

Are Calcium Isotopes a Reliable Monitor of Trophic Level in Marine Settings?

M. T. CLEMENTZ,^{a*} P. HOLDEN^b AND P. L. KOCH^a

^a Department of Earth Sciences, University of California, Santa Cruz, California, USA

^b Research School of Earth Sciences, Institute of Advanced Studies, The Australian National University, Canberra, Australia

ABSTRACT Recent research has shown that calcium isotopes are fractionated by metabolic processes, leading to a decrease in $^{44}\text{Ca}/^{40}\text{Ca}$ ratio with increasing trophic level. If so, calcium isotopes could provide information on trophic relationships within foodwebs millions of years older than what we have been able to study thus far with alternative methods (i.e., nitrogen isotopes ($\delta^{15}\text{N}$), Sr/Ca). To explore whether $\delta^{44}\text{Ca}$ values provided marine trophic level information, we measured the $\delta^{44}\text{Ca}$ composition of tooth enamel and bone from modern marine mammals representing a 2.5 order range in trophic level. Marine mammal enamel $\delta^{44}\text{Ca}$ values clustered into two groups — mammals foraging on vegetation or invertebrates exhibited higher $\delta^{44}\text{Ca}$ values than those foraging on fish or other marine mammals. We next examined whether this correlation was preserved in the fossil record by examining a 15 Ma marine fauna from southern California and observed that the relationship between $\delta^{44}\text{Ca}$ values of specimens followed the same pattern as observed in modern faunas, but the mean $\delta^{44}\text{Ca}$ values were significantly different from modern $\delta^{44}\text{Ca}$ values for mammals of similar trophic level. We conclude that the relative spacing of $\delta^{44}\text{Ca}$ values amongst fossil taxa can serve as a valuable tool for defining trophic level of extinct organisms and can provide critical information on relationships within ancient foodwebs. Copyright © 2003 John Wiley & Sons, Ltd.

Key words: calcium isotopes; trophic level; marine ecosystems; palaeoecology

Introduction

The application of stable isotope analysis to palaeontological studies has provided a wealth of information on the ecology of extinct organisms and the structure of ancient ecosystems. The applicability of stable isotope proxies depends upon the preservation of potential substrates for analysis, however. For diet source reconstruction based on carbon isotopes there are a variety of biogenic materials (i.e., hair, collagen, bone, enamel) with differing temporal windows of reliable preservation (<200 kyr to

>50 Myr). For trophic level reconstruction, nitrogen isotopes ($\delta^{15}\text{N}$) and elemental concentrations (Ba/Ca, Sr/Ca) are two proxies that have been employed in archaeological and palaeontological studies (Bocherens *et al.*, 1996; Burton *et al.*, 1999). However, substrates for $\delta^{15}\text{N}$ analysis (i.e., hair, muscle, collagen) are typically not preserved in the fossil record for durations >200 kyr, limiting the approach to relatively young samples, though occasional reports of $\delta^{15}\text{N}$ differences among truly ancient vertebrates are intriguing and need further work (e.g., Bocherens *et al.*, 1988; Ostrom *et al.*, 1993). Diagenetic alteration of Ba and Sr concentrations during fossil preservation limits the reliability of this proxy over time as well (Sillen, 1986; Price *et al.*, 1992). Therefore, critical information on the structure of ancient foodwebs >200 kyr old is unavailable until a new trophic level

* Correspondence to: Dept. of Earth Sciences, University of California-Santa Cruz, 1156 High Street, Santa Cruz, CA 95064, USA. e-mail: clementz@es.ucsc.edu

proxy is developed that has a greater preservation potential.

Recently, Skulan *et al.* (1997) proposed that calcium isotope ratios (^{44}Ca to ^{40}Ca) vary with trophic level in modern ecosystems. They sampled an assortment of modern terrestrial and marine organisms, both vertebrates and invertebrates, and noted a significant drop in $\delta^{44}\text{Ca}$ values with increasing trophic level. Though the exact mechanism for this relationship is not fully understood, it appears that calcium isotopes are fractionated during biomineralization of bone, enamel, and other skeletal materials, but are not fractionated during ingestion, resulting in $\delta^{44}\text{Ca}$ values for soft tissues matching the $\delta^{44}\text{Ca}$ value of an animal's diet while the hard parts are $\sim 1.5\%$ depleted relative to diet. Within both marine and terrestrial foodwebs, this fractionation step was found to be manifested as an $\sim 1.0\%$ drop per trophic step, but the relative $\delta^{44}\text{Ca}$ values of taxa at the same trophic level differed between land and sea. Terrestrial species had consistently lower $\delta^{44}\text{Ca}$ values than marine species as a result of differences in the baseline $\delta^{44}\text{Ca}$ values of waters and soils within these ecosystems (Zhu & MacDougall, 1998). They concluded that $\delta^{44}\text{Ca}$ values may not only identify trophic relationships amongst organisms within an ecosystem, but may also allow discrimination between organisms inhabiting marine and terrestrial environments.

