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Stable isotopes, ecological integration and environmental change: wolves record atmospheric carbon isotope trend better than tree rings

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Large-scale patterns of isotope ratios are detectable in the tissues of organisms, but the variability in these patterns often obscures detection of environmental trends. We show that plants and animals at lower trophic levels are relatively poor indicators of the temporal trend in atmospheric carbon isotope ratios ($\delta^{13}C$) when compared with animals at higher trophic levels. First, we tested how differences in atmospheric $\delta^{13}C$ values were transferred across three trophic levels. Second, we compared contemporary $\delta^{13}C$ trends (1961–2004) in atmospheric CO_2 to $\delta^{13}C$ patterns in a tree species (jack pine, *Pinus banksiana*), large herbivore (moose, *Alces alces*) and large carnivore (grey wolf, *Canis lupus*) from North America. Third, we compared palaeontological (approx. 30 000 to 12 000 ^{14}C years before present) atmospheric CO_2 trends to $\delta^{13}C$ patterns in a tree species (*Pinus flexilis*, *Juniperus* sp.), a megaherbivore (bison, *Bison antiquus*) and a large carnivore (dire wolf, *Canis dirus*) from the La Brea tar pits (southern California, USA) and Great Basin (western USA). Contrary to previous expectations, we found that the environmental isotope pattern is better represented with increasing trophic level. Our results indicate that museum specimens of large carnivores would best reflect large-scale spatial and temporal patterns of carbon isotopes in the palaeontological record because top predators can act as ecological integrators of environmental change.

Keywords: stable isotopes; CO_2; $\delta^{13}C$; ecological integrator; palaeoenvironment; wolf

1. INTRODUCTION

There is considerable interest in inferring environmental attributes and climate histories based on stable isotope ratio data derived from animals (Koch 1998; Kohn & Cerling 2002; Hedges *et al.* 2004; Leng 2004; Hoppe *et al.* 2006; West *et al.* 2006). Such inferences are based on the logic that since producer isotopic values reflect, in part, their growing conditions (Dawson *et al.* 2002), then consumer isotopic values in turn reveal information about the producer's environment. However, few experimental isotopic studies have examined the validity of such inferences and/or established general patterns in the transfer of environmental information across trophic levels.

A useful approach to understand the fidelity of environmental records at the producer level inferred from consumer-derived isotope data is to conduct complementary observational and experimental analyses. Although such an approach is frequently used to infer dietary histories from stable isotope records from animal tissues (Macko *et al.* 1982; Hobson & Schwarcz 1986; Hilderbrand *et al.* 1996; Ostrom *et al.* 1997), similarly derived climate proxies often lack corresponding experimental studies. The call for more comparative experiments to properly reconstruct dietary, trophic level and body condition based on stable isotope analysis (Gannes *et al.* 1997) is equally pertinent to reconstructing climate histories and habitat use.

In contemporary and palaeontological climate studies, the stable isotope values of carbon ($\delta^{13}C$) have proved particularly useful. For example, ecosystem changes such as carbon starvation in trees growing under conditions of reduced atmospheric CO_2 concentration have been studied using $\delta^{13}C$ analysis of fossil plant tissues (Ward *et al.* 2005). Also, in the context of current climate change issues (Karl & Trenberth 2003; Kennedy 2004) and research on global carbon cycling (Falkowski *et al.* 2000), atmospheric $\delta^{13}C$ chronologies are of interest to researchers modelling past and future atmospheric CO_2 scenarios (Ciais *et al.* 1995, 2005; Rayner *et al.* 1999; Nakicenovic & Swart 2000).

Since plants assimilate atmospheric CO_2 directly, it has been argued that shifts in atmospheric $\delta^{13}C$ values would be most accurately recorded in plant tissues (Dawson *et al.* 2002; Long *et al.* 2005). Consumer tissues are thought to more poorly reflect isotopic patterns in atmospheric CO_2 owing to variations in diet, assimilation efficiencies of dietary components, isotope fractionation (a change in isotope ratios between source substrate and products), tissue turnover and differential allocation of nutrients among tissues (Gannes *et al.* 1997; Long *et al.* 2005). However, ecological theory suggests that organisms at higher trophic levels act as integrators, linking lower

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pathways in space and time (de Ruiter *et al.* 2005). Primary producer tissue reflects an integration of the environmental conditions over the time of tissue growth. Herbivores feed upon numerous primary producers, and carnivores ingest tissues from multiple herbivores. Hence, stable isotope values derived from organisms at higher trophic positions may effectively increase sample size, thereby reducing variation (i.e. a carnivore represents multiple herbivores, which in turn represent many primary producers).

Here, we present the results of one experiment and two observational studies designed to independently investigate patterns of $\delta^{13}C$ records across trophic levels. In the experimental test, we expected the variability in $\delta^{13}C$ values to decrease with increasing trophic level based on the untested idea that effective sample size is greater at higher trophic levels, and thereby should statistically provide less variable reflections of environmental factors. The observational studies explored the ability of contemporary species to act as ecological integrators of trends in atmospheric $\delta^{13}C$ values and concentrations over historical and palaeontological time scales. In each study, we focus on the dominant global atmospheric variable ($\delta^{13}C$ value and CO_2 concentration) responsible for temporal variability in plant and animal $\delta^{13}C$ values over a given time period. The dietary sources of carbon for insect and mammalian consumers are predominately plant-derived carbohydrates (for herbivores) and protein-rich body tissues (for carnivores; Hedges 2003). Both insect and mammalian carnivores ingest a range of prey body tissues; but, as whole-body consumers, we might expect insect carnivores to use a wider range of dietary carbon sources. Thus, we do not directly compare the experimental insect and observational mammalian consumer datasets, but instead report similar trends in isotopic variance with relation to trophic level.

