

## ENVIRONMENTAL INFLUENCES ON DIETARY CARBON AND $^{14}\text{C}$ AGES IN MODERN RATS AND OTHER SPECIES

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**ABSTRACT.** Diet can play a significant role in anomalous radiocarbon ages derived from bone and other tissues when the food web incorporates depleted  $^{14}\text{C}$  reservoirs, such as the marine environment. Dietary effects from a post-bomb carbon variation have also been found in modern invertebrates and populations of *Rattus exulans* (Beavan and Sparks 1998). We now present the effect on absolute percent modern (pMC) and the conventional radiocarbon age (CRA) of a modern aquatic/terrestrial food web in a volcanic zone of the North Island, New Zealand. At Lake Taupo, geothermal venting transfers  $^{14}\text{C}$  depleted carbon to lake waters, which aquatic plants fix into the food chain; depleted  $^{14}\text{C}$  is shown to then pass on to shellfish, waterfowl, and Norway rat (*Rattus norvegicus*). The geothermally induced  $^{14}\text{C}$  variations from modern atmospheric pMC and CRA can increase apparent  $^{14}\text{C}$  ages by >2000 years.

### INTRODUCTION

Shells and bones of organisms that feed exclusively from specific radiocarbon-depleted reservoirs (i.e. marine or limestone-based aquatic environments) can return anomalous  $^{14}\text{C}$  ages as a function of diet (Albero et al. 1986; Bard et al. 1993; Lanting and van der Plicht 1998; Goodfriend et al. 1999; Arneborg et al. 1999), and are typically identified by enriched  $^{15}\text{N}$  and  $^{13}\text{C}$  values (Schoeninger and DeNiro 1984). There is also a potential for diet-induced  $^{14}\text{C}$  age errors in omnivorous organisms that mix contemporaneous  $^{14}\text{C}$  and  $^{14}\text{C}$ -depleted carbon reservoirs.

Our examination of variability in  $^{14}\text{C}$  ages due to diet arose from an issue concerning  $^{14}\text{C}$  dates from sub-fossil Polynesian rat (*Rattus exulans*) that range from ~700 BP to >2000 BP (Holdaway 1996). Because *R. exulans* is a human commensal introduced to New Zealand, the oldest dates for this species would be a proxy for earliest human contact and the introduction of mammalian predators to New Zealand more than 2000 years ago. Human contact with New Zealand as early as 2000 BP, however, exceeds the current paradigm of around 700 BP for human discovery and colonization of New Zealand (Roberts 1991; Anderson 1991; McFadgen et al. 1994).

At one South Island, New Zealand archaeological site, we found that anomalous  $^{14}\text{C}$  ages for both *R. exulans* and fresh-water aquatic birds appeared to have been induced by diet (Beavan-Athfield and Sparks 2001). In a further examination of depleted-carbon transfer processes in food webs, we report on the  $^{14}\text{C}$  variation found in a modern food web at Lake Taupo, North Island, New Zealand.

### THE STUDY AREA

Lake Taupo (Figure 1) is a warm-monomictic lake in the Taupo Volcanic Zone (TVZ), with bicarbonate anion predominant (White et al. 1980). Geothermal activity expels mantle-derived  $\text{CO}_2$  from several vents in the lake bottom with typical enriched stable carbon ratios ( $^{13}\text{C}$ ) of  $-3.4\text{‰}$  to  $-2.3\text{‰}$  (de Ronde, personal communication October 2000), compared to atmospheric  $\text{CO}_2$  values of  $-7.6\text{‰}$ . Mantle  $\text{CO}_2$  contributes to the extremely old Conventional Radiocarbon Ages (CRA, Stuiver and Polach 1977) of inorganic carbon found in waters from various sites on Lake Taupo, ranging from 1833 BP to more than 4000 BP, and  $^{13}\text{C}$  from  $-4.7\text{‰}$  to  $0.9\text{‰}$  (Table 1). For this study, we chose an aquatic-based food web in a marsh site on the southern shore of Lake Taupo, at Tokaanu Bay, Turangi

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(Figure 1). Additional water samples were taken from Lakes Pohue and Tutira in Hawke's Bay to examine carbon contribution to lake water outside of the TVZ (Figure 1, inset).

