DOI: 10.1111/j.1570-7458.2007.00572.x

MINI REVIEW

Stable isotope methods in biological and ecological studies of arthropods

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Accepted: 13 February 2007

Key words: marking, labelling, enrichment, natural abundance, resource turnover, 13-carbon, 15-nitrogen, 18-oxygen, deuterium, mass spectrometry

Abstract

This is an eclectic review and analysis of contemporary and promising stable isotope methodologies to study the biology and ecology of arthropods. It is augmented with literature from other disciplines, indicative of the potential for knowledge transfer. It is demonstrated that stable isotopes can be used to understand fundamental processes in the biology and ecology of arthropods, which range from nutrition and resource allocation to dispersal, food-web structure, predation, etc. It is concluded that falling costs and reduced complexity of isotope analysis, besides the emergence of new analytical methods, are likely to improve access to isotope technology for arthropod studies still further. Stable isotopes pose no environmental threat and do not change the chemistry or biology of the target organism or system. These therefore represent ideal tracers for field and ecophysiological studies, thereby avoiding reductionist experimentation and encouraging more holistic approaches. Considering (i) the ease with which insects and other arthropods can be marked, (ii) minimal impact of the label on their behaviour, physiology, and ecology, and (iii) environmental safety, we advocate more widespread application of stable isotope technology in arthropod studies and present a variety of potential uses.

Introduction

This article is a synthesis of potential stable isotope methodologies used to study biology and ecology in an arthropod (and mainly entomological) context. It is intended to provide an introduction to the use of stable isotopes in entomological studies and provide a springboard for further research. The science of stable isotopes and how these can be used in various ecological and biological studies is covered.

Stable isotopes occur naturally in the environment. They are safe and non-radioactive, and do not decay, which make them useful natural tracers. An isotope of an element has the same atomic number as the element but a different number of neutrons and thus a different atomic weight. For example, approximately 1% of all carbon atoms are ^{13}C, the rest ^{12}C, whereas approximately 0.4% of all nitrogen atoms, are ^{15}N atoms with the rest ^{14}N atoms.

There are two approaches to using stable isotopes. Natural abundance studies use naturally occurring differences in isotopic signatures to follow flows and processes. Enrichment studies deploy labelled compounds enriched in particular isotopes, which are added to the system and followed.

Natural abundance studies: innate differences in isotopic signature are the result of different rates of reaction at an enzymatic level, which can result in slight variations in isotopic composition in nature, and these natural signatures can be used in ecological studies to trace food-web structure, migration patterns, feeding preferences, etc. (Hobson & Clark, 1992; Ostrom et al., 1997; Wassenaar & Hobson, 1998; Fantle et al., 1999; Hood-Nowotny et al., 2005).

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Enrichment studies: there is a wide variety of commercially available stable isotope-enriched compounds that have higher concentrations of the rarer isotope than the natural background (or natural abundance). These can be easily integrated into feeding regimes of target insects and can be used, for example, in capture–recapture, feeding preference, and resource allocation. It is important to note that natural abundance and enrichment studies are often subject to different assumptions, terminologies, and caveats.

Over the past 20 years, the cost and complexity of stable isotope analysis has decreased dramatically. The cost of highly labelled ^{13}C glucose, for example, is approximately $100 per gram. Samples are easy and safe to dispatch and as a result, there are now a number of laboratories offering isotope analysis at affordable prices, ranging from $5–80 per sample depending on market forces and the nature of the isotope. The availability of this service allows for an outsourcing approach, with the associated benefits, for example, of not having to invest in capital equipment and associated infrastructure.

It should be noted that unlike painting, dusting, etc., stable isotope methods are non-invasive and samples require only minimal preparation following collection, which makes the cost of the process as a whole comparable to methods such as polymerase chain reaction (PCR). The developments in mass spectrometry have widened the accessibility and scope of stable isotope science, which has led to an increase in their use in an eclectic mix of scientific disciplines, from archaeology, nutrition, geology, and physiology, through to forensics (Hood-Nowotny et al., 2005).

Many of the biological processes and reactions that have been investigated using radioactive tracers could use stable isotopes instead, with the distinct advantage that there are no environmental impact or (bio)safety issues associated with using stable isotope-labelled material. Because stable isotopes pose no environmental risks, it is possible to release stable isotope-labelled insects into the environment and to trace their movement or to apply specific stable isotopes in a variety of ecosystem-labelling experiments. Stable isotopes are particularly useful in ecophysiology, which is the science of how whole populations and communities behave in relation to environmental constraints. These provide a means to follow pathways with minimal disturbance or impact to the system (Le Maho, 2002).

There is a vast range of articles describing the use of stable isotopes in biological and ecological studies per se. However, given the power and potential of isotopic techniques as methodological tools, the literature focusing on arthropods is surprisingly limited. As this may be a result of researchers being unfamiliar or uncomfortable with isotope technology, this article will attempt to address this issue. We will first briefly introduce the basics of isotopes, including the measurement of isotopes and how isotope values are reported and interpreted. This will be followed by descriptions of how isotopes can be used in a series of applications in biology and ecology, which will encompass growth, distribution, and processes influencing the abundance of organisms, in addition to intra- and interspecies interactions and the transformation and flux of energy and matter. More specifically, we will seek to explain how stable isotopes can be used for marking in population and range studies, studying migration, analysing food-web structure, investigating feeding preferences and resource allocation and the dynamics of tissue turnover, and studying mating and competition.

Isotope basics

The natural abundance of the rarer isotopic form is usually expressed as a simple percentage of all isotopic forms. For example, the natural abundance of ^{15}N in air is 0.3663%. This means that one atom in every 273 atoms of nitrogen is the rarer ^{15}N isotope and 272 atoms are the ^{14}N form. The most commonly used isotopes in ecological studies are hydrogen (^{2}H natural abundance 0.01492%), carbon (^{13}C natural abundance 1.108%), nitrogen (^{15}N natural abundance 0.3663%), oxygen (^{18}O natural abundance 0.204%), and occasionally strontium (^{84}Sr 0.56%, ^{86}Sr 9.86%, or ^{87}Sr 7.0%) or sulphur (^{34}S natural abundance 4.25%).