2. MATERIAL AND METHODS

First, we describe our experimental study in which we tested how differences in atmospheric $\delta^{13}C$ values were transferred across a three-trophic level food chain (cabbage plant–caterpillar–insectivore). We manipulated the atmospheric $\delta^{13}C$ values in semi-contained model systems and then measured the $\delta^{13}C$ values for the growth chamber air and the organismal food chain. This experiment tested the theoretical prediction that the variability in $\delta^{13}C$ values would decrease with increasing trophic level because consumers act as ecological integrators of environmental $\delta^{13}C$ conditions. Second, we describe our two analyses of $\delta^{13}C$ chronologies from organisms at three trophic levels (producer–herbivore–carnivore). In the first analysis, we relate historic to modern organismal chronologies to corresponding changes in atmospheric $\delta^{13}C$ values. In the second analysis, we compare fossil plant and animal $\delta^{13}C$ chronologies with late Pleistocene changes in the CO_2 concentration of the atmosphere.

(a) *Experimental study*

Producers were grown in open top chambers (OTCs) in which atmospheric $\delta^{13}C$ values were manipulated. Insect herbivores fed *in situ* on producers within the OTCs and were subsequently fed to an insectivore. $\delta^{13}C$ values were measured for the OTC environment and for organisms at all three trophic levels, thereby permitting the assessment of how precisely atmospheric $\delta^{13}C$ values are reflected at each trophic position.

Cabbage (*Brassica oleracea* var. *capitata*) was chosen for this study owing to its rapid growth rate, small size, ease of cultivation, seed availability and palatability to common insect herbivores. Approximately two to three weeks after greenhouse germination twelve 15 cm pots, each containing one plant, were placed into each of ten 0.5 m^3 OTCs. The OTC construction followed that of Drake *et al.* (1989). OTCs were arranged into five blocks with each block containing one of two randomly assigned treatments: one relatively ^{13}C-enriched (hereafter 'heavy') OTC and one ^{13}C-depleted (hereafter 'light') OTC. Heavy and light ^{13}C treatments were obtained by elevating CO_2 above ambient levels in all OTCs using two isotopically different CO_2 sources mixed with atmospheric air: heavy treatment OTCs ($n=5$) received additional CO_2 with a $\delta^{13}C$ value of $-11‰$ and light treatment OTCs ($n=5$) received additional CO_2 with a $\delta^{13}C$ value of $-35.5‰$. The mean (± 1 s.d.) CO_2 concentration within each OTC (528 ± 10 ppm) was monitored automatically via continuous sampling of chamber air and an infrared CO_2 gas analyser for 3 min at least every 5 hours (Drake *et al.* 1989). CO_2 flow to each OTC was adjusted via manual rotometer. Cabbages were watered daily and fertilized once per week (100 ml containing 25 mg of Miracle Grow 15-30-15 fertilizer).

Egg-laying female cabbage butterflies (*Pieris rapae*) were net captured locally and held (approx. two weeks) in a net enclosure ($2 \times 2 \times 2$ m) with a sufficient food source (*Lantana camara*). Eight days after plants were placed in OTCs, 1–2 butterflies were transferred to each OTC and contained by temporarily screening the chamber top. Egg laying on multiple plants in each chamber was confirmed visually; if egg laying did not readily occur (i.e. within approx. 1 hour), butterflies were released and replaced with one to two new individuals until egg laying was observed. Caterpillars were allowed to feed *in situ* for the duration of the experiment (approx. eight weeks).

Eggs of spined soldier bugs (*Podisus maculiventris*) were obtained from Rincon-Vitova Insectaries, Inc. (Ventura, CA). Soldier bugs were reared until the fifth instar on the experimentally raised *P. rapae* caterpillars at 25 and 20°C, coinciding with a 16 : 8 hours light : dark cycle in an environmental chamber. Larvae were housed and fed daily in 100×15 mm plastic Petri dishes containing moistened filter paper, which was replaced daily. Two Petri dishes, each containing 2–5 soldier bug larvae, corresponded to each OTC to ensure adequate survival and archive tissue. Soldier bug larvae were fed caterpillars (randomly selected from plants) of the same instar daily.

Air samples for isotopic analysis were collected from each OTC four times during the experiment: once when plants were first placed into each chamber and then one, four and eight weeks subsequently. Air from each chamber was collected between 22.00 and 24.00 hours in septum-capped 10 ml vials, previously evacuated with argon gas.

Cabbage leaf tissue, caterpillars and soldier bug larvae corresponding to each growth chamber were randomly selected for elemental and isotopic analysis once soldier bug larvae reached the fifth instar. Two caterpillars of the most recent instar fed to the soldier bugs (i.e. fourth) were randomly selected from chambers; one for elemental analysis and the other for isotope analysis. The entire cabbage leaf on

which the caterpillars were found was collected for analysis. Caterpillars tended to feed on newer leaves, hence leaf samples were of a similar age. Caterpillars were isolated for at least 24 hours before analysis to evacuate gut contents. All samples, plant and insect, were double rinsed with distilled water, air dried and then lyophilized. Leaf tissues were ground to a fine powder in a mill (model 8000M, SPEC CertiPrep, Inc., Metuchen, NJ).

Lipids were removed from insect samples because during biosynthesis lipids can become depleted in ^{13}C (DeNiro & Epstein 1977) resulting in lower $\delta^{13}C$ values for lipids versus proteins and carbohydrates; hence, whole-body $\delta^{13}C$ can vary appreciably with lipid levels even if diet is constant. To extract lipids prior to isotope analysis, insect samples (i.e. caterpillars and soldier bugs) were soaked in 1 : 1 chloroform : methanol solution three times, sonicated for 10 min each time and then rinsed with distilled water and air-dried (Focken & Becker 1998; Sotiropoulos *et al.* 2004). Once dry, insect samples were ground to a fine powder using a mortar and pestle.

Isotopic analysis was conducted at the University of Michigan Biological Station's Analytical Laboratory on a ThermoFinnigan Delta^{plus} Continuous-Flow Stable Isotope Ratio Mass Spectrometer interfaced with a GasBench II and a Costech 4010 Elemental Analyzer. Gas sample injection volume was 100 µl to generate output voltage peaks of approximately 400 mV at $m/e=44$. Five injections were done per gas sample. Stable isotope values are reported in standard δ notation and are referenced to Vienna PeeDee Belemnite. Precision based on repeated measures of internal standards was ± 0.25‰ for $\delta^{13}C$.