## MATERIALS AND METHODS

### Sample Collection

At Tokaanu Bay we collected lake water, four aquatic plant species (*Lachnagrostis filiformis*, *Lagarosiphon major*, *Glossostigma sp. unidentified species*), New Zealand scaup (*Aythya novaeseelandiae*) a small diving duck which feeds on macro-invertebrates, shellfish and aquatic vegetation (Marchant and Higgins 1990), and two rats (*R. norvegicus*) were collected in February and November, 2000, within a 1500 m<sup>2</sup> area of the Tokaanu marsh, between the Tokaanu Tailrace and the western banks of the marsh on the Tongariro River delta (Figure 1). Water samples were collected from lake shore sites in less than 1 m of water, in clean 500 mL Pyrex flasks which were submerged until full and sealed while under water to avoid atmospheric exchange with the sample. Aquatic plants were collected by hand from <1 m of water at Tokaanu marsh, and separately bagged for identification. New Zealand scaup was collected from the Tokaanu Tailrace as a fresh carcass. Norway rats (*R. norvegicus*) were snap-trapped about 50 m apart in the Tokaanu marsh. We also include in our analyses fresh water mussel (*Hyridella menziesi*) collected live from Lake Taupo in 1983, and samples of Lake Taupo water collected along a north south axis at various times since 1958 (Table 1).

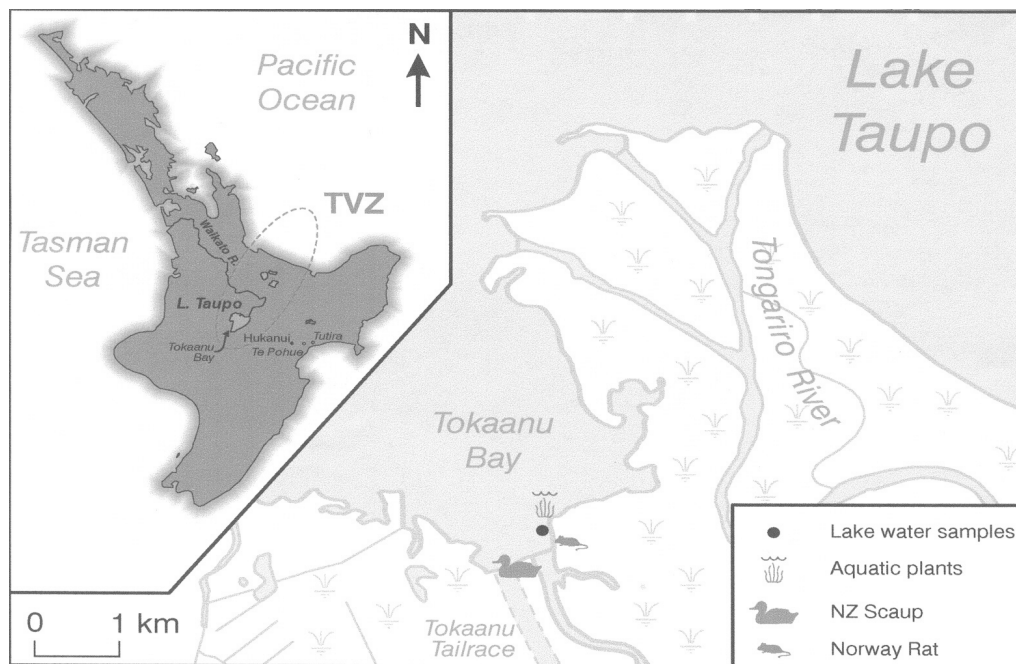


Figure 1 Map of the North Island of New Zealand showing area of the Taupo Volcanic zone, the Waikato River and Lakes Te Pohue and Tutira. Inset shows the marshland sampling site at Tokaanu Bay, on the southern shore of Lake Taupo.