Measurement of isotopes

Stable isotope analysis of samples is traditionally undertaken using isotope ratio mass spectrometry (IRMS) coupled to an elemental analyser (Figure 1). Samples are usually collected in dried form and are finely ground and weighed into a small cylindrical tin cup (8 mm in height × 5 mm in diameter). However, in entomological studies, it is often possible to analyse a whole insect or insect part by loading it in the tin cup and drying it directly, thereby substantially reducing sample preparation time. These organic samples are initially combusted at high temperature (1800 °C) and converted to gas (N_2, CO_2, H_2, and SO_2) in an appropriate preparation system linked to the IRMS. The sample is scrubbed and constituent gases are separated on a gas chromatograph column and bled into the mass spectrometer. Under vacuum, the gases are ionized on a hot filament, accelerated, and separated by a magnetic field based on their mass to charge ratio (m/z). The separated ions are collected in Faraday cups, where the ratios of the isotopes of interest are determined. The output of the mass spectrometer is a ratio, which can be internally converted to an atom percentage value or a delta value (see below), depending

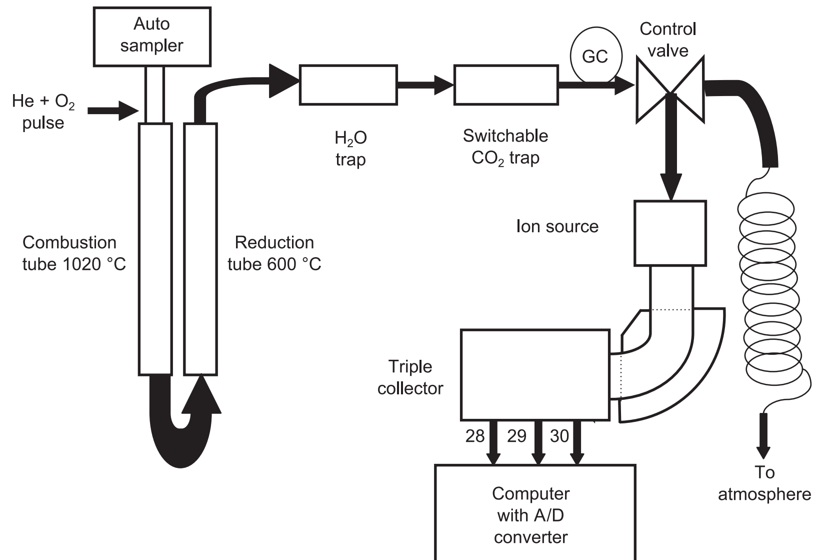

Figure 1 Graphic representation of elemental analyser linked to mass spectrometer (after Hood & Blair, 2001).

on the standards used and the experimental requirements. Current fully automated IRMS systems are easily capable of accurately determining isotope ratios in approximately 10 µg of carbon and 5 µg of nitrogen. Depending on the machine and its configuration, this usually takes 5–10 min per sample.

There is a range of other successful spectroscopic techniques to measure stable isotopes in gases and liquids, many of which are laser or infra red based. These techniques are likely to expand the scope and affordability of isotope measurement capabilities even further in the future, as they facilitate rapid analysis at picomole concentrations and are generally simpler systems than the mass spectrometer described above. For a review of the current status in this field see Kerstel & Gianfrani (2005).

Isotope units and terminology

The measurement of isotopic composition for a particular element is commonly based on the ratio of the less abundant isotope of interest to the more abundant isotope. For nitrogen, for example, this is:

Atom % $^{15}N = [^{15}N/(^{14}N + ^{15}N)] \times 100$ or

Atom % ^{15}N = [moles of ^{15}N/(moles of ^{15}N + moles of ^{14}N)] × 100.

In enrichment experiments, values are generally reported in atom % ^{15}N or atom percent excess (APE; i.e., enrichment minus universal value for natural abundance): APE = sample atom % – reference atom %, whereas atom % ^{15}N – natural abundance of air N = atom % excess value. The atom % ^{15}N abundance of atmospheric air is 0.3663 and the universal standard for N. A fertilizer with an enrichment of 5.000 atom % ^{15}N can also be described as 4.6337 atom % ^{15}N excess.

In natural abundance studies, values are reported as ratios referenced against international standards in delta (δ) units parts per thousand (‰). There are a number of internationally recognized conventional reference standards ranging from air to limestone [Pee Dee Belemnite (PDB)] that are listed in Table 1.

A lower-case δ value is defined as the isotopic ratio of a sample standardized to the isotopic ratio of a defined reference: $[(R_S - R_R)/R_R] \times 1000 = \delta$, which can also be written as $[(R_S/R_R) - 1] \times 1000 = \delta$, where R_S is the isotopic ratio of the sample and R_R is the isotopic ratio of the reference standard with R = [atom % ^{15}N/atom % ^{14}N].

Thus, a sample of 0.3700 atom % ^{15}N would be R_S = 0.3700/99.63 = 0.0037137, the R_R of the reference (in this case air is 0.3663/99.6337 = 0.003676) $\{[(^{15}N/^{14}N)_{sample}/(^{15}N/^{14}N)_{reference}] - 1\} \times 1000 = [(0.003713/0.003676) - 1] \times 1000 = 10.27\ \delta\ ^{15}N$‰ vs. air.

Samples that are depleted in the heavier isotope due to discrimination against the heavier isotope have negative δ values.

