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# Controlling for anthropogenically induced atmospheric variation in stable carbon isotope studies

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**Abstract** Increased use of stable isotope analysis to examine food-web dynamics, migration, transfer of nutrients, and behavior will likely result in expansion of stable isotope studies investigating human-induced global changes. Recent elevation of atmospheric $CO_2$ concentration, related primarily to fossil fuel combustion, has reduced atmospheric $CO_2$ $\delta^{13}C$ ($^{13}C/^{12}C$), and this change in isotopic baseline has, in turn, reduced plant and animal tissue $\delta^{13}C$ of terrestrial and aquatic organisms. Such depletion in $CO_2$ $\delta^{13}C$ and its effects on tissue $\delta^{13}C$ may introduce bias into $\delta^{13}C$ investigations, and if this variation is not controlled, may confound interpretation of results obtained from tissue samples collected over a temporal span. To control for this source of variation, we used a high-precision record of atmospheric $CO_2$ $\delta^{13}C$ from ice cores and direct atmospheric measurements to model modern change in $CO_2$ $\delta^{13}C$. From this model, we estimated a correction factor that controls for atmospheric change; this correction reduces bias associated with changes in atmospheric isotopic baseline and facilitates comparison of tissue $\delta^{13}C$ collected over multiple years. To exemplify the importance of accounting for atmospheric $CO_2$ $\delta^{13}C$ depletion, we applied the correction to a dataset of collagen $\delta^{13}C$ obtained from mountain lion (*Puma concolor*) bone samples collected in California between 1893 and 1995. Before correction, in three of four ecoregions collagen $\delta^{13}C$ decreased significantly concurrent with depletion of atmospheric $CO_2$ $\delta^{13}C$ ($n \geq 32$, $P \leq 0.01$). Application of the correction to collagen $\delta^{13}C$ data removed trends from regions demonstrating significant declines, and measurement error associated with the correction did not add substantial variation to adjusted estimates. Controlling for long-term atmospheric variation and correcting tissue samples for changes in isotopic baseline facilitate analysis of samples that span a large temporal range.

**Keywords** $^{13}C$ · Carbon dioxide · Correction factor · Isotopic baseline · *Puma concolor*

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## Introduction

Seminal works have documented global changes in flora and fauna through the Earth's history (Cerling et al. 1997, 1998; Iacumin et al. 2000; Burton et al. 2001). Recently, many studies have used stable isotope ratios to explore the effects of global warming, increased $CO_2$ concentrations, and invasions of exotic species on plant and animal physiology, community composition, and ecosystem function (Vander Zanden et al. 1999; Arens et al. 2000; Barber et al. 2000; Bergengren et al. 2001; Hibbard et al. 2001; Kritzberg et al. 2004; Pace et al. 2004).

The ratio $^{13}C/^{12}C$ plays a prominent role in studies of global warming and increased $CO_2$ concentrations. Because of fractionation during photosynthesis, $CO_2$ from biogenic sources is depleted in $^{13}C$ relative to $CO_2$ from inorganic sources (O'Leary 1981), and recent large-scale influxes of $CO_2$ from organic sources (e.g., fossil fuel combustion) have decreased atmospheric $CO_2$ $\delta^{13}C$ ($^{13}C/^{12}C$, Friedli et al. 1986; Francey et al. 1999). Such a decrease provides a marker that can be used to construct atmospheric budgets and calculate input of biogenic, including anthropogenic, carbon relative to input of inorganic carbon (Keeling et al. 1989). Since plants assimilate $CO_2$ from the atmosphere, plant tissue $\delta^{13}C$ relates to atmospheric $CO_2$ $\delta^{13}C$ (O'Leary 1981). Consequently, atmospheric geoscientists and paleoclimatologists have used plants to infer past atmospheric conditions (Arens et al. 2000), for example, using tree ring cellulose $\delta^{13}C$ as an indicator of annual atmospheric $CO_2$ $\delta^{13}C$ (Freyer 1979; Mazany et al. 1980; Stuiver et al. 1984; Stuiver and Braziunas 1987; Lipp et al. 1991). Further, time-specific measures of atmospheric $CO_2$ $\delta^{13}C$ have been inferred from kernels of maize grown during known years (Marino and McElroy 1991), and long-term records of $\delta^{13}C$ have been reconstructed from sediment cores of peat (White et al. 1994). Additionally, $\delta^{13}C$ of carbonates in aquatic invertebrates such as sponges (Druffel and Benavides 1986; Böhm et al. 1996), coral (Nozaki et al. 1978), and mollusks (McConnaughey et al. 1997) have been shown to relate to oceanic dissolved inorganic carbon (DIC), which relates to atmospheric $CO_2$ $\delta^{13}C$; and long-term records of these organisms could be used to infer historic atmospheric conditions.

