FACTORS INFLUENCING ^{14}C AGES OF THE PACIFIC RAT *RATTUS EXULANS*

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ABSTRACT. An isotopic database for the Pacific/Polynesian rat *(Rattus exulans)* and foods that it scavenges is used to examine diet-induced ^{14}C age variation in omnivores. We discuss a suite of 26 $\Delta^{14}C$ determinations and ^{13}C and ^{15}N analysis for modern Pacific/Polynesian rat bone gelatin and available food items from Kapiti Island, New Zealand (40°51′S, 174°75′E). These analyses provide the first isotopic data for modern specimens of the species, collected as part of a larger project to determine potential sources of bias in unexpectedly old ^{14}C age measurements on subfossil specimens of *R. exulans* from New Zealand. Stable C, N and ^{14}C isotopic and trapping data are used to trace carbon intake via the diet of the rats in each habitat. Data from specimens linked to five specific habitats on the island indicate that modern populations of *R. exulans* are not in equilibrium with atmospheric values of $\Delta^{14}C$, being either enriched or depleted relative to the atmospheric curve in 1996/97, the period of collection. The $\Delta^{14}C$ values recorded for *R. exulans* are associated with diet, and result from variation in $\Delta^{14}C$ values found in animal-protein food items available to a scavenging omnivore. The titer of carbon deviating from atmospheric values is believed to be derived from the essential amino acids in the protein-rich foods of the rat diet.

Present evidence suggests that the depletion required to affect ^{14}C ages limits the possibility that diet introduces dramatic offsets from true ages. Marine diets, for example, would have a variable effect on ages for terrestrial omnivores, contraindicating the application of a standard marine correction for such specimens. We suggest that to identify the extent to which diet may influence the ^{14}C age in a given specimen of terrestrial omnivore, the separation and dating of essential amino acids *vs.* a non-essential amino, such as glycine, be applied.

INTRODUCTION

The Pacific/Polynesian rat (*Rattus exulans*) was spread throughout the Pacific from Asia during *ca.* 400 yr of Polynesian exploration. Because the first appearance of *R. exulans* anywhere in New Zealand is associated with human occupation, its bones can be utilized as a proxy zoological tracer of human arrival, even when recovered from sites not associated with human occupation.

There is active debate concerning the timing of contact with New Zealand by prehistoric voyagers. Some archaeological studies support a relatively short prehistory, with arrival at a period between 700 yr BP and 1000 yr BP (Anderson, Allingham and Higham 1996). Conclusions drawn in many palynological studies infer that sharp changes in pollen assemblages are due to forest burn-off practices of prehistoric agriculture coinciding with this period (Elliot *et al.* 1995; McGlone, Mark and Bell 1995; Davidson 1984; Mildenhall 1979). A case has also been made for a longer prehistory, with arrivals between *ca.* 1950 yr BP and 1450 yr BP (Bulmer 1989; Sutton 1987; Kirch 1986; Groube 1968).

In 1995 and 1996 a suite of 19 samples of subfossil *R. exulans* from six natural sites in the North and South Islands of New Zealand was ^{14}C dated by accelerator mass spectrometry (AMS) at the Rafter Radiocarbon Laboratory, New Zealand. The samples were submitted as part of a separate project to determine dates for the appearance of *R. exulans* and its impact on the New Zealand ecosystem. Age determinations for the bone samples were found to be significantly older than allowed by the short prehistory paradigm, with ages ranging from 1204 BP to 2155 BP (Holdaway 1996). While stable isotope data excluded a significant contribution of marine food to the diet of these specimens, even with such a diet a simple marine correction would be insufficient to shift ages away from the expected maximum of *ca.* 900 yr BP.

The Rafter Radiocarbon Laboratory has subsequently undertaken a program to review these apparently anomalous dates for *Rattus exulans*. The study took into consideration various possibilities for

alteration of expected ages, among which was that dietary items ingested by *R. exulans* have strongly depleted ^{14}C values not readily apparent in $\delta^{13}C$ and $\delta^{15}N$ diet analysis, such as for intake of marine proteins. The basis of this dietary effect is the biochemical pathways of carbon, where essential amino acids in the diet transfer their carbon isotope signatures to bone proteins. Dietary offsets from true age could be an issue in omnivores or marine feeders, but the extent of such an effect was unknown. Based on work by Ambrose and Norr (1993) we hypothesized that the ^{14}C information carried by nonessential amino acids reflects near-contemporary value, whereas the essential amino acids from protein in the diet can carry carbon that may deviate from atmospheric equilibrium. To examine diet-induced variation of ^{14}C ages, we chose a modern population of *R. exulans* whose food items and bone gelatin were analyzed.

Here we present the data from our program dealing with a possible dietary contribution to anomalous ages, using isotopic data from modern samples of *R. exulans*. Results from an additional review of the effectiveness of bone treatments and laboratory methods will be presented in a forthcoming paper.