Population studies using enrichment techniques

Labelling insects with stable isotopes is an effective marking method for population studies and tracking using mark–release–recapture techniques (MRR). Commercially available compounds enriched with stable isotopes such as

Table 1 R isotopic ratios of reference materials used for delta scales

Element	R isotopic ratio of the reference materials used for the delta scales	Reference materials used for the delta scales
Carbon $^{13}C/^{12}C$	0.0112372	Pee Dee Belemnite (PDB)
*Carbon $^{13}C/^{12}C$	0.011224	Vienna Pee Dee Belemnite (VPDB)
Nitrogen $^{15}N/^{14}N$	0.0036765	Air (AIR)
Hydrogen $^{2}H/^{1}H$	0.00015576	Vienna Standard Mean Ocean Water (VSMOW)
Oxygen $^{18}O/^{16}O$	0.0020052	VSMOW
Sulphur $^{34}S/^{32}S$	0.0450045	Canyon Diablo Troilite (CDT)
*Sulphur $^{34}S/^{32}S$	0.0441626	Vienna Canyon Diablo Troilite (VCDT)

Gröning (2004). *PDB and VPDB are considered equivalent and CDT and VCDT are considered equivalent.

glucose or amino acids can be easily integrated into the feeding regimes of laboratory-reared specimens prior to release at reasonable cost (Table 2). It is also possible to grow labelled food in ^{15}N-labelled fertilizers or ^{13}C-enriched atmospheres and then feed the labelled food to the target organism. Appropriate isotope feeding management is required to ensure that (i) the isotope label is fixed into structural body tissue of the target organism, (ii) it is not 'lost' through metabolic turnover, and (iii) there are no toxic effects of labelled compound addition (i.e., the addition of the compound rather than the label per se may cause detrimental effects). Preliminary laboratory-based insect-specific and isotope-specific studies to determine the most appropriate feeding-management strategies are therefore essential to optimize labelling. Stable isotope marking of insects fulfils the marking criteria set out by Hagler & Jackson (2001), including retention, not affecting the insect's fecundity or behaviour, durable, non-toxic, easily applied, clearly identifiable, and inexpensive. The latter could be contested in the case of stable isotope analysis. However, the cost of analysis is comparable to other modern chemical or molecular analyses. In insect studies in particular, the costs of the enriched feeding compounds are not prohibitively expensive and the comparatively small size of most insects means that whole insects or specific insect body parts can be analysed, which reduces sample preparation time and thus cost. For example, we have estimated that it would cost between $150–250 to label 1 000 000 *Anopheles* mosquitoes with ^{13}C-labelled glucose in the larval stages and because mosquitoes fit neatly into the tins cups, there is minimal sample preparation time. In the case of mosquitoes, the larval diet is spiked with a low concentration of highly labelled glucose solution, which is retained throughout adult life (Hood-Nowotny et al., 2006).

Table 2 Approximate costs (in 2005) of enriched compounds that could be useful in biological and ecological studies of arthropods

Compound	Approximate enrichment (%)	Approximate cost per gram in $
^{13}C-mixed fatty acids	98	200
Amino acids		
Glycine-^{13}C	98	95
Aspartic acid-^{13}C	98	300
Leucine-^{13}C	98	200
Valine-^{13}C	98	275
Glycine-^{15}N	98	95
Glycine-$^{13}C/^{15}N$	98	900
$^{13}C_6$ D-glucose	98	160
^{13}C-UL-fructose	98	315
Water		
$^{2}H_2O$	98	0.3
$H_2{}^{18}O$	97	195
$^{15}NH_4Cl$	99	40
$K^{15}NO_3$	99	25

The principle of MRR techniques is that insects are collected from the field, labelled in situ or produced in laboratory colonies, subsequently released back into the field, and recaptured after a specific period or within a specific geographical range. The presence of the marker is examined and the proportion of marked individuals determined using population models such as the closed system Lincoln–Petersen model (Service, 1993) or the open system Jolly–Seber approach (Hargrove, 2001). One of the limitations of the isotope technique is that the analysis methods are usually destructive, thus methods that rely on repeated capture of marked individuals (Jackson, 1939) are not possible.

Arthropod movement

Knowing how far, where, and when insects will move is central to our understanding of their ecology and biology and a precondition for effective conservation or control efforts. It is possible to trace migration, drift, and range

of insects in their natural habitat, using either natural abundance techniques, which rely on predictable isotopic variations across landscapes or enrichment techniques, which require the addition of an isotope label to the system.

Arthropod movement studies using the addition of an isotopically enriched label

Labelling a distinct portion of an ecosystem with stable isotopes is a useful, minimally invasive method to study insect dispersal from an ecophysiological perspective (Macneale et al., 2004, 2005). Using this approach, Hershey et al. (1993) resolved the drift paradox, a phenomenon of adult upstream flight that compensates downstream movement of larval populations of mayflies (*Baetis* spp.) in an Artic river. A section of the river was continuously labelled with ^{15}N fertilizer over a 2-week period. The epilithic algae, a major food source for the mayflies, rapidly assimilated the ^{15}N label. Both nymph and adult populations were sampled up- and downstream from the labelling zone. Knowing where the label was applied and capturing (labelled) individuals at different locations along the stream allowed the researchers to use modelling procedures to estimate how far the mayflies had flown or had been dispersed by wind, etc. It was concluded that one-third to one-half of the adult population actively fly or become passively dispersed between 1.6 and 1.9 km upstream. Similar studies are also possible in terrestrial settings. These types of labelling experiments may be of particular use in mosquito ecology studies. For instance, in northern Sudan it is believed that the Nile acts as a natural barrier to mosquito dispersal across the river. This hypothesis could be easily tested by labelling some known breeding sites with ^{18}O-labelled water and subsequently collecting mosquitoes on the opposite side of the river and analysing their ^{18}O content to determine whether cross-river dispersal occurs. Labelling of water at low isotope enrichments is easy and safe and labelling plants with isotopes is also relatively simple. This is achieved by either growing them in labelled fertilizer or by growing them in $^{13}CO_2$-labelled environments.

Natural abundance methods for arthropod movement studies

Naturally occurring stable isotope markers are useful as they do not require the pre-marking of individuals. Specific geographical regions have distinctive isotope profiles as a consequence of biogeochemical processes. These can be used to trace the origin and movement of insects. The most commonly used isotopes are the isotopes of hydrogen (δ^2H), oxygen ($\delta^{18}O$), carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$), and strontium ($\delta^{87}Sr$). δ^2H generally decreases with increasing latitude and altitude due to rainfall patterns and temperature differences over large geographical ranges. $\delta^{18}O$ is higher in summer than winter above latitudes of 30° and generally decreases with distance from the sea [for detailed maps of isotopic signature of rainfall see Bowen et al. (2005) and http://isohis.iaea.org]. $\delta^{18}O$ and δ^2H of precipitation and snow varies systematically across the globe (Bowen et al., 2005). $\delta^{13}C$ decreases with increasing latitude and altitude, and drier habitats are more enriched (less negative) than wetter habitats with predominantly C_3 plant species. $\delta^{87}Sr$ signatures vary dependent on concentration of calcium in the soil where calcium-rich soils are more enriched compared to calcium-poor soils, and older soils are more enriched than younger soils. It should be noted that $\delta^{87}Sr$ signatures may also be influenced by nuclear fall-out.