Table 1 Stable isotope values and radiocarbon activity of water, aquatic plants, and animals from Lake Taupo, New Zealand

	$\delta^{15}\text{N}\text{‰}$	$\delta^{13}\text{C}\text{‰}$	pMC <sup>a</sup>	CRA <sup>b</sup> (BP)	Lab nr
Lake Taupo water					
Tokaanu Bay <sup>c</sup>		-6.4	62.8 ± 0.8	3694 ± 100	NZA12392
Tokaanu Bay <sup>c</sup>		-5.9	59.4 ± 0.4	4143 ± 55	NZA12393
Taupo: Turangi inlet <sup>d</sup>		-4.7	67.4 ± 0.5	3167 ± 64	NZ3248
Taupo-mid lake <sup>e</sup>		0.9	74.6 ± 0.5	2325 ± 58	NZ6401
2 Mile Bay <sup>f</sup>		0.3	77.9 ± 0.5	1983 ± 48	NZ5743
2 Mile Bay <sup>f</sup>		0.6	79.3 ± 0.5	1833 ± 48	NZ5744
Taupo: Waikato River outlet <sup>c</sup>		-4.7	60.2 ± 0.5	4071 ± 65	NZ3247
Other sites					
Lake Tutira, Hawkes Bay <sup>g</sup>		-6.2	106.1 ± 0.7	-523 ± 55	NZA12430
Lake Te Pohue, Hawkes Bay <sup>g</sup>		-7.7	100.6 ± 0.7	-101 ± 55	NZA12433
Aquatic plants, Tokaanu Bay <sup>c</sup>					
<i>Lachnagrostis filiformis</i>	3.1	-25.4	94.2 ± 0.8	429 ± 70	NZA12022
<i>Lagarosiphon major</i>	-0.7	-16.6	57.1 ± 0.4	4452 ± 55	NZA12023
Unidentified <i>sp</i>	-1.6	-24.1	63.2 ± 0.6	3639 ± 75	NZA12047
<i>Glossostigma sp</i>	5.7	-24.7	87.8 ± 0.7	997 ± 65	NZA12048
Fresh water mussel flesh <sup>d</sup> <i>Hyridella menziesi</i>					
	nd	-21	77.7 ± 0.4	1998 ± 43	NZ6378
NZ Scaup <i>Aythya novaeseelandiae</i>					
Liver	8.4	-13.9	72.9 ± 0.5	2487 ± 50	NZA11169
Bone	8.6	-15.7	71.3 ± 0.6	2674 ± 65	NZA11209
Muscle	8.4	-15.9	70.9 ± 0.5	2719 ± 50	NZA11165
Skin	9.2	-17.7	68.9 ± 0.5	2946 ± 60	NZA11210
Norway Rat <sup>c</sup> <i>R. norvegicus</i>					
Rat #1, femur and tibia	6.5	-22.1	102.7 ± 0.7	-261 ± 60	NZA12024
Rat #2, femur and tibia	11.6	-20.3	76.2 ± 0.6	2139 ± 55	NZA12025

<sup>a</sup>pMC = absolute percent modern, relative to NBS HOxI, corrected for decay since 1950 (Stuiver and Polach 1977)

<sup>b</sup>CRA = conventional radiocarbon age

<sup>c</sup>Collected February 2000

<sup>d</sup>Collected November 1958 (Rafter et al. 1972)

<sup>e</sup>Collected August 1983 (Currie 1984)

<sup>f</sup>Collected February 1978 (H Polach, unpublished data)

<sup>g</sup>Collected November 2000

### Physical and Chemical Preparation

*R. norvegicus* and *New Zealand scaup bone*: Tibiae and femora from carcasses 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 hr. Collagen was filtered from the solution and gelatinized with 0.01M HCl in a nitrogen atmosphere at 90 °C for 16 hr. The gelatin was then double-filtered through Whatman™ GF/C and 0.45 µm Acro-disc™ filters, and lyophilized to weigh yields. An average of 4 mg of this bone gelatin was com-

busted in an evacuated and sealed quartz tube with CuO and Ag wire at 900 °C for 2 hr. After cooling, the quartz tube was cracked on a vacuum line and the CO<sub>2</sub> cryogenically distilled. The distilled CO<sub>2</sub> was graphitized and analyzed by accelerator mass spectroscopy (AMS) dating.

*Aquatic plants:* After identification, vegetable matter was washed in 0.01M HCl and rinsed to neutral in deionized water, then vacuum dried at 30 °C. Two to three milligrams of material was combusted and graphitized as above.

*Fresh water mussel:* Fresh-water mussel flesh was collected and analyzed in August of 1983 (Currie 1984). The sample was processed by washing with deionized water, freeze-drying, and preparing for gas counting in the manner of Jansen (1984).