Methodology

Collection

To identify dietary pathways for sources of depleted carbon in rat bones, we selected a modern population of *Rattus exulans* for isotopic analysis. *R. exulans* were collected during April and June 1996 on five trap lines set on Kapiti Island (40°51′S, 174°75′E), a wildlife sanctuary administered by the New Zealand Department of Conservation. The trap lines ran through five of eight distinct habitats as described on Kapiti by Miskelly (1992). Line 2, a coastal shrub/grassland on the northeastern spur of the coastline, incorporated a brackish lagoon with nesting waterfowl; Line 4 was in a mixed tawa/hinau forest on the northwest coast ridge of the island's summit, and incorporated a low-density nesting colony of sooty shearwater, *Puffinus griseus*; Line 6 was in a mid-altitude, mixed forest of native trees; Line 7 was in kohekohe forest; and Line 8 was in a grassland/shrub succession on the northwest coast of the island.

Rats were trapped and retrieved within 12 h of death. Their femurs and tibiae were removed and bagged with an identification number indicating trap line, date of collection and gender, and were stored frozen until analysis. Food items available to scavenging rats were also collected in 1996. Carcasses of black-backed gull (*Larus dominicanus*), gannet (*Sula serrator*) and little blue penguin (*Eudyptula minor*) were sampled for flesh, skin, feather and bone. A reconnaissance of trap line habitats was made in February, 1997 to collect invertebrates, decayed wood, fungus and vegetable matter, all of which are possible food items in the diet of *R. exulans* in those habitats.

Physical and Chemical Preparation

Rattus exulans bone samples: Tibiae and femora from rats were defleshed, freeze-dried, and scraped to remove cartilage and bone marrow. All bones were then washed and sonicated in deionized water, and dried in a vacuum oven at 30°C. Each sample was pulverized in a Retch mill to <450 μm, and demineralized in 0.5M HCl while stirred at room temperature for 1 h. Collagen was filtered from the solution and gelatinized with 0.01M HCl in a nitrogen atmosphere at 90°C for 16 h. The gelatin was then double-filtered through GF/C and 0.45 μm Acrodisc® filters, and lyophilized to weigh yields. An average of 4 mg of this bone gelatin was combusted and the CO_2 cryogenically distilled, with a 100 mb split taken for ^{13}C analysis. The remaining CO_2 from combustion was graphitized and analyzed by AMS dating.

Bird flesh. An average of 200 mg of muscle was sampled from avian breasts, washed in deionized water and 0.5M HCl, freeze-dried and pulverized. The flesh was hydrolyzed in 6M HCl, and filtered. The hydrolysate was combusted, the CO_2 distilled, an aliquot taken for ^{13}C and the remainder graphitized for AMS analysis.

Invertebrates. Invertebrates were washed in deionized water and 0.5M HCl, rinsed to neutral and vacuum-dried in a 30°C oven, then combusted in a sealed tube, and the CO_2 distilled, graphitized and analyzed by AMS.

Vegetable matter and fungi. Samples of leaf material, berries and bracket fungus were broken up, washed in deionized water and 0.5M HCl, rinsed to neutral, freeze-dried and pulverized. Samples were then combusted, and the CO_2 cryogenically distilled and graphitized as above.

Amino acid analysis. Hydrolysates of flesh and bone samples were analyzed by the Renal Research Unit of Wellington School of Medicine on a Waters Pico*Tag® HPLC system by the method of Negro *et al.* (1987).

Variation in ^{13}C Due to Lipids

Concerns about small sample sizes of available rat bone (average 160 mg each) and possible contamination effects from lipid extraction procedures required alternative methods of assessing the effect of lipids on $\delta^{13}C$ analysis. A second leg bone from each of two specimens undergoing full analysis was defatted using the method of Radin (1981) and analyzed for yield, $\Delta^{14}C$ and $\delta^{13}C$. Results from these preliminary experiments on the change to $\delta^{13}C$ values in bone gelatin show $\delta^{13}C$ enrichments of 0.3 to 1.7‰ in lipid-extracted samples. The $\Delta^{14}C$ values between lipid-extracted and non-lipid-extracted samples indicate no change within error of analysis.

^{13}C and ^{15}N Analysis

^{13}C. Splits of CO_2 from cryogenic distillation of each sample after combustion were analyzed at the Institute for Geological and Nuclear Sciences on an NAA 6-60 RMS mass spectrometer, with machine error ±0.1 ‰.

^{15}N. Subsamples of gelatin of bones or hydrolysate of flesh samples were analyzed at the University of Waikato Stable Isotope Unit on a Europa Scientific Dumas elemental analyzer interfaced with an Europa Tracermass mass spectrometer. Instrument error is reported as ±0.5‰.

Results

We analyzed all samples for ^{14}C to examine the range of variation from atmospheric equilibrium in different specimens. Here $\Delta^{14}C$ is defined conventionally as the deviation of the ^{14}C concentration from the absolute ^{14}C standard, 0.95 times the activity of the HOxI oxalic acid standard, corrected for decay since 1950 (Stuiver and Polach 1977). Variation in the $\Delta^{14}C$ of organisms, relative to atmospheric values during their lifetime, has been associated with at least two phenomena: differences in the carbon signature of the environment (reservoir effects), and "lag times" due to slow tissue turnover or accumulation of tissue that integrates changing atmospheric values over time (*e.g.*, inbuilt age in long-lived trees.) In Figure 1 we show the Southern Hemisphere atmospheric curve for 1990 through 1996, and the data for all ^{14}C analysis of materials.