The oxygen and hydrogen isotope signatures of an insect will accurately reflect the signature of its water source, which is usually dependent on the signature of the weighted average of local precipitation. It should be noted, however, that processes of evaporation and infiltration can lead to isotopic discrimination, and ground water and water bodies may thus have significantly different signals from the weighted average of precipitation. The carbon isotope signatures reflect the insect's diet, although the $\delta^{15}N$ signature can also be affected by water availability and nutritional stress. Both will give some indication of the organism's position in the food web (see section on food-web structure).

Use of natural abundance methods to study long-distance dispersal

Latitudinal differences in $\delta^{13}C$ and δ^2H of food and water sources have been used to determine the natal origin of monarch butterflies (*Danaus plexippus* L.) overwintering in Mexico (Hobson et al., 1999). Initially, isotopic values in the butterflies were compared with their host plants and water sources, simply by rearing butterflies on test milkweed plants irrigated with water of known isotopic composition. Second, it was assessed whether geographical patterns in the δ^2H and $\delta^{13}C$ values were evident. A co-ordinated effort of rearing and sampling of adult butterflies, cultured from eggs reared on rain-fed milkweed host plants, was undertaken at different locations across the butterfly's ecological range throughout the USA. Hobson et al. (1999) then tested whether the resolution of the isotopic patterns was sufficient to infer natal origins, and isotopic maps were drawn. In an additional article (Wassenaar & Hobson, 1998), the signature of the host plant and adults were analysed for δ^2H and $\delta^{13}C$ and the natal origins of the

butterflies overwintering at 13 locations across Mexico were extrapolated using isotopic signals.

These studies allowed essential information about the migration patterns and breeding ranges of the butterflies to be established. This technique is somewhat limited, however, to long-range migrations or to studies of movement across isotopic boundaries. Although the number of long-range migratory insects is limited, it may also be possible to use the technique to study the transport of permanent and periodic ectoparasites such as lice, keds (Hippoboscidae), or ticks on migratory birds or mammals. Conversely, the insect ectoparasite may be a convenient subsample for analysis of the migratory history of the host species. The natural abundance isotope technique could possibly be used to determine the origin of invasive species pests and to determine whether insects are transitory migrants or belong to established resident populations. For example, it could be possible to determine whether mosquitoes found on aircraft are native or of non-native origin (Committee on Air Quality in Passenger Cabins of Commercial Aircraft et al., 2002); the effectiveness of these techniques is dependent on the availability, resolution, and inherent uncertainty of the isotopic data maps. Stable isotopes are proving to be increasingly useful tools in wildlife forensics and movement ecology (Bowen et al., 2005; Holden, 2006).

Studies of natural enemy movement in a pest-management context using stable isotopes is discussed by Prasifka & Heinz (2004). They discuss how the difference in isotopic signals of C_3 and C_4 plants can be used to study the movement of predators and parasitoids in multicropping systems. Terrestrial vascular plants differ in their ^{13}C:^{12}C ratios because of their photosynthetic pathways, C_3, C_4, or CAM (crassulacean acid metabolism). C_3 plants are so-called because the first product in their photosynthetic Calvin cycle pathway is a three-carbon compound (Calvin, 1962). These plants are generally native in temperate climates having low light intensity and have typical isotopic values of around –27 ± 2‰ but range between –25 and –35‰ vs. PDB, depending on the species and environmental conditions (O'Leary, 1988). Plants such as maize, millet, and sugar cane have C_4 Hatch–Slack photosynthetic pathways (Hatch & Slack, 1966) and thrive in high light intensity environments. These have isotopic values of around –13.1 ± 1.2‰ and range between –7 to –18‰ vs. PDB, with maize about –11‰ vs. PDB (O'Leary, 1988). CAM plants such as succulents or desert plants are generally adapted to low water environments, and have isotopic values in between those of C_3 and C_4, in the range of –10 to –20‰ vs. PDB (O'Leary, 1988). These inherent differences in isotopic signatures can be useful in diet-switching experiments and when studying the ecology of insects.

Gould et al. (2002) used the isotopic differences in C_3 and C_4 to prove that, for pink bollworm (*Helicoverpa zea* Boddie), local corn can act as a refuge during summer months and that in autumn nearly all moths captured originated from C_4 hosts although there were none available in the area. This confirmed other research suggesting that the populations migrated in from more northerly areas, which subsequently allowed development of effective pest-management strategies with Bt cotton in the area.

Diet analysis and feeding behaviour of arthropods

Isotopic techniques to study feeding behaviours have distinct advantages over traditional techniques such as gut content analysis and observation, as these allow for long-term studies and are generally less time consuming. Using both natural abundance and labelling techniques, it is possible to study food-web structure, feeding behaviour, food preference, and assimilation.

Analysis of food-web structure using natural abundance techniques

Isotopic analysis of organisms in a food web provides information about trophic relationships and will reflect what a particular organism has eaten, thereby providing an integrated measure of diet over time. It has been observed that during food assimilation and excretion, there are isotopic shifts in enrichment. Based on an extensive body of literature, McCutchan et al. (2003) suggest that consumers are typically enriched by about 2.3 ± 0.18‰ $\Delta\delta^{15}N$ (mean ± SE) and 0.5 ± 0.13‰ $\Delta\delta^{13}C$ (mean ± SE) (where Δ denotes the change in isotope ratio between diet and consumer). These are slightly different from the values reported earlier in the literature, $\Delta\delta^{15}N$ +3‰ and $\Delta\delta^{13}C$ +1‰, respectively, which were based on smaller data sets (DeNiro & Epstein, 1978, 1980; O'Leary, 1988). Isotopes of S are also increasingly being used in a multi-isotope approach and trophic shift in high-protein diets has been shown to be around 2.0 ± 0.65‰ and in low-protein diet –0.5 ± 0.56‰ (O'Leary, 1988; Kwak & Zedler, 1997; McCutchan et al., 2003; Bowen et al., 2005). ^{15}N enrichment increases predictably with trophic level and is used extensively in food-web construction. Identifying primary producers in the food web is dependent on the ability to differentiate potential sources isotopically and may require the use of a multi-isotope or a multidisciplinary approach.