Here, we expand on this work (Skulan *et al.*, 1997; Skulan & DePaolo, 1999), focusing on trophic relationships among modern marine mammals. Ten species from four families were selected, and bone or enamel samples were collected from each specimen. Our goal was to determine if the patterns observed in previous studies hold for a multi-species study that crossed phylogenetic boundaries. We were particularly concerned with testing whether the method allowed discrimination among marine mammals at low trophic levels (i.e., plant-feeders versus invertebrate feeders). We then assessed whether predicted patterns were preserved in fossil marine mammals up to 15 Myr old. Finally, we sampled a specimen of *Desmostylus*, an extinct mammal related to elephants and sirenians whose ecology is poorly constrained by morphological information to see if $\delta^{44}\text{Ca}$ values could shed light on its role within ancient coastal ecosystems.

Methods and materials

Tables 1 and 2 list the ten modern and five fossil species of marine mammals we included in our study as well as the $\delta^{44}\text{Ca}$ values for marine organisms reported in Skulan *et al.* (1997) and Skulan & DePaolo (1999). To augment the number of modern marine herbivores included in our study, we analysed samples of the extinct sea cow, *Hydrodamalis gigas*, which was hunted to extinction within the past 250 years. Estimates of trophic level for modern species were based on work by Pauly *et al.* (1998).

From each specimen, 1 to 2 mg of bone or enamel was collected and ground to a fine powder using either a drill or agate mortar and pestle. Samples were soaked in a NaOCl solution overnight to remove organic material and then rinsed five times with deionized water. After rinsing, samples were transferred to acid-washed Teflon vials and dissolved in 1.5 ml of 2.5 N HCl at 120 °C overnight to ensure complete dissolution. Sample solutions were then pipetted into 1.5 ml acid-washed centrifuge tubes for storage. Approximately 1 μg of Ca from each sample was injected back into the previously used Teflon vial for analysis. Next, 270 μl of a Ca double spike (^{42}Ca and ^{48}Ca) solution was injected into each vial, which was then placed onto a hot plate at 120 °C and allowed to dry down completely.

Calcium isotope variations were measured on a VG 54/WARP multi-collector thermal ionization mass spectrometer in the Department of Earth Sciences, University of California Santa Cruz. To load samples for analysis, 2 μl of HNO_3 was added to each vial to dissolve the sample, then 1 μl of this solution was collected and pipetted onto a rhenium filament and dried for ~ 2 min at 800 °C. We then dried 1 μl of H_3PO_4 onto the filament to hold the sample in place, as well as 1 μl of tantalum solution to enhance peak intensity. We measured the abundances of ^{40}Ca , ^{42}Ca , ^{44}Ca , and ^{48}Ca in each sample 100 to 200 times. As a means of assessing potential contamination in our ^{40}Ca measurements from ^{40}K , we measured the abundance of ^{39}K in each sample prior to and immediately after analysis; in all reported sample values, contamination was negligible. Methods to correct for isotopic

Table 1. $\delta^{44}\text{Ca}$ values of modern marine vertebrates and invertebrates. Trophic level values are estimated based on Pauly *et al.* (1998)

