issue in stable isotope ecology.

A formula relating % lipid to carbon:nitrogen ratios (C:N) and resultant $\delta^{13}C$, developed by McConnaughy & McRoy (1979) has gained favour as a simple method to normalize $\delta^{13}C$ data without having to extract lipids. We analyzed isotope ratios of stream invertebrates (n = 68) and various fish tissues (n = 57) pre- and post-lipid extraction to determine if the normalization formula (created from a marine food web) was also appropriate for freshwater organisms. For invertebrates, differences between extracted and non-extracted tissues were fairly small (average change = 1.0‰) compared with some marine organisms. Lipid normalized $\delta^{13}C$ was significantly lower than lipid extracted $\delta^{13}C$ (paired t-test, p = 0.001) due to the formula's normalization to a constant but non-zero lipid content that corresponds to a C:N of 4. For fishes, the normalization formula was effective at approximating lipid extraction for high lipid tissues such as gonads and liver with C:N > 5, but was less effective in adjusting $\delta^{13}C$ when C:N was lower.

We also reviewed the literature to determine the prevalence of lipid extraction in stable isotope ecology. Approximately 10% of published studies extracted lipids prior to stable carbon analysis; this low value was partly due to the preferential use of low-lipid tissues such as bird feathers and fish muscle. However, given the potentially high lipid content of many food web components (e.g. zooplankton), we propose new guidelines for decisions on lipid extraction and normalization when using stable carbon isotopes. For example, because of the potential importance of dietary lipids, we advocate avoiding lipid-extracting prey items when consumer tissues are left intact. We also suggest that, due to different responses to lipid extraction by different taxa, lipid-normalization equations may be required on a taxon-specific basis.

Reference:

McConnaughy, T., and McRoy, C.P. 1979. Food-web structure and the fractionation of carbon isotopes in the Bering Sea. Marine Biology 53: 257-262.

Technical considerations when using stable hydrogen isotopes in aquatic ecology

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Stable hydrogen isotopes (δD) hold great potential as a third isotope in discriminating amongst organic matter sources for consumers in aquatic systems. However, issues such as differential hydrogen exchange among tissues and the presence of highly depleted lipids could confound interpretation of δD data.

We conducted experiments to assess the proportion of hydrogen available to exchange with ambient water vapour in a variety of tissues commonly sampled in aquatic food webs. These tissues, which included fish scales, fin and muscle, benthic invertebrates, sea lice, algae and leaf litter, were incubated under vacuum with labeled waters (δD = -135‰, +115‰). Different fish tissues exhibited different proportions of exchangeable H, with muscle tissue having less % exchangeable (12-18 %) than previously measured values for keratins (19-22 %) and scales having very little exchangeable H (6 %).

We also analyzed δD in lab-reared brook trout (*Salvelinus fontinalis*) and feed to estimate diet-tissue fractionation, pre- and post-lipid extraction. When tissues were analyzed prior to lipid extraction, trout muscle (-121.3 ± 3.2‰ S.D.) had vastly different δD values compared with feed (-151.6 ± 1.4‰ S.D.). However, following lipid extraction, feed samples (-105.5 ± 4.9‰ S.D.) were not significantly different than those of trout (-108.6 ± 3.6‰ S.D.), consistent with prior reports of negligible diet-tissue deuterium fractionation in animals (Hobson et al. 1999).

These observations have implications for methodology and the development of new standards for δD measurements. Proper application of techniques will allow more efficient use of stable hydrogen isotopes in constructing aquatic food webs.

References:

Hobson, K.A., Wassenaar, L.I., and Taylor, O.R. 1999. Stable isotopes (δD and δ^{13}C) are geographic indicators of natal origins of monarch butterflies in eastern North America. Oecologia 120: 397-404.