Kwak & Zedler (1997) highlight that trophic estimates and trophic position of consumers derived from isotopic data alone should be interpreted with some caution, as isotopic signatures of the consumers are a function of source mixing as well as trophic enrichment. Observed isotopic

shifts maybe the result of sample preparation. For example, bulk samples may exhibit less isotopic shift than specific tissues such as muscle tissue (McCutchan et al., 2003). Carnivores and fluid feeders may exhibit different isotopic shift patterns to consumers with plant or algal diets (McCutchan et al., 2003). It should be noted that any possible contribution from biological atmospheric nitrogen fixation in a system should be accounted for, as these organisms will have an isotopic signature that resembles atmospheric nitrogen (0‰ ^{15}N). Isotopic signatures can also be used to identify atmospheric nitrogen input into the food web, as demonstrated by Tayasu et al. (1998) in their study of biological fixation of atmospheric nitrogen as a nitrogen source in termites. The contribution of microsymbionts to insect nutrition is a topical question and is often only inferred from genetic studies. Cook & Davidson (2006) concluded that stable isotopes can be useful for quantifying the true nutritional benefits of microsymbionts and could be done using, for example, $^{15}N_2$ gas.

The isotopic food-web construction methodology has been extensively used in marine and terrestrial habitats (Wada et al., 1991; Kwak & Zedler, 1997; Tayasu et al., 1998; McCutchan et al., 2003; Wissel & Fry, 2005), despite the uncertainties inherent in the methodology discussed above and in a number of publications, which question the use of a 'typical' mean enrichment across trophic levels (McCutchan et al., 2003; Ruess et al., 2004; Spence & Rosenheim, 2005; Cook & Davidson, 2006).

Scheu & Falca (2000) studied food webs in forest soil ecosystems, which included a number of insect species, and showed that in one of the forests studied the food web spanned four trophic levels. They concluded that ^{15}N:^{14}N ratios in most cases reflected trophic structure despite isotopic differences among species (Neilson et al., 1998; Ponsard & Arditi, 2000), and they also observed differences between starved and non-starved individuals (Scrimgeour et al., 1995; Schmidt et al., 1997). Recent work has shown that it may also be possible to study the qualitative and quantitative shifts in food webs, due to environmental disturbances, with stable isotopes (Caquet, 2006). Isotopically labelling primary consumers with enriched compounds can also be useful in food-web studies, as this approach may provide clearer pathway distinctions in complex systems (see below).

Feeding behaviour

Little is known about specific insect–food source relationships. Tracing energy flows from plant to insect, or insect to insect, usually requires tedious methods such as direct observation, gut content analysis, pigment tracing, or radioactive methods. Stable isotopes offer a safe, rapid, and direct technique to study feeding behaviour both in the laboratory and the field. Natural abundance methods in which primary food sources have inherently different isotopic signatures, such as C_3 and C_4 plants, may be used to study food preferences of insect species (Petelle et al., 1979; Webb et al., 1998; Prasifka & Heinz, 2004). Food preferences of a variety of native insects were determined from the differences in isotopic signatures of C_4 and C_3 crop plants. Leaf hoppers (Cicadellidae) were shown to prefer the C_3 species with isotopic values in the range of the C_3 plant values, while lace bugs (Tingidae) and tortoise beetles (Chrysomelidae) had values that were indicative of feeding from C_4 plants (Petelle et al., 1979).

Using multiple isotope ratios of nitrogen and carbon can provide clearer differences in host diets. Markow et al. (2000) distinguished between four different host plants of *Drosophila* using isotopic signals, and were then able to isotopically segregate seven wild-caught *Drosophila* species, suggesting it would be a useful technique to study *Drosophila* resource ecology.

Differences in feeding strategies within species may also be revealed. For example, Trimble & Sagers (2004) showed that *Azteca constructor* Emery ants were more opportunistic foragers of phylosphere fauna at lower elevations in Costa Rican forests while at higher elevations they relied more heavily on the resources supplied by their host-plant species. In addition, specific contribution of orchid extrafloral nectars to ant diets have been calculated using isotopic techniques (Fisher et al., 1990). It is also possible to determine larval food sources using natural abundance isotope techniques. Kiyashko et al. (2004) demonstrated that dipteran larvae (*Stictochironomus pictulus* Meigen) fed mainly on methanotrophic bacteria had ^{13}C values ranging from –57.4 to –62.4 ^{13}C ‰ vs. PDB values that are characteristic of methanotrophic bacteria.

In species that have distinct aquatic larval stages, the signature of the structural tissue with low metabolic rate may reveal important information about the size and nature of larval breeding sites, as it is likely that larger water bodies will be less enriched in ^{18}O and ^{2}H than smaller ephemeral water bodies. Besides this, the ^{13}C signature of incoming terrestrial organic matter will also be different from that of native aquatic plant species. This could, for example, be useful in studying the location and characteristics of the breeding sites from which adult mosquitoes have emerged.

