

POLISH JOURNAL OF ECOLOGY (Pol. J. Ecol.)	54	4	563–584	2006
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State-of-the-art review

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THE USE OF STABLE ISOTOPE ANALYSES IN FRESHWATER ECOLOGY: CURRENT AWARENESS

ABSTRACT: Ecological research using stable isotopes has progressed rapidly during the last 20 years and although more studies are including the addition of isotopically labelled compounds at tracer levels, the overwhelming majority rely on measurements of natural abundance ratios. Access to isotope ratio mass spectrometry has increased, spurred on by awareness of the techniques and increasing demand, and consequently cost of sample analysis has dropped. Today stable isotopes of carbon ($^{13}\text{C}/^{12}\text{C}$), nitrogen ($^{15}\text{N}/^{14}\text{N}$), sulphur ($^{34}\text{S}/^{32}\text{S}$), oxygen ($^{18}\text{O}/^{16}\text{O}$), and hydrogen ($^2\text{H}/^1\text{H}$) can be determined routinely. Perhaps one of the most appealing attributes of isotopic signatures is their potential use to find patterns and determine mechanisms across a range of scales from the molecular level through to characterising whole food webs, reconstructing palaeoenvironments, tracing nutrient fluxes between ecosystems and identifying subsidies, or migrations of organisms.

Ecologists from every discipline who are unlikely to have been trained as isotope chemists have added stable isotope analysis (SIA) to their “toolbox”, but often increasing use leads to increasing abuse. The usefulness of SIA arises from predictable physical and enzymatic-based discrimination between biological and non-biological materials leading to different isotopic compositions. Without some ecological understanding of these, interpretation of isotope-derived data can often be flawed. Here, I explore how SIA re-

cently has been used for research in aquatic ecology, reviewing how some of these techniques have allowed elucidation of key processes in aquatic systems such as the contribution of allochthony to lake food webs, and discuss the “state of the art”. Included are some thoughts on where our knowledge in aquatic ecology remains deficient and how continued development and future application of SIA and interdisciplinary methodologies may be applied.

KEY WORDS: baselines, carbon, food webs, fractionation, lipids, nitrogen, trophic levels

1. INTRODUCTION

Interest in the application of stable isotope techniques to answer ecological questions in freshwater sciences has increased dramatically over the last few decades. Many ecologists now realise the power of such tools, especially when not viewed as a panacea, but when used to complement more conventional techniques. Research interest has been driven in part by rapid advances in the technology and perhaps by better communication between the manufacturers of the hardware and the biologist end users, culminating in much greater accessibility and affordability. There is a comprehensive

list of laboratories from around the world offering stable isotope measurements at commercial rates; these can be found at the ISOGEOCHEM website (<http://www.isogeochem.com>) which also informs visitors of stable isotope suppliers, and has an email discussion group which acts as a forum for scientists from all disciplines interested in the use of stable isotopes. Within the UK for example, the Natural Environment Research Council maintains three Stable Isotope Facilities providing organic and light stable isotope ratio mass spectrometry to the UK life sciences community. Each facility offers a different portfolio of analytical techniques and focuses on differing applications: the Organic Mass Spectrometry Facility at the University of Bristol; the Life Sciences Community Stable Isotope Facility located within the Scottish Universities, Environmental Research Centre at East Kilbride; and the ^{15}N Stable Isotope Facility located within the Centre for Ecology and Hydrology (CEH) at Lancaster University. Increasing interest and associated use has led to the costs of mass spectrometry systems becoming more accessible for individual research institutes and university departments to afford. In 1998, the first in a series of bi-annual international conferences entitled “Applications of Stable Isotope Techniques to Ecological Studies (ISOECOL)” was hosted in Saskatoon, Canada, and one of the five themed sessions was dedicated to aquatic ecosystems (Hobson and Wassenaar 1999). Since then, the number of attendees at these particular meetings has burgeoned and aquatic-related research still holds a prominent status (~45% of the oral presentations in 2004 hosted in Wellington, New Zealand). Individual countries have been hosting smaller, annual conferences and workshops for a similar number of years, such as the Stable Isotope Mass Spectrometer Users Group (SIMSUG) within the UK. The more intimate nature of these smaller conferences allows for useful cross-fertilisation of research ideas between diverse disciplines such as quality control, forensics, veterinary physiology and aquatic ecology, and between representatives of the research and development teams of the mass spectrometer manufacturers and the end user scientists. The increasing number of re-

search papers published in peer review journals incorporating some aspect of stable isotope work reflects the perceived usefulness of stable isotope measurements to freshwater scientists (Fig. 1).

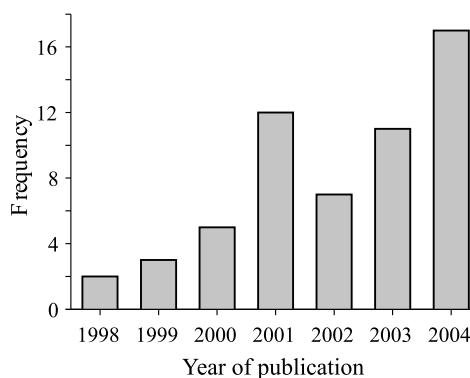


Fig. 1. The number of papers incorporating some aspect of stable isotope analysis recently published in the journal *Freshwater Biology*.

Perhaps part of the intrinsic appeal of stable isotope techniques, not only for freshwater scientists, lies in the range of scales over which they can be applied. These span from the microscopic to the global: investigating the microbial mitigation of methane (Conrad 2005) or the nutrition and physiology of benthic and pelagic meiofauna (Carman and Fry 2002, Pel *et al.* 2004); examining the spatial scales of carbon flow in river food webs (Finlay *et al.* 2002, Covich 2006) or from benthic, littoral and pelagic zones within lakes (France 1995, Adams *et al.* 2003) to invertebrate and vertebrate consumers; using isotopic additions to “label” individual insect imagos and trace dispersal between metapopulations (Hershey *et al.* 1993, 2006, Briers *et al.* 2004) or analysing strontium isotopes in anadromous fish otoliths to characterise natal origin at the continental scale (Kennedy *et al.* 2000); to using global patterns in deuterium isotopes in precipitation for wildlife forensic use (Bowen *et al.* 2005). Applications have moved on from simply descriptive studies of the food web of individual water bodies. Through the use of stable isotopes we now have a better understanding of the role of allochthonous terrestrial mate-

