The use of stable isotopes to determine trophic status and seasonal variability in the diet of *Acomys subspinosus*

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Submitted in partial fulfilment of the degree of B.Sc. (Hons) in Plant Ecology
University of Cape Town, 2004

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Abstract

The recent documentation of seed dispersing rodents of large nut-fruited plants in the Cape has lead to questions relating to how dependant these rodents are on these seeds as part of their diet and whether there is a seasonal difference in the diet of these rodents when seeds are not available. In the Cape the only rodent that has thus far been identified as a scatter hoarding rodent is *Acomys subspinosus*. To determine the importance of seeds and the effect of seasonality in the diet of *A. subspinosus* trapping in various locations was done to collect hair samples from *A. subspinosus* as well as other small mammals. Hair samples were analysed for $\delta^{13}C$ and $\delta^{15}N$ content using mass spectrometry. *Proteaceae* and *Restionaceae* seeds were also analysed using the mass spectrometer to determine their $\delta^{13}C$ and $\delta^{15}N$ signals for seeds are. Data from previous studies on foliage $\delta^{13}C$ and $\delta^{15}N$ content was used to determine whether seeds are enriched relative to leaves. This study has shown that leaves are lower in than seeds. It has also shown that the $\delta^{15}N$ and $\delta^{13}C$ values from rodents are higher than that of seeds and thus the use of stable isotopes in trophic ecology is a valid tool in determine granivory from herbivory or insectivory. There was no significant evidence to prove that *A. subspinosus* has seasonal variation in its diet based on inner and outer hair isotope analysis (t=1.1084, p<0.2808). A difference in the $\delta^{15}N$ content of *A. subspinosus* hair samples taken from rodents trapped in summer (in the Cedarburg) and those trapped in spring (in Elgin) was found (t=2.1788, p<0.005). The difference could have been due to different habitats but variation in season $\delta^{15}N$ signals cannot be ruled out. *A. subspinosus* also had rather low $\delta^{15}N$ and $\delta^{13}C$ values which may indicate that seeds are not very important in their diets.

Introduction

Recently it has been documented that some rodents disperse seeds of nut-fruited Cape plants (Midgley et al, 2002). This has lead to questions about how dependant the rodents are on seeds. Rodents that disperse seeds in the Cape are scatter hoarders and therefore they bury seeds individually in order to recover them later to either eat them or re-cache them. Thus, investigations in to the diet of these rodents are necessary to determine whether seeds form part of their long-term diet or whether these seeds are only a part of their diet when they are available. The ecology and dietary information of many small mammals of South Africa is not well understood or documented (Apps, 1986). In Smithers' mammals of Southern Africa, virtually nothing is known about the diet of the seed dispersing rodent *Acomys subspinosus*. Thus it is impossible to determine how important nuts are in the diet of these animals. It is also not known whether *A. subspinosus* is an opportunistic granivore depending on local conditions such as the presence of nut-fruited *Proteaceae* and *Restioanaceae*.

One of the main problems in determining the diet of animals is getting results from experimental and field observations due to its impracticality (Baugh et al, in press). This is especially problematic when animals are small and their behaviour is easily affected by the presence of humans. Observing foraging behaviour of small mammals, like rodents, is thus especially problematic. Certain indirect measures of dietary information can be flawed. Data from stomach contents and faeces analysis can have biases, which includes differential digestibility of prey (Kelly, 1999). Thus, a mechanism of inferring dietary composition of various food sources with, as little bias as possible is pivotal in trophic ecology.

One way to determine what animals are consuming and how this changes over time can be the use of stable isotopes (West et al, 2004). One can use $\delta^{15}N$ and $\delta^{13}C$ values to infer contributions of different food sources to animal diets (Smith

et al, 2002). The use of isotopes in determining the diet of various animals has been successful ever since the discovery of the two pathways of carbon by C_3 and C_4 plants and subsequent trophic fractionation of both Carbon (C) and Nitrogen (N). The use of these stable isotopes is based on fractionation that takes place at different trophic levels. $\delta^{15}N$ and $\delta^{13}C$ are heavier than their more abundantly occurring isotopes, $\delta^{14}N$ and $\delta^{12}C$, and thus $\delta^{15}N$ and $\delta^{13}C$ are discriminated against by plants. With each increase in trophic level structure, $\delta^{15}N$ and $\delta^{13}C$ values should increase, due to fractionation in food sources of granivores, insectivores, omnivores, carnivores and numerous other feeding type animals (Pauly, 1998).

For N there is no single process that creates fractionation of isotopes, while for C Rubisco, water use efficiency and trophic level status can create fractionation (Kelly 1999). But N isotopes are useful for identifying the contributions of several types of plants to food chains (Kelly, 1999); it may also be used to determine the trophic level of an animal.

The uses and application of stable isotopes in trophic ecology

Isotope fractionation has been used in the reconstruction of mammalian diets of the past, including ancient human populations. The reconstruction has mainly been achieved through the use of ever growing hair, bone, and dental material (Ayliffe et al, 2004). Detailed chronological information can be can be extracted from biological archives such as feathers, baleen, tooth enamel and hair (West et al, 2004, Kelly, 1999, Macko et al, 1999). Hair is useful for determining modern and historical diets of animals as it provides insight into variability of prey selection and one can thus make inferences about trophic ecology (Baugh et al, in press). The use of hair to determine dietary changes on a seasonal scale or finer is possible but the interpretation of isotope signals from hairs are hampered by the limited understanding of assimilation of dietary isotope compositions into animal tissues on timescales ranging from days to weeks (Ayliffe et al, 2004). Different

animals also have differing hair growth rates and these could further hinder the use of isotopes in interpreting short-term dietary changes. In humans an isotopic change can be observed, using beard hairs, in as little as 6- 12 days, because it takes about 6-12 days for growing hair to emerge from skin (Macko, 1999). Thus, to determine the spatial scales at which one can analyse isotopes from hairs, one needs to have some idea of hair growth rates from the species in question.