Enhancing performance in elemental analysis of solids and liquids

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The well established elemental analysis of C, N, S by Dumas combustion and O and H by high-temperature techniques has been extensively used over the past decades also in isotope analysis by coupling with an isotope-ratio mass-spectrometer (IRMS). Due to the extension into further application fields the demand for special applications increased. Besides multi-element analysis and high sample throughput for routine lab work one of the always demanding challenges were small amounts of sample material or small concentrations of the desired elements accompanied by appropriate precision.
Previous efforts to measure small N containing solid samples have used trapping techniques or numerical correction of the N blank. A small volume reactor and reduced flows combine low cost hardware changes for existing conventional EA types. With the addition of a blank reducing device the limitation to for N containing samples can be set to below micro molar amounts.
Common equilibration techniques for water measurements require millilitres of sample material. With the chromium reduction method there is a continuous flow technique that allows small water samples but restricts measurements to H isotope determination of water. The advantage of high-temperature pyrolysis with glassy carbon is the simultaneous conversion of water samples or organic liquids like ethanol to H2 and CO which can be subsequently measured in a single run using the fast magnet jump method of the mass spectrometer. This method allows sub micro litre amounts of sample. Both continuous flow methods suffer from memory effects. Changes of the glassy carbon reactor setup as well as a modification of the Helium supply (bottom feed connector) as proposed by Gehre et al. (2004) can significantly decrease reactor memory effects while the bottom feed connector additionally improves peak shape and the number of sample throughput of solid samples.

References:
Gehre et al. (2004): Continuous flow 2H/1H and 18O/16O analysis of water samples with dual inlet precision. Rapid Commun. Mass Spectrom. (18), p. 2650-2660.

Carbon and nitrogen isotopes as proxy parameters for urban and rural atmospheric pollution

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The present level of atmospheric pollution in urban conurbations demands improvement in monitoring and control of air quality. Therefore, EU-regulation 1999/30/EC has become effective in January 2005. In order to understand the spatial heterogeneity and discriminate variable sources of atmospheric pollutants it is necessary to establish a large scale continous sampling grid. Passive sampling using artificial devices or natural vegetation biomonitoring allows acquisition of well-defined samples at affordable costs.

The overall study investigates airborne PAH, heavy metal loads and magnetic properties (a proxy for PM pollution) of pine needles (Lehndorff and Schwark, 2004, Urbat et al., 2004, Lehndorff et al., 2006). We here discuss the $\delta^{13}C$ and $\delta^{15}N$ composition of pine needles as proxy for CO_x- and NO_x-concentration in air, respectively. Pinus nigra needles are used as passive samplers, due to the ubiquitous occurrence of this ornamental tree in urbanized and rural areas. Needle ages of up to five years provide a time-integrated record of atmospheric pollution. In addition, time resolved sampling of needle cohorts allows specific analysis of changes in plant physiology and accumulation processes.

Climatic, nutritional and physiological factors are known forces to determine isotopic composition of plants. However, N- and C-oxide concentration of ambient air is assumed to be the main agent influencing the isotopic composition of pine needles via stomatal uptake and incorporation into biosynthate in urban areas.

Results to be shown in this contribution include microscopic investigations and analysis of $\delta^{13}C$ and $\delta^{15}N$ composition of pine needles from 130 locations. A regional sampling grid comprising 71 samples covers an area of 3000 km^2. The area is characterized by highly variable land use including forests, arable land, pastures, lignite open pit mining, industrial and residential areas. The metropolitan area of Cologne City encompasses 405 km^2, randomly sampled at 59 locations. To adequately represent local air quality needles were taken from 3 trees at each location. Additionally, needle cohorts of up to five years age were analysed separately at 6 locations. Here the whole vegetation period was covered by quarterly sampling to study accumulation as well as seasonal climatic effects.

Previous studies indicated that isotopically light CO_x derived from fossil fuel combustion upon photosynthetic fixation may lead to more negative $\delta^{13}C$ values in urban vegetation (Lichtfouse et al., 2003). On a regional scale this observation can be confirmed in our study but an inverse trend was noticed for metropolitan areas. This inversion is attributed to interference of stomatal uptake of CO_x in environmentally stressed pine needles. In a comparable manner $\delta^{15}N$ in rural areas exhibits heavy values with a trend to lighter $\delta^{15}N$-signatures towards urban centres. Within the City of Cologne heavier $\delta^{15}N$-values are measured and related to enhanced NO_x emission and reduced air mixing. This interpretation is well supported by a positive correlation of direct NOx measurements (Environmental Institute of Cologne) with the $\delta^{15}N$ of urban pine needles.