Taxonomic group	Modern species	Habitat preferences/locality	Trophic level	$\delta^{44}\text{Ca} \pm 2\sigma^1$
Marine Invertebrates				
Gastropoda	Limpet (<i>Bathycarnea</i> sp.)	Marine Offshore	2.0	-0.4 ± 0.1^3
	Marine Snail (<i>Conus puncticulatus</i>)	Marine Nearshore	2.0	-0.4 ± 0.1^3
Cephalopoda	Squid	Marine Offshore	3.2	$(0.7 \pm 0.2)^3$
Pelecypoda	Marine Mussel (<i>Mytilus</i> sp.)	Marine Nearshore	2.2	$(0.8 \pm 0.1)^3$
Echinodermata	Starfish	Marine Nearshore	2.2	0.2 ± 0.2^3 0.1 ± 0.1^3
Marine Vertebrates				
Chondrichthyes	Manta Ray	Marine Nearshore	2.7	-0.2 ± 0.1^3
Osteichthyes	Sardine	Marine Nearshore	2.7	-0.6 ± 0.1^3
	Anchovy	Marine Nearshore	2.7	-0.2 ± 0.2^3 0.1 ± 0.2^3
	Groupers	Marine Nearshore	3.3	$(0.5 \pm 0.3)^3$ -0.6 ± 0.1^3
	Barracuda	Marine Nearshore	3.3	-0.5 ± 0.1^3
Sirenia	Dugong (<i>Dugong dugon</i>)	Marine Seagrass/ Australia	2.0	$-1.3 \pm 0.5\%^2$
	Steller's Sea Cow (<i>Hydrodamalis gigas</i>)	Marine Kelp Beds/ Alaska	2.0	$-1.0 \pm 0.1\%$
	Manatee (<i>Trichechus manatus</i>)	Marine and Freshwater/ Florida	2.0	$-2.2 \pm 0.1\%$
Mustellidae	Sea Otter (<i>Enhydra lutra</i>)	Marine Kelp Beds California	3.4	$-1.0 \pm 0.1\%$
	Sea Otter (<i>Enhydra lutra</i>)	Marine Kelp Beds California	3.4	$-0.9 \pm 0.2\%^3$
Pinnipedia	Walrus (<i>Odobenus rosmarius</i>)	Marine Nearshore/ Alaska	3.4	$-2.3 \pm 0.3\%^2$
	California Sea Lion (<i>Zalophus californianus</i>)	Marine Nearshore/ California	4.1	$-2.5 \pm 0.1\%$
	Harbour Seal (<i>Phoca vitulina</i>)	Marine Nearshore/ California	4.0	$-2.8 \pm 0.1\%^2$
	Northern Fur Seal (<i>Callorhinus ursinus</i>)	Marine Offshore/ California	4.2	$-1.2 \pm 0.2\%^3$
Cetacea	Harbour Porpoise (<i>Phocoena phocoena</i>)	Marine Nearshore/ California	4.1	$-2.2 \pm 0.1\%^2$
	Dall's Porpoise (<i>Phocoenoides dalli</i>)	Marine Nearshore/ California	4.1	$-2.3 \pm 0.1\%^3$
	Pilot Whale (<i>Globicephala macrorhynchus</i>)	Marine Offshore/ California	4.3	$-2.6 \pm 0.1\%$
	False Killer Whale (<i>Pseudorca crassidens</i>)	Marine Offshore/ California	4.4	$-2.6 \pm 0.1\%$
	Sperm Whale (<i>Physeter catodon</i>)	Marine Offshore/ California	4.4	$-2.4 \pm 0.1\%$

¹ 2σ represents $2 \times$ the standard error within a run for each sample.

² Mean value and standard deviation for two separate runs of the same sample. Single run values are *D. dugon* ($-0.9 \pm 0.3\%$, $-1.6 \pm 0.1\%$), *O. rosmarius* ($-2.1 \pm 0.3\%$, $-2.5 \pm 0.1\%$), *P. vitulina* ($-2.7 \pm 0.1\%$, $-2.8 \pm 0.3\%$), *P. phocoena* ($-2.2 \pm 0.2\%$, $-2.2 \pm 0.1\%$).

³ Values reported in Skulan *et al.* (1997) and Skulan & DePaolo (1999); values reported in parantheses represent measurements made on tissue as opposed to bone or enamel.

Table 2. $\delta^{44}\text{Ca}$ values of fossil taxa analysed in this study. Trophic level values are estimated based on information from extant ecological analogues