Separate elemental analysis was done to assess gross nutritional quality of producers and herbivores. Ground cabbage leaf tissues and caterpillar tissues without lipid extraction were combusted in a Fisons NA1500 Elemental Analyzer at Michigan Technological University's Ecosystem Science Centre to determine total carbon and nitrogen.

Homogeneity of variance tests and *t*-tests were used to test for differences in equality of variance across trophic levels, in isotopic fractionation between atmosphere and plant, with each trophic step, and in nutritional quality between heavy and light treatment producers and herbivores. These comparisons tested the *a priori* hypotheses that $\delta^{13}C$ variability would decrease with increasing trophic level, hence differences where $p<0.05$ were considered significant.

(b) *Observational study—Isle Royale*

We compared trends (1961–2004) in the $\delta^{13}C$ values of atmospheric CO_2 to $\delta^{13}C$ values in a species of tree (jack pine, *Pinus banksiana*), large herbivore (moose, *Alces alces*) and large carnivore (grey wolf, *Canis lupus*) from North America. Annual $\delta^{13}C$ values of atmospheric CO_2 for 1962–1975 ($n=8$) were obtained from high-precision ice core records (Francey *et al.* 1999) and for 1978–2002 ($n=25$) from direct atmospheric measurements (Keeling *et al.* 2005). Jack pine $\delta^{13}C$ data for 1974–1994 were obtained from tree ring ($n=93$) cellulose from Manitoba and Saskatchewan (Ehleringer *et al.* 1998). Moose ($n=59$) and wolf ($n=47$) bone collagen samples for 1961–2004 were drilled from Isle Royale (Lake Superior, USA) specimens using a handheld Dremel microdrill. All available Isle Royale wolf specimens were sampled; moose specimens were selected indiscriminately from all samples available (more than 4000) to mimic field collection. Fifty micrograms of samples were decalcified in 0.5 N HCl for 1–2 days at 4°C and were then rinsed and dried. Samples were lipid extracted in five rinses of a 2 : 1 chloroform and methanol solution, with sonication of each rinse for 0.5 hours. For stable isotope analysis, collagen samples (1.0 mg) were weighed into precombusted tin capsules. Stable carbon isotope ratios were measured using an elemental analyser coupled with a mass spectrometer (Europa Hydra 20/20) at the University of California Davis Stable Isotope Facility. The standard deviation for replicates of a gelatin standard analysed with the collagen samples was <0.2‰ for carbon.

Since most plants in boreal ecosystems assimilate atmospheric CO_2 through the C_3 photosynthetic pathway, our Isle Royale $\delta^{13}C$ records are not complicated by changes in the relative abundances of C_3 and C_4 plants (Ehleringer & Monson 1993). We intentionally chose a tree species (i.e. jack pine) from a mainland location to reduce the confounding effect of elevation on carbon isotope discrimination (Sparks & Ehleringer 1997). Consequently, the variation exhibited in the jack pine chronology used here is likely to be less (i.e. more conservative) than would be expected for Isle Royale jack pine, where elevation changes (up to 238 m) exceed those of the mainland sites (Ehleringer *et al.* 1998).

Moose do not prefer jack pine as browse species, but this is of negligible consequence to our analysis because the range in $\delta^{13}C$ values of plants that Isle Royale moose consume (-20 to -30‰; Tischler 2004) encompasses the $\delta^{13}C$ values of the mainland jack pine. Isle Royale moose and wolves are linked through a strong predator–prey interaction; on average, moose comprise approximately 90% of wolf diet (Thurber & Peterson 1993) and kills are well used (i.e. soft tissue, hide, brain and long-bone marrow are all consumed). Based upon conservative estimates of moose kill rates at Isle Royale (Vucetich *et al.* 2002), a likely minimum of 150–200 moose contribute to a wolf's diet over the course of its lifetime (approx. 5 years; Vucetich & Peterson 2004).

We highlight that the amount of time represented by producer and consumer samples varies depending upon what tissue is analysed. In general, wolf and moose bone collagen $\delta^{13}C$ values reflect a longer time span (multiple years) of resource usage than individual tree ring cellulose $\delta^{13}C$ values (one tree ring=1 year of atmospheric CO_2 assimilation). Two-thirds of all Isle Royale wolves die before the age of 5 years (Vucetich & Peterson 2004) and only 4 of the 47 wolves sampled in this study were certainly older than 5 years. The mean life expectancy at age 1 year for Isle Royale moose is 7.3 years (Peterson 1977) and 22 of the 59 moose sampled in this study were young (less than 2 years), rapidly growing individuals, the remainder were from older individuals (mean age 12.3 years). To make consumer and producer tissue records comparable, we smooth the jack pine tree ring $\delta^{13}C$ data using a 5-year running average, a timeframe that is equivalent to the average turnover time of bone collagen (Hobson & Clark 1992) and wolf life expectancy. Note that Hobson & Clark's (1992) analysis of ^{13}C turnover in tissues involved the use of growing animals, and therefore likely underestimates carbon turnover time in adults. We also explored the effects of 8- to 10- and 12-year running averages because of the older ages of a portion of the moose specimens.

(c) *Observational study—La Brea and Great Basin*

We compared trends (approx. 12 000–30 000 of ^{14}C years before present (kyr ago BP)) in the concentration of atmospheric CO_2 to $\delta^{13}C$ values of late Pleistocene Great

Basin (Arizona, Utah, Nevada) pack rat midden limber pine (*Pinus flexilis*) needle cellulose ($n=31$; Van de Water *et al.* 1994) and La Brea tar pit (southern California) juniper (*Juniperus* sp.) wood cellulose ($n=11$; Ward *et al.* 2005), and La Brea bison (*Bison antiquus*, $n=31$) and dire wolf (*Canis dirus*, $n=73$) bone collagen (Coltrain *et al.* 2004). Atmospheric CO_2 concentrations for 12–30 kyr ago BP ($n=120$) were obtained from ice core records (Neftel *et al.* 1988; Staffelbach *et al.* 1991; Marchal *et al.* 1999) and are plotted with the plant and animal records to graphically demonstrate the synchrony of atmospheric CO_2 changes with plant and animal $\delta^{13}C$ chronologies.