No δ^{15}N trophic shift between mother and offspring deer mice (*Peromyscus maniculatus*)

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Stable isotopes offer a useful method for analyzing diet in small mammals, but natural variations over the course of an animal's life must first be investigated. Nitrogen isotopes become enriched by 3 to 5 ‰ with each trophic level increase. During lactation, offspring feed on milk produced by their mothers, and the δ^{15}N values of their tissues should reflect this increase in trophic level. Enrichments in ^{15}N of 1 to 4 ‰ have been observed during and after lactation in a variety of large mammalian species.

We have examined the δ^{15}N values of tissues from mothers and offspring deer mice (*Peromyscus maniculatus*) before, during and after lactation. Pregnant females were captured in the wild, brought into the laboratory and fed a controlled diet. Blood, muscle, liver and hair were sampled from mothers and offspring at three life stages: on the date of birth (Neonate), 16 days following birth (Dependent), and 25+ days following birth (Independent). Milk samples were also taken at 7 and 14 days following birth. Isotopic results are presently available for the blood and milk samples. From these data, we have observed that newborn mice reflected the laboratory diet much more quickly than adults, indicating fast turnover rates in growing young. During lactation, the δ^{15}N values of offspring were only slightly higher (+0.2 ‰) than their mothers and quickly returned to baseline levels once the young were weaned onto the laboratory diet. Our most parsimonious explanation of this behaviour is nitrogen cycling by rapidly growing young during lactation.

Geographical Patterns of Human Diet Derived From Stable Isotope Analysis of Fingernails

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Carbon and nitrogen isotope ratios of human fingernails were measured on 490 individuals in the western-USA and from 273 individuals in southeastern-Brazil living in urban areas and from 53 individuals living in a moderately isolated area in the central Amazon region of Brazil and existing mostly on locally grown foods. In addition we measured the carbon and nitrogen isotope ratios of common food items to assess the extent to which these isotopic signatures remain distinct for people eating both omnivorous and vegetarian diets and living in different parts of the world, and the extent to which dietary information can be interpreted from these analyses. Fingernail $\delta^{13}C$ values (mean $\pm$ standard deviation) were -15.4 $\pm$ 1.0 and -18.8 $\pm$ 0.8 ‰ and $\delta^{15}N$ values were 10.4 $\pm$ 0.7 and 9.4 $\pm$ 0.6 ‰ for southeastern-Brazil and western-USA populations, respectively. Despite opportunities for a "global supermarket" effect to swamp out carbon and nitrogen isotope ratios in these two urbanized regions of the world, differences in the fingernail isotope ratios between southeastern-Brazil and western-USA populations persisted and appeared to be more associated with regional agricultural and animal production practices. Omnivores and vegetarians from Brazil and the USA were isotopically distinct, both within and between regions. In a comparison of fingernails of individuals from an urban city and isolated communities in the Amazonian region, the urban region was similar to southeastern Brazil whereas individuals from isolated non-urban communities showed distinctive isotopic values consistent with their diets and with the isotopic values of local foods. Although there is a tendency for a "global supermarket" diet, carbon and nitrogen isotopes of human fingernails hold-dietary information directly related to both food sources and dietary practices in a region.

Advanced Laser techniques to Investigate Carbon isotopE discrimination during decomposition: The ALICE project

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Methods involving stable isotopes have been successfully applied since decades for various application fields (Astronomy, geology, geochemistry, microbiology, community and ecosystem ecology). Tracing and measuring 13C natural abundance in ecosystem compartments greatly enhanced understanding of the C fluxes along food webs and in the plant-soil-atmosphere C exchange when compartments present different C isotopic signatures (i.e. atmospheric CO2 vs photosynthetic leaves, C3 vs C4; etc.), with minimum disturbances of the system. However, the assumption that no isotopic discrimination occur during respiration is commonly made in numbers of C isotope-based ecological studies (Subke et al., 2004) . But verifications of such assumption are sparse and not enough reliable.