Fossil genus	Specimen #	Habitat/locality	Trophic level	$\delta^{44}\text{Ca}$
Proboscidean				
<i>Gomphotherium</i>	UCMP-100026	Terrestrial	2.0	$-1.7 \pm 0.1\text{‰}$
Equid				
<i>Merychippus</i>	UCMP-23730	Terrestrial	2.0	$-1.3 \pm 0.1\text{‰}$
<i>Merychippus</i>	UCMP-154920	Terrestrial	2.0	$-1.6 \pm 0.1\text{‰}$
Desmostylian				
<i>Desmostylus</i>	UCMP-82372	?	?	$-1.6 \pm 0.1\text{‰}$
Sirenian				
<i>Dusisiren</i>	LACNHM-127971	Marine Nearshore	2.0	$-1.3 \pm 0.1\text{‰}$
Pinniped				
<i>Allodesmus</i>	UCMP-83061	Marine Nearshore	~4.0	$-1.0 \pm 0.1\text{‰}$
<i>Allodesmus</i>	UCMP-No #	Marine Nearshore	~4.0	$-1.0 \pm 0.1\text{‰}$
Cetacean				
Odontocete	UCMP-154487	Marine Nearshore	~4.0	$-2.0 \pm 0.1\text{‰}$

fractionation during measurement and calculation of $\delta^{44}\text{Ca}$ values are based on Zhu (1999). Results are presented as $\delta^{44}\text{Ca}$ values, where $\delta^{44}\text{Ca}$ is the normalized per mil (‰) difference in $^{44}\text{Ca}/^{40}\text{Ca}$ between a sample and our laboratory standard ($^{44}\text{Ca}/^{40}\text{Ca} = 0.021713 \pm 0.000002$; $n = 4$):

$$\delta^{44}\text{Ca} = \left(\left(\frac{^{44}\text{Ca}}{^{40}\text{Ca}_{\text{sample}}} \div \frac{^{44}\text{Ca}}{^{40}\text{Ca}_{\text{standard}}} \right) - 1 \right) * 1000$$

The lab standard we used was the same as that used by researchers at Scripps Institution of Oceanography; the $^{44}\text{Ca}/^{40}\text{Ca}$ value that we generate for this standard is in close agreement with that calculated by Scripps ($^{44}\text{Ca}/^{40}\text{Ca} = 0.0217155 \pm 0.0000012$). Unfortunately, we did not analyse seawater as a reference for making interlaboratory comparisons as suggested by Zhu & MacDougall (1998). However, we were able to analyse two taxa of marine mammals that were also analysed by Skulan *et al.* (1997); the values we obtained were nearly identical to those of Skulan *et al.* (1997) (Table 1) and indicate that we can make direct comparisons between our sample values and those generated by Skulan *et al.* (1997) and Skulan & DePaolo (1999).

To assess the dependence of $\delta^{44}\text{Ca}$ values upon trophic level, we calculated the statistical significance of a linear regression through the data. Significance of differences in $\delta^{44}\text{Ca}$ values ($P < 0.05$) associated with dietary type were assessed using either a one-factor analysis

of variance (ANOVA) for comparisons among several groups, followed by a post-hoc pairwise comparison test (Tukey), or a Student's t-test for comparisons between two groups. All statistical tests were calculated using the software program SYSTAT 9.0.

Results

Values for modern mammals range from -1.0‰ for *H. gigas* to -2.8‰ for the single harbour seal we analysed (Table 1), which span a 2.0 level range in trophic position. We found that our samples fit within the range of marine mammal $\delta^{44}\text{Ca}$ values reported by Skulan *et al.* (1997) and Skulan & DePaolo (1999), and for the two species that were analysed by both groups, $\delta^{44}\text{Ca}$ values were within analytical error (Table 1). Statistical analysis of our data revealed a strong negative correlation between trophic level and $\delta^{44}\text{Ca}$ values (linear regression, slope = -0.630 ± 0.147 , $P = 0.003$), which closely matched the correlation seen for marine values reported by Skulan *et al.* (1997) and Skulan & DePaolo (1999) (linear regression, slope = -0.743 ± 0.178 , $P = 0.002$). Combining the two data sets enhanced the correlation (linear regression, slope = -0.930 ± 0.165 , $P = 0.000$), so the remaining statistical analyses were performed on the combined data set. Values differed significantly among the three basic dietary types: herbivores, molluscivores, and carnivores (one way ANOVA, $F = 9.272$, $P =$

0.001), and pairwise comparison found that both herbivores and molluscivores were statistically different from carnivores (Tukey test, $P < 0.01$) but were not statistically different from each other (Tukey test, $P = 0.621$). Combining herbivores and molluscivores into one group and comparing to carnivores yielded a stronger discrimination (Student's *t*-test, $t = 4.210$, $P = 0.000$) and a difference in mean values of 1.4‰.