rial as an energy subsidy to rivers (Clapcott and Bunn 2003, McCallister *et al.* 2004) and lakes (Grey *et al.* 2001, Pulido-Villena *et al.* 2005) and conversely, the subsidy of terrestrial predators by aquatic-derived prey (Collier *et al.* 2002, Paetzold *et al.* 2005); whether top-down, or bottom-up processes dominate in grassland and forested streams (Nystrom *et al.* 2003) or lakes (Bertolo *et al.* 2005); and how anthropogenic-derived nitrogen deposition may induce biological and biogeochemical changes in relatively pristine ecosystems (Jones *et al.* 2004). The overwhelming majority of studies still utilise natural abundance ratios, most commonly of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$), but increasingly of hydrogen (δD), oxygen ($\delta^{18}\text{O}$) and sulphur ($\delta^{34}\text{S}$). However, in recent years there have been plenty of excellent examples of isotope addition tracer studies which have furthered considerably our knowledge of ecosystem structure and function (e.g. Hershey *et al.* 1993, Kling 1994, Sanzone *et al.* 2002, Pace *et al.* 2004). Furthermore, compound specific techniques allowing the isotopic analysis of individual lipids, amino acids, carbohydrate sugars and hydrocarbons for example, are also becoming more widely applied. The facilities for such analyses are still relatively uncommon and analytical costs currently may be prohibitive for many scientists; but this is a repeat of the bulk stable isotope situation 25 years ago and hopefully will change in the near future. The purpose of this review is not to give an overview of the isotope terminology, principles and applications to freshwater ecology; readers are referred to the numerous excellent review papers and books aimed at ecologists in general for this (e.g. Peterson and Fry 1987, Coleman and Fry 1991, Lajtha and Michener 1994). Rather, it aims to summarise the “state of the art” for freshwater ecologists while highlighting that with increasing use of a technique may come increasing abuse; to collate some of the stable isotope studies which have particularly advanced understanding in freshwater ecology; and finally considers some areas where our fundamental knowledge is still lacking, but which stable isotope applications may help to address.

2. “HUMBLE” TROPHIC ECOLOGY

Arguably, the most common application of stable carbon and nitrogen isotope measurements is to establish a trophic linkage between a consumer and its diet. Stable isotopes can be used to trace the fates of different energy or nutrient sources provided those sources exhibit distinct and robust isotopic signatures, and the isotopic signatures are transferred conservatively or are changed predictably as they move between trophic levels.

This is particularly relevant to aquatic ecologists, where more often than not, the aquatic environment prevents direct observation of what prey items are actually ingested, and/or the organisms involved are often microscopic and conventional gut content analysis is simply impossible. Even when the organisms of study are sufficiently large upon which to perform gut content analysis, stable isotope measurements may provide more information because the gut is often empty (e.g. large piscivores such as trout, Grey *et al.* 2002, Clarke *et al.* 2005), or the prey is too digested (gastric mill of crayfish, Whitley and Rabeni 1997), or indeed liquefied (e.g. suctorial predators, McCutchan *et al.* 2003) to permit identification. Further disadvantages of gut content analyses often listed in stable isotope studies include that procuring gut contents is often destructive to the organism, that gut contents only indicate what has actually been ingested rather than assimilated and that they only provide a recent “snapshot” of the diet depending upon the rate of digestion (Gearing 1991). However, it can be argued that procuring samples for stable isotope analyses is also destructive in many cases; the tissue of choice for large organisms such as fish is still muscle – Pinnegar and Polunin 1999, although some recent studies have advocated use of substitutes such as scales (Perga and Gerdeaux 2003), adipose tissue (McCarthy and Waldron 2000, Dempson and Power 2004), or fin clips (Jardine *et al.* 2005).

More importantly, the most lauded property of a stable isotope signature is that it integrates the dietary signature, providing a time-integrated indication of diet. However, therein lies a problem because the least un-

derstood aspects of isotope physiology are those governing the rate of turnover and hence expression in animal tissues, especially in natural systems, and we are forced to rely heavily upon assumptions, some of which are often conveniently overlooked. Gannes *et al.* (1997) stressed the importance of supplementing field with laboratory studies to further validate the assumptions associated with reconstructing diets, trophic relationships, and body condition. Almost 10 years later, there is still a paucity of results from laboratory stable isotope studies reaching publication, although such data regularly are included in conference presentations (but see Goedkoop *et al.* 2006). Perhaps this is in part due to the fact that authors often attempt to publish the laboratory work as a separate paper relative to their field studies; a counterproductive strategy as it is rare that such papers contribute to the broader understanding of our subject, appearing too focussed on one particular waterbody or species/taxon when they are separated from the context of their related field study.

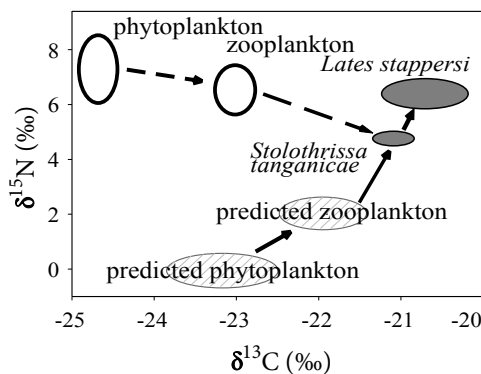


Fig. 2. Stable isotope bi-plot of a simplified Lake Tanganyika pelagic food web. Solid arrows link dominant species of the pelagic food chain and show “classical” stepwise ^{13}C - and ^{15}N -enrichment stemming from predicted phytoplankton and zooplankton isotope values to planktivorous (*Stolothrissa tanganyicae*) and piscivorous (*Lates stappersi*) fish. Actual phytoplankton and zooplankton isotope values link to fish via the dashed arrows and reflect quicker assimilation of a distinct nitrogen isotope signature immediately prior to the specific sampling event. Redrawn from O’Reilly *et al.* (2002).

A standardised isotopic $^{15}\text{N}/^{13}\text{C}$ bi-plot may portray a “complete” food web but if the individual biological components (for example ranging from phytoplankton to large piscivorous fish with their associated differences in isotopic turnover rates) used to construct the relationships were only collected at one time period without any idea of temporal variability within that particular system, then interpretation of the data representing trophic linkages is extremely limited and potentially misleading (see Fig. 2 based upon the following example). The importance of time averaging of isotopic signatures across different trophic levels to the interpretation of food webs derived from isotope data was exemplified by a study at Lake Tanganyika by O’Reilly *et al.* (2002). Lake Tanganyika has a rather simplified pelagic food web stemming from phytoplankton to a zooplankton community dominated by one copepod species. The copepods are preyed upon by the numerically dominant *Stolothrissa tanganyicae* Regan, which in turn forms the prey base for *Lates stappersi* Boulenger. Thus, the rather linear food chain should have been clearly represented by classical stepwise ^{13}C - and ^{15}N -enrichment between trophic levels. O’Reilly *et al.* (2002) actually recorded that phytoplankton exhibited the highest $\delta^{15}\text{N}$, followed by zooplankton. They attributed the apparently highest trophic position being occupied by phytoplankton to assimilation from a very recent upwelling event introducing ^{15}N -enriched nitrogen to the epilimnetic waters. Such a ^{15}N -enriched signal rapidly had become assimilated into the primary producers and to a certain extent, primary consumers but had not yet been assimilated into the muscle of secondary consumers. In fact, as this example shows, consumers are rarely in isotopic equilibrium with their food sources, so such instantaneous comparisons between consumer and diet $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ provide limited information which must be interpreted carefully regarding trophic variations, even if the dietary trophic position is known.