Stable C isotope experiments currently rely on the conventional isotope ratio mass spectrometry (IRMS) for measuring the 13C abundance. IRMS is, in spite of its high analytical precision, one of the limiting factor for experimental designs, in particular for continuous monitoring in field studies. In these last years laser spectrometry demonstrated to be a valid alternative to IRMS. Based on the fact that CO2 absorption patterns strongly depend on isotopic substitution, highly sensitive laser spectrometers can be developed in order to measure the 13C/12C isotope ratio in gaseous samples containing carbon dioxide. In this context, at the Environmental Science Department, an innovative diode-laser-baser methodology has been recently developed, enabling continuous measurements of both CO2 concentration and isotopic composition (Gianfrani et al., 2004; Castrillo et al., 2006). Such potentialities could certainly enlarge the possibilities of experimental settings, thus opening new fields of investigation.

The "ALICE" project, funded by the Marie Curie Fellowship for the Transfer of Knowledge Development Host Scheme, aims to implement an advanced laser spectrometry technology in order to study the isotopic composition and fractionation of respired CO2 from various substrates and micro-organisms. The final stage of the project will lead to field applications of the laser spectrometer. The expected results from these works will represent a very significant advance in (i) the verification of the assumption of no isotopic fractionation during respiration and (ii) also in measurements that were impossible without the laser spectrometer. The poster presents two experimental settings illustrating these two aspects.

Fractionation of Copper Isotopes during Dissolution of Malachite

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The field of transition metal stable isotope geochemistry has gained pace in recent years thanks to the development of multi-collector ICP-MS, which has made it possible to precisely measure fractionation of <1 ‰ in elements heavier than 40 amu. It is now crucial to develop the theoretical framework and empirical database to enable the use of transition metal isotopes for studying the biogeochemical cycling of these elements through the ecosystem. Copper is an important element in the environment being both an essential micronutrient for living organisms and highly toxic in elevated concentrations. To constrain the fractionation of copper isotopes by biological systems it is first necessary to establish the magnitude of fractionation taking place abiotically. In the aquatic ecosystem copper (II) compounds and soluble aqueous complexes control the proportion of bioavailable copper.

The copper phase malachite ($Cu(OH)_2CO_3$) is a common mixed ligand solid found under surficial conditions and its solubility is an important factor controlling the copper concentration in solution. Systematic experiments have been performed to determine the fractionation of copper isotopes during proton-promoted dissolution of malachite under controlled conditions. Batch reactions were set up with powdered malachite and $HClO_4$ at pH 4 and allowed to proceed at a constant temperature of 25°C for 10 minutes, 1 hour, 24 hours and 72 hours. For each experiment dissolved copper samples were taken and filtered to remove any particulates and the remaining solid malachite was collected and dried. Both were analysed by multi-collector ICP-MS for their isotopic ratio ($^{65}Cu/^{63}Cu$) and the fractionation factor $\Delta^{65}Cu$ ($Cu(II)_{(aq)}$-$Cu_{malachite}$). There was negligible fractionation between the dissolved and solid phases across all time periods, yielding an overall $\Delta^{65}Cu$ ($Cu(II)_{(aq)}$-$Cu_{malachite}$) = -0.03 ± 0.12 (1 SD, n=16, 4 replicates for each time period).The constancy of the isotope ratios indicates equilibrium conditions have been reached within the first 10 minutes and no kinetic fractionation is observed. Additional experiments were run for 24 hours at 4°C and 50°C, and the temperature was found to have no effect on the fractionation factors within experimental error. These findings are a fundamental part of this ongoing study to establish the stable isotope behaviour of copper during processes involving organic components.

The use of stable isotopes in supplementary feeding experiments

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One of the difficulties with supplementary feeding experiments is assessing the use of supplementary food by individuals, especially in large-scale experiments with multiple food sources. Traditional approaches such as feeder observation are time consuming and and other methods such as gut and faecal analysis also present problems. A possible solution to this is to estimate the relative use of supplementary versus natural food resources via stable isotope analysis. This method has the benefit that it directly estimates resource use and can provide information over different temporal periods.

This poster presents results of how labels can be added to commonly fed supplemental food items to allow them to become isotopically distinct from natural food items. Stable isotope analysis can then be used to estimate the use of supplemental versus natural food in the diet of individual animals.