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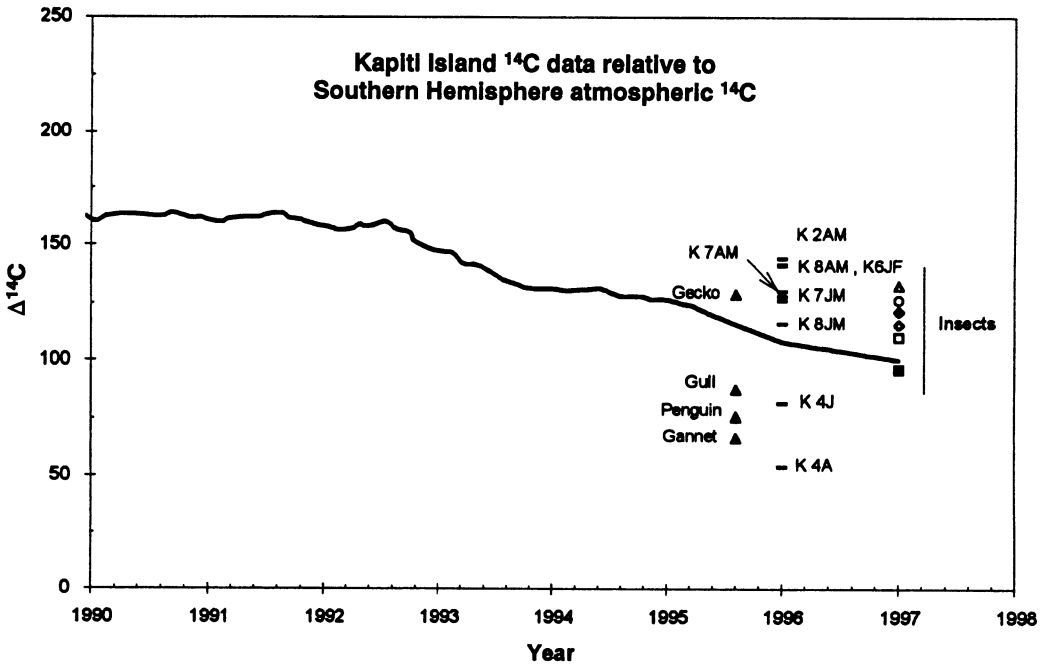

Fig. 1. $\Delta^{14}C$ of Kapiti Island specimens plotted against time of collection. Data points for gecko and seabirds have been displaced to the left for clarity, and are contemporaneous with the kiore data (K series). The continuous curve is the smoothed Southern Hemisphere atmospheric $\Delta^{14}C$ curve (M. R. Manning and W. H. Melhuish, personal communication 1993). Uncertainties in the Kapiti Island data are typically ± 9‰.

Plant Materials and Atmospheric ^{14}C

Plant materials from Kapiti Island and vicinity were analyzed to determine agreement with the Southern Hemisphere $\Delta^{14}C$ atmospheric values. The potential for using seasonal plant material to monitor $\Delta^{14}C$ values in the atmosphere was recognized in early bomb carbon studies (see Nydal 1963; Tauber 1967) and has had more recent application in determinations of variation in $\Delta^{14}C$ in contemporary and pre-bomb vegetative matter (Druffel and Griffin 1995; O'Brien and Stout 1978). Values for plant materials were also used in our determination of factors influencing rat bone values, as young shoots, fruits and grass seed make up an important component of the *Rattus exulans* diet (Bunn and Craig 1989; Speed 1986).

The $\Delta^{14}C$ values of three different plant materials, karaka berry (*Corynocarpus laevigatus*), mahoe berry (*Melicytus ramiflorus*) and a pohutukawa leaf (*Metrosideros excelsa*), collected on Kapiti Island in March 1997, are listed in Table 1. The weighted mean of the measured $\Delta^{14}C$ values is 94.8 ± 8.0 ‰. The latest date for which measured atmospheric $\Delta^{14}C$ values at Wellington are available is March 1995. Extrapolating the atmospheric curve to March 1997 gave an expected $\Delta^{14}C$ of about 100‰, which was essentially the same as the averaged contemporary vegetation value.

Interesting information about differential carbon turnover rates, or "lag times" (Druffel and Griffin 1995) was provided by the sample of shelf fungus *Polystictus*, from which an unidentified species of wood mite and a Carabid beetle were also collected. The $\Delta^{14}C$ from a well-mixed, powdered sample of the fungus, collected from a fallen tree on Kapiti, was +213 ± 11‰ (NZA 7510). As a fungus, *Polystictus* does not photosynthesize but takes up carbon along with nutrients from the organic sub-

strate. The apparent 20.8 yr lag time in transfer of tropospheric ^{14}C to the analyzed material, the fungus, is actually a measure of the integration of previously fixed atmospheric values incorporated by the host tree.