The following three recent papers have critically evaluated temporal variation in isotope signatures for different aquatic organisms. Matthews and Mazumder (2005a, 2005b) addressed various aspects of temporal

variation in stable isotope values measured from zooplankton. Firstly by examining zooplankton elemental composition in relation to zooplankton $\delta^{13}\text{C}$ and to the putative prey (POM) $\delta^{13}\text{C}$, they showed that body C:N composition (which they postulated reflected varying lipid storage over time) explained more temporal variation in zooplankton $\delta^{13}\text{C}$ than did variation in the prey $\delta^{13}\text{C}$ (Matthews and Mazumder 2005a). Considering the potential lipid content of the different zooplankton species which may store lipids at different times of the year appears then to be important in order to make useful assessments on reliance of different food resources, as will be seen in a later section dedicated to the “fat debate”. Matthews and Mazumder (2005b) then went on to examine the consequences of large temporal variability of zooplankton $\delta^{15}\text{N}$ for modelling higher predator trophic position and isotopic variation; they found that food source $\delta^{15}\text{N}$ variability coupled with growth rate differences between individual consumers contribute to consumer intrapopulation isotopic variation and led to correlations between consumer size and $\delta^{15}\text{N}$. From the perspective of much larger organisms such as fish, then one has to be careful as to exactly what temporal information concerning assimilated diet is stored within the isotopic signature of a tissue such as muscle (which was previously established from laboratory experimental evidence as the tissue of choice, most closely reflecting the isotope ratios found in the diet – Pinnegar and Polunin 1999). Perga and Gerdeaux (2005) questioned the validity of whether muscle reflected diet in a continuous manner in their study organism, the European whitefish (*Coregonus lavaretus* L. s. l.), because ectotherms typically exhibit rather discontinuous growth over the year in the wild; periods of somatic growth, gonadic growth and basal metabolism during which no growth occurs. Perga and Gerdeaux (2005) argued that muscle of such ectotherms would only truly reflect somatic growth, whereas liver tissue, which would be constantly involved in protein turnover, would reflect not only somatic growth but isotopic routing into gonadic growth and routine metabolism too. When comparing whitefish muscle and liver $\delta^{15}\text{N}$ to zooplankton prey $\delta^{15}\text{N}$ over a 20 month

period, the amplitude of liver isotope variation was much greater than that of muscle, and more closely tracked that of the available prey suggesting that muscle isotope values indeed only reflected the somatic growth period of seven months in that particular lake; and since the somatic growth period for fish varies with latitude (e.g. Conover 1990) it is important to consider just how much temporal dietary information is recorded in, for example, perch muscle tissue collected from a lake in northern Sweden compared to perch from a lake in southern England. I believe we should see more aquatic studies using comparisons of different tissues with differing turnover rates (where organism size allows) as advocated by Hobson (1999). Furthermore, as Perga and Gerdeaux (2005) highlight, the majority of controlled tissue turnover experiments have been conducted using endotherms (although there are a number of good fish examples e.g. Hesslein *et al.* 1993, Sweeting *et al.* 2005). Those studies that have been conducted tend to control for food supply and temperature and hence result in constant fish growth from which it is difficult to extrapolate to field conditions, and is therefore one avenue for future research.

3. IMPORTANT FACTORS TO CONSIDER WHEN DESIGNING A STABLE ISOTOPE STUDY OR WHEN INTERPRETING ISOTOPE-DERIVED DATA

3.1. Trophic fractionation

An important assumption for many stable isotope studies is the “predictability” of the transfer of an isotopic signature from one trophic level to another and if the trophic fractionation is not well quantified, then the reliability of stable isotopes to trace trophic pathways (sources, trophic levels) is compromised. For carbon and nitrogen isotopes, commonly assigned and widely discussed mean trophic fractionation factors are +0–1‰ and +3‰ respectively, drawn from the early laboratory studies of DeNiro and Epstein (1978, 1981) and Minagawa and Wada (1984). Although these values were originally derived from studies incorporating many different taxa from different environ-

ments, several recent reviews of this particular subject have highlighted that even after ca. 25 years further study and collection of new data, they have proven remarkably robust and are generally representative for aquatic consumers (e.g. Post 2002, McCutchan *et al.* 2003, Vanderklift and Ponsard 2003). What these latter studies do indicate however is the potential range in trophic fractionation factors. McCutchan *et al.* (2003) concluded that much of the variation in apparent trophic fractionation for C, N and S could be attributed to differences in diet or the method of sample preparation and advised that estimates used in field studies should reflect not only what is known about trophic fractionation on particular diets among the consumers in question but also how such estimates are affected by preparation methods for subsequent stable isotope analysis. For example, the mean trophic fractionation of N varied depending upon whether consumers were maintained upon an invertebrate diet (1.4‰), a plant-derived diet (2.2‰) or a high protein diet (3.3‰); and was higher for C when consumers were analysed as muscle (1.3‰) compared to when the whole organism was analysed (0.3‰). McCutchan *et al.* (2003) highlight the fact that currently there are very few estimates of trophic fractionation for several important groups of consumers such as freshwater invertebrates.

It is important then to acknowledge potential variation in trophic fractionation because it is key to successful interpretation of consumer dietary source and trophic level, and the isotopic mixing models used for estimating dietary contributions are especially sensitive to the parameters used (see later section on mixing models). There are not only interspecific differences in trophic fractionation, but intraspecific differences as well. Adams and Sterner (2000) provided a timely and detailed analysis of how diet stoichiometry can impact upon apparent trophic fractionation using *Daphnia* and directly manipulating the elemental composition of their *Scenedesmus* prey. *Daphnia* fed *Scenedesmus* with a high C:N ratio, i.e. a low quality/protein diet, exhibited greater apparent trophic fractionation which they attributed to internal recycling of N within the consumers and which is effectively a starvation

effect. This conclusion is at odds with the findings of McCutchan *et al.* (2003) where predators typically exhibited higher trophic fractionation compared to primary consumers. Diets of predators are relatively protein rich, and thus it appears that N trophic fractionation is higher when dietary N exceeds or is below requirements for optimal growth, and the commonly assumed mean value (i.e. ~3‰) reflects consumers fed upon diets approaching an optimal for growth. From a meta-analysis, Vanderklift and Ponsard (2003) suggested that the most influential factors affecting N trophic fractionation were the biochemical form of nitrogen excretion and the nutritional status of the consumer.

Unfortunately many of the experiments designed to determine the trophic fractionation between a consumer and a diet and which contribute much of the data for analyses of Post (2002), McCutchan *et al.* (2003) and Vanderklift and Ponsard (2003) have not considered variable diet stoichiometry so it is difficult to assign that proportion of the variance observed to what may now be considered experimental design flaws. It is also now recognised that ration size, dietary lipid content and feeding rate impact directly upon the value of trophic fractionation which can be calculated, as detailed studies of carp and tilapia have shown (Focken and Becker 1998, Gaye-Siessegger *et al.* 2004a, 2004b). If one thinks back to the plea from Gannes *et al.* (1997) for more controlled feeding and dietary switching experiments with a wide range of organisms to allow for better determination of trophic fractionation and turnover rates, it would appear prudent to account for diet stoichiometry and feeding rate or ration size in such experiments.