The range in $\delta^{44}\text{Ca}$ values for fossil taxa (1.4‰) was slightly lower than for modern specimens (1.6‰), but fossil $\delta^{44}\text{Ca}$ values were within the range of values for modern specimens (Table 2). Within marine species, $\delta^{44}\text{Ca}$ values for the herbivore, *Dusisiren*, and the pinniped, *Allodesmus* were higher than that of the carnivorous odontocete, which yielded the lowest $\delta^{44}\text{Ca}$ value for all fossil specimens. Comparison of $\delta^{44}\text{Ca}$ values for terrestrial (*Gomphotherium*, *Merychippus*) and marine (*Dusisiren*) herbivores found that terrestrial specimens typically yielded lower $\delta^{44}\text{Ca}$ values than marine herbivores. The value for *Desmostylus* was comparable to values reported for terrestrial herbivores.

Calcium isotopes in modern marine foodwebs

Our results confirm that a distinct relationship exists between the $\delta^{44}\text{Ca}$ value of an animal's bone

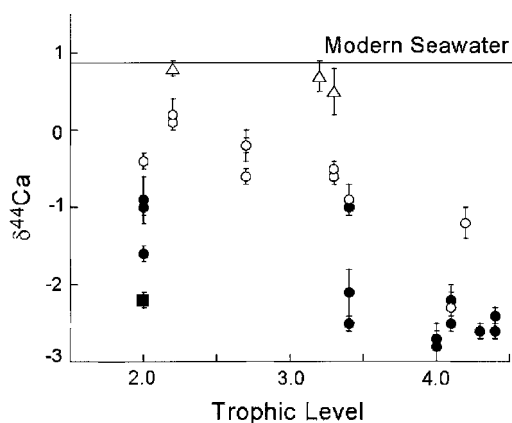


Figure 1. Plot of trophic level versus $\delta^{44}\text{Ca}$ values of modern marine animals (●) and Florida manatee (■) analysed in this study and by Skulan *et al.* (1997) and Skulan & DePaolo (1999) for both bone (○) and tissues (△). Error bars represent 2σ values calculated for either separate runs of the same sample or single run variation (see Table 1).

or enamel and its trophic position. However, the relationship between trophic level and $\delta^{44}\text{Ca}$ does not appear to be linear in marine systems. Instead, the only significant decrease in $\delta^{44}\text{Ca}$ values occurs between herbivores/molluscivores (sea cows/otters) and higher level marine carnivores; differences in marine consumer $\delta^{44}\text{Ca}$ values do not appear to be significant near the base of the food chain. One factor that might contribute to this pattern is the observation by Skulan & DePaolo (1999) that the $\delta^{44}\text{Ca}$ values of the soft tissues of marine invertebrates at different trophic levels (e.g., filter-feeding mussels and carnivorous squid) were nearly identical and similar to that of ocean water (Figure 1). The calcium-rich shells of these invertebrates were also similar, but depleted in ^{44}Ca relative to ocean water. While direct measurements of marine plant tissue are currently lacking, it is very likely they have $\delta^{44}\text{Ca}$ values similar to ocean water and invertebrate tissues. Thus mammalian herbivores and molluscivores (who do not consume the ^{44}Ca -depleted shells of their prey) would have similar $\delta^{44}\text{Ca}$ values for enamel and bone. Only consumers ingesting a large amount of hard tissues, such as bone or cartilage, would be expected to show significantly lower $\delta^{44}\text{Ca}$ values.

Another interpretation of the data could be based on differences in seawater ingestion by the marine mammals we analysed. Mammals ingesting a greater amount of seawater would be predicted to yield higher $\delta^{44}\text{Ca}$ values than those that were obtaining most of their water from metabolizing their food. If so, this would mean that the differences we have observed reflect osmoregulatory differences rather than trophic level differences among species. Measurements of seawater consumption by marine mammals are typically low, since most marine mammal species obtain most of their water from their prey, either preformed or as metabolic water (Pilson, 1970; Hui, 1981), suggesting that diet is still the main factor controlling the $\delta^{44}\text{Ca}$ value of marine mammal bone and enamel. However, the two dietary categories we have identified (herbivore/molluscivore and carnivore) may correlate with differences in seawater consumption. Marine vegetation and invertebrates typically have much higher electrolyte concentrations than marine vertebrates (i.e., fish, marine mammals,

etc.), which may necessitate increased consumption of seawater in order to deal with the high salt load of their diet (Costa, 1982) and would result in higher $\delta^{44}\text{Ca}$ values for this diet category when compared to carnivores. At present, our data set is too small to differentiate the influence of seawater consumption from trophic level on marine mammal $\delta^{44}\text{Ca}$ values, but our analyses do support an interpretation that $\delta^{44}\text{Ca}$ values are impacted by dietary differences among marine mammals.