Since plants form the foundation of most food chains, and consumer $\delta^{13}C$ correlates with $\delta^{13}C$ of diet (DeNiro and Epstein 1978; Peterson and Fry 1987; Kelly 2000), animal tissue $\delta^{13}C$ can indirectly relate to atmospheric $CO_2$ $\delta^{13}C$. Long-term trends of $\delta^{13}C$ of fossilized faunal tissue have been correlated with $\delta^{13}C$ of atmospheric $CO_2$ over the past 40,000 years (Iacumin et al. 1997; Iacumin et al. 2000; Richards and Hedges 2003). Further, Cerling and Harris (1999) observed a decline in mammalian tooth enamel $\delta^{13}C$ values consistent with declines observed in atmospheric $CO_2$ $\delta^{13}C$.

In ecosystem studies, stable isotope analysis has been used to study food-web dynamics, migration, transfer of nutrients between ecosystems and animal behavioral ecology (Kling et al. 1992; Ben-David et al. 1997, 1998a, 2004; Hobson 1999; Kelly 2000; Post 2003; Pace et al. 2004). For example, in trophic studies, $\delta^{15}N$ is useful for determining trophic position of a consumer because $\delta^{15}N$ becomes enriched (i.e., ratio of $^{15}N : {}^{14}N$ increases) as nitrogen passes through food webs; hence, consumers with greater $\delta^{15}N$ values typically occupy higher trophic positions (DeNiro and Epstein 1981; Peterson and Fry 1987; Kelly 2000). Unlike $\delta^{15}N$, $\delta^{13}C$ is not appreciably changed as carbon moves through food webs (Peterson and Fry 1987; Post 2002), however, in terrestrial ecosystems, because $^{13}C$ is enriched in C4 plants relative to C3 plants, $\delta^{13}C$ is useful for determining photosynthetic pathway of plants that form the foundation of terrestrial trophic systems (Peterson and Fry 1987). Likewise in lakes, $\delta^{13}C$ of algae and detritus in littoral regions is enriched relative to $\delta^{13}C$ of phytoplankton from pelagic regions, and $\delta^{13}C$ of consumers can be used to discriminate between these two energy sources (France 1995). Further, isotope analyses have demonstrated links between terrestrial and aquatic ecosystems, with aquatic systems being supplemented by carbon of terrestrial origin (Pace et al. 2004) and terrestrial systems being supplemented with carbon of aquatic origin (Ben-David et al. 1998b).

With the example set by previous work (Cerling et al. 1997, 1998; Iacumin et al. 2000; Burton et al. 2001) and the current increase in use of stable isotope analysis, it is likely that a rapid expansion of studies of recent human-induced global changes will follow. Though changes in atmospheric $CO_2$ $\delta^{13}C$ provide a useful tool for estimating biogenic contributions to atmospheric $CO_2$ concentrations, large-scale depletion of atmospheric $CO_2$ $\delta^{13}C$ produces a substantial change in isotopic baseline for stable carbon isotope studies and represents a potentially serious confounding factor for ecosystem studies. Atmospheric $CO_2$ provides, directly or indirectly, the fundamental carbon source for most terrestrial and aquatic food chains, and comparison of tissues samples collected over a temporal span can yield biased results unless atmospheric change in $CO_2$ $\delta^{13}C$ is controlled. Establishing an appropriate isotopic baseline is a critical component of stable isotope investigations (Cabana and Rasmussen 1994; Vander Zanden et al. 1997; Post 2002), and although potential bias from atmospheric depletion of $CO_2$ $\delta^{13}C$ has been identified by other researchers (Cerling and Harris 1999), temporal changes in atmospheric $CO_2$ $\delta^{13}C$ have not been fully addressed in most of the literature on isotopic ecology.

Herein, we construct a correction factor, based on a recently published, high-precision record of atmospheric $CO_2$ (Francey et al. 1999), to facilitate tissue sample comparison while controlling for atmospheric change. We then demonstrate its use by applying this correction factor to collagen $\delta^{13}C$ data from a 100-year series of mountain lion (*Puma concolor*) bone samples collected in California, and we address potential effects of precision of this correction factor on comparison of samples collected through time. Finally, we identify limitations in the applicability of the correction factor and indicate areas in which additional research is needed.