3.2. Baseline indicators

To compare stable nitrogen isotope data among systems, especially when using $\delta^{15}\text{N}$ as an indicator of trophic level, it is important to incorporate a reference to the "isotopic baseline" from each individual system. Cabana and Rasmussen (1996) examined the $\delta^{15}\text{N}$ of filter-feeding mussels from a number of different Canadian lakes to determine the sources of nitrogen entering each system. The difference in mussel tissue $\delta^{15}\text{N}$ spanned

>10‰ among lakes, reflecting differential inputs from sewage derived nutrients assimilated by the phytoplankton upon which the mussels fed. Cabana and Rasmussen suggested that such relatively long-lived primary consumers could be substituted as a “baseline” to correct for temporally variable signatures of primary producers before calculating the trophic positions of higher consumers based upon their $\delta^{15}\text{N}$. Indeed, six years later, Post (2002) stated that one of the most difficult methodological issues facing the effective application of stable isotopes to food web studies was the assignment of an appropriate isotopic baseline. In Post (2002), he demonstrates how snails typically reflect the isotopic signature of biofilms and associated detritus forming the littoral food web base whereas filter-feeding unionid and zebra mussels reflect those of the seston at the base of the planktonic food web (cf. Cabana and Rasmussen 1996). Snails, like mussels, have well defined feeding strategies and are relatively slow growing compared to the primary producers they aim to represent, so they integrate temporal isotopic variation over a relatively long period. However, the plankton and littoral can be highly heterogeneous environments (Thackeray *et al.* 2004) and substantial small-scale spatial variability in isotope ratios can be expected in planktonic and epiphytic primary producers (MacLeod and Barton 1998, Jones *et al.* 2004). It is worth considering that if one is working on a morphologically diverse system then it will be important to gather sufficient baseline indicator organisms from diverse substrates to be representative of location. Thus, Harrod *et al.* (2005) collected >50 individual *Potamopyrgus jenkinsii* (Lumbye) from rocks, sand, sediment and vegetation to reflect the substantial environmental heterogeneity among their study sites which spanned a salinity gradient from fresh to marine.

However, the choice of baseline indicator organism ultimately depends upon the question of interest, and it is not necessarily important to use a primary consumer to integrate the inherent temporal isotopic variability of primary producers. How well a baseline indicator organism reflects the resource upon which it is reliant depends ultimately upon

the tissue turnover rate and the consequent isotopic turnover rate which is correlated with body mass (Peters 1983). This has parallels to the argument of whether stable isotope measurements provide a temporal integration compared to the “snapshot” perspective of gut content analysis. Thus, several multiple-lake food web analyses have used pelagic zooplankton to develop a baseline (Vander Zanden and Rasmussen 1999, Post 2002) or as the baseline itself (Cabana and Rasmussen 1994). Bulk zooplankton samples are only suitable if the taxa comprising the whole truly reflect the isotopic values of the primary food source because individual taxa may exhibit considerable isotopic variation (Grey *et al.* 2000, Matthews and Mazumder 2003). Better then to select specific taxa from a bulk zooplankton sample, and although zooplankton do not integrate over the same temporal scale as mussels, Matthews and Mazumder (2003) argue that a time series of *Daphnia* at a carefully chosen temporal resolution may be useful to detect fine scale seasonal patterns of, for example, anthropogenic activities on water bodies. Toward the other temporal extreme exhibited by freshwater organisms, Perga and Gerdeaux (2003, 2004) used scales of the zooplanktivorous whitefish *Coregonus lavaretus* as an integrator of pelagic carbon to compare between lakes of differing size and trophic state.

Furthermore, although organisms such as unionid bivalves appear to be the most representative “baseline indicators”, they are not available in all freshwater systems and/or are difficult to collect because of associated conservation status and so alternatives must be sought. Chironomid larvae have been used in some studies because they are assumed to filter feed on the “rain” of primary production gradually settling out from the water column above them (Vadeboncoeur *et al.* 2001), and in some lakes this is undoubtedly the case as the isotopic data may suggest (group A in Fig. 3). However, in other lakes, a number of species of chironomids have demonstrated extremely high intraspecific isotopic variability; *Chironomus plumosus* (L.) exhibited $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values varying by up to 35‰ and 15‰ respectively between individuals of the same instar, collected from

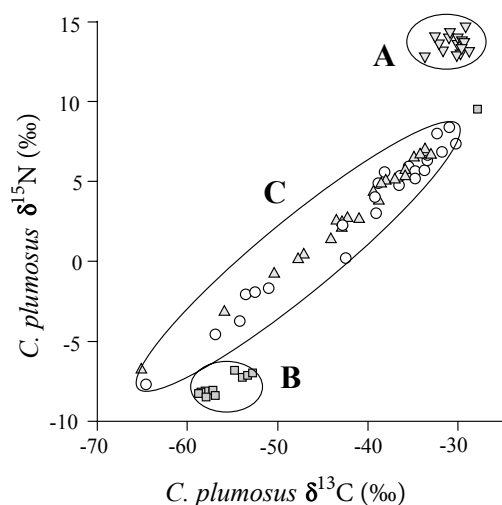


Fig. 3. Stable isotope bi-plot for individual *Chironomus plumosus* larvae collected from four lakes: A – Großer Binnensee, reflecting assimilation from phytoplankton; B – Plußsee, reflecting assimilation from methanotrophic biomass; and C – Wyresdale Park Lake and Esthwaite Water, reflecting a mixed diet and differential contributions from both methanotrophic and phytoplankton sources to individual larvae. Redrawn from Grey *et al.* (2004a).

the same depth, in the same lake, on the same date, due to differential assimilation of methanotrophic bacterial biomass (see group C, Fig. 3, redrawn from Grey *et al.* 2004a). Although the ultimate resource for methanogenesis in these typically productive lakes may be autotrophic biomass consigned to the detrital food web, the kinetic isotope effects involved in methanogenesis and methanotrophy result in the subsequent biomass generated exhibiting markedly different isotopic signatures from the substrate (Whiticar 1999). In such lakes it would be difficult to use a pooled isotope signature from the dominant chironomid species as an isotopic baseline as it would bear scant resemblance to the primary production that may be fuelling the rest of the food web. Obviously, the observed variation in chironomid $\delta^{15}\text{N}$ cannot also be viewed as representative of larvae feeding over five trophic levels! Furthermore, Grey *et al.* (2004b) have demonstrated substantial temporal variability ($>15\%$) in chironomid $\delta^{13}\text{C}$ throughout the year, which must also be accounted for before use as an effective baseline. Thus, as Post (2002) suggested, it

is critical to understand the natural history of any consumer that may be considered for use as an isotopic baseline.