Akamatsu et al. (2004) used the inherent isotopic difference between aquatic and terrestrial diets to determine food preference of riparian spiders [*Argiope bruennichii* (Araneidae), *Nephila clavata* (Tetragnathidae), and *Tetragnatha praedonia* (Tetragnathidae)]. In aquatic environments,

the lower diffusion velocity of CO_2 in water means that carbon fractionation is relatively small thus aquatic plants usually have higher $\delta^{13}C$ values compared with terrestrial plant species (Yoshioka, 1997). In addition, $\delta^{14}N$ values may differ due to differences in nitrogen sources. The contribution of aquatic or terrestrial sources to the spider's diet was determined using two-source mixing models:

$$\delta^{13}C_{spider} - \Delta = \delta^{13}C_{aquatic\ insect} \times f + \delta^{13}C_{terrestrial\ insect} \times (1 - f),$$

where Δ is isotopic fractionation in carbon during the feeding process, and f is the relative contribution of the respective insects to the diet. Using these techniques, it was shown that more than 50% of the diet of web-building spiders consisted of aquatic insects. This method is subject to a number of assumptions: primarily, that the system is in isotopic equilibrium; second, that the diet composition and isotopic values of the food resources are constant; and finally, that the isotopic turnover of consumer tissue is rapid and reflects temporal changes.

McCutchan et al. (2003) suggest that care should be taken when the isotopic differences between two different food sources is small and when comparing isotopic shifts of samples prepared in different ways. They demonstrated that the calculated contribution from a specific source may differ by as much as 30%, depending on whether a trophic shift of +0.3 or +1.1‰ is assumed in experiments where food sources differ by only 4‰.

These problems could be overcome using an alternative end-point mixing model, as this does not require numerical values for fractionation, which are required by source-based models, because it assumes fractionation is similar for all species. The end-point mixing model compares observed values of a consumer with hypothetical values to determine if the consumer has eaten 0 or 100% of a particular resource. Using additional information from other resident species that feed on known specific diets, it then attempts to calculate diet constituents from this information (Lancaster et al., 2005).

Investigations of feeding strategies using enrichment techniques

Natural abundance techniques are limited, as they are dependent on the presence of isotopic differences of food sources to determine feeding strategies. Labelling of primary producers or prey with enriched isotopes is an alternative method to study food preference or food-web complexities, especially in tritrophic and multitrophic systems. Almost identical labelled and unlabelled primary producers or compounds differing in key components can be grown or formulated (Hood et al., 1999). This technique has been used to study feeding preference of adult *Anopheles arabiensis* Patton mosquitoes, fed sugar diets supplemented with methylparaben, an antimicrobial agent widely used in food and pharmaceutical products. In a simple two-treatment experiment, mosquitoes were offered a choice of unadulterated sugar water and sugar water with methylparaben added. ^{13}C glucose was added as a tracer in Treatment 1 to the unadulterated sugar, and in Treatment 2 to the methylparaben-amended sugar. Similar isotopic values of the mosquitoes in the two treatments would have inferred no preference; however, higher enrichments were seen in mosquitoes in Treatment 1, suggesting there was significant aversion to the methylparaben-amended sugar waters, particularly from the females, which are known to be more sensitive to odours (MQ Benedict, RC Hood-Nowotny, PI Howell & EE Wilkins, unpubl.).

Labelling plants with nitrogen is relatively simple, as it is possible to fertilize soil or nutrient solutions with labelled fertilizer nitrogen in a variety of forms, or glycine (Unsicker et al., 2005), which the plant will inevitably take up. It is also possible to label the plant only using stem/tree injection or leaf-labelling techniques that introduce ^{15}N into the plant only, allowing soil communities to be studied (Hood & Blair, 2001). ^{13}C labelling of plants using chamber or tent systems is relatively simple and could also be used for such studies (Hood et al., 2004).

Prey–predator relationships in field settings were studied using ^{15}N-labelled aphids (*Sitobion avenae* Fabricus). A range of predator species (Carabidae, Linyphiidae, Staphylinidae, and Coccinellidae) were identified and competitive relationships were established between species (Nienstedt & Poehling, 2000, 2004a,b). Fischer et al. (2003) studied the symbiotic relationship of ants (*Pheidole bicornis* Forel) and their Piperaceae host-plant species in natural settings. In a pulse experiment, ants were fed with highly labelled ^{15}N glycine in situ and the label traced into the ant colony. In further pulse chase experiments, Fischer et al. (2003) followed the fate of nitrogen excreted by the ants and transferred to the host-plant species. Even whole lakes have been labelled with ^{13}C, demonstrating an ecophysiological approach (Pace et al., 2004). These studies highlighted the utility of isotope labelling in natural settings and how this can be used to study resource allocation and turnover.

To study tritrophic or multitrophic systems, it could be useful to apply multiple and cross-labelling strategies. For example, a series of treatments are set up, in which the primary producer is labelled using ^{13}C or ^{15}N or not labelled. Primary and secondary consumers are then fed on the primary producer of the three treatments separately. These are then switched so that each treatment has a ^{13}C-labelled, ^{15}N-labelled, and an unlabelled trophic

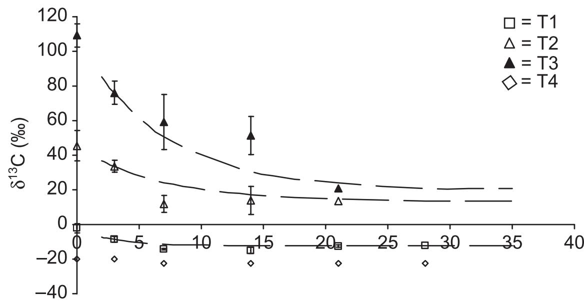

Figure 2 δ^{13}C of whole mosquito against time after emergence (days). T1–T3, 0.5, 2.5, or 5 mg of 99 atom % ^{13}C dried ground glucose per gram of larval food, mixed dry; T4, control unlabelled larval food only. Bars are ± 1 SD. The dashed lines are modelled data (after Hood-Nowotny et al., 2006).

level. By measuring the isotope label in the tertiary consumer, it is possible to determine whether there has been tritrophic feeding.