We are currently using this technique in two large-scale field projects assessing the impacts of supplemental food on the breeding success of blue tits and great tits. Blood, feather and claw samples will be taken from birds fed on supplemental food items. The results will provide some indication of reliance on supplemental foods and the subsequent impact this has on future survival and breeding success.

Variation in oxygen isotope fractionation during cellulose synthesis: molecular and biosynthetic effects

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The oxygen isotopic composition of plant cellulose is commonly used for the interpretations of climate, ecophysiology and dendrochronology in both modern and palaeo-environments. Further applications of this analytical tool will depend on our in-depth knowledge of the isotopic fractionations associated with the biochemical pathways leading to cellulose. Here we test two important assumptions regarding isotopic effects due to the location of the oxygen in the carbohydrate moiety and the biosynthetic pathway towards cellulose synthesis. We show that the oxygen isotopic fractionation of the oxygen attached to carbon 2 of the glucose moieties differs from the average fractionation for the oxygen attached to carbon 3-6 from cellulose synthesized by seedlings of two different species (*Triticum aestivum* L. and *Ricinus communis* L.) by at least 9‰. Our observation that the fractionation for the respective oxygen in cellulose synthesized by the *Triticum* seedlings, which have starch as their primary carbon source, is different than the corresponding fractionations in *Ricinus* seedlings, within which lipids are the primary carbon source, shows that the biosynthetic pathway toward cellulose affects oxygen isotope partitioning. Our findings may explain the species dependent variability in the overall oxygen isotope fractionation during cellulose synthesis.

Effects of formalin and ethanol preservation on the $\delta^{18}O$ signatures of brook charr and Atlantic salmon otoliths

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The use of otolith chemistry as a tool for inferring the ecology of fishes implicitly assumes that handling and preservation procedures do not alter the isotopic composition of the otolith. Storage and handing is known to affect measured metal concentrations. No similar information is available on possible $\delta^{18}O$ isotope alterations arising from standard preservation procedures. To test for potential preservation effects, sagittal otoliths from 30 brook charr (*Salvelinus fontinalis*), and Atlantic salmon smolts (*Salmo salar*) were removed and treated in replicate treatment and control experiments. For each specimen a single otolith was removed as a control and analysed for $\delta^{18}O$. The remaining otolith was left in the cranial cavity of the preserved fish. Specimens were randomly assigned to one of six preservation media-temperature storage treatments for 120 days (ethanol: hot, ambient, and cold; formalin: hot, ambient, and cold), after which the remaining otolith was removed, analysed for $\delta^{18}O$ and compared to control values using a 3-way (temperature, species, preservative) ANOVA. Of the seven possible single factor and interaction effects, only the species and preservative-species interaction effects were significant ($P<0.05$). Results suggest that for a given species, differentially preserved otoliths (temperature, preservative) may be used in comparative analyses, but that comparisons between species may not be made even when samples are commonly stored. Possible causes for observed species differences are discussed in relation to the physical properties of the otolith (e.g., porosity and density) that may be related to differences in ration (low versus high) that hold consequences for growth rate. The interpretation of causes further suggests caution in regard to intra- and inter-population comparison of differentially preserved fish, specifically where data are used to make inferences about individual thermal histories.

An Automated Cryo-focusing Approach for Sulfur Isotope Analysis of Organic and Other Low-level Sulfur Materials