3.3. Tubby or not tubby, fat is the question

A further factor to consider, not only with baseline indicator organisms such as snails, mussels and chironomids which are relatively sessile, but with all other organisms that are potentially under study, is the question of lipid accumulation or synthesis. Lipid synthesis from dietary constituents results in ^{13}C -depletion (DeNiro and Epstein 1977). It is thus important not only to consider whether components of a food web may have significant proportions of lipids in the tissues analysed and whether these could bias interpretation of trophic relationships, but also whether the organism of study actually synthesises lipids from diet (e.g. fish) or accumulates them directly from diet (e.g. zooplankton) – see Matthews and Mazumder (2005a). Accumulation or synthesis may be temporally variable in relation to reproduction or aestivation of aquatic organisms (see Fig. 4) and thus only significant at certain times of the year. Although it has long been recognised that lipid $\delta^{13}\text{C}$ may differ considerably from that of protein or carbohydrate, opinions

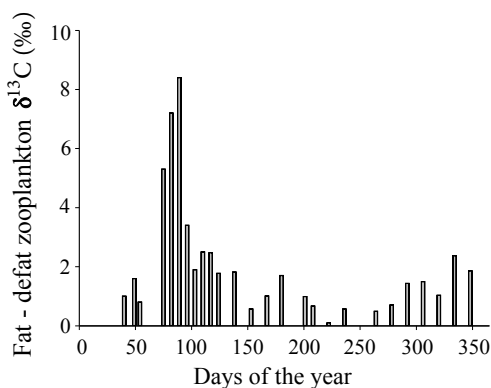


Fig. 4. Temporal variability in zooplankton lipid content represented by the difference in $\delta^{13}\text{C}$ values from untreated and lipid-free samples: zero difference between the two values indicates negligible lipid content. The lipid content of zooplankton collected in late March – early April altered the bulk isotopic signature by $>8\%$. Zooplankton was collected from Plußsee.

differ as to the best method to account for their presence and it is an area of study currently generating considerable interest (Kiljunen *et al.* 2006). Some workers advocate chemical removal of lipids (extraction) to establish a clearer picture of the relationship between an organism and its putative diet, arguing that “lipid free” tissues truly reflect assimilated carbon (Power *et al.* 2003). However, lipid extraction procedures tend to alter stable nitrogen isotope integrity (Sotiropoulos *et al.* 2004) and in these days of continuous flow isotope ratio mass spectrometry when both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ are routinely derived from one analysis, accounting for the lipid content effectively doubles analysis cost and time. Hence, several mathematical “lipid-normalisation” methods have been proposed, which are reliant upon the strong relationship between lipid content and tissue C:N ratios; that which is most often quoted is from McConnaughey and McRoy (1979). Kiljunen *et al.* (2006) extensively reviewed the studies that used the McConnaughey and McRoy approach and found very little evidence of validation of the method; despite the fact that the equations were derived from rather limited C:N and lipid content data from marine fish and crustaceans, they found that it has been applied to a wide range of organisms including freshwater invertebrates. Furthermore, Kiljunen *et al.* (2006) used empirical data from a wide range of fish and invertebrates and found the model to be less than satisfactory when compared to lipid-free isotope data, especially for invertebrates. This is likely due to the fact that invertebrates tend to be analysed whole, whereas muscle tissue is typically excised from larger organisms such as fish. The higher C:N ratios recorded from invertebrates may arise from non-lipid compounds such as chitin or glycogen, which would not be removed by chemical extraction techniques. So, it appears that “normalisation” methods can be used to account for lipid content in organisms such as fish (although Kiljunen *et al.* (2006) recommend some modifications to original models and advocate validation), but should not be used indiscriminately for invertebrates.

4. MIXING MODELS

The aforementioned factors are important to consider when applying isotope mixing models to quantitatively, as well as qualitatively, describe data. I do not wish to dwell on this matter because there are many critiques of mixing models and their application available in the recent literature (see Phillips and Koch 2002, Robbins *et al.* 2002, Phillips and Gregg 2003, Phillips *et al.* 2005). Suffice to say that these models are extremely sensitive to variation in parameter input; Phillips and Koch (2002) highlighted that the weakest link in the application of mixing models to dietary reconstruction or energy sourcing related to the use of appropriate values for trophic fractionation, which was discussed earlier. Intuitively then, in a similar manner, using lipid-corrected or non-corrected $\delta^{13}\text{C}$ data in such a model will introduce considerable variation in the output.

5. SAMPLE NUMBERS AND INTRASPECIFIC VARIABILITY

Ecologists have tended to view stable isotope analyses as convenient means of integrating diverse processes within food webs. Of course, this may be most appropriate to achieve certain goals but the reporting of inter-individual isotopic variability can actually provide valuable insights into feeding behaviour and identify hitherto unrecognised energy transfer pathways such as methanotrophy (Grey 2002). A further consequence of the increased availability and affordability of stable isotope measurements is that the number of samples analysed per study has typically increased, and instead of pooling many organisms to be analysed as one representative sample, there are now many studies incorporating intraspecific variability, if not between individuals, then at least between size classes representing potential ontogenetic transitions (e.g. Beaudoin *et al.* 1999, Grey 2001, Grey *et al.* 2004a, Harrod *et al.* 2005). Intraspecific variation in the $\delta^{15}\text{N}$ of largemouth bass juveniles closely reflected the timing of an ontogenetic shift by different individuals to piscivory, which was confirmed by concurrent studies of gut contents

and otolith microstructures by Post (2003). Increased variance within a sample set may sometimes hinder interpretation but is often of inherent interest and individual variation in diet has important implications for the study of not only ecological but also evolutionary interactions, ethology and population dynamics. Two recent studies have critically evaluated the use of variance in stable isotope data. In a theoretical paper, Bearhop *et al.* (2004) proposed the use of variance of stable isotope ratios as an alternative method for the assessment of trophic niche width when combined with conventional approaches such as gut content analyses because the use of isotope data overcomes the problems commonly associated with other indices of niche dimensions. These include failure to integrate diet over ecologically significant time-scales; lack of a single scale allowing for comparisons between populations or species; and difficulty in combining dietary prey diversity and evenness in an ecologically meaningful way. Bearhop *et al.* (2004) further suggest that their approach is best suited to relatively closed systems such as islands or freshwater lakes and may provide substantial insight into the underlying processes of impacts made by alien species introductions (see later section and Gheradhi 2006) but they are keen to stress that the potentially confounding effects of organismal physiology should be considered when applying their approach. Individual dietary specialisation was the focus of a study by Matthews and Mazumder (2004) driven by a model based upon dietary composition. Matthews and Mazumder (2004) suggest that stable isotope ratios can only be used for detecting individual specialisation once variability due to age, sex and ecomorph has been accounted for. Intrapopulation isotopic variability of consumers (at least in $\delta^{13}\text{C}$ used in their study) was dependent upon the isotopic range and distribution of food sources in the study system.

6. SUBSIDIES, THE ROLE OF ALLOCHTHONOUS MATERIAL, AND ISOTOPE ADDITIONS

Stable isotopes as natural or augmented tracers have certainly furthered our under-

standing of the important role of terrestrially derived material to lake food webs. The impetus for this research probably stems from studies of the dark, humic-stained waters of the temperate boreal zone where light penetration and hence autotrophic production is limited (Jones 1992, Meili 1992). However, all lakes potentially can be classified as humic, receiving some degree of subsidy from the surrounding catchment (Jones 2005). Direct evidence for the importance of allochthonous production remained elusive until it was realised that terrestrial organic matter would be likely to exhibit a different basal resource isotope signature compared to those found in many aquatic systems. In a series of studies of the pelagic food web of Loch Ness, it was first demonstrated that by careful separation of the autochthonous production (predominantly diatoms) from the particulate organic matter dominated by allochthonous material and subsequent stable isotope analysis of each fraction, that the pelagic zooplankton biomass comprised approximately 50% allochthonous carbon (Jones *et al.* 1998). Then Grey *et al.* (2001) went on to demonstrate that although reliance upon allochthonous resources was highly seasonal and species-dependent, nearly 40% of bulk zooplankton biomass was subsidised from the catchment on an annual basis. Furthermore, the influence of allochthonous material could be traced up the food web to brown trout (*Salmo trutta* L.) and Arctic charr (*Salvelinus alpinus* L.). However, the charr appear to accumulate the majority of their biomass during the summer (see earlier discussion regarding temporal integration of isotope ratios in muscle tissue based upon Perga and Gerdeaux 2005) by feeding upon the abundant cladocerans so charr reflect a relatively greater reliance upon autochthonous production than trout (Grey 2001, Grey *et al.* 2002).