Two species had lower $\delta^{44}\text{Ca}$ values than expected based on the general pattern we observed. The manatee and walrus had $\delta^{44}\text{Ca}$ values $\sim 1.0\%$ lower than expected for marine herbivores/molluscivores. Low $\delta^{44}\text{Ca}$ values in the manatee could be related to habitat preferences. Unlike the dugong and Steller's sea cow, which are/were limited to marine habitats, the manatee can tolerate a wide range of salinities and inhabits freshwater, estuarine, and marine environments. As freshwater $\delta^{44}\text{Ca}$ values tend to be $\sim 1.0\%$ lower than those of seawater (Zhu & MacDougall, 1998), it is not surprising that manatee $\delta^{44}\text{Ca}$ values are lower than those of marine sirenian species. For this reason, the manatee $\delta^{44}\text{Ca}$ value was excluded from the correlation between marine trophic level and $\delta^{44}\text{Ca}$ values. For the walrus, low $\delta^{44}\text{Ca}$ values may reflect supplementation of its primarily mollusc-based diet with other types of prey. Field observations, stomach content data, and stable isotope analysis suggest these animals may feed on higher trophic level prey species from time to time, including other marine mammals (Lowry & Fay, 1984; Muir *et al.*, 1995), which would result in $\delta^{44}\text{Ca}$ values similar to more carnivorous pinniped species. Because of the potential range in diets and habitats exploited by marine mammals, we will continue to quantify species-level $\delta^{44}\text{Ca}$ variation for marine mammals to better gauge the relationship between trophic level and $\delta^{44}\text{Ca}$ values.

Trophic relationships in a middle miocene marine ecosystem

Overall, $\delta^{44}\text{Ca}$ values in modern marine mammals do appear to record information about trophic level (at least higher in the foodweb), but how

robust is this proxy for interpretation of foodweb relationships in the fossil record? Preservation of expected $\delta^{44}\text{Ca}$ differences among fossil taxa with unambiguous dietary or habitat preferences is a powerful test of the method, especially when considering the possibility for large variations in marine $\delta^{44}\text{Ca}$ values over time (De La Roche & DePaolo, 2000). As expected, herbivorous mammals (i.e., *Merychippus*, *Gomphotherium*, *Dusisiren*) had high $\delta^{44}\text{Ca}$ values, and terrestrial herbivores had slightly lower values than marine herbivores (Table 2; Figure 2). However, the small difference between marine and terrestrial herbivores is identical to the difference in $\delta^{44}\text{Ca}$ values between two individuals of *Merychippus* ($\sim 0.3\%$), which implies that within-species variation must be quantified before reliable conclusions can be reached when comparing between species. Though no terrestrial carnivores were available for comparison, we did analyse two species of marine carnivores, the pinniped *Allodesmus* and a specimen of a Middle Miocene odontocete. For the odontocete, $\delta^{44}\text{Ca}$ values were extremely low and nearly identical to $\delta^{44}\text{Ca}$ values for similar-sized, modern toothed whales (Tables 1 and 2), supporting the conclusion that primary dietary information is preserved in the $\delta^{44}\text{Ca}$ value of tooth enamel. In contrast, $\delta^{44}\text{Ca}$ values for *Allodesmus* are extremely high and suggest this species may have foraged on marine invertebrates, such as squid. This isotopic interpretation of the ecology of *Allodesmus* is congruent with earlier morphologically-based assessments that cite the squid-eating northern elephant seal as the most likely modern analogue (Mitchell, 1966).