TABLE 1. Dates on Modern Vegetation

Lab code (NZA-)	Material	$\delta^{13}C$	$\Delta^{14}C$
7511	Karaka berry, *Corynocarpus laevigatus*	−28.6	+107.8 ± 9.2
7512	Mahoe berries, *Mellicytus ramiflorus*	−27.7	+79.7 ± 10.3
7514	Pohutukawa leaf, *Metrosideros excelsa*	−26.2	+93.9 ± 9.4
7510	Shelf fungus, *Polystictus sp.*	−22.8	+213.4 ± 11.2

Faunal Materials

The *R. exulans* scavenges invertebrate species and the carcasses of vertebrates, as well as taking grass seed and tender plant shoots and fruit. It has also been implicated in the predation of chicks and eggs of nesting birds (Booth *et al.* 1996; Lovegrove 1996; Imber 1964). We analyzed a number of possible food items (after Fuller 1994) that were available to the rat population of Kapiti.

Marine Birds

New Zealand Department of Conservation restrictions limited our collection of marine bird materials to found carcasses of animals. A gull was collected on Kapiti, and carcasses of a penguin and gannet from the immediate area were donated to the project. We were unable to sample the sooty shearwater, which nests in a trap line area on Kapiti, or its eggs. For dietary inputs to rats who scavenge marine birds such as the shearwater, we use the values for penguin and gannet, which have exclusively marine diets, and black-backed gull, a marine and terrestrial scavenger (Table 2).

In Figure 1 the food values ($\Delta^{14}C$) are compared with the Southern Hemisphere atmospheric ^{14}C curve. For the penguin and gannet, stable isotope values on the flesh hydrolysate confirm the marine basis of their diets. The $\Delta^{14}C$ for the penguin and for the gannet were lower than the average value for surface ocean waters sampled at Plimmerton, a nearby mainland coastal site, *viz.* +90 ± 5.0‰. A

TABLE 2. Dates on Vertebrate and Invertebrate Protein Sources

Lab code (NZA-)	Material	$\delta^{15}N$	$\delta^{13}C$	$\Delta^{14}C$
7049	Black-back gull, *L.dominicanus*	+11.7	−22.0	+87.5 ± 9.3
7048	Little Blue penguin, *E. minor*	+13.6	−17.9	+75.6 ± 9.2
7050	Gannet, *Sula serrator*	+11.3	−17.2	+66.8 ± 8.9
7059	Gecko, unidentified species	nd	−23.4	+128.9 ± 8.0
7412	Weta, *Hemiandrus,* north coast	+19.7	−25.7	+110.0 ± 9.3
7413	Beetle, *Mimopeus* sp.	nd	−26.5	+132.5 ± 9.5
7414	Beetle, Carabidae sp, north shore	+10.5	−27.8	+126.4 ± 9.9
7415	Beetle, Carabidae sp, tawa/hinau forest	+10.3	−26.4	+126.7 ± 9.6
7416	Weta, *Hemiandrus*, tawa/hinau forest	+6.2	−23.6	+96.0 ± 9.3
7417	Beetle, *Armadillion* sp, kohekohe forest	nd	−25.1	+121.5 ± 10.9
7508	Weta, *Gymnoplectron*, north coast	nd	−24.8	+115.8 ± 9.9
7509	Wood mite, unidentified	nd	−24.1	+111.4 ± 9.2
7513	Beetle Carabidae sp, from shelf fungus	nd	−24.9	+137.4 ± 10.9

possible reason for the variance from water values is that fish eaten by the birds come from sites with values more depleted than at Plimmerton. In contrast, the black-backed gull had a $\Delta^{14}C$ comparable to surface water values at Plimmerton, yet stable isotope values suggest a varied diet that may well include scavenging from anthropogenic and other terrestrial sources; similar isotopic variation in $\delta^{13}C$ has been noted for Kelp gulls taking supplementary food from refuse dumps (Steele and Hockey 1990).

Invertebrates

Stomach content analysis of *Rattus exulans* from Kapiti (Fuller 1994) indicates that *R. exulans* are largely insectivorous in forest habitats. Invertebrates from the island had a range of $\Delta^{14}C$ values associated with their different feeding habits and possibly the rate of tissue carbon turnover in some long-lived (3–10 yr) species, such as wetas of the family Stenopelmatidae.

The values for invertebrates (Table 2) were generally enriched relative to the atmospheric value of $\Delta^{14}C$ by 100 ± 9‰ for the period. Included in these samples are three individuals of Carabidae spp., carnivorous/scavenger beetles that feed upon invertebrates and vertebrate carcasses. The $\Delta^{14}C$ for a Carabid beetle from the island's north coast and a Carabid from tawa/hinau forest on the summit ridge are indistinguishable within error. A third Carabid taken from the shelf fungus had a higher $\Delta^{14}C$, suggestive of slightly different feeding habit than the previous samples, perhaps related to feeding on insects living in the fungus.