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Sulfur (S) isotope analysis has not received the same level of interest in ecological investigations as traditional carbon (C) and nitrogen (N) isotope approaches. One reason for this is the low concentration of S in most organic materials, which often presents difficulties related to sample mass requirements, the scale of the question (true replicates versus composite samples), and subsequent limitations to interpretation. We have re-visited the idea of cryo-focusing SO_2 and present an automated version of the technique with hopes of increasing the accuracy, flexibility, and potential interest in S isotope analysis. The analytical set-up consists of an elemental analyzer (EA) and isotope ratio mass spectrometer operating in continuous flow mode, however, a Thermo GasBench II is interfaced in lieu of the traditional ConFlo III and acts as an automated sample gas handling device. Samples are combusted in the EA using operating conditions normal for S isotope analysis, but the sample stream is diverted through a metering valve and subsequently through the six-port valve in the GasBench II that is connected to an automated pass through cold trap. This step allows venting of nearly all of the N_2 and CO_2 sample gases, after which the cold trap is immersed in liquid nitrogen for condensation of sample SO_2 gas. Following trapping, the six-port valve is actuated and the cold trap is raised from liquid nitrogen and allowed to thaw. Dry helium sweeps the sample SO_2 through a capillary GC column and open split in the GasBench II, and on to the ion source. The system is completely automated, with gas handling and cyro-focusing controlled by the mass spectrometer software. Cyro-focusing has enabled us to analyze organics and other matrices with S concentrations as low as 100 ppm, representing a reduction in sample size by nearly two orders of magnitude. Precision varies with sample matrix, but under most circumstances is comparable (±0.3‰) to traditional continuous flow analyses of sulfates and sulfides. Accuracy is achieved by two-point calibration using internationally distributed standards with accepted values relative to V-CDT. A benefit of reduced sample size is reduction in the amount of water formed *in situ* during the combustion process. Further, venting of matrix gases enhances the purity of sample SO_2 and mitigates a major problem that plagues normal continuous flow analysis. We provide examples of S isotope data from a wide array of sample matrices that have been analyzed using this technique, including individual aquatic invertebrates, carbonate-associated sulfate in ostracod tests, collagen extracts from bone and teeth, ungulate blood, and tropical soils.

Experimental methods for the extraction and stable isotopic analysis of sub-microlitre quantities of water from leaf samples

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A number of methods have been published for analysing the stable isotopic composition of water, including water in leaf samples. These include direct distillation of water from the leaf and mass balances between dry material and total leaf (as was practiced by Kim Gan in this laboratory). In this study we want to handle the smallest quantities of leaf with the least possible amount of labour.

Several methods (including that of Kim Gan and co-workers) attempt to flush ambient vapour from the sample tube before sealing in a leaf fragment. This may not be necessary. 1 m^3 of air at 25°C contains about 25 g of moisture when saturated. A 6 mm O.D. (3 mm I.D.) glass tube 80 mm long has a volume of 5.7×10^{-7} m^3 = ((25*5.7×10^{-7})/18)/2 = 4.0×10^{-7} moles water at 50% relative humidity. 1 mm^3 water (1 microlitre) = .001 g / 18 = 5.6×10^{-5} moles water. The ratio of leaf water to ambient vapour is 140:1. Clearly even a reasonable estimate of ambient vapour composition will result in a very small error. In fact the error may be smaller than damage to the leaf water composition that might result from flushing it with a dry gas. We will also collect vapour and analyse it isotopically.

We wish to analyse both water isotope systems: hydrogen and oxygen. Two options are available for water oxygen – direct pyrolysis of the water over carbon or equilibration with CO_2 and analysis of that gas. Our preferred method for hydrogen isotopes is to inject the water directly onto a hot chromium column and reduce it to produce pure H_2.

We will cryogenically distil water from leaf samples using a deep freeze. The sealed tubes containing the leaves and air have one end placed in a heated and insulated aluminium block, thermostatically controlled to 5°C, while the other end of the tube projects into a freezer at -15°C. Over several days the vapour pressure differences between the two ends of the tube result in all the water being extracted from the leaf in the heated end and frozen into the cold end. This method (with a longer distillation period) appears to work equally well for such troublesome items as thick stems. Once the distillation is complete the tube is cracked in two and the ice (now melted) is micro-pipetted into small, lined vials suitable for an auto-injector. The AS 800 autosamplers that we use can then inject the water directly into pyrolysis columns (carbon, 1400°C) or onto chromium (1050°C) in a helium carrier. The resulting gases are separated by a GC and analysed on either a GV Instruments Isoprime (H_2 or CO) or a Micromass Isochrom (CO only). Typically we perform multiple injections of water for oxygen isotope analysis (like many laboratories) to compensate for memory effects and improve precision. If the volumes of water are extremely small there may not be enough for multiple injections, at about 0.7 μl per analysis. For this reason it may be preferable to equilibrate CO_2 with the leaf water in the tube and analyse the CO_2 directly, avoiding memory effects. At present we propose to place the leaf sample in a 6 mm borosilicate tube rapidly, then quickly flush CO_2 into the tube and heat seal it. The tube will then be placed in a constant-temperature enclosure and left to equilibrate for several days – the reaction being accelerated by the plant's own carbonic anhydrase. After equilibration the glass tube will be placed in a flexible plastic tube in-line with the GC and mass spectrometer, and cracked. The gas will be dried and passed with the He carrier gas through a GC which will separate the CO_2 from the other components and carry it to the mass spectrometer for analysis.