Diet assimilation and turnover

Bulk isotopic values of organisms raised on monotonous diets reflect the isotopic value of the diet, while the bulk isotopic value of organisms using more than one dietary source are a weighted average of the isotopic values of the different sources. Isotopic ratios in the tissues of organisms are a consequence of food source signature and tissue-specific fractionation and turnover processes, and therefore can reveal substantial information about the diet of the individual (Ostrom et al., 1997; Chamberlain et al., 2004). Uptake and turnover of specific resources can be calculated if a number of organisms are initially uniformly labelled and the change in enrichment, due to dilution effects of the label from unlabelled sources and loss of label through excretion or respiration, is monitored over time (Figure 2) (O'Brien et al., 2000; Hood-Nowotny et al., 2006). It has been established that turnover rates vary between body tissues (Tieszen et al., 1983) and that different tissues have distinctly different isotopic signatures relative to bulk values (Hobson & Clark, 1992). By switching the isotopic signature of the diet, it is possible to study the nutrient uptake of carbon, nitrogen, or sulphur, turnover, and assimilation in even the smallest of insects such as Collembola (Chamberlain et al., 2004). The isotopic values are retained in an isotopic memory related to the source values, growth, and the rate of turnover (Tieszen et al., 1983). Examination of isotopic values of different tissues reveals the temporal history of diet intake, whereas metabolically active pools such as blood provide information on recently acquired food resources and tissues that are more inert (such as cuticle) on longer timescales (e.g., skeletal tissue). Diet-switching experiments have been used to study the temporal dynamics of different tissues of two predacious ladybeetles *Harmonia axyridis* and *Coccinella septempunctata* (Coccinellidae), using aphids fed on C_3 soybean or C_4 maize. Isotopic signature in the body fat and reproductive organs changed rapidly, suggesting high metabolic rates and, as expected, change in the isotopic signature was slower in the more metabolically inert tissues such as wings and cuticle (Gratton & Forbes, 2006).

In insects with distinct larval stages, structural tissue may reveal information about larval food sources and habitats, as evidence suggests that signatures from larval diets are 'fixed' in the insect (Hood-Nowotny et al., 2006). For example, the European corn borer (*Ostrinia nubilalis* Hübner) essentially retain their larval food δ^{13}C signatures in the wing tissues with only minimal variations (<5%) owing to adult diets (Ponsard et al., 2004). This information could be used to trace back the juvenile habitat of the insect and control or protect the site. It has been demonstrated that in insects that grow from a nymph form and moult, for example, locusts, structural chitin turned over fastest of all components studied (Webb et al., 1998). These physiological differences stress that species-specific complimentary isotope studies are important in establishing underlying mechanisms prior to field application of isotope techniques.

Compound-specific studies

The rates of dietary incorporation into specific compounds, such as specific lipids or amino acids, can be established using compound-specific mass spectrometry, in which compounds are combusted following separation on a gas chromatography column (GC-C-MS). These types of study can lead to a better understanding of the biochemistry of an organism, especially those that are almost impossible to study using conventional observation and feeding studies such as Collembola (Chamberlain et al., 2004). Using these switching-type experiments, it is also possible to distinguish between assimilation and synthesis pathways. For example, if all lipids are produced by de novo synthesis, a similar value δ^{13}C of all lipids would be expected as they are all derived from the common constituent acetyl coenzyme A; any deviation from this value would suggest uptake as opposed to synthesis (Chamberlain et al., 2004). A similar principle has also been used to study defence compounds in chrysomelild beetles (*Gastrophysa viridula* Degeer and *Phaedon cochleariae* Fabricius; Soe et al., 2004) and sex pheromone biosynthesis in moths (*Planotortrix excessana* Walker; Bjostad & Roelofs, 1986).

Table 3 Summary of isotopic techniques used in entomological studies

Method	Insect	Reference
Insect marking		
Enrichment-^{13}C	Mosquitoes	Hood-Nowotny et al., 2006
Enrichment-^{15}N	Locusts	Unsicker et al., 2005
Movement		
Enrichment-^{15}N	Stoneflies	Macneale et al., 2004, 2005
Enrichment-^{15}N	Mayflies	Hershey et al., 1993
Natural abundance	Butterflies	Wassenaar & Hobson, 1998; Hobson et al., 1999
Diet analysis and feeding behaviour		
Predation		
Enrichment-^{15}N	Aphids	Nienstedt & Poehling, 2000, 2004a,b
Natural abundance	Ladybird beetles/aphids	Ostrom et al., 1997
Feeding preference		
Natural abundance	Ants	Trimble & Sagers, 2004
Natural abundance	Termites	Tayasu et al., 1998
Natural abundance	Diptera	Kiyashko et al., 2004
Natural abundance	Ants	Fischer et al., 1990
Natural abundance	*Drosophila*	Markow et al., 2000
Dietary allocation and turnover		
Natural abundance	Butterflies	Fisher et al., 2004
Natural abundance	Moths	O'Brien et al., 2000
Enrichment-^{15}N	Ants	Fischer et al., 2003
Natural abundance	Lady beetles	Gratton & Forbes, 2006
Food web analysis		
Natural abundance		Scrimgeour et al., 1995; Ponsard & Arditi, 2000; Scheu & Falca, 2000
Diet		
Natural abundance	Locusts	Webb et al., 1998
Natural abundance	Collembola	Chamberlain et al., 2004
Mating		
Natural abundance	European corn borer	Ponsard et al., 2004; Malausa et al., 2005 Dalecky et al., 2006
Biosynthesis		
Natural abundance	Beetles	Soe et al., 2004
Enrichment-^{13}C and ^{2}H	Moths	Bjostad & Roelofs, 1986

Mating and competition

The study of insect mating has traditionally been based on observation and/or use of fluorescent or radioactive markers (Dame & Schmidt, 1964; Smittle et al., 1969). In insects with large spermatophores (a solidified droplet of sperm and nutritional compounds, which is transferred from the male to the female during mating), it is possible to use the natural differences in C_3 and C_4 diets to study sperm transfer (Ponsard, 2004). However, in smaller insects, labelling techniques offer the possibility of tracing labelled sperm into unlabelled females. Similar studies using radio isotopes demonstrated the proof of principle (Smittle et al., 1969). Stable isotopes have the advantage over radioisotopes that they do not cause detrimental effects, although deuterium at concentrations of more than 15% of the diet can prove fatal due to its large relative mass compared to the lighter isotope and consequent enzymatic fractionation.

Differences in the isotopic signature of spermatophores of European corn borers (*O. nubilalis*) reared on different C_3 and C_4 host-plant species were used to determine whether moths from a specific host species mate only with other moths from the host species (assortative mating) or whether there is cross-host-species mating. Initial experiments showed that the signature of the spermatophores reflected the male larval-host-plant type (Ponsard, 2004). In field experiments it was also shown that moths that develop on different host plants exhibit almost absolute reproductive isolation (Malausa et al., 2005).