*NZ scaup tissues:* Flesh, skin and liver samples were washed in distilled water and freeze-dried. Approximately 2–3 mg of lyophilized tissue was then combusted and graphitized as above for AMS analysis.

*Lake water:* 120 mL of lake water and anti-bumping granules were placed in a sidearm reaction vessel flushed with N<sub>2</sub>. After evacuation, approximately 6 mL of orthophosphoric acid in the sidearm was introduced to the water and stirred at room temperature until the reaction ceased. Evolved CO<sub>2</sub> was cryogenically distilled, collected and measured for yield, and graphitized as above.

#### **Elemental Analysis of Bone Gelatin and Flesh**

Samples for <sup>15</sup>N and <sup>13</sup>C were analyzed on a Europa Geo 20/20, interfaced to an ANCA-SL elemental analyzer. Isotopes are in continuous flow mode. Carbon and nitrogen isotopes were analyzed simultaneously from an average 1.5 mg of dry sample. The carbon dioxide and nitrogen gases were resolved using chromatographic separation on a GC column at 85 °C, and analyzed for percent abundance and delta element value. C/N is calculated from percent abundance. Analysis of each sample is done in duplicate; reported values are drift-corrected and an average of the duplicate. Machine error values are: ± 0.1‰ for carbon, ± 0.3‰ or better for nitrogen. Standards are: flour (<sup>13</sup>C: -25.3‰; <sup>15</sup>N: 3.01‰) and ANU sucrose/IAEA-N2 (<sup>13</sup>C: -10.4‰; <sup>15</sup>N: 20.34‰) which are run after every six duplicates of sample.

#### **RESULTS AND DISCUSSION**

Results of <sup>14</sup>C and stable isotope analyses are shown in Table 1. What is immediately striking is the range of variability in <sup>14</sup>C content evident in a number of what might be considered “modern” sources, including the Lake Taupo water itself. The overall <sup>14</sup>C depletion reflects the input of volcanic CO<sub>2</sub> through venting in the lake floor. This can be contrasted with Lakes Tutira and Pohue, which are some 200 km to the east of the TVZ boundary, and show predominantly atmospherically derived carbon.

At Lake Taupo, aquatic plant photosynthesis fixes the dissolved inorganic carbon and transfers depleted <sup>14</sup>C from lake water to the base of the food web, returning CRAs on four species of plant ranging from 429 BP to more than 4000 BP (pMC = 79.3% to 60.2%). The carbon isotope transfer is similar to that seen in plankton living in the <sup>14</sup>C-depleted waters of some European lakes (Geyh et al. 1998). In Tokaanu Bay, the sampled aquatic plants also grow at varying levels at or near the lake surface, and the variation in apparent <sup>14</sup>C age and pMC of some species such as *Lachnagrostis* and *Glossostigma* may indicate some modern atmospheric CO<sub>2</sub> exchange. Variable fractionation of δ<sup>13</sup>C also

seen in these aquatic plants may be according to species type and rates of metabolism, as well as by the isotopic composition of the dissolved inorganic carbon substrate (Osmond et al. 1981).

Mussel flesh shows a similar mixed-reservoir effect, with a depleted  $\delta^{13}\text{C}$  ratio ( $-21.3\text{‰}$ ) relative to lake water bicarbonate ( $0.3\text{‰}$  to  $-5.9\text{‰}$ ). The depletion may be due to two possible factors. Filter feeding on particulate matter that is a mixture of carbon from allochthonous terrestrial material at atmospheric equilibrium ( $\text{pMC} = 110\text{‰}$ ,  $\delta^{13}\text{C} = -0.7\text{‰}$ ) and algae which drew carbon from lake water depletions would moderate both the enriched  $\delta^{13}\text{C}$  and the  $^{14}\text{C}$  depletion effect of lake water. Alternatively, a terrestrial source may not be required to deplete values of  $\delta^{13}\text{C}$  in the mussel flesh. Aquatic plants which were sampled produced a mean  $\delta^{13}\text{C}$  of  $\approx -21\text{‰}$  (Table 1), thus mussel flesh values may be associated with filter feeding of particulate matter such as algae, an aquatic plant type which we did not sample during this project.