Although Loch Ness is by no means an extraordinary lake in terms of the contributions from allochthonous versus autochthonous sources, the opportunity to distinguish and trace such contributions with confidence using natural abundance stable isotope ratios is rather rare. To circumvent this problem it is possible to enhance the isotopic composition of certain ecosystem components via

experimental isotope addition, usually at the nutrient resource level and thus amplify the contrast between isotopic signatures. Thus, Pace *et al.* (2004) experimentally manipulated the ^{13}C -content of the dissolved inorganic carbon of two lakes via additions of $\text{NaH}^{13}\text{CO}_3$ to effectively label the primary production and make that component more isotopically distinct compared to the allochthonous sources of carbon. Stable isotope analyses of the higher organisms within the food web allowed Pace *et al.* to trace the fate of the ^{13}C -addition via daphnids into YOY bass and on the basis of the modelled isotope-derived data, evaluate that *Daphnia* received 20-50% of its carbon from a source other than primary production. The export of aquatic production and hence subsidy of terrestrial ecosystems has also been demonstrated by experimental isotopic enrichment of the aquatic larvae of terrestrial insects prior to their emergence (e.g. Sanzone *et al.* 2002). Thus, subtle additions of isotopic tracers, which have no nutritive value and negligible physiological impact upon a "natural" ecosystem, can be very useful in identifying such pathways of energy transfer.

7. APPLIED ISSUES

In this next section, I will use examples from the fields of fisheries and aquaculture and recent applications of stable isotope measurements to investigate a variety of applied issues. Intensive cage cultivation techniques are increasingly found in freshwater systems and the potential detrimental environmental impacts of these are numerous and well documented, especially those regarding eutrophication (e.g. Kibria *et al.* 1997). Feeds used in such aquaculture ventures often have distinct isotopic signatures relative to the natural local resources and biota; the majority is derived from low order, marine consumers (G. MacFarlane – unpublished) and therefore traceable using stable carbon, sulphur and possibly nitrogen isotopes. Thus, several studies have evaluated the contributions from natural and allochthonous (aquaculture feed) resources to shrimp and mussel species within polyculture systems (Yokoyama *et al.* 2002, Burford *et al.* 2004), or indeed, the subsidy of the natural receiving com-

munity by aquaculture waste (Grey *et al.* 2004c). Expansion of the commercial salmonid aquaculture industry and associated numbers of escapees resulting from both intentional and inadvertent releases has led to concerns regarding the negative impacts of escapees (e.g. Gross 1998), either through resource competition and/or reduction of the genetic and ecological adaptiveness of wild stocks; any study investigating the potential impacts of escaped farmed fish requires the ability to identify accurately farmed individuals from wild natives. Thus, Dempson and Power (2004) demonstrated that muscle taken from wild-caught salmonids were sufficiently isotopically distinct from aquaculture specimens to allow for separation of the two groups but admitted that the use of the method may be confounded by known isotopic variation in feeds used, and the length of time elapsed since escape resulting in dilution of the "farm" isotopic signature with natural food due to tissue isotopic turnover. A further controversial topic surrounding the commercial salmonid industry has been the inferred decline of wild salmonid stocks allegedly via sea lice infections originating from aquaculture ventures, although a direct causal link has never been established (Tully and Nolan 2002). Thus, Butterworth *et al.* (2004) have demonstrated that $\delta^{15}\text{N}$ signatures differentiated between populations of sea lice (*Lepeophtheirus salmonis* (Krøyer)) originating from farmed and wild stocks for exactly the same reasons as the study of Dempson and Power (2004) but unfortunately did not go on to ultimately identify and quantify the source of lice found upon wild salmonids. Again, extended residence time of "farmed" lice on a wild host would result in dilution of the "farmed" tracer, so it is important to understand likely isotopic turnover rates in such ectoparasites before their true usefulness as a tool can be gauged.

The isotopic distinction between marine, brackish and fresh waters has been extremely useful in assessing and evaluating wild anadromous fish stocks. With a knowledge of the likely isotopic turnover rate in fish tissues, it has proved possible to discriminate between freshwater resident and migratory individuals of brook trout, *Salvelinus fontinalis* Mitchill (Doucett *et al.* 1999) and

brown trout, *Salmo trutta* (McCarthy and Waldron 2000), and further, to estimate the contribution of sympatric anadromous and freshwater resident trout to juvenile production (Doucett *et al.* 1999) and assess the extent to which their spawning grounds overlap (Charles *et al.* 2004). Similarly, Harrod *et al.* (2005) have used stable carbon and nitrogen isotopes to demonstrate individual variation in European eel (*Anguilla anguilla* L.) populations, suggesting that individual eels could be classified as freshwater, brackish or marine resident. Interestingly, their results also suggest substantial movement between habitats. Their results mirror those generated by recent studies of eel otolith microchemistry (e.g. Tsukamoto and Arai 2001), and such facultative catadromy challenges the widely accepted version of eel life cycles, having significant implications for researchers working on eel biology and management. The potential existence of different migratory components is likely to be particularly problematic for those attempting the stock assessment of eels in open marine and coastal ecosystems.

Otoliths, the accretionary structures located within the inner ear of teleosts, have long been used by fisheries scientists to determine fish age. However, otolith microchemistry can be tied to the accretion chronology and thus it is possible to reconstruct many of the physical and chemical characteristics of the environment that the particular fish has inhabited (Campana 1999). Kennedy *et al.* (2000) have outlined how fish from particular geographic regions can be distinguished from those from another region if the isotopic ratios in the water and food between regions are sufficiently distinct, in particular using natural strontium isotope ratios, which typically vary between tributaries passing over areas of isotopically distinct bedrock. Strontium isotopic ratios are thought to provide increased precision and be more widely useful than elemental strontium composition because elemental composition is affected by temperature, salinity, growth and maturation and/or genetics of fish. Similarly, otolith $\delta^{34}\text{S}$ can be used to identify hatchery raised fish, reconstruct nutrition histories and migrations (Weber *et al.* 2002), or otolith $\delta^{18}\text{O}$ can be used to

infer metabolic and temperature histories because otolith aragonite precipitates at or close to oxygen isotope equilibrium with the surrounding water (Guiger *et al.* 2003).

Our understanding of contaminant accumulation in fish populations has been improved by augmenting contaminant analyses with complementary stable isotope analyses. Traditional studies of contaminant biomagnification in food webs used discrete trophic classifications based upon inferred feeding behaviour or gut content analyses but interpretation is complicated for species exhibiting considerable trophic plasticity both within and among water bodies. However, $\delta^{15}\text{N}$ as a continuous measure of trophic position neatly circumvents this problem (e.g. Cabana and Rasmussen 1996), and has allowed for numerous studies investigating the accumulation of xenobiotic compounds such as toxaphene (Kidd *et al.* 1995), PCBs (Kidd *et al.* 1999) or mercury (Syväranta *et al.* 2006) in fish species at levels considered hazardous to human health. From a conservation perspective, Kidd *et al.* (2001) used the stable carbon and nitrogen isotopes as indicators of source and trophic position respectively and demonstrated that organochlorines such as DDT were accumulated in the larger, fattier fish toward the top of the Lake Malawi food web which is of course internationally recognised for its diverse fish communities. Perhaps more importantly, their study identified that pelagic fish species were more prone to accumulation than species reliant upon benthic production, information that could be used for management or conservation strategies.