## Materials and methods

### Atmospheric model

To correct for changes in $\delta^{13}C$ of atmospheric $CO_2$, we modeled a high-precision record of atmospheric $CO_2$ $\delta^{13}C$ from direct atmospheric measurements and from air trapped in Antarctic ice (Francey et al. 1999). The Francey et al. (1999) data were used because (1) they represent a complete, long-term record of postindustrial atmospheric conditions; (2) precision estimates for each point, useful for variance estimates in our analyses, are reported; (3) measurement errors present in earlier datasets (e.g., Friedli et al. 1986) have been corrected; (4) trends correspond closely with data represented elsewhere (e.g., Keeling et al. 1989); and (5) high-latitude $CO_2$ $\delta^{13}C$ values do not differ from annual global means

(Keeling et al. 1989) and therefore serve as a reliable proxy for generalized global trends.

We modeled the Francey et al. data (1999) from 1880 to 1996 using a negative exponential model:

$$\delta^{13}C = k - e^{at^2}, \tag{1}$$

where $k$ and $a$ were estimated parameters and $t$ was an index to year in which 1 represented 1880.

### Example data set

We used a directory of museum collections of mammals (Hafner et al. 1997) to identify and query 33 museums from the USA regarding their holdings of mountain lions from California. From responses to these inquiries we compiled a database of 280 mountain lion specimens collected in California (Long and Sweitzer 2001) and analyzed bone samples of 194 individual mountain lions collected between 1893 and 1995. Bone collagen was used for isotopic analysis because of its availability in museum collections; further, slow turnover rate of bone collagen makes this tissue advantageous for providing an integrated lifetime average of dietary input (Tieszen and Boutton 1989). For most mammals, fractionation enriches tissue $\delta^{13}$C much less than other stable isotopes such as $^{15}$N, and tissue $\delta^{13}$C is often enriched only 1–6% over dietary $\delta^{13}$C (Kelly 2000).

Museum curators often value skulls more highly than postcranial skeletal material, and as isotope values of collagen show very little to no variation between different bones within a single organism (DeNiro and Schoeninger 1983), we preferentially sampled the latter when available. Using a rotary tool equipped with a small cutting blade, approximately 1 g of postcranial skeletal material was sampled from the axis of a long bone or a portion of rib. For specimens represented by skulls only, a tile-cutting bit was used to section bone from the tentorium inside the cranium.

Extraction, purification, and lipid removal of bone collagen followed Matheus (1997). One milligram of recrystallized collagen was loaded into an 8×5 mm tin capsule (Elemental Microanalysis Limited) for isotopic analysis, which was performed on a Europa Scientific continuous flow isotope ratio mass spectrophotometer (University of California Davis, Stable Isotope Facility and University of Alaska Fairbanks, Stable Isotope Laboratory). Isotope data are expressed as parts per thousand deviation from a PDB limestone standard (Kelly 2000).

To reduce spatial variability and to control for potential differences in climate and community structure, which may affect $\delta^{13}$C of animal tissue, we used collection location data to categorize each mountain lion sample into one of ten physiognomically similar geographic subdivisions of California (Hickman 1993), hereafter referred to as ecoregions (Fig. 1). Analyzed specimens were distributed among the ecoregions, including Northwest California ($n = 36$), Cascade Ranges ($n = 9$), Sierra Nevada Mountains ($n = 61$), Central Western California ($n = 48$), and Southwest California ($n = 33$). Because the Cascade Range region was relatively underrepresented with museum specimens, tissue samples from this ecoregion were grouped with geographically contiguous Northwest California, and these combined data are hereafter referred to as Northern California ($n = 45$). Additional specimens ($n = 7$) were distributed among three other ecoregions (Great Central Valley, Mojave Desert, and Great Basin), but because of small sample sizes, we excluded these samples from analyses.

Before application of the correction factor, we assessed temporal variation in collagen $\delta^{13}$C within each ecoregion using Spearman rank correlation ($R_s$), due to non-normal distribution of collection dates. Further, by using a nonparametric test, we did not assume a linear relationship between collection date and bone collagen $\delta^{13}$C. Statistically outlying $\delta^{13}$C values were checked using studentized residuals. Two samples were determined to be outliers ($t = 3.53$, $P < 0.05$) and were excluded from subsequent analysis.