For the New Zealand scaup, four different tissue samples from the same individual show CRAs with a standard deviation of 189 years, much greater than the statistical uncertainty ( $T' = 35.1$ ,  $\chi^2_{v=3;p=0.05} = 7.8$ ) (Ward and Wilson 1978). The  $^{14}\text{C}$  variations appear to be strongly correlated with variations in  $\delta^{13}\text{C}$  (Figure 2), suggesting that the carbon is drawn from two dominant sources, each with its characteristic  $^{14}\text{C}$  and  $^{13}\text{C}$  signatures. The partitioning of the dietary carbon between the different tissues implied by Figure 2 is likely to be associated with different rates of tissue turnover and variability in diet over time (Hobson et al. 1999).

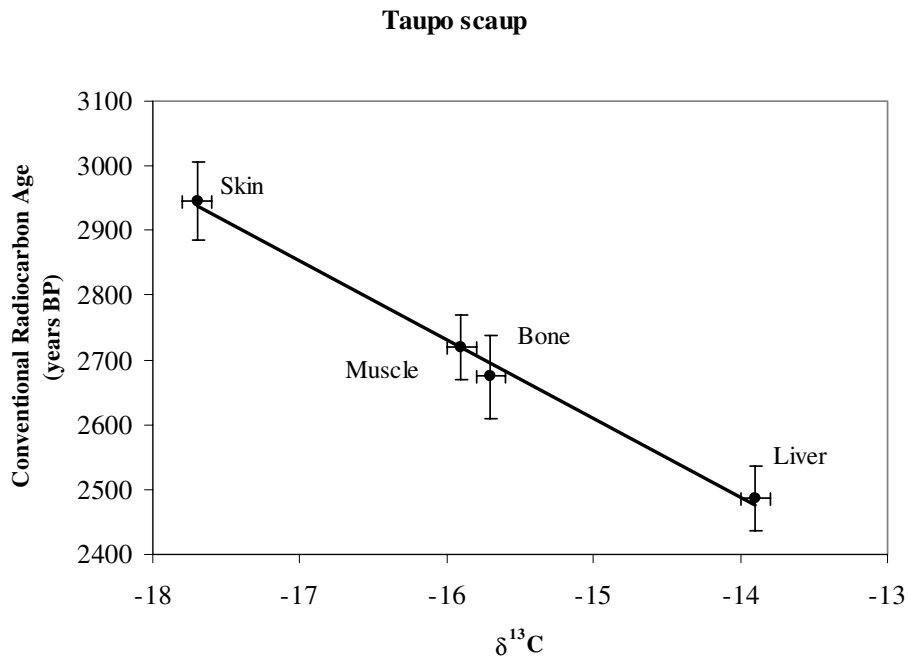


Figure 2 Correlation between  $^{14}\text{C}$  age and  $\delta^{13}\text{C}$  of the tissue samples from the Lake Taupo scaup (Table 1). Trendline is a least squares fit to the data points. CRA= conventional radiocarbon age (Stuiver and Polach 1977).

The most extreme case of variability occurs for the two Norway rats (*R. norvegicus*). Two individuals trapped within 50 meters of each other in February 2000 have CRAs of  $2139 \pm 55$  BP (pMC  $76.2 \pm 0.6$ ) and  $-260 \pm 60$  BP (pMC  $102.7 \pm 0.7$ ), respectively. Repeat measurements confirmed this result. *R. norvegicus* is an omnivorous scavenger and can include aquatic waterfowl eggs, nestlings and adult carcasses in its diet (Bettesworth 1972), explaining the older CRA observed if this specimen fed upon fowl such as local scaup, whereas the second *R. norvegicus*' diet has a  $^{14}\text{C}$  content similar to present-day atmosphere (Southern Hemisphere 1999/2000 est. pMC  $\approx 110\%$ .) Similar effects, though less marked, have been reported in modern populations of *Rattus exulans* (Beavan and Sparks 1998). The  $\delta^{13}\text{C}$  for the two rats, while different, do not point to such a large difference in the CRAs, although there is a significant difference between the  $\delta^{15}\text{N}$  values, indicating a diet higher in animal protein for the rat with a  $>2000$  BP apparent age.