Among the invertebrates collected were several wetas (Orthoptera: Stenopelmatidae), belonging to the genera *Gymnoplectron* and *Hemiandrus*. Two of these weta from the north coast scree have similar terrestrial-like $\delta^{13}C$ values, but enriched $\delta^{15}N$ of 19.7‰. A second *Hemiandrus* specimen, from the tawa and hinau forest, had by contrast a $\delta^{15}N$ of 6.2‰. While the $\delta^{15}N$ varied by 13.5‰ among samples from the north coast *vs.* the tawa forest, the $\Delta^{14}C$ values are similar within measurement error. Isotopic analysis of these omnivorous weta shows that the $\delta^{13}C$ range is reasonable for metabolic enrichment of plant values by the feeder. Enriched nitrogen values, though, for the coastal specimens may be explained by a larger percentage of these wetas' diet being small invertebrates or carrion, rather than vegetation.

These food items are used to define the parameters of isotope values that would be taken up by a *R. exulans* feeding on terrestrial prey, and have a relation to the observed ^{14}C values for rats, a relationship that we further examine.

Isotope data for *Rattus exulans* specimens are summarized in Table 3, and the $\Delta^{14}C$ data is plotted the Southern Hemisphere atmospheric ^{14}C curve in Figure 1. Values of $\Delta^{14}C$ for rat bone gelatin indicate that the individuals fed largely on items like the animal proteins listed above, which were shown to incorporate non-equilibrium ^{14}C.

There were notable variations in the $\Delta^{14}C$ values for *Rattus exulans*. The specimens K4A and K4J (Trap Line 4, in the shearwater nesting colony) are depleted relative to the atmospheric curve. K4A is the most significant depletion found, at +54.4‰. While we initially suspected the depletions may have come through feeding upon sooty shearwater eggs or carcasses, the stable isotope values for K4A and K4J ($\delta^{13}C$ = –21.5‰, $\delta^{15}N$ = +11.6‰, and $\delta^{13}C$ = –20.6‰, $\delta^{15}N$ = +12.9‰, respectively) would not have signalled a marine component to the diet, yet ^{15}N values are higher than expected for terrestrial mammals (see Schoeninger and DeNiro 1984).

Rat specimens from other forest and grassland trap lines show enrichments in $\Delta^{14}C$. The highest enrichment was in K2AM, from coastal shrub and grassland. The $\delta^{13}C$ and $\delta^{15}N$ are within values

TABLE 3. Dates on Modern *Rattus exulans* from Kapiti Island

Lab code (NZA-)	Location, date, gender	Code	$\delta^{15}N$	$\delta^{13}C$	$\Delta^{14}C$
7034	Line 4, April, female	K4AF	+11.6	−21.3	+54.4 ± 8.7
7032	Line 4, July, female	K4JF	+11.3	−20.6	+81.7 ± 8.8
7407	Line 8, July, male	K8JM	+4.6	−21.6	+116.3 ± 9.7
7408	Line 7, July, male	K7JM	+5.2	−20.4	+125.9 ± 9.4
7403	Line 2, April, female	K2AF	+11.0	−22.3	+127.9 ± 10.3
7405	Line 7, April, male	K7AM	+8.2	−21.4	+129.9 ± 9.4
7406	Line 8, April, male	K8AM	+4.4	−20.4	+140.9 ± 9.5
7409	Line 6, July, female	K6JF	+5.3	−21.5	+141.7 ± 10.4
7404	Line 2, April, male	K2AM	+6.5	−22.3	+144.1 ± 9.6

expected for terrestrial mammals (Schoeninger and Moore 1992). A second specimen from the coastal shrub/grassland trap area, K2AF, is 16.2‰ lower in $\Delta^{14}C$ than the K2AM specimen taken 750 m away on the same trap line and more enriched in $\delta^{15}N$. The differences in $\Delta^{14}C$ values may be attributable to the proximity of K2AF on the trap line to a black-backed gull colony at the coastal grass, a scavenging opportunity not available to K2AM, as *Rattus exulans* ranges are limited to between 22 m and 70 m (Williams 1975). Mixed feeding of K2AF may have incorporated some black-back gull tissue into a mainly insect and plant diet, which might slightly deplete ^{14}C and enrich ^{15}N. Additional support for this explanation is that enriched nitrogen values are often associated with higher trophic levels of feeding (Schoeninger and Moore 1992).

Other $\Delta^{14}C$ enrichments were found in ridgetop grassland specimens K8AM and in K6JF from the mid-altitude tawa and hinau forest. In sample K8JM, from the same ridgetop grassland, the $\Delta^{14}C$ value was 116.3 ± 9.7‰ (NZA 7407), 25.4‰ less than K8AM, 375 m away on the same trap line. We cannot yet explain the large difference between specimens, except to note that the distance between the two would allow for the rats to have ranged in different areas, with different food sources available to the two individuals. However, stable isotope values do not vary significantly between the two specimens.

Our final rat specimens, K7AM and K7JM from the kohekohe forest trap line, gave equivalent isotope concentrations.