We intentionally chose a carnivore inferred to have wide dietary breadth in order to examine the relationship between variance in $\delta^{13}C$ values and trophic level in a complex ancient food web. While bison likely contributed to dire wolf diet, they were not the only megafaunal prey available to La Brea carnivores. Isotopic food web reconstructions for La Brea mammalian megafauna (Coltrain *et al.* 2004) show that dire wolf diet was variable, and potentially included all of the abundant grazing and browsing herbivore species at the tar pits (e.g. horse, bison, camel, ground sloth, mastodon). The cranial morphology and levels of tooth breakage in La Brea dire wolves also suggest that they were either generalized predators of large prey or scavengers (Binder *et al.* 2002).

We compare $\delta^{13}C$ values of co-occurring La Brea fauna and juniper trees, but due to the paucity of the juniper dataset we also include $\delta^{13}C$ values from an extensive Great Basin limber pine dataset (Van de Water *et al.* 1994). The juniper data are included solely for visual comparison and are not included in statistical analyses. La Brea bison were grazers (Coltrain *et al.* 2004) and likely did not consume juniper or limber pine. Therefore, our study does not involve directly interacting plants and animals, but instead focuses on how organismal $\delta^{13}C$ records reflect atmospheric changes at the regional scale.

Juniper and limber pine were directly dated by accelerator mass spectrometry ^{14}C analysis. Van de Water *et al.* (1994) normalized the limber pine $\delta^{13}C$ values to modern sea level, but concluded that latitudinal corrections were not necessary. Bone collagen dates reflect the age of the pit from which they were collected. Pit ages in kyr ago BP were 61 and 67 (12 kyr ago BP), 13 (15 kyr ago BP), 3 (15 kyr ago BP), 60 and 91 (26–28 kyr ago BP) and 77 (28–33 kyr ago BP). Thus, for statistical analyses (limber pine data only) and graphical presentation, the limber pine and juniper data were binned according to the age ranges of the La Brea tar pits. Herbivore $\delta^{13}C$ data for pits 3, 60, 61, 67 and 91 were from Coltrain *et al.* (2004); dire wolf $\delta^{13}C$ data from pits 3, 60 and 91 were from Coltrain *et al.* (2004). Collagen $\delta^{13}C$ data from the remaining pits are presented here for the first time.

Fossil bone collagen extraction and preparation for the new $\delta^{13}C$ data presented here followed the methods in Fox-Dobbs *et al.* (2006). In brief, approximately 120 mg bone samples were crushed to a coarse powder, continuously rinsed with solvents (petroleum ether and acetone, 24 hours each) in a soxhlet extractor to remove tar, and then decalcified as above. The collagenous residue was gelatinized in 0.01 M HCl at 57°C for 12 hours and then passed across a 1.5 μm glass-fibre filter, with retention and lyophilization of the filtrate. Carbon isotope analysis was as previously described for the Isle Royale samples and done at the University of California Davis Stable Isotope Facility.

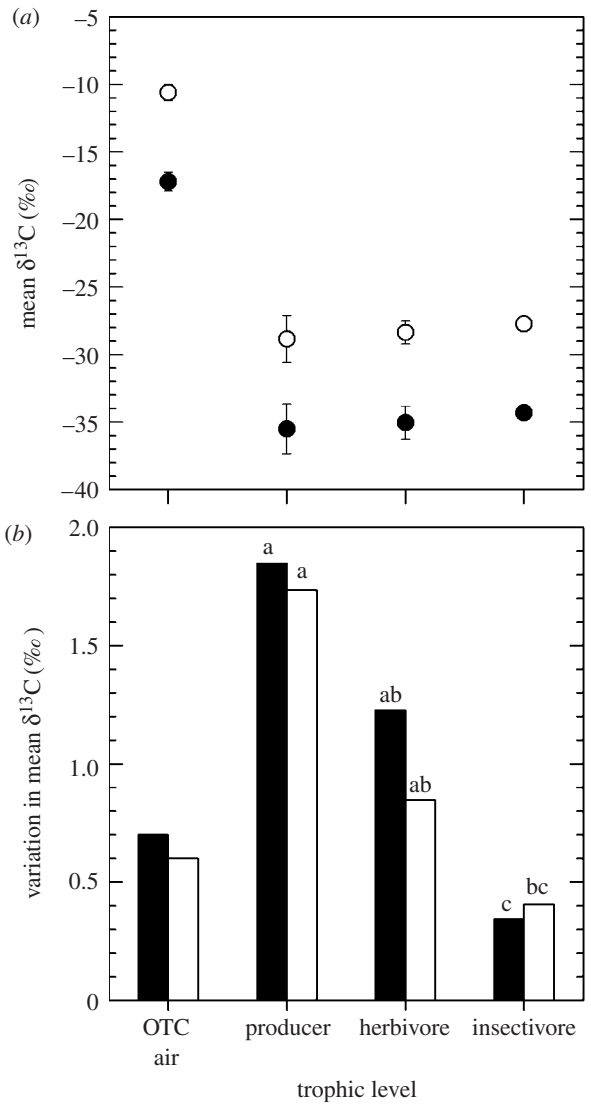

Figure 1. $\delta^{13}C$ values for experimental growth environment and across trophic levels; symbols and bar fills correspond to relatively heavy (open) or light (closed) CO_2 treatment sources. (*a*) Points (mean ± 1 s.d.) indicate $\delta^{13}C$ values for CO_2 of open top chamber (OTC) air and tissues at each trophic level. Note OTC air treatment difference is reflected at each trophic level. (*b*) Bars indicate standard deviations of mean $\delta^{13}C$ values measured for CO_2 of OTC air and tissues at each trophic level. Bars labelled with different lower case letters are significantly different.

Standard deviations of the mean $\delta^{13}C$ values were calculated for 12 and 15 kyr ago BP samples for each trophic level; means for other time periods were not analysed statistically due to small sample sizes. Homogeneity of variance tests ($\alpha=0.05$) were used to test differences in equality of variance between trophic levels by sample age.