The range of  $^{14}\text{C}$  variability reported here may explain other, apparently anomalous,  $^{14}\text{C}$  ages from the same area. For example, at Whakamoenga Cave on the northeast shore of Lake Taupo, the bones of moa from a human occupation layer at an archaeological site have a CRA of  $4747 \pm 120$  BP and  $\delta^{13}\text{C}$  of  $-24.8\text{‰}$  (NZA577; McFadgen 1995). The age predates assumed limits on the age of human settlement in New Zealand. It also predates the last Taupo eruption of 1720 BP (Sparks et al. 1995), which would have destroyed any biological material in this area. While moa are presumed to have been largely herbivorous, grazing which included lakeshore macro-invertebrates cannot be ruled out.

The bone and tissue results from Tokaanu Bay also show that overall stable isotope ratios appear affected by omnivorous diets. While enriched  $^{15}\text{N}$  and  $^{13}\text{C}$  ranges can be used to indicate dietary links to reservoirs depleted in  $^{14}\text{C}$  (e.g. marine environments, limestone environments, Schoeninger and DeNiro 1984), previous work on carbon transfer from marine or aquatic to terrestrial food webs show that the  $^{13}\text{C}$  and  $^{15}\text{N}$  ratios of a mixed diet can effectively mask the contribution of certain  $^{14}\text{C}$  depleted sources (Beavan and Sparks 1998; Beavan-Athfield and Sparks 2000). Stable isotopes do not reliably signal mixed reservoir feeding in terrestrial omnivores, due to the variation in the isotopic composition of the different food sources exploited and the proportion of each food type in the total diet (Chisholm et al. 1983; Hobson et al. 1999).

This mixed-range isotope effect is observed in some modern *R. exulans*, where depleted  $^{14}\text{C}$  indicated partial dietary contributions from a marine reservoir, but the bone retained apparently terrestrial-range  $^{13}\text{C}$  ( $-20.4\text{‰}$  to  $-21.3\text{‰}$ ) and nominally enriched  $^{15}\text{N}$  ratios (mean  $+11.4\text{‰}$ , Beavan and Sparks 1998). A similar effect is seen at Tokaanu Bay for the anomalously old Norway rat (Table 1), which had a diet inclusive of an unknown proportion of aquatic-linked, high animal-protein food, such as scaup.

In general, the effect of localized sources of depleted carbon on food webs in New Zealand is largely unknown. Geothermal activity contributes to the water of at least 26 other lakes in the 6000 km<sup>2</sup> of the TVZ (Timperly and Vigor-Brown 1986). Old  $^{14}\text{C}$  ages for the Waikato River outlet of Lake Taupo (Table 1) suggest a transfer of  $^{14}\text{C}$  depleted water to non-geothermal areas hundreds of km to the northwest of the TVZ, a river along which more than 250 archaeological sites are located. The effects of geothermal,  $^{14}\text{C}$ -depleted water on the aquatic/terrestrial food web in the region has not yet been tested and is likely to be highly variable, due to mixing with additional watershed inputs along the Waikato's course. The significance of the local geological substrate is also unclear. When looking for other environmental effects, we tested water samples from two additional lakes in the North Island, Te Pohue and Tutira, to the east and well outside of the TVZ, in a region of Pliocene age coquina limestone strata (Naish et al. 1998). We found that the  $^{14}\text{C}$  age of bicarbonate derived from water in these lakes return modern (post-bomb)  $^{14}\text{C}$  values (pMC =  $100.6 \pm 0.8$  and  $106.1 \pm 0.7$ ,



respectively; Table 1), suggesting natural incorporation of post-bomb atmospheric  $^{14}\text{C}$  which significantly offsets possible effects from dead carbon in the limestone geological substrate.

## CONCLUSIONS

We suggest that old carbon reservoirs in the New Zealand environment are a potential source of error for  $^{14}\text{C}$  dates of organisms, depending on their diet. Stable isotope ratios in omnivores may mask  $^{14}\text{C}$  contributions from marine and geothermal reservoirs. To counter this we recommend a careful consideration of  $^{14}\text{C}$  results from all types of bone and other tissue from omnivorous organisms. Testing regional carbon reservoirs, and comparing  $^{14}\text{C}$  ages of other materials associated with bone (Holdaway and Beavan 1999, Beavan-Athfield et al. 1999) can highlight or eliminate potential dietary effects. Local environment effects should therefore always be considered when interpreting  $^{14}\text{C}$  ages of *R. exulans* and other terrestrial or aquatic omnivores throughout New Zealand. This effect is not confined to New Zealand and should be considered when interpreting  $^{14}\text{C}$  dates on bone in general.

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