### Application of the correction factor

To correct $\delta^{13}$C values of bone collagen in our sample based on changes in atmospheric CO$_2$ $\delta^{13}$C, we calculated change in atmospheric CO$_2$ $\delta^{13}$C between 1880 and the year in which a tissue sample was obtained and added this value to tissue $\delta^{13}$C values. To estimate precision of adjusted $\delta^{13}$C values for bone tissue, we added the variance of measurement error from the analysis of bone samples to the variance of the estimate of the predicted $\delta^{13}$C value from the negative exponential model using a Taylor series approximation (Seber 1982):

$$\widehat{\text{var}}\left(\hat{\delta}^{13}\text{C}_{\text{adjusted}}\right) = \widehat{\text{var}}\left(\hat{\delta}^{13}\text{C}_{\text{bone}}\right) + D(\hat{\theta})I'(\hat{\theta})D(\theta), \tag{2}$$

where $D(\hat{\theta})$ is the vector of partial derivatives of the negative exponential model with respect to parameters $k$ and $a$, and $\hat{I}(\hat{\theta})$ is the estimated variance–covariance matrix coefficients in the negative exponential model. To reassess temporal variation after controlling for atmospheric variation, we conducted Spearman rank correlations on corrected bone collagen $\delta^{13}$C.

## Results

### Atmospheric model

The negative exponential model of atmospheric CO$_2$ $\delta^{13}$C explained a significant amount of variation in the Francey et al. (1999) data ($F_{2,88} = 4800.8$, $P < 0.001$,

**Fig. 1** Specimens of mountain lions (*Puma concolor*) collected in California, USA, between 1893 and 1995 characterized by five ecoregions. Completely overlapping collection locations are not registered separately. Data from the Cascade Ranges and Northwestern California were grouped for analyses

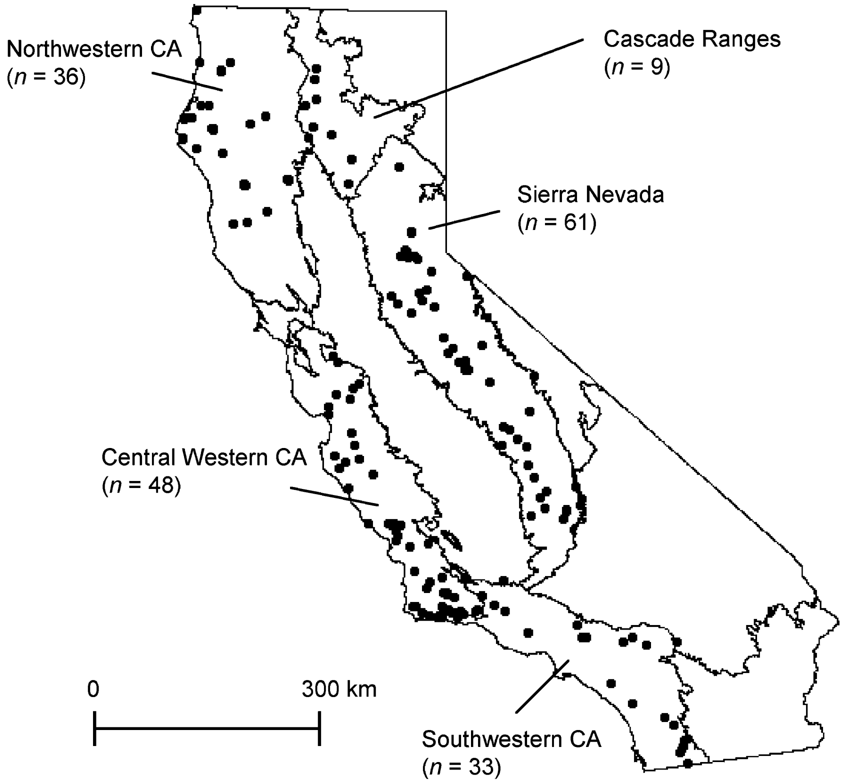


Fig. 2). Parameter estimates and associated standard errors were found to be $k = -5.5656$ ($1.2606\times10^{-2}$) and $a = 6.0932\times10^{-5}$ ($5.8652\times10^{-7}$). Residuals exhibited homoscedasticity, and 95% confidence intervals of predicted values encompassed the actual values for all but two data points.