Evaluating ^{15}N and ^{13}C isotope ratio analysis to investigate diet choice in wireworms (Coleoptera: Elateridae)

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Wireworms are the larvae of click beetles (Coleoptera: Elateridae) and especially those found within the genus *Agriotes* are known in temperate climates as severe pests of maize, potatoes, and other crops. Previous investigations have shown that these wireworms may feed on weeds and soil organic matter as well. Unfortunately, assessing wireworms' dietary choice under field conditions is not a simple task as elaterid larvae are fluid feeders, leaving no microscopically discernible food fragments to be found in gut dissection. Stable isotope analysis offers a promising way to track wireworms' dietary choices under field conditions. However, to guide us how stable isotope data gathered in the field should be interpreted, experiments analysing how wireworms' specific life history traits affect their stable isotope signatures are needed.
Here we evaluated experimentally how ^{13}C and ^{15}N isotopes at natural abundances can be used to study the diet of wireworms within the genus *Agriotes*. Using larvae reared from egg to the sixth instar under standardized conditions we (i) tested whether isotopic signatures differ among body segments and exuvia, (ii) determined the trophic shift and its variability between the *Agriotes* larvae and their plant diet, (iii) evaluated the consequences of the trophic shift's variability for determining larvaes' diet by a resampling-analysis (iv) investigated the wireworms' response in stable isotope signatures to extended periods of starvation, (v) investigated the shift in wireworms' ^{13}C and ^{15}N signatures following a diet switch, and (vi) tested the approach to use isotopic signatures of specific compounds to resolve the temporal history of dietary intake.
We found that mean $\delta^{13}C$ signatures decreased significantly from head to the ninth abdominal segment. The wireworms' exuvia was not significant different in its isotopic content compared to the wireworm itself, providing a means to continuously record a larva's feeding history in a non-invasive way. A species-specific trophic shift was found for *Agriotes obscurus* (1.62±0.24‰ SE) and *A. sputator* (1.08±0.27‰ SE) larvae in $\delta^{15}N$, but no such shift occurred in $\delta^{13}C$. These findings highlight that the use of "mean enrichment estimates" for disentangling trophic relationships is restricted and should not be applied without preliminary tests to the predator-prey combination under investigation. Considering the species' mean trophic shift, our resampling-analysis shows that a minimum sample size of three and four individuals in *A. obscurus* and *A. sputator*, respectively, is needed to reduce the risk of a false-positive assignment to two trophic levels below α=5%. Interestingly, the $\delta^{13}C$ and $\delta^{15}N$ signatures of *A. obscurus* larvae which were starved for up to 128 days did not change compared to wheat-fed ones. The wireworms' $\delta^{13}C$ values responded within two weeks after they were switched from a wheat to a maize diet, the latter being enriched in ^{13}C. By analysing the whole-body, the lipid compound, and the remaining fat-free body of these larvae, we found that the $\delta^{13}C$ signal of the new diet was reflected more slowly in the fat-free body compared to the lipid compound and the whole body. This offers a way to separate wireworms eating a mixed diet from those that have shifted diets previously.