Aquatic communities are susceptible to invasions (Gherardi 2006). A quantitative analysis of dietary and spatial overlap between recently introduced and indigenous species can be used to determine the prospects for coexistence or exclusion. Ecologists are still largely unable to predict the impacts and consequences of species invasions (Lodge 1993, Pimm 1991), primarily because natural systems (e.g. food webs) are complex and variable (Polis and Strong 1996). Stable isotope derived data has not only been used to characterise changes to food web structure in response to these invasions, but such data may also be useful in predicting potential

impacts of invasions or introductions, and aligning management strategies. Stable isotope measurements can be used to characterise trophic structure post-introduction of an exotic species, which is probably the most common case reported in the literature because there is often a paucity of pre-introduction stable isotope data (but there is potential for using recalcitrant structures – see below). Hence, Clarke *et al.* (2005) augmented gut content analysis and demonstrated that there was considerable dietary overlap and reliance upon kokanee (*Oncorhynchus nerka* (Walbaum)) as prey, despite a considerable reduction in their numbers, between native bull trout (*Salvelinus confluentus* (Suckley)), cutthroat trout (*O. clarki* (Richardson)) and northern pikeminnow (*Ptychocheilus oregonensis* (Richardson)), and the introduced lake trout (*Salvelinus namaycush* (Walbaum)) and rainbow trout (*O. mykiss gairdneri* (Richardson)) in a large oligotrophic lake. However, it is of more interest if there are pre-invasion stable isotope data with which to compare directly but unfortunately such cases are rare. Vander Zanden and Rasmussen (1999) inferred that bass (*Micropterus dolomieu* Lacépède and *Ambloplites rupestris* (Rafinesque)) invasion of two separate Canadian lakes impacted upon the pathway of energy transferral leading to the top native predator, the lake trout. Where bass were present, there were fewer prey fish species present and the predatory lake trout $\delta^{15}\text{N}$ was lower suggesting a lower trophic position induced by a plankton-derived diet. In reference lakes without the bass invader, the lake trout $\delta^{15}\text{N}$ was higher indicating a greater proportion of biomass attributable to piscivory. Using such trophic relationships between lake trout, bass, zooplankton and minnow prey fish delineated by stable isotope measurements, Vander Zanden *et al.* (2004) went on to develop a model to predict future occurrences and impacts of smallmouth bass for a suite of 788 Ontario lakes. Food web analysis based upon stable isotope measurements for 18 lakes suggested that lake trout were “buffered” from the impacts of bass on minnows in lakes where there was an ample supply of pelagic prey fish; thus the lakes could be classified according to their vulnerability to bass invasion based upon the predictabil-

ity of bass occurrence and their subsequent impacts. Lakes then classified as vulnerable could be prioritised for efficient use of management resources.

8. FUTURE CHALLENGES FOR STABLE ISOTOPE ANALYSES TO FURTHER OUR KNOWLEDGE

There are countless avenues to be explored with stable isotope analysis. Integrating dietary studies using other methods such as fatty acid or molecular analyses, tying intraspecific isotope variability to morphometrics and/or genetics, and better understanding the role of parasites in food webs are just some that come to mind. In this final section, I touch briefly on some recent advances in methodology which have aided the study of the microbial role in aquatic food webs, and then also on the potential for analysis of preserved samples.

Microorganisms are primarily responsible for the biogeochemical cycling of the Earth's elements and yet despite this, we still have very limited taxonomic knowledge of the organisms which are responsible for specific processes; that which we do is usually related to the incredibly small percentage of microbes which can be isolated and cultured. The “black box” of the microbial loop, a large diversity of organisms exhibiting trophic plasticity and a wide array of putative food sources, and the importance of contributions from the chemolithoautotrophic communities both in the water column and in the sediments, are areas of research that are benefiting from the application of stable isotope analyses. However, it is worth noting at this juncture that the trophic fractionation for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures, which appears relatively dependable for larger consumers, appears not to hold for bacteria. Methanogenesis and methanotrophy are good examples where the end product, i.e. the biomass, derived from the raw materials exhibits markedly different signatures from the substrate (Whiticar 1999). Unfortunately, bulk stable isotope analyses are of limited use because of the sample mass requirements and the difficulties in separating matter from the multitude of cells and detritus typically constituting such samples to provide a pure

sample of the particular organisms or materials of interest. There are sample preparation procedures that can alleviate the problem of producing sufficient quantities of pure samples prior to introducing the sample into the elemental analyser. These include techniques such as density gradient colloidal silica separation (Hamilton *et al.* 2005), flow-cytometric cell sorting (Pel *et al.* 2004), or SPLITT (Split-flow thin fractionation, King *et al.* 2006). Advanced preparation techniques used in conjunction with a miniaturisation of the elemental analyser components can allow for bulk stable isotope analyses of small samples $\geq 1 \mu\text{g N}$ and $2 \mu\text{g C}$ such as pooled samples of 5 to 60 individual meiofauna (Carman and Fry 2002).

However, the development of compound specific isotope ratio mass spectrometry has enabled the combined use of specific lipids as taxonomic biomarkers and their isotopic signatures as quantitative descriptors of the sources and sinks of carbon flow in aquatic systems; the majority of examples stem from the marine environment (Jahnke *et al.* 1995, Abraham *et al.* 1998, Jahnke *et al.* 2001) but are of course directly transferable to the freshwater environment. Used in conjunction with isotopic labelling (Pel *et al.* 1997, Boschker *et al.* 1998, Middleburg *et al.* 2000, Chamberlain *et al.* 2004, Pel *et al.* 2004), the compound specific approach provides a means to couple function with the identity of different microorganisms (Weisse 2006). A further alternative approach involving isotopically labelled substrates uses molecular biological methods to isolate the ^{13}C -DNA produced from ^{12}C -DNA by density gradient centrifugation, which can then be characterised taxonomically using gene probing and sequencing (Radajewski *et al.* 2000). Although primarily used in soil science so far, stable isotope probing (SIP) has great potential in freshwater ecology for identifying those microbes that are actively involved in mediating specific processes.