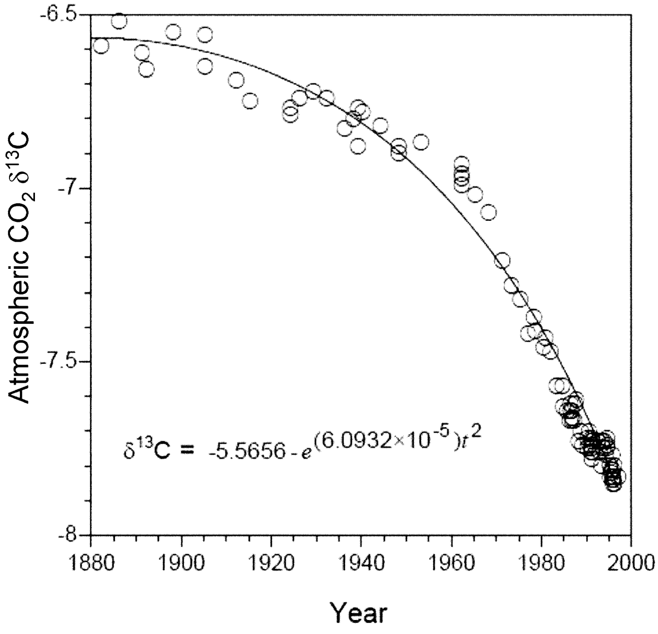


**Fig. 2** Change in atmospheric CO$_2$ $\delta^{13}$C since 1880 (data from Francey et al. 1999), with Eq. 1 fit to the data ($F_{2,88} = 4{,}800.8$, $P < 0.001$)

### Example data set

Consistent with observations that temporal changes in $\delta^{13}$C of atmospheric CO$_2$ affect $\delta^{13}$C of consumer tissue, mountain lion bone collagen $\delta^{13}$C was negatively correlated with collection date in three of four ecoregions ($n \geq 32$, $P \leq 0.01$, Fig. 3). Mountain lion bone collagen $\delta^{13}$C in the Sierra Nevada ecoregion demonstrated no significant trend ($n = 61$, $P = 0.14$, Fig. 3).

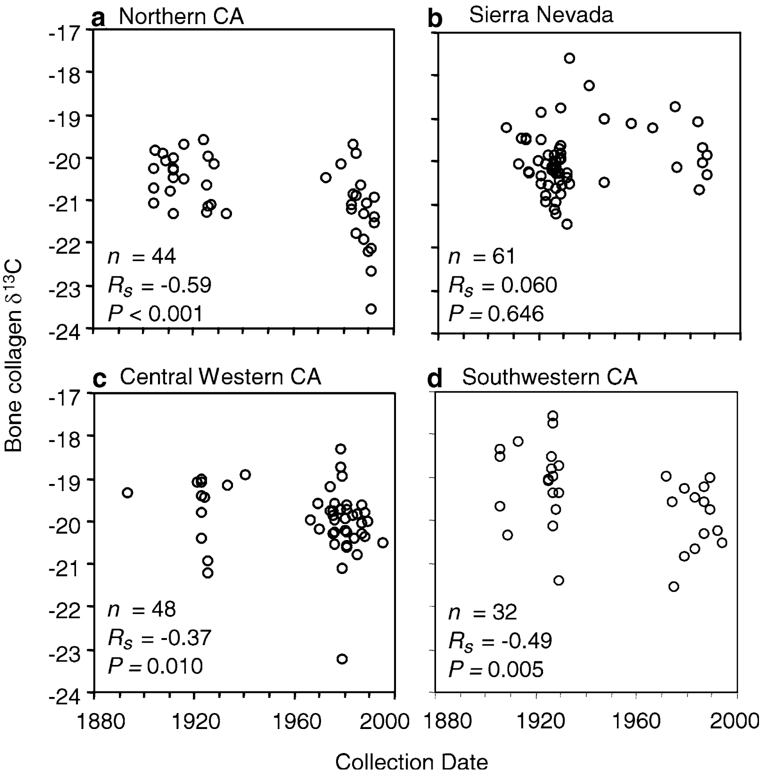
### Correction factor application

Application of the correction factor to the mountain lion bone collagen $\delta^{13}$C data removed the trend from three ecoregions that had shown significant declines ($n \geq 32$, $P \geq 0.18$, Fig. 4), and adjusted bone collagen $\delta^{13}$C values of mountain lions from the Sierra Nevada demonstrated a significant temporal increase ($n = 61$, $P < 0.001$, Fig. 4). Adjusted $\delta^{13}$C estimates from bone tissues had CVs < 2%.