Subarctic Marijuana 'Migration': A multi stable isotope (C,N,O and H) forensic study of Alaskan marijuana

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A large proportion of Alaska (AK) Bureau of Drug and Alcohol Enforcement officers' time has in the past been spent controlling the production and distribution of marijuana. Marijuana in AK can originate from within the state (e.g. Fairbanks and the Matsu Valley) and from a number of areas outside the state (e.g. Latin America, Canada and the lower 48 states of the U.S.A.). Although Latin America has been reported to supply a large proportion of the marijuana in the lower 48 states of the U.S.A. the proportions from different potential geographic areas that supply more remote areas of the globe, such as Fairbanks, AK, are not well known. This is primarily because marijuana confiscated from individuals cannot often simply be traced back to the source from which it was originally grown. We are developing a forensic method (Drug Enforcement Agency license number RW0324551) by analyzing multiple stable isotopes (C,N,O and H) preserved in marijuana samples, confiscated in Fairbanks, AK and supplied to us by the University of Alaska Fairbanks (UAF) Police Department, to identify the likely geographic source from which the marijuana originated. To date, the stable oxygen and hydrogen isotope composition ($\delta^{18}O$ and δD vs. V-SMOW) of 36 marijuana plant samples have been found to range from 10.0‰ to 27.6‰ and -197.1‰ to -134.9‰ respectively. The large range of data suggests that the samples originated from multiple sources ranging from low to high latitudes. A large range in $\delta^{15}N$ values from the samples was also evident (-7.0‰ to 14.8‰). Most intriguing of all was the unexpected large range in the stable carbon isotope compositions ($\delta^{13}C$) of the samples (-62.2‰ to -24.4‰). Twelve of the 36 samples were found to have an exceedingly low $\delta^{13}C$ (-36.1‰ to -62.2‰) compared to the typical $\delta^{13}C$ of other plants using C_3 photosynthesis. Interior growing conditions (e.g. hydroponic and green house) and a variety of CO_2 sources (e.g. CO_2 from tanks and fermentation CO_2 generators) supplied to growing marijuana (in addition to atmospheric CO_2) that are sometimes used to improve marijuana yields may account for these exceptionally low $\delta^{13}C$ values. Our project has implications for the development of state of the art forensic tools for application in more remote areas such as AK, where the resources associated with law enforcement have to be allocated over a wide geographic area.

Broad ranges in natural variation of stable isotopic compositions veil simplistic migratory system

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Stable isotope ratio measurements have long been used in studies of trophic ecology and researchers are increasingly using them to infer seasonal linkages in studies of migratory animals. Most of these studies have relied on assumptions that have not been adequately tested under field conditions. For example, most studies of migratory birds assume little or no variation in δ-values associated with feathers grown at the same location, yet very few such studies measure values for feathers of known origin (e.g. freshly moulted) to anchor their inferences. Although much more work has been done to probe the assumptions associated with studies of trophic ecology, many empirical studies likewise assume little or no variation in the values of the dietary items (or trophic levels) used for tissue synthesis.

We took advantage of a unique moult-migration scenario to explore the variation present in isotope values for carbon, nitrogen, hydrogen, and oxygen measured in feathers and food items of a migratory bird under non-manipulated conditions. Eared grebes (*Podiceps nigricollis*) migrate to the Great Salt Lake in Utah, USA each fall where they moult flight feathers and add needed fat before flying further south to spend the winter. During this non-sequential moult, the birds are flightless, using the lake as protection against predation. The birds consume primarily a single food resource during this period. Therefore, the feathers are presumably derived from resources that are extremely localized in terms of both geographic and trophic structure. Because of the unique constraints on this system, we suspected our results would define a realistic "low end" of the range in variation from a natural system.

We measured δ-values for stable isotopes of carbon, nitrogen, hydrogen, and oxygen in freshly grown feathers collected from grebes at the Great Salt Lake in January, 2005. Feathers ranged from -163 to -29 for δ^2H, and from -6 to 14 for δ^{18}O (n=70). δ^{13}C ranged from -28 to -12 and δ^{15}N ranged from 7 to 18 for feathers (n=67). We compared these data with δ-values for dietary items collected on site at the same time. We relate feather values to diet values and both feather and diet values to δ^2H and δ^{18}O values measured for 1/local surface water, 2/values predicted from the meteoric water line, and for 3/values predicted from spatially-smoothed, temporally averaged precipitation data. Based on these comparisons, we discuss possible explanations for the observed variation, and identify future directions that would contribute to a more mechanistic understanding. Our findings contain important implications for both food web and migration studies that use natural variations in isotope compositions.

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