Besides analysis of marine mammals with well-constrained ecologies, we also analysed the extinct mammal, *Desmostylus*, a distant relative of modern elephants and sirenians whose ecology is enigmatic (McLeod & Barnes, 1984; Inuzuka *et al.*, 1995). About the size of a hippopotamus, the broad crushing molars of *Desmostylus* have been interpreted as adaptations for foraging on either abrasive vegetation or molluscs. With respect to abrasive vegetation, *Desmostylus* could have ingested nearshore algae and their mud covered holdfasts, estuarine sea grasses, or terrestrial vegetation. Unfortunately, our analysis of modern marine mammals shows molluscivores and herbivores can not be discriminated from

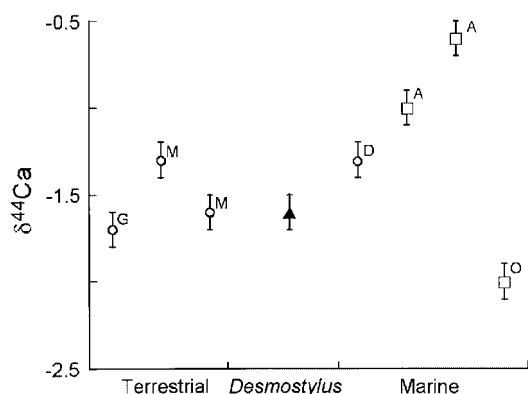


Figure 2. Plot of trophic level versus $\delta^{44}\text{Ca}$ values of fossil taxa including herbivores (O), carnivores (\square), and *Desmostylus* (\blacktriangle). A = *Allodesmus*, D = *Dusisiren*, G = *Gomphotherium*, M = *Merychippus*, and O = *Odontocete*. Error bars represent 2σ values calculated for single run variation (see Table 2).

one another with calcium isotopes. Yet, the low $\delta^{44}\text{Ca}$ value for *Desmostylus* is most similar to values for terrestrial herbivores, much lower than the value for the potential marine molluscivore, *Allodesmus*, and even lower than the value for the marine herbivore, *Dusisiren* (Figure 2). Recently developed constraints from other isotopic systems help clarify the situation for *Desmostylus*. Carbon isotope data show that this individual consumed sea grass, oxygen isotope data suggesting that *Desmostylus* (as a genus) was strongly aquatic, and strontium isotope data show that this individual inhabited estuarine/marine waters (Clementz *et al.*, in press). In light of these results, our single $\delta^{44}\text{Ca}$ value for *Desmostylus* could have resulted from incorporation of a terrestrially-derived Ca isotope signal via foraging within estuarine waters. Clearly, more isotopic data are needed to support this conclusion, and non-isotopic data (e.g., enamel microwear) will be required to distinguish mollusc crushing from consumption of coarse, possibly mud-covered aquatic vegetation.

Conclusions

The results of this study confirm that marine mammal bone and tooth enamel $\delta^{44}\text{Ca}$ values contain dietary level information that can discriminate herbivorous and molluscivorous marine mammals from higher trophic level carnivores,

and can therefore provide information on relationships among consumers within marine foodwebs. Though the exact mechanism for this isotopic fractionation of Ca is not fully understood, similar values for distantly related marine mammals feeding upon similar diets suggest that this mechanism is not affected by physiological differences between groups and that $\delta^{44}\text{Ca}$ values of animals can be compared across phylogenies. Furthermore, retention of expected spacing of $\delta^{44}\text{Ca}$ values between 15 Ma primary and tertiary marine consumers supports application of this proxy for interpretation of foodweb relationships within palaeontological studies. Finally, application of this technique to a palaeoecological study of the extinct mammal *Desmostylus* confirmed the utility of this proxy when dealing with organisms whose habitat and dietary preferences are poorly constrained by other analytical methods.

Acknowledgements

We thank the following individuals and institutions for collection and access to specimens for analyses: Doug Long, California Academy of Sciences; Pat Holroyd, University of California Museum of Paleontology; Larry Barnes, John Heyning, and David Janiger, Los Angeles County Natural History Museum; Dave Casper, Long Marine Laboratory; Amanda Toperoff, Moss Landing Marine Laboratory; Diane Gifford-Gonzalez and Richard Baldwin, University of California, Santa Cruz Archaeological Collections, and the California Department of Fish and Game. We especially thank Dr Adina Payton, Stanford University, and researchers at SCRIPPS Institute of Oceanography for granting us access to their laboratory standard to calibrate and begin our analyses. A National Science Foundation (NSF) Predoctoral Fellowship and Achievement Rewards for College Scientists Fellowship supported MTC when much of this research was conducted. Analytical and travel costs were covered by NSF grants EAR 9725854 and 0087742, and a grant from the Dr Earl H. Myers and Ethel M. Myers Oceanographic and Marine Biology Trust Fund.