## Discussion

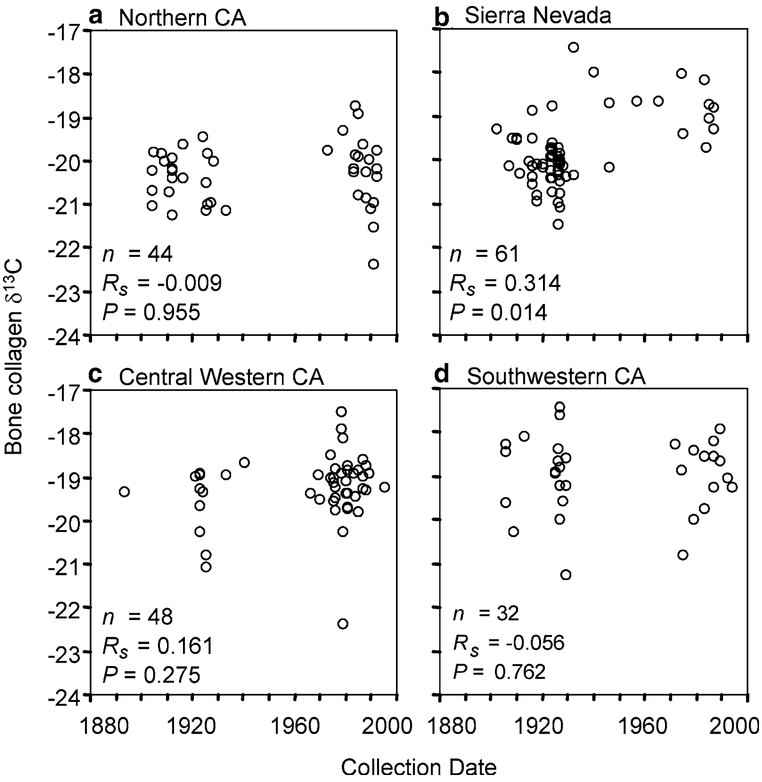
Changes in $\delta^{13}$C of atmospheric CO$_2$ have been shown to alter $\delta^{13}$C of plant (Marino and McElroy 1991; Arens et al. 2000) and animal tissue (Richards and Hedges 2003), but implications of recent declines in atmospheric CO$_2$ $\delta^{13}$C for application of stable isotope analysis in

**Fig. 3** Correlation of mountain lion bone collagen $\delta^{13}$C with collection date for specimens from four ecoregions of California: (**a**) Northern CA, (**b**) Sierra Nevada, (**c**) Central Western CA, and (**d**) Southwestern CA



exploration of recent global changes have largely been overlooked (but see Cerling and Harris 1999; Barber et al. 2000). Using mountain lion bone collagen as an example, we documented significant temporal declines in collagen $\delta^{13}$C values throughout much of California, and these declines were consistent with patterns associated with depletion of atmospheric $CO_2$ $\delta^{13}$C (Cerling and Harris 1999).

**Fig. 4** Correlation of corrected mountain lion bone collagen $\delta^{13}$C with collection date. Specimens are categorized by four ecoregions of California, and Eq. 1 (see Fig. 2) has been applied to bone collagen $\delta^{13}$C values to control for atmospheric variation



Comparison of results from our analyses before and after correction was applied illustrates the need to account for atmospheric variation in long-term stable isotope studies. Application of the correction factor changed the significance of results in all four ecoregions tested. After correction, three ecoregions demonstrated no significant trend. In the Sierra Nevada ecoregion, we observed a significant temporal increase in mountain lion bone collagen $\delta^{13}$C, a pattern likely relating to prey-switching behavior in response to changing prey availability in this region (Long 2001). Without correction, significant ecological changes may have been assumed in three ecoregions that demonstrated significant change in tissue $\delta^{13}$C. On the other hand, potentially important ecological changes may have been overlooked in the Sierra Nevada ecoregion, where no trend was detected before correction.