3. RESULTS

(a) *Experimental*

The heavy and light CO_2 additions resulted in significantly different OTC air, leaf, caterpillar and soldier bug $\delta^{13}C$ values (figure 1). Standard deviations of mean insectivore $\delta^{13}C$ values were significantly less variable than herbivore values for the light treatment ($F_{1,8}=12.7$, $p=0.03$, $n=5$) and less variable than producers for both treatments (heavy, $F_{1,8}=18.3$, $p=0.016$, $n=5$; light, $F_{1,8}=28.9$, $p=0.007$, $n=5$; figure 1).

Producer and herbivore C : N ranged between 21.6–21.8 ($n=10$) and 4.8–5.2 ($n=10$), respectively, and did not differ between treatments (producers, $t_8=0.11$, $p=0.91$; herbivores, $t_8=1.25$, $p=0.25$). This result indicates that the gross forage quality for herbivores and prey for insectivores did not likely differ across treatments.

Isotope discrimination values (mean ± 1 s.d.) for producers (heavy $=18.3\pm2.0$‰, $n=5$; and light $=18.3\pm2.6$‰, $n=5$) did not differ across treatments ($t_8=0.04$, $p=0.97$), indicating equivalent gas exchange and photosynthetic activity between treatments. Trophic shifts (mean ± 1 s.d.) for herbivores (heavy $=0.4\pm1.2$‰, $n=5$; and light $=0.4\pm2.0$‰, $n=5$) and insectivores (heavy $=0.6\pm0.8$‰, $n=5$; and light $=0.7\pm1.3$ ‰, $n=5$) did not differ across treatments ($t_8=0.02$, $p=0.98$; and $t_8=0.14$, $p=0.89$, respectively). These fractionation results associated with biochemical discrimination against ^{13}C fall within typical variation ranges for C_3 plants (Dawson *et al.* 2002) and trophic shifts for animals (McCutchan *et al.* 2003).

(b) *Observational*

Least squares regression analysis indicated that $\delta^{13}C$ chronologies for each trophic level were significantly related to time, reflecting the decline in $\delta^{13}C$ values observed in atmospheric CO_2 ($p<0.01$ for each trophic level). Examination of numerical measures of leverage for the wolf, moose and jack pine data did not reveal cases with excessive influence. Atmospheric carbon dioxide has increased 31% since pre-industrial times, from 280 parts per million by volume (ppmv) to more than 370 ppmv today, and is currently increasing at an annual rate of approximately 0.76% due to anthropogenic inputs (Karl & Trenberth 2003). Fossil fuel and biomass combustion release isotopically light (relative to atmospheric CO_2 $\delta^{13}C$ values) CO_2 into the atmosphere, which explains the observed depletion trend in the $\delta^{13}C$ chronologies (figure 2; Keeling *et al.* 2005).

Accepting the atmospheric $\delta^{13}C$ depletion trend as a factual environmental signal, we consider the R^2 value a suitable indicator of signal-to-noise ratio at each trophic level (figure 2). The $\delta^{13}C$ chronology R^2 value is $5\times$ higher for wolves than the 5-year smoothed tree rings; $2.5\times$ times higher for moose than the 5-year smoothed tree rings; and the slope of the wolf regression is closest to the slope exhibited for atmospheric CO_2 regression (figure 2). An 8- and 9-year smoothing interval lowered the tree ring R^2 to 0.10 and 0.11, respectively. With 10-year smoothing, the tree ring R^2 remained at 0.12 (the same as in the 5-year smoothing) and the 12-year smoothing raised the R^2 slightly to 0.14. Slope coefficients for these larger smoothing intervals ranged from -0.018 to -0.021, which were not significantly different from the tree ring slope of -0.021 found for the 5-year smoothing interval.

There is a noteworthy decrease in the collagen $\delta^{13}C$ values of fauna (herbivore and carnivore) from the La Brea tar pits during the last glacial–interglacial transition (approx. 15 to 12 kyr ago BP; shaded box figure 3). A similar shift in $\delta^{13}C$ values is recorded with greater noise (i.e. increased variation) in the $\delta^{13}C$ values of contemporaneous Great Basin limber pine trees. These shifts record a past event of major change in the global CO_2 concentration of the atmosphere (figure 3; see §4 below).