9. HINDCASTING

In a previous section, the use of otoliths (and their associated chrono-series of stable isotopes essentially locked into a recalcitrant structure) to reconstruct fish natal origins

or migratory histories was described. Stable isotope values from such recalcitrant structures, and indeed other preserved samples may be used to infer some measure of temporal change that may not be directly related to the organism from which the data is derived. There have been relatively few retrospective studies using stable isotopes of archived materials, perhaps in part, due to uncertainty concerning the effects of long term preservation on samples which have required chemical fixation but this does not apply to structures such as scales, otoliths and operculae from fish, unionid shells, or chironomid head capsules and diatom frustules which can be recovered from sediment cores. Within the past five years, there have been a number of studies examining the effects of preservation with the explicit purpose of tapping the potential historical archives held in museum specimens, or long-term collections of "fixed" zooplankton samples such as those held by the Freshwater Biological Association and Centre for Ecology and Hydrology (zooplankton archives for Windermere extend to 1934). Some of these studies have focused solely on aquatic organisms (Bosley and Wainright 1999, Arrington and Winemiller 2002, Sarakinos *et al.* 2002, Feuchtmayr and Grey 2003, Sotiropoulos *et al.* 2004, Sweeting *et al.* 2004). Thus we now have a much better understanding of the effects of the commonly used preservatives on stable isotope integrity; still lacking is a definitive test of long term effects compared to controlled samples prepared from fresh materials and not frozen from all these different preservative types (but see Ogawa *et al.* 2001, Sweeting *et al.* 2004). Feuchtmayr and Grey (2003) noted from their study of preservative effects upon zooplankton that freezing can result in marked differences (up to 1‰ in $\delta^{13}\text{C}$) compared to material dried and prepared immediately for stable isotope analysis; they suggested that it was probably a function of the actual preparation procedure resulting in loss of leached carbon compounds after mechanical rupture of the cells. Retention of all components of frozen material should allow for direct comparison. Consistent preparation of frozen archived material can provide excellent time series but such archives are relatively rare (see

Kiriluk *et al.* 1999). Other chemical preservatives appear to affect elemental composition and hence stable isotope integrity rather rapidly after addition (e.g. Sweeting *et al.* 2004) and so if all samples have been preserved and stored consistently for >1 year, any temporal effect of preservative should have been removed. Maguire and Grey (2006) analysed a time-series of formalin preserved zooplankton samples from Lough Erne spanning 28 years and found no consistent trends in the isotopes or elemental composition that would signal a preservative effect; indeed they were able to compare freshly prepared to preserved samples at two time points (1 and 6 years old) and found no temporal effect of storage. Ogawa *et al.* (2001) tested muscle tissue from several species of fish, which were formalin-fixed over a period extending from 9-117 weeks, and found insignificant changes in $\delta^{15}\text{N}$. It would appear then that there is a wealth of untapped potential in preserved time-series samples for many retrospective analyses. Both Kiriluk *et al.* (1999) and Maguire and Grey (2006) examined time-series for trophic changes induced by environmental disturbances because there were no pre-disturbance measures made at the time; both studies found marked isotopic shifts induced by zebra mussel (*Dreissena polymorpha* Pallas) invasion and establishment. From the change in walleye (*Stizostedion vitreum* (Mitchill)) $\delta^{13}\text{C}$ collected annually from the western basin of Lake Erie it was inferred that the fish had switched to more pelagic foraging, probably related to increased water clarity resulting from mussel filtration (Kiriluk *et al.* 1999). Stable isotope measurements of zooplankton from humic-stained Lough Erne departed from values typical for autochthonous feeding and tended towards those more typical of reliance upon allochthonous resources after establishment of the zebra mussels (Maguire and Grey in press). Similarly, Gorokhova *et al.* (2005) used preserved samples of zooplankton and fish to compare food web structure before and after invasion by the predatory cladoceran, *Cercopagis pengoi* (Ostroumov); herring trophic position had shifted from 2.6 to 3.4 indicating the addition of trophic steps leading to herring biomass after invasion by the zooplankton.

There are more examples of time-series stable isotope studies derived from recalcitrant structures but this type of study is still relatively rare considering the availability of extensive archives. Fish scales which have had the inorganic fraction removed can be useful not only for retrospective studies, but also because scales can be used as a non-destructive surrogate for muscle tissue, and they encapsulate the complete life history of the individual fish (Estep and Vigg 1985, Wainright *et al.* 1993, Perga and Gerdeaux 2003). Perga and Gerdeaux (2003) established a protocol for the preparation of scale material, demonstrated that scale was a reliable substitute for muscle and then used archived whitefish scales to retrospectively examine a period of re-oligotrophication of the large, deep Lake Geneva; a close correlation between whitefish scale $\delta^{13}\text{C}$ and phosphorus loading was considered as an indication of the changing nutrient status.

It would appear then that there is great potential in retrospective studies using stable isotopes to examine environmental or anthropogenic induced change. However, as with all stable isotope data, we return to the fact that interpretation is of paramount importance, and with archived material there must be sufficient supplementary ecological data to support any inferences made. For example, the change in fish scale $\delta^{13}\text{C}$ in the above study potentially could have been interpreted as resulting from a diet switch, but the authors had negated this interpretation by analysis of complementary gut content data (Perga and Gerdeaux 2003). Other examples of hindcasting using stable isotope data from recalcitrant structures include temperature reconstructions using $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ in mussel shells and otoliths. Wurster and Patterson (2001a) determined sub-weekly climate records by micromilling contemporary samples of fingernail clam (*Sphaerium simile* (Say)) and zebra mussel shell calcium carbonate $\delta^{18}\text{O}$, which accurately reflect the surrounding water $\delta^{18}\text{O}$. They found that both types of mollusc recorded climate variables with a high degree of integrity and would thus serve as excellent palaeoclimate proxies. Furthermore, they established that fingernail clams from a small waterbody exhibited greater variation about the seasonal

pattern than did the zebra mussels which came from a much larger waterbody, leading them to conclude that smaller waterbodies were more sensitive to storm perturbations and should be a focus for establishing subtle changes in climate history. In a concurrent study, Wurster and Patterson (2001b) milled $\delta^{18}\text{O}$ from the otoliths of freshwater drum (*Aplodinotus grunniens* Rafinesque) which had been recovered from an archaeological site in the USA (dated to 5500 years before present) and provided evidence that the mid-Holocene suffered greater climate variability and was substantially wetter than was previously thought. The latter study demonstrates the temporal scales at which stable isotopes studies can be applied. The announcement of a special conference held in early 2006 specifically focussing on the use of stable isotopes as temporal tracers of ecological change reflects a growing interest in the techniques.

10. SUMMATION

This paper was not intended to be an extensive review of all the stable isotope techniques, combinations and applications available to freshwater ecologists. Rather, I wished to draw the reader's attention to some of the recent success stories and scientific advances arising from applications of the "tool"; to some of the pitfalls from assuming that stable isotope analysis is some sort of panacea and can be applied indiscriminately; and to highlight some of the future potential yet to be exploited. Again, I return to the plea from Gannes *et al.* (1997) that stable isotope data are no substitute for ecological knowledge but should be treated more as complementary sources of information to couple with conventional data and further our understanding of freshwater ecology.

ACKNOWLEDGEMENTS: I am indebted to Roger Jones (formerly Lancaster, currently Jyväskylä University) for "enriching" my career with the concept of stable isotope analyses and for letting me loose on the Loch Ness food web; to Winfried Lampert for the opportunity to pursue some "extremely negative" ideas at the Max Planck Institute for Limnology; and to Susan Waldron in particular, and countless other stable isotope enthusiasts for the many discussions in

the last 10 years. I am also grateful to Maciej Gliwicz and the organisers of the SEFS4 meeting for the fabulous opportunity to inspire European freshwater scientists in the application of stable isotopes to freshwater ecology.

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(Received after revising December 2005)