That atmospheric depletion in $CO_2$ $\delta^{13}$C was detectable in a generalist, apex predator indicates that results of atmospheric change are transmitted up trophic chains and are pervasive even in systems with large environmental variation in multiple processes. Effects of depleted atmospheric $CO_2$ $\delta^{13}$C have been clearly documented in a variety of plant tissues (Marino and McElroy 1991; Arens 2000), and because plants assimilate carbon directly from the atmosphere, the depletion pattern is most accurately recorded in plant tissue. At higher levels in trophic chains, additional sources of isotopic variation are introduced (e.g., mixed diet), but long-term changes in atmospheric $CO_2$ $\delta^{13}$C are indirectly recorded in herbivore tissue (Cerling and Harris 1999; Iacumin et al. 2000; Richards and Hedges 2003), and as suggested here, in carnivore tissue. Therefore, correcting for atmospheric change in stable isotope studies is important regardless of trophic position.

Cerling and Harris (1999) measured $\delta^{13}$C of grasses and ungulate tooth enamel from samples collected in the 1960s and in the 1990s. To compare these samples, they accounted for atmospheric variation by correcting for an approximate 1‰ change in $\delta^{13}$C of atmospheric $CO_2$ between 1956 and 1997, based on data presented by Keeling et al. (1979) and Friedli et al. (1986). Our correction technique is similar, but uses a model based on long-term data from 1880 until modern times and incorporates the precision of this model in the variance estimate for the corrected $\delta^{13}$C ratio.

Application of a correction factor introduces additional variability in ratio estimates and, consequently, reduces precision. However, source data for the correction factor were precise (typically error $<0.5\%$ of point estimates, Francey et al. 1999), and our negative exponential model fit the data well (Fig. 2). In our study, measurement error associated with the correction factor was much less than variation exhibited in bone tissue $\delta^{13}$C values. Therefore, measurement error, determined from Eq. 2, did not substantially reduce the precision of the estimates.

Although data analyzed here are exclusively terrestrial, similar patterns appear in aquatic systems. For example, long-term depletion of atmospheric $CO_2$ $\delta^{13}$C has lowered $\delta^{13}$C of dissolved inorganic carbon, which in turn has lowered $\delta^{13}$C of precipitated carbonate in aquatic invertebrates (Nozaki et al. 1978; Druffel and Benavides 1986; Böhm et al. 1996). Further, aquatic phototroph tissue $\delta^{13}$C relates largely to DIC $\delta^{13}$C (Osmond et al. 1981), and because, as in terrestrial systems, aquatic consumer tissue $\delta^{13}$C is determined largely by $\delta^{13}$C of their food (Post 2003), effects similar to those observed in terrestrial systems should be apparent in aquatic systems. Consequently, correction for atmospheric change in stable carbon isotope investigations is also important for aquatic investigations.

In both aquatic and terrestrial systems, however, time lags between atmospheric change and ecological assimilation of atmospheric $CO_2$ may represent a potentially serious confounding complication. For instance, if isotopic changes in atmospheric $CO_2$ require a long time to equilibrate between systems (Libby et al. 1964), direct application of our correction factor may not be appropriate. In terrestrial systems, though tissue $\delta^{13}$C of plants reflect current atmospheric $CO_2$ $\delta^{13}$C, lags may exist (1) if there are delays in assimilation of plant material into trophic webs, and (2) if there are delays in assimilation of dietary material into tissue, particularly tissues with low-turnover rates in especially long-lived species. For instance, as bone collagen has low metabolic turnover relative to other tissues such as hair, blood, and muscle, $\delta^{13}$C of bone collagen is generally considered to represent an integrated lifetime average of dietary input (Tieszen and Boutton 1989). Though specific estimates in animals are rare, Hobson and Clark (1992) showed isotopic turnover of bone collagen $\delta^{13}$C in Japanese Quail (*Coturnix japonica*) to be 173.3 days. However, this rate is likely longer in adults of long-lived species such as Asian elephants (*Elephas maximus*, Sukumar and Ramesh 1992) and humans (Libby et al. 1964).

In addition to metabolic lag in tissues, long residence time of carbon in low trophic levels may delay reflection of atmospheric trends at higher trophic levels. In many systems, annual plant growth, which relates isotopically to current atmospheric conditions, forms the bulk of diet for primary consumers, and in these cases, time lags would be minimal. Exceptions, such as deep-water lentic systems with detritus-based trophic webs and long detrital residence time, would delay atmospheric trends. For example, time lags in incorporation of algal $\delta^{13}$C values were recorded in nematodes under experimental conditions, and these time lags were consistent with the mixing rates of the sediments (Moens et al. 2002). Once carbon is sequestered by primary consumers, further substantial delays are unlikely, as soft tissues with quick metabolic turnover rates typically provide much of the carbon that is assimilated by higher order consumers (Tieszen and Boutton 1989). However, researchers need to be aware of potential violations of these assumptions, and correct for long-turnover rates when appropriate.