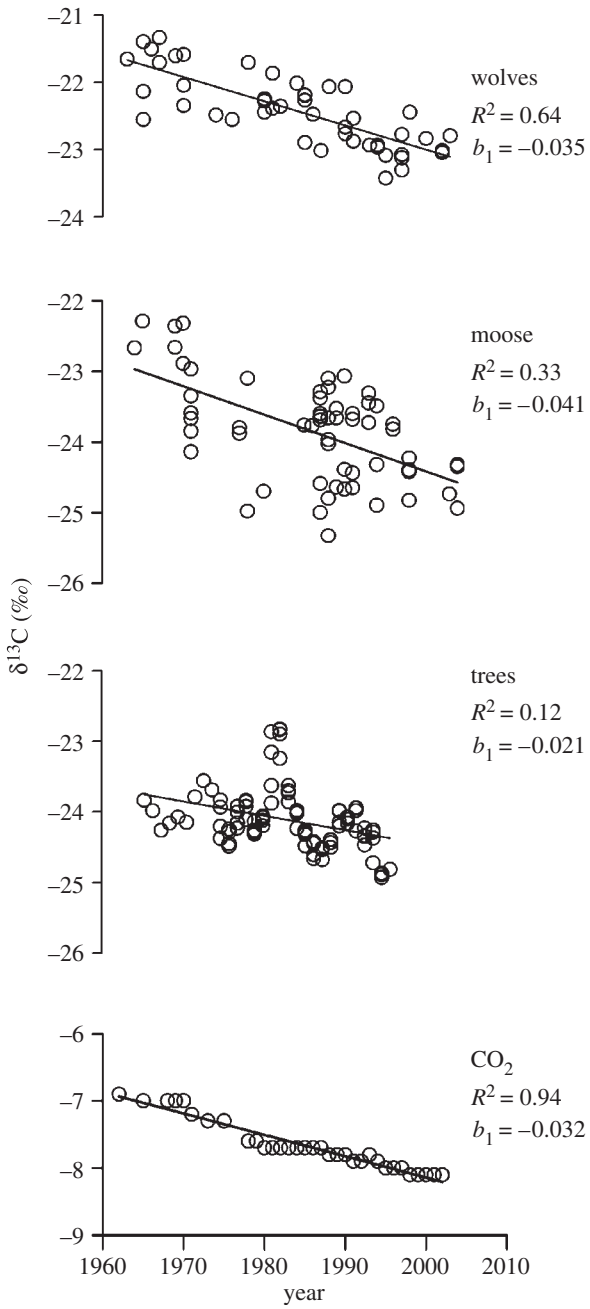

Figure 2. The relationship between year and $\delta^{13}C$ values for global atmospheric CO_2, North American jack pine (*P. banksiana)* tree ring cellulose, and Isle Royale moose (*A. alces*) and wolf (*C. lupus*) bone collagen. $\delta^{13}C$ data are reported in per mil (‰) relative to Pee Dee Belemnite (PDB) limestone standard. Each panel includes individual $\delta^{13}C$ values (open symbols) and a simple linear regression line for the relationship between time and $\delta^{13}C$ values. Jack pine data are a 5-year running average (see §2). Note increasing trend in correlation from trees to moose to wolves.

The 12 and 15 kyr ago BP herbivore and carnivore tissue samples exhibited significantly less $\delta^{13}C$ variation than the Great Basin limber pine trees ($F_{2,55}=11.33$, $p<0.001$ and $F_{2,31}=3.31$, $p=0.025$ for 12 and 15 kyr ago BP samples, respectively; figure 4).

4. DISCUSSION

The experimental results (figure 1) and observational analyses (figures 2–4) provide independent evidence indicating that changes in the $\delta^{13}C$ value and

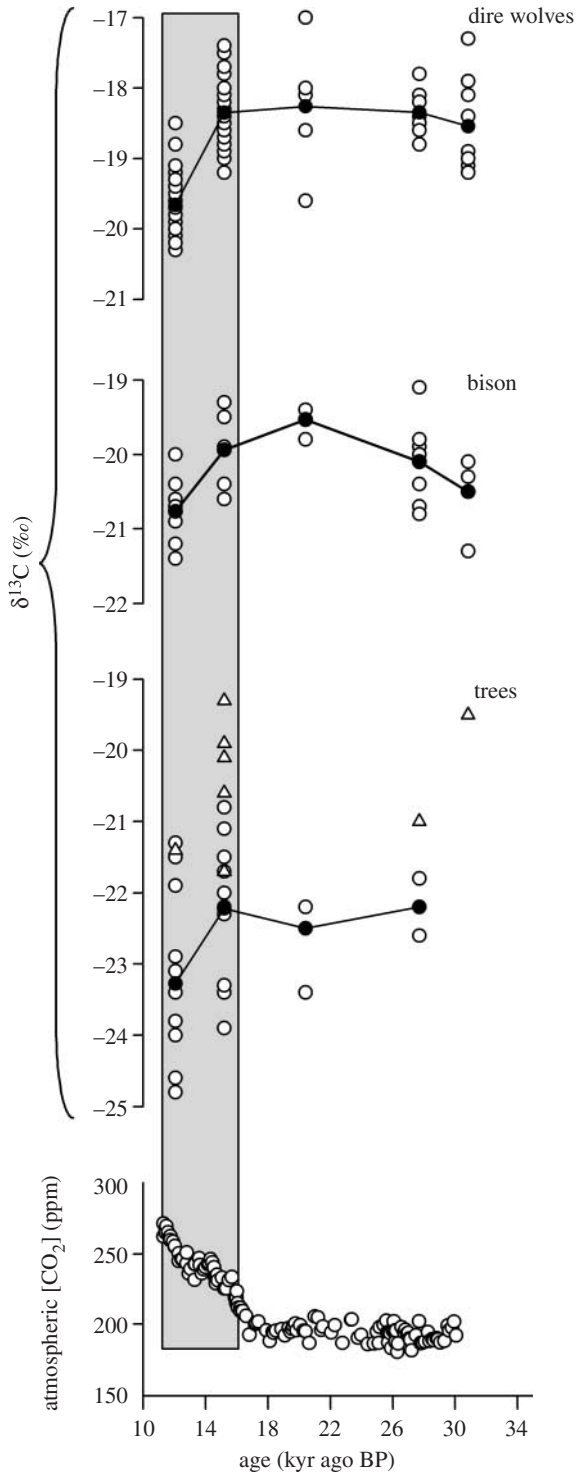

Figure 3. The relationship between year and $\delta^{13}C$ values for Great Basin pack rat midden limber pine needle (*P. flexilis*; tree circle symbols) and La Brea tar pit juniper wood (*Juniperus* sp.; tree triangle symbols) cellulose, and La Brea bison (*B. antiquus*) and dire wolf (*C. dirus*) bone collagen $\delta^{13}C$ data are reported in per mil (‰) relative to PDB limestone standard. Each panel includes individual $\delta^{13}C$ values (open symbols) with lines connecting mean $\delta^{13}C$ values (closed symbols) between time periods. We also include ice core-derived atmospheric CO_2 concentrations (Neftel *et al.* 1988; Staffelbach *et al.* 1991; Marchal *et al.* 1999). The plant and animal $\delta^{13}C$ values are plotted versus ^{14}C age (kyr ago BP), and the CO_2 concentrations are versus ice core gas age (kyr ago BP). Shaded box highlights the rapid post-last glacial maximum (LGM; 12–15 kyr ago BP) decrease in plant and animal $\delta^{13}C$ values and the concurrent increase in atmospheric CO_2 concentration.