Studies of male mosquitoes labelled with ^{13}C have shown that it is possible to trace the fate of labelled sperm into female spermathecae (Helinski et al., 2007). These techniques are particularly useful in mating competition studies, particularly in semifield settings that attempt to mimic more holistic systems and that include the presence of predators, where the presence of fluorescent markers may influence mating behaviour or predation.

Case study

The broad scope for the potential use of stable isotopes in ecological and biological studies of insects is evident from the literature presented above and is summarized in Table 3. Exploring the potential uses of stable isotopes in a specific context, such as the malaria/mosquito system, demonstrates how isotopic techniques could be used to understand fundamental questions (Figure 3) such as the ecology of sugar feeding, mating, host seeking, survival, and oviposition. To study sugar feeding behaviour, the distinctive isotopic signatures of some plants (such as biological nitrogen fixers and C_3 and C_4 plants) could be used as tracers. Using either natural signatures or isotopically enriched plants, determination of species preference is also possible. Resource turnover rates of a labelled population can be established in semifield and field settings, thereby providing simple but vital information in complex systems.

Using highly labelled isotopes for determination of sperm transfer in the laboratory has been demonstrated and these techniques could easily be scaled up to semifield systems. In addition, studying the range and dispersal of labelled sperm through a population is possible. As most stable isotopes are non-toxic and are routinely used for diagnostic purposes in medical research, mosquito-feeding trials, in which human adults are 'labelled up' through supplementary feeding with stable isotopes, may be useful for host-preference studies, drug and repellent testing, etc., in 'real' environments. A labelled blood source also provides an easily identifiable point source for post-feeding dispersal studies. Tracing of labelled blood to determine resource allocation to the eggs or other tissues could also provide useful physiological information.

Stable isotope marking allows the study of food-web structure, resource flow, and predator pressure. Natural signatures can also be used to provide invaluable information about these processes from a holistic perspective. Care should be taken, however, to understand the processes contributing to the isotopic signatures in the system.

Identification or assessment of breeding sites using both natural and artificially induced isotopic signatures may provide detailed information allowing a strategic approach to larval control. Isotopes could also be a useful tool to elucidate larval feeding strategies. Isotopic information stored in slow turnover tissues of mosquitoes, such as chitin, may provide valuable information about the natal origin of the mosquitoes.

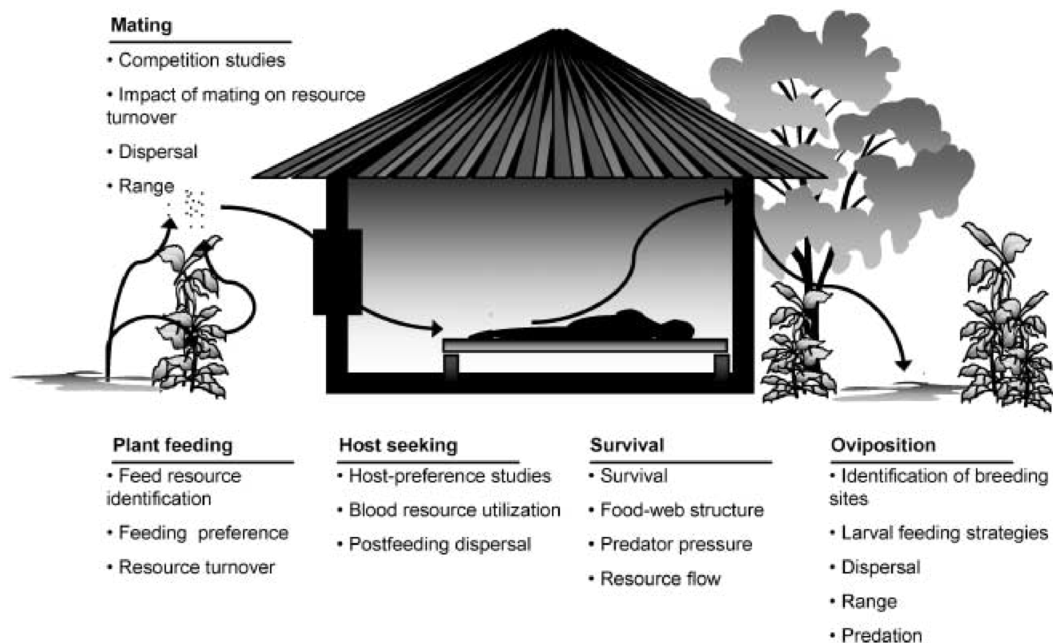

Figure 3 Potential application of stable isotopes in malaria mosquito research.

Conclusions

As apparent from this Mini Review, there is a wide range of potential applications of stable isotopes in arthropod studies, suggesting a budding branch of learning, which, coupled with the development of simpler and cheaper technologies for isotope analysis, could provide the opportunity of many of the fundamental unknowns of entomological research to be addressed using an ecosystems approach. Although the biological and physical processes that lead to differences in the natural abundance of stable isotopes provide a valuable tool for entomologists and ecologists, care should be taken to account for all the processes that may influence the pattern of isotopic distribution in the insect's environment. This in itself should promote a more holistic understanding of these processes. Most isotope studies benefit immensely from more reductionist laboratory-based experiments to understand specific processes within the system.

The opportunity for the blossoming of ecosystem entomological research through the application of stable isotope science is evident; it will require an open-learning, interdisciplinary approach, motivated by the desire to understand the role of insects in a complex world.

There are a number of learning resources available to both the isotope novices and veterans alike. Iso-geochem is a forum that will field all sorts of questions and queries (http://list.uvm.edu/cgi-bin/wa?A0=ISOGEOCHEM) and is read by the majority of isotope scientists (not only geologists). In addition, in most European countries and the USA, there are mass spectrometry users groups who usually meet annually to discuss the use of stable isotopes in diverse fields. For details of the European groups, see the JESIUM website (http://chemsrv0.pph.univie.ac.at/jesium/).

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