In our analysis of mountain lion tissue, we corrected date of all samples based on collection date, though this surely introduced variation associated with time lags. However, mountain lions are not a particularly long-lived species; Beier (1993) estimated that most mountain lions do not live past 5–6 years of age, with maximum longevity of approximately 12 years. Hence, when analyzing bone collagen, we expect lag from atmospheric $CO_2$ to tissue $\delta^{13}C$ to be, on average, 5 years or less. Correcting for a lag of 5 years, even in our most recent sample from 1995, where atmospheric change is most extreme, changes $\delta^{13}C$ by less than 0.15‰ and, over the range of our investigation, introduces negligible variation. However, in studies of longer-lived species, with significantly greater life expectancies, or in studies examining tissues such as tooth dentine, which has turnover rates much slower than bone collagen (Richards et al. 2002), correction for time lags may be appropriate.

In aquatic systems, a third source of lag may be present, as turnover rate between atmospheric $CO_2$ $\delta^{13}C$ and DIC $\delta^{13}C$ can be quite variable, especially in large bodies of water. For example, Böhm et al. (1996) found no isotopic equilibrium between oceanic surface water DIC and atmospheric $CO_2$, and because equatorial North Atlantic Ocean surface water DIC has only approximately $10 \pm 4$ years to equilibrate with the atmosphere before being recycled into deeper waters, DIC $\delta^{13}C$ consistently reflected only 50–65% of the atmospheric decline (Böhm et al. 1996). Therefore, depletion of tissue $\delta^{13}C$ in oceanic organisms, especially deep-water organisms, will likely be less than depletion of terrestrial tissue $\delta^{13}C$. In smaller bodies of water, equilibration between atmospheric $CO_2$ $\delta^{13}C$ and DIC $\delta^{13}C$ can occur more rapidly, and where dissolved $CO_2$ quickly equilibrates with atmospheric $CO_2$ our correction should be appropriate.

Further factors complicating temporal $\delta^{13}C$ correction in aquatic systems relate to variable sources of carbon input. For example, using $\delta^{13}C$ Post (2002) identifies three sources of DIC that may be incorporated into trophic webs in lakes: dissolution of atmospheric $CO_2$, remineralization of allochthonous or autochthonous organic carbon, and weathering of carbonates. Dissolution of atmospheric $CO_2$, directly, and most remineralization of organic carbon, indirectly, relate to atmospheric $CO_2$. In systems where these sources of carbon dominate, such as small and medium-sized lakes (Post 2002), temporal correction for atmospheric change is appropriate. Pace et al. (2004) suggested that 22–50% of dissolved organic carbon in lakes is derived from terrestrial origins, and if differences exist in assimilation rates of atmospheric $CO_2$ between terrestrial inputs and autochthonous production, temporal $\delta^{13}C$ correction may be complicated in aquatic systems. Further, carbon derived from weathering of carbonates (e.g., limestone), does not relate to current atmospheric conditions, and in large lake systems where substantial amounts of assimilated DIC may originate from this carbon source (Post 2002), direct application of our correction factor is not appropriate. Related to these potential complications, we recommend additional research on applicability of our proposed isotopic correction for aquatic systems, specifically in relation to turnover time, equilibration rates, and multiple sources of carbon input.

The ability to use stable isotope analysis to explore human-induced changes in food-web dynamics, predator–prey interactions, patterns of migration, transfer of nutrients between ecosystems, and animal behavioral ecology as well as alterations of structure and function of ecosystems (Bergengren et al. 2001; Hibbard et al. 2001) has the potential to provide insights into ecological processes that alter regional patterns of biodiversity (Petchy et al. 1999; Post et al. 1999). Ability to correct tissue samples for changes in isotopic baseline resulting from long-term alteration of atmospheric $CO_2$ $\delta^{13}C$ will facilitate analyses that span a large temporal range. Although effects of increased variability from the correction factor may be problematic in some cases, observation of negligible variation in our case is encouraging. Potentially confounding effects related to carbon turnover rate and, in aquatic systems, variable input of carbon sources, warrant additional research relating to appropriate temporal correction. In general, because of the pervasive nature of change in atmospheric $CO_2$ $\delta^{13}C$, we recommend controlling for this source of variation for any $^{13}C$ study in which tissue samples span even a moderate time frame.

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