DISCUSSION

Enrichment in ^{14}C for most rat bone gelatin, and depletion for rats from Trap Line 4, indicate that the species has fed on items other than grass seed and plants, which would have had ^{14}C in atmospheric equilibrium. The ^{14}C isotopic analysis for food items, in addition to stable isotope analysis, apparently has the potential to track the uptake of carbon from various food sources that are a part of a scavenging rat's diet. We have paid particular attention to the ^{14}C values of animal-protein foods available to the rat in our examination of the sources and pathways of carbon at variance with atmospheric and found in rat bone protein.

When drawing conclusions from measurements, understanding biochemical pathways is as important as assessing the food webs from which organisms derive their carbon. For example, Druffel and Griffin (1995) made an important observation about the different biochemical pathways that must exist for chicken egg yolks and albumen to differ in $\Delta^{14}C$ relative to the shell. However, their suggestion that this variation was due to the inclusion of dead calcium carbonate in hen feed is challenged by Long, Hendershott and Martin (1983) and von Schirnding, van der Merwe and Vogel

(1982), who observed that carbon from such a mineral inclusion would be lost as CO_2 due to dissolution of carbonate by stomach acids. Carbon in eggshell is believed to be derived from blood bicarbonate, as is the mineral part of bone apatite, at values relative to respiration of organic nutrients (Long, Hendershott and Martin 1983). In Long's experiments, the inclusion of dead ^{14}C in avian feeds produced no measurable effect on eggshell carbonate nor organic matter.

In our examination of diet-induced $\Delta^{14}C$ variance, we have found that organisms may be up to 60‰ at variance from atmospheric $\Delta^{14}C$ because of carbon inputs via the food web. In the invertebrates analyzed, $\Delta^{14}C$ values reflect the known feeding habits (carnivore or herbivore diet) of the specimen. The omnivorous/scavenger species of Carabidae, for instance, had $\Delta^{14}C$ values enriched over plant material and more closely associated with the values of herbivore insects and carcasses assumed to have been eaten by the beetle. For herbivorous weta, which live 3–10 yr, a factor contributing to the slightly enriched $\Delta^{14}C$ over fresh plant material values may be the inclusion of carbon derived from older leaf litter and lichens, or carbon ingested in previous years, reflecting slow tissue turnover.

Differences in the ^{14}C values of *Rattus exulans*, which on average have a one-year life span, are expected to be a direct function of diet. The routing of carbon from dietary protein to bone protein was first proposed by Chisholm, Nelson and Schwartz (1982), Chisholm *et al.* (1983) and Krueger and Sullivan (1984). While collagen can incorporate carbon from non-protein sources under conditions of a low-protein diet, the direct routing of dietary carbon to bone protein is normally via the essential amino acids, which are most abundant in proteinaceous dietary items, as shown by Ambrose and Norr (1993). In their study, the essential amino acids strongly influenced the $\delta^{13}C$ signature of bone protein by metabolic processes that are not fully understood, but that seem to be a nonlinear function of the percentage of protein in the diet. The carbon from essential amino acids in animal foods also carries ^{14}C, which could be at variance with contemporary atmospheric carbon if the feeder is taking sustenance from a non-equilibrium carbon reservoir (*e.g.*, a seabird feeding on marine fish, or a worm living on humus). We have calculated that essential amino acids in these bone samples of *R. exulans* carry *ca.* 30% of the carbon in the bone protein. As there is no evidence thus far for a fractionation of ^{14}C that would metabolically enrich values in tissue, we assume ^{14}C values integrated from proteinaceous diet items are reflected in those values analyzed for bone gelatin.

These assumptions allow us to model the impact of dietary ^{14}C on the previously measured samples. The basis of this approach is shown in Tables 4 and 5, which show amino acid analyses of hydrolyzed bone protein from two Kapiti *Rattus* specimens, K4J and K8JM. From the relative molar amounts of amino acids and the number of carbon atoms in each molecule, it is possible to calculate the effectiveness of each component acid at influencing the overall carbon isotope signature for the bulk protein. If the $\delta^{13}C$ and $\Delta^{14}C$ of each amino acid were known, a more precise calculation would be possible to obtain the corresponding values for the resultant bone protein. We have not yet obtained such detailed data, but we may still illustrate the effects to be expected by identifying the essential (derived solely from diet) and the nonessential (metabolized) amino acids, assigning "typical" isotope signatures to each component from known parameters in diet, and comparing the calculated protein signatures with the values actually measured.

Specimen K8JM (Table 4) is taken to be representative of a largely terrestrial-based diet. Here, the nonessential amino acids are metabolized in the body from energy substrates (carbohydrates, sugars from plant materials) and glycine syntheses, while the essential amino acids come largely from protein-rich sources, such as invertebrates, in the diet. As inputs to the calculation, equivalent $\Delta^{14}C$ val-

TABLE 4. Amino analysis of modern *Rattus* specimen K8JM, enriched in ^{14}C. Measured isotope signatures: $\delta^{13}C = -21.6‰$, $\Delta^{14}C = +116.3 \pm 9.7‰$ (NZA 7407). Model calculation: $\delta^{13}C = -22‰$, $\Delta^{14}C = +107.4‰$.