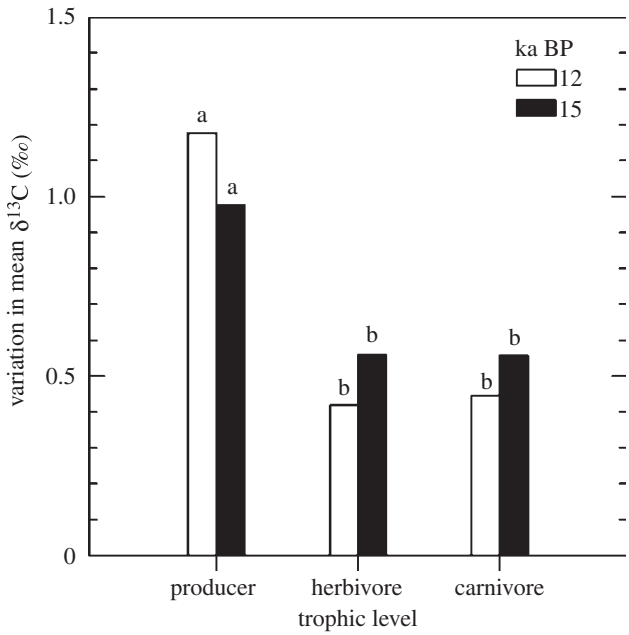

Figure 4. $\delta^{13}C$ values for palaeontological tissues from producers (Great Basin *P. flexilis* and La Brea *Juniperus* sp.), herbivore (La Brea *B. antiquus*) and carnivore (La Brea *C. dirus*). Bars indicate one standard deviation of mean $\delta^{13}C$ values measured for cellulose of producers and bone collagen of herbivores and carnivores; bar fill corresponds to sample age in ^{14}C thousands of years before present (kyr ago BP). Bars labelled with different lower case letters are significantly different.

concentration of atmospheric CO_2 propagate up a food chain with an increasing signal-to-noise ratio at higher trophic levels. We regard the experiment as a proof of concept analysis whose purpose was to verify a pattern that is probably capable of exploitation in a useful manner. The illustration of this potentially useful pattern is novel and was subsequently found to exist in two natural, observational investigations.

Forage quality and insect feeding rate may interact (Coviella & Trumble 1999; Barbehenn *et al.* 2004) and both can influence results in stable isotope studies (Focken & Becker 1998; Focken 2001). Given that no difference was detected in producer and primary consumer C : N ratios between treatments in the experimental study, and that consumers were allowed to feed ad libitum, it is unlikely that undetected differences in gross forage nutrition or feeding rate influenced our results. When the tree ring data (figure 2) are not smoothed with multi-year running averages (i.e. 5, 8–10, 12 years), their R^2 decreases to 0.08 (i.e. 8×lower than that of wolves and 4×lower than that of moose). Hence, even when conservatively accounting for the effects of unequal time represented by producer and consumer tissues, and Isle Royale topography, consumer tissues still reflect the environmental trend better than producer tissues.

Carbon isotope discrimination values for producers in the experiment did not differ across treatments indicating that the treatment $\delta^{13}C$ difference explains the $\delta^{13}C$ differences exhibited at each trophic level. Variation in carbon isotope discrimination of producers is caused by combined genetic and environmental factors that influence leaf-level gas exchange through morphological and functional plant responses. Consequently, numerous

factors (e.g. soil moisture, humidity, irradiance, temperature, nitrogen availability, leaf size, age, and thickness, stomatal density, canopy height, gender, altitude and genotype) lead to between-individual differences in the isotope ratios of primary producer tissues of the same species (reviewed in Dawson *et al.* 2002). Although the improved signal-to-noise ratio pattern (figures 2 and 3) at upper trophic levels compared with producers had been considered theoretically plausible (figure 1), it was considered biologically unlikely because consumer diet change, body condition, variable tissue turnover rates and migration were mechanisms thought to obscure isotopic patterns by creating substantial variation in stable isotope ratios of individual herbivores and carnivores (Gannes *et al.* 1997; Dawson *et al.* 2002; Long *et al.* 2005). For example, variable diet combinations for Isle Royale moose (e.g. aquatic and terrestrial components; Tischler 2004) and wolves (e.g. moose and beaver, *Castor canadensis*), and individual physiology (e.g. starvation, lactation) can lead to significant between-individual differences in isotope ratios.

However, our findings provide evidence that top consumers act as ecological integrators, linking lower pathways in space and time. That is, producers incorporate elements over time, averaging isotopic fluctuations across their environment. Herbivores sample multiple producers, averaging across their spatial range, and predators aggregate variation in prey. As a result, higher trophic level species provide higher signal-to-noise ratio for trends in isotope chronologies, such as the $\delta^{13}C$ value of atmospheric CO_2 (figures 2 and 3). Among animal ecophysiologists and ecologists using stable isotope methods this logic is loosely referred to as trophic averaging and temporal or spatial integration. Although trophic integration of atmospheric isotopic change is well documented in some cases (e.g. ^{14}C in Bada *et al.* 1990), to our knowledge this is the first experimental test and observational investigation of the patterns of integration.

While our experimental results demonstrate support for a general concept, we also consider the ecological relevance of the design (Bernardo 1998; Morin 1998). The experimental difference in $\delta^{13}C$ treatments (6.6‰) may not reflect the current or recent ecological range. Although changes in atmospheric $\delta^{13}C$ of a similar magnitude have been inferred over geological time scales (Mora *et al.* 1996), recent $\delta^{13}C$ trends show less change over the past two centuries. Atmospheric $\delta^{13}C$ records measured from ice cores exhibit approximately 1.5‰ depletion over the past 200 years, typically attributed to the combustion of isotopically light fossil fuels (Trudinger *et al.* 1999). Considering our results with this historical perspective, $\delta^{13}C$ chronologies developed from higher trophic position animals would arguably detect similar trends based on the lower variance (0.3–0.4‰; figure 1*b*) at the secondary consumer level. Producer tissue, in contrast, would be less likely to exhibit the historical atmospheric depletion given that the variability observed in their tissue values (1.7–1.8‰; figure 1*b*) exceeds the net depletion (1.5‰) seen in other studies (Trudinger *et al.* 1999; Sugawara *et al.* 2003).