References

- Bocherens H, Fizet M, Cuif JP, Jaeger JJ, Michard JG, Mariotti A. 1988. First measurements of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ natural isotopic abundance on fossil dinosaurian organic matter; application to determining the diet of *Anatosaurus*, *Ornithischia*, *Hadrosauridae*. *Comptes Rendus de l'Academie des Sciences, Serie 2, Mecanique, Physique, Chimie, Sciences de l'Univers, Sciences de la Terre* **306**(20): 1521–1525.
- Bocherens H, Pacaud G, Lazarev PA, Mariotti A. 1996. Stable isotope abundances (^{13}C , ^{15}N) in collagen and soft tissues from Pleistocene mammals from Yakutia: implications for the paleobiology of the Mammoth Steppe. *Palaeogeography, Palaeoclimatology, Palaeoecology* **126**: 31–44.
- Burton JH, Price TD, Middleton WD. 1999. Correlation of bone Ba/Ca and Sr/Ca due to biological purification of calcium. *Journal of Archaeological Science* **26**: 609–616.
- Clementz MT, Hoppe KA, Koch PL. A paleoecological paradox: the habitat and dietary preferences of the extinct tethythere, *Desmostylus*, inferred from stable isotope analysis. *Paleobiology*. In press.
- Costa DP. 1982. Energy, nitrogen, and electrolyte flux and sea water drinking in the sea otter *Enhydra lutris*. *Physiological Zoology* **55**(1): 35–44.
- De La Roche CL, DePaolo DJ. 2000. Isotopic evidence for variations in the marine calcium cycle over the Cenozoic. *Science* **289**(5482): 1176–1178.
- Hui CA. 1981. Seawater consumption and water flux in the common dolphin *Delphinus delphis*. *Physiological Zoology* **54**(4): 430–440.
- Inuzuka N, Domning DP, Ray CE. 1995. Summary of taxa and morphological adaptations of the *Desmostylia*. *The Island Arc* **3**: 522–537.
- Lowry LF, Fay FH. 1984. Seal eating by walrus in the Bering and Chukchi Seas. *Polar Biology* **3**: 11–18.
- McLeod SA, Barnes LG. 1984. Fossil desmostylians. In *The Natural Sciences of Orange County. Memoirs of the Natural History Foundation of Orange County*, Butler B, Grant J, Stadum CJ (eds). The Foundation: Huntington Beach, Ca, USA, **1**: 39–44.
- Mitchell ED. 1966. The Miocene Pinniped *Allodesmus*. *University of California Publication in Geological Sciences* **61**: 1–46.
- Muir DCG, Segstro MD, Hobson KA, Ford CA, Stewart REA, Olpinski S. 1995. Can seal eating explain elevated levels of PCBs and organochlorine pesticides in walrus blubber from eastern Hudson Bay (Canada)? *Environmental Pollution* **90**: 348–355.
- Ostrom PH, Macko SA, Engel MH, Russell DA. 1993. Assessment of trophic structure of Cretaceous communities based on stable nitrogen isotope analyses. *Geology* **21**(6): 491–494.
- Pauly D, Trites AW, Capuli E, Christensen V. 1998. Diet composition and trophic levels of marine mammals. *ICES Journal of Marine Science*. **55**: 467–481.
- Pilson MEQ. 1970. Water balance in California sea lions. *Physiological Zoology*. **43**: 257–269.
- Price TD, Blitz J, Burton J, Ezzo JA. 1992. Diagenesis in prehistoric bone: problems and solutions. *Journal of Archeological Science*. **19**: 513–529.
- Sillen A. 1986. Biogenic and diagenetic Sr/Ca in Pliopleistocene fossils of the Omo Shungura Formation. *Paleobiology*. **12**: 311–323.
- Skulan J, DePaolo DJ. 1999. Calcium isotope fractionation between soft and mineralized tissues as a monitor of calcium use in vertebrates. *Proceedings of the National Academy of Sciences* **96**: 13 709–13 713.
- Skulan J, DePaolo DJ, Owens TL. 1997. Biological control of calcium isotopic abundances in the global calcium cycle. *Geochimica et Cosmochimica Acta* **61**: 2505–2510.
- Zhu P. 1999. *Calcium isotopes in Marine Environment*. PhD dissertation. Scripps Institute of Oceanography: University of California-San Diego; 1–74.
- Zhu P, MacDougall JD. 1998. Calcium isotopes in the marine environment and the oceanic calcium cycle. *Geochimica et Cosmochimica Acta* **62**: 1691–1698.