Amino acid*	MW†	Carbons‡	Moles§	$\delta^{13}C$#	$\Delta^{14}C$#
Aspartic acid	114	4	1.249	−22	99
Glutamine	151	5	2.140	−22	99
Hydroxyproline	129	5	2.092	−22	99
Serine	87	3	0.863	−22	99
Meth. sx.	147	5	0.013	−22	99
Arginine*	156	6	1.263	−22	127
Threonine*	101	4	0.528	−22	127
Glycine	57	2	7.779	−22	99
Alanine	71	3	2.800	−22	99
Proline	97	5	2.822	−22	99
Valine*	99	5	0.781	−22	127
Methionine*	131	5	0.205	−22	127
Isoleucine*	113	6	0.393	−22	127
Leucine*	113	6	0.901	−22	127
Phenylalanine*	147	9	0.447	−22	127
Hydroxylysine	144	6	0.235	−22	99
Lysine*	128	6	0.774	−22	127
Histidine*	137	6	0.194	−22	127
Tyrosine	163	9	0.178	−22	99

*Essential amino acids marked with asterisk
†Molecular weight in protein
‡Number of carbon atoms carried
§Number of moles of amino acid present in analysis
#Input values for model

ues of +99‰ are used for the energy substrate component, and +127‰ for the protein/essential amino acid component, a value reflecting an average of the $\Delta^{14}C$ for invertebrates. For the $\delta^{13}C$, we have used a common value of −22‰, based on an average of values of −25‰ for plants and −26‰ for insects, with a metabolic enrichment of +3.5‰ applied, following the observation of Ambrose and Norr (1993). Our model of partitioned carbon values from a diet believed to be terrestrial plant and insect based predicts that the values would be $\Delta^{14}C = 108.0‰$ and $\delta^{13}C = -22‰$. Measured values for the specimen K8JM are $\Delta^{14}C = 116.3 \pm 9.7‰$ and $\delta^{13}C = -21.6 \pm 0.1‰$.

Table 5 shows a similar analysis for *Rattus* specimen K4J, for which a marine-based food source (shearwaters, *Puffinus griseus*) was available. The isotope signatures analyzed for penguin and gannet tissue were used for the essential amino acid component, at $\Delta^{14}C = 72‰$ and $\delta^{13}C = -17.0‰$, and we use the previous, modern/terrestrial isotopic values for nonessential amino acids. Here, calculated bone protein values are $\Delta^{14}C = 89.9‰$ and $\delta^{13}C = -19.5‰$, which compares well with the actual measurement, $\Delta^{14}C = 81.7 \pm 8.8‰$ and $\delta^{13}C = -20.6 \pm 0.1‰$. Good agreement between calculation and measurement need not be expected for such a simplistic set of assumptions, but nevertheless the ^{14}C and ^{13}C values obtained through the model agree to within the experimental error. In particular, the assumption that either all of the essential or all of the nonessential amino acids can be assigned a common isotope signature is unlikely to be entirely correct; the variability of the individual amino acid isotopic ratios has been previously observed by Hare and Estep (1983) and Macko *et al.* (1983).

TABLE 5. Amino analysis of modern *Rattus* specimen K4J, depleted in ^{14}C. Measured isotope signatures: $\delta^{13}C = -20.6‰$, $\Delta^{14}C = +81.6 \pm 9.7‰$ (NZA 7032). Model calculation: $\delta^{13}C = -19.4‰$, $\Delta^{14}C = +89.9‰$.

Amino acid*	MW	Carbons	Moles	$\delta^{13}C$	$\Delta^{14}C$
Aspartic acid	114	4	0.515	−22	99
Glutamine	151	5	0.861	−22	99
Hydroxyproline	129	5	0.919	−22	99
Serine	87	3	0.384	−22	99
Meth. sx.	147	5	0.017	−22	99
Arginine*	156	6	0.550	−14	72
Threonine*	101	4	0.236	−14	72
Glycine	57	2	3.467	−22	99
Alanine	71	3	1.158	−22	99
Proline	97	5	1.251	−22	99
Valine*	99	5	0.316	−14	72
Methionine*	131	5	0.104	−14	72
Isoleucine*	113	6	0.180	−14	72
Leucine*	113	6	0.373	−14	72
Phenylalanine*	147	9	0.191	−14	72
Hydroxylysine	144	6	0.107	−22	99
Lysine*	128	6	0.358	−14	72
Histidine*	137	6	0.086	−14	72
Tyrosine	163	9	0.061	−22	99