The integrating effect by secondary consumers is a neglected means of developing improved proxies of ecosystem change given ample museum collections and the recent advances in isotopic measurements. Moreover, consumer tissues, in contrast to producer tissues, are abundant in the fossil record (e.g. teeth, bone, chitin) and reliable stable isotope values are routinely measured in biogenic tissues that have been preserved for millions of years (e.g. Cerling *et al.* 1997; Hedges *et al.* 2004; McFadden & Higgins 2004; Asara *et al.* 2007). Notably, bone collagen has been isolated from fossil bones 68 Myr old (Schweitzer *et al.* 2007).

Tissue and organism metabolic rates are important considerations in selecting archival tissues for developing isotopic proxies of environmental change. Mass-specific metabolic rate decreases exponentially with increasing mass (Schmidt-Nielsen 1983). Thus, carbon turnover in the tissues of smaller organisms will be swifter than in tissues of more massive organisms. Tissue type, due to differences in tissue metabolisms, is also critically linked to turnover rates. For example, bone, muscle, liver and blood plasma are four tissues that turn over at relatively very slow, slow, medium and fast rates, respectively (Fry 2006). Consequently, consumption of a mix of species of different sizes and/or tissues with variable metabolic rates results in the ingestion of a mix of carbon isotope signals. We would expect then that small organisms and metabolically fast tissues to rapidly equilibrate to isotope changes in their environment, whereas larger organisms and metabolically slow tissues will exhibit a lag in isotopic response time. Smaller animals and metabolically fast tissues may be more variable and gain less resolution with higher trophic levels. In contrast, larger organisms and metabolically slow tissues essentially increase temporal integration, which may result in more resolution with higher trophic levels.

The La Brea and Great Basin fossil case study (figure 3) illustrates how the $\delta^{13}C$ chronologies of consumers from multiple trophic levels record a past event of major ecosystem change. Specifically, patterns in La Brea consumer chronologies likely reflect how the rapid increase (from approx. 190 to 250 ppmv) in atmospheric CO_2 concentration after the LGM (Neftel *et al.* 1988; Staffelbach *et al.* 1991; Marchal *et al.* 1999) affected C_3 plant photosynthetic physiology. Previous studies (Leavitt & Danzer 1992; Van de Water *et al.* 1994) have linked the decrease in producer $\delta^{13}C$ values between 15 and 12 kyr ago BP (i.e. shaded box in figure 3) to increased discrimination against ^{13}C during photosynthesis at higher CO_2 concentrations. Substrate limitation (i.e. carbon starvation due to low atmospheric CO_2 concentrations) appears to have been prevalent in LGM trees from western North America (for a detailed discussion, see Van de Water *et al.* 1994; Ward *et al.* 2005). Change in the $\delta^{13}C$ value of atmospheric CO_2 probably does not explain the figure 3 patterns (shaded box) because in producer and consumer tissues the isotopic signal generated by the increase in CO_2 concentration (approx. 1‰ decrease) exceeds the concurrent change in the $\delta^{13}C$ value of atmospheric CO_2 at the glacial–interglacial transition (approx. 0.25 ‰ increase; Leuenberger *et al.* 1992; Smith *et al.* 1999).

Our results are the first to demonstrate how the glacial–interglacial shift in plant $\delta^{13}C$ values was recorded in primary (bison) and secondary (dire wolf) consumer tissues from the same geographical region. Trends in top predator chronologies that are also present in primary consumer chronologies probably reflect climate or vegetation changes, not dietary shifts. La Brea dire wolves

and bison are equally suitable for detecting baseline isotopic changes, which is remarkable considering the difference in dietary breadth of an opportunistic carnivore versus an obligate grazer. A decrease in the $\delta^{13}C$ values of fossil collagen from late Pleistocene European herbivores (horse, bison, elk) is similar in magnitude and timing to that of the La Brea fauna, indicating that the response of C_3 plants to changing CO_2 concentrations was globally synchronous (Richards & Hedges 2003; Stevens & Hedges 2004). The findings of our La Brea and Great Basin case study have implications for future palaeoclimatic and palaeoenvironmental reconstructions, since spatial and temporal patterns in plant tissue $\delta^{13}C$, $\delta^{15}N$ and $\delta^{18}O$ values can be driven by climatic factors (Koch 1998). For example, trends in plant water use efficiency (Van de Water *et al.* 1994; Dawson *et al.* 2002) are correlated with soil moisture content and ambient temperature patterns (Ehleringer & Monson 1993). Therefore, changes in plant tissue $\delta^{13}C$ values due to varying aridity would probably propagate through food webs, in which case the higher signal-to-noise ratio in top predator chronologies would better reflect large-scale climate change.

We note that rising variance of ecological parameters has been identified as foreshadowing ecological regime shifts (Carpenter & Brock 2006). However, Carpenter & Brock (2006) discuss that changes in variance due to impending regime shifts may be difficult to distinguish from other drivers of variance such as exogenous noise that affects ecosystems. If rising variance is indeed a leading indicator of ecological transition then, given the results of our study, stable isotope ratio time series from herbivore and carnivore populations may provide chronologies that are sensitive to major ecological transitions, but less affected by other noisy ecosystem components.

In conclusion, our results indicate that there is greater justification for selecting higher trophic position species in research explicitly interested in developing proxy environmental records and inferring habitat parameters from stable isotope chronologies. There are, however, important caveats to this recommendation, namely considering diet breath and home range. Animals with home ranges that cover broad geographical regions or cross important ecophysiological boundaries will be inappropriate proxies for understanding local environmental change. Care should be taken to discern the degree of omnivory in focal consumers if inferring environmental variables from stable isotopes is the main objective. Consumers with relatively narrow diets and limited ranges, such as are found on Isle Royale, will probably reflect environmental changes better than consumers in complex food webs. In particular, species feeding on both producers using different photosynthetic modes, or on a mix of foods derived from marine, freshwater and terrestrial sources should be avoided. Plants with different modes of photosynthesis exhibit contrasting carbon isotope ratios (Griffith 1991; Dawson *et al.* 2002), and foods derived from marine and freshwater sources often exhibit contrasting isotopic composition (Chisholm *et al.* 1982). Yet, contrary to previous expectation, the analyses presented here show that informative inferences about environmental variables based on carbon isotope chronologies are possible with species exhibiting moderate diet breadth and consumer-derived isotopic values can show environmental trends with significantly less noise than producer values.

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