The carbon values of well-preserved samples of the hair protein, keratin, have been showed to correlate well with diet (Macko, 1999) and thus hair is a good candidate for reconstructing paleo-diets. This means the use of hair to reconstruct the diet of extant animals should be valid as well. Furthermore Tiezen and Fagre (1993) found that N isotope values correlate well with diet and is typically enriched by about 3% above dietary material (Macko, 1999).

Hair is composed of a protein complex, called keratin, formed from amino acids that are derived from both exogenous (diet, environmental water) and endogenous sources (metabolic turnover of endogenous tissues) (West et al, 2004). Thus the isotopic composition of hair potentially provides information on the animal's diet, nutritional status and location and movements and because keratin is not easily degradable (Macko et al, 1999) hair samples can be preserved for seasonal or long-term changes in isotope signals. The isotopic composition of hair has been used to reconstruct the diet in a variety of taxa (Macko et al 1999, Kelly, 1999, Stapp 2002).

In a study by Midgley (2002) *Acomys subspinosus* was identified as a rodent that buried and retrieved seeds under lab conditions in a scatter hoarding experiment. Thus *A. subspinosus* is known to cache seeds as a stored resource. Although other plant species within the fynbos are suspected of as possibly being rodent dispersed by seed caching, no significant work has been done to determine which rodent species may be responsible for the scatter hoarding.

Aims

The overall aim of this project is to use hair of small mammals to obtain isotope signals from different animals to allow dietary analysis. Here I will address a few aspects relating to mass spectrometry in the use of dietary reconstruction. To do this I will determine whether there is a difference in the $\delta^{15}N$ and $\delta^{13}C$ signals of leaves and seeds of nut-fruited families (Restionaceae and Proteaceae).

The effects of seasonal variation of *A. subspinosus* will also be investigated with the main objective being to determine if $\delta^{15}N$ and $\delta^{13}C$ signals change seasonally. It is well known that nut fruited species release nuts *en masse* in summer (Rebelo 1995). Thus I asked whether isotope signals from hair of *A. subspinosus* trapped in summer were different to ones trapped earlier in the year. This will allow me to determine the extent to which *A. subspinosus* relies in nuts throughout the year. Finally I will attempt to determine where *A. subspinosus* fits in on a spectrum from grazer to insectivore in terms of $\delta^{15}N$ and $\delta^{13}C$ values. This will also allow a perspective to determine whether *A. subspinosus* is dependant on nuts.

The effect of habitat on $\delta^{15}N$ and $\delta^{13}C$ signals will be broadly discussed, as sufficient data hasn't been collected to draw up conclusive results. A preliminary look at the effect of habitat will be discussed. The geographic distribution of *A. subspinosus* has been difficult to unravel. Bond et al (1980) could not explain the distribution of *A. subspinosus* based on vegetation structure. Thus the presence or absence of large nut-fruited members of the *Proteaceae* could be the important factor in the distribution of *A. subspinosus* because *A. subspinosus* relies on these seeds as food sources. Thus another aim is to determine if the $\delta^{15}N$ and $\delta^{13}C$ signals of *A. subspinosus* can be explained by the presence or absence of nut-fruited *Proteaceae* or *Restionaceae*.

Methods

Hair data collection

Hair samples from rodents trapped with Sherman® traps in various locations (Table 1) were used for the analysis. Most of the samples are from the Cedarberg where trapping took place from the 20th to the 23rd December 2003. Samples from Sir Lowry's pass were collected from the 10th to the 12th of September 2004, while samples from the Koeberg area were collected from the 23rd-25th September 2004. Samples from the Elgin region were collected from the 10th to the 12th September and over one trap night on the 26th September 2004.

Hair was cut from rodents and placed into an envelope, the species name, place of capture and date of capture was recorded. At some sites the mass of the rodent was also recorded. For *A. subspinosus* hair samples were separated into outer and inner hair to get an idea of seasonal variations and fluctuations in diets.

Isotope analysis

In the lab, hairs collected were washed with 90% ethanol. The ethanol was allowed to evaporate from the hairs before they were weighed and placed into aluminium cups. The mass of the hairs used for Carbon and Nitrogen isotope analysis ranged from 0.45mg to 0.60mg. Hair samples from individual rodents were used to determine Carbon and Nitrogen Ratios of the hairs.

Fynbos seeds were also analysed to get an idea of the nitrogen and carbon signal for these species. Table 2 shows the different seeds used in the isotope analysis. The husks of seeds were removed and inner material was used for isotope analysis. Three replicates of each seed species were used and samples ranging from 2.8mg-3mg were weighed out into tin cups. Samples were analysed for δ^{15}Nand δ^{13}C in a Carlo Erba NA elemental analyser coupled with a Delta-S continuous flow isotope ratio-mass spectrometer (Thermo Finnigan flash EA 1112 series); and were combusted at 1600 C. The accuracy of the mass spectrometer was monitored

using standards. Merck gel (0.45mg to 0.6mg) and new nasturtium (2mg) standards were used during this experiment.

Isotope ratios are expressed as follows:

$$\delta^{N}E = (R_{sample}/ R_{standard} - 1) \times 1000$$

Where N is the mass of the heavy isotope of Element E and R is the ratio of heavy to light isotope ($^{13}C/^{12}C$ or $^{15}N/^{14}N$). The standard is the international standard of the Peedee belemnite marine limestone ($