*Essential amino acids marked with asterisk; other columns as in Table 4

This simple model examines the possibility that diet introduces carbon that could seriously offset true ages in subfossil specimens. In the case of an anomalously old sample from the Holdaway (1996) suite, we can use the model to estimate the amount of ^{14}C depletion required in a protein food source to produce the observed ^{14}C ages (Table 6). Here we have assumed that the rat is actually only 400 yr old; the nonessential amino acids are derived from 400-yr-old carbon from energy substrates, while the essential amino acids are supplied from an unspecified protein source that is strongly depleted in ^{14}C, but with a typically terrestrial $\delta^{13}C$. The target total $\Delta^{14}C$ is *ca.* −200‰, corresponding to a conventional ^{14}C age (CRA) of *ca.* 1700 BP. The amino acid analysis used in the calculation is that for the Holdaway (1996) *Rattus exulans* subfossil specimen (CRA 1747 ± 69 BP, NZA 5922). The nonessential amino acids are assumed to have $\Delta^{14}C$ of −54‰, *i.e.,* the chronological age of the rat at *ca.* 400 BP. The calculation shows that the protein source requires a $\Delta^{14}C$ of −600‰, corresponding to a CRA of 7300 BP, to duplicate the measured age. However, it is likely that the rat will have access to a variety of protein sources, and the calculated depletion represents an average value. Hence, some proteins could be contemporary, but this would require that a significant component be depleted by more than −600‰. In the extreme case, further calculation shows that the required depletion can be achieved by a mixture of 82% contemporary carbon and 18% totally depleted carbon via dietary protein. Whether or not such numbers are reasonable requires support from further field studies, but it should be noted that highly $\Delta^{14}C$-depleted fauna have been observed in natural environments, *e.g.,* snails dwelling in artesian springs (Riggs 1984).

From our modeling, it appears that the type of depletion required in the diet to affect ^{14}C ages severely limits the possibilities for diet to introduce dramatic offsets from true ages. Marine diet inclusions for terrestrial-based animals, for example, would have a variable effect on true age, as the depleted carbon from a marine-based source would be partitioned among other dietary inputs. This

TABLE 6. Amino analysis of subfossil *Rattus* specimen from the Holdaway (1996) suite, CRA = 1747 ± 69 BP. Measured isotope signatures: $\delta^{13}C = -20.8‰$, $\Delta^{14}C = -200.0 \pm 6.8‰$ (NZA 5922). Model calculation: $\delta^{13}C = -20.4‰$, $\Delta^{14}C = -200.3‰$, CRA = 1751 BP.

Amino acid*	MW	Carbons	Moles	$\delta^{13}C$	$\Delta^{14}C$
Aspartic acid	114	4	0.439	–22	–54
Glutamine	151	5	0.852	–22	–54
Hydroxyproline	129	5	0.898	–22	–54
Serine	87	3	0.325	–22	–54
Meth. sx.	147	5	0.003	–22	–54
Arginine*	156	6	0.492	–16	–600
Threonine*	101	4	0.186	–16	–600
Glycine	57	2	3.342	–22	–54
Alanine	71	3	1.147	–22	–54
Proline	97	5	1.156	–22	–54
Valine*	99	5	0.242	–16	–600
Methionine*	131	5	0.032	–16	–600
Isoleucine*	113	6	0.111	–16	–600
Leucine*	113	6	0.261	–16	–600
Phenylalanine*	147	9	0.143	–16	–600
Hydroxylysine	144	6	0.108	–22	–54
Lysine*	128	6	0.260	–16	–600
Histidine*	137	6	0.021	–16	–600
Tyrosine	163	9	0.029	–22	–54

*Essential amino acids marked with asterisk; other columns as in Table 4

suggests that caution is required when applying a marine correction to materials other than marine shell or animals living and feeding solely in a marine environment. Conventional ^{14}C ages for marine shell or animals with a fully marine substrate (*e.g.*, sea turtle, fish) have marine corrections applied to account for the known difference in $\Delta^{14}C$ between the ocean and atmosphere. Other materials, such as bone and tissue from organisms that have been found to have an enriched or "marine-like" $\delta^{13}C$ and $\delta^{15}N$, have been assumed to require similar types of marine corrections. However, the application of a marine correction in these circumstances should either not be used or be used with caution. For terrestrial animals that have an omnivorous diet—even where marine foods make up the richest portion of the protein intake—only ~30% of the carbon *via* essential amino acids in the bone protein can carry depleted carbon signatures.

CONCLUSION

We observed variations in ^{14}C in our modern populations of *Rattus exulans* that could be associated with diet. To examine whether similar variations over habitat occur elsewhere, this project will be repeated on a second offshore island, Taranga (Hen Island) in the Hauraki Gulf, New Zealand. For the subfossil samples of *R. exulans*, or other subfossil bones of terrestrial omnivores, we require an alternative method to gauge the presence and effects of dietary carbon, especially as stable isotope values may not readily indicate dietary variation. For this reason we consider that HPLC separation of essential amino acids and their comparative analysis with other bone protein fractions is required to assess the presence and impact of disequilibrium dietary carbon. Our current research involves the application of amino acid separation techniques to extract individual essential amino acids, to group these in an aliquot with sufficient carbon for AMS analysis, and to compare these with a non-

essential amino, such as glycine, from the same extraction procedure. As a nonessential amino acid such as glycine is formed in the body from CO_2 and ammonia by the action of a glycine synthase, the glycine should have a $\Delta^{14}C$ approximately in equilibrium with blood bicarbonate levels and with atmospheric levels during the lifetime of the individual. Variance between the $\Delta^{14}C$ of essential amino acids and glycine should reveal the presence of carbon from diet that is in disequilibrium with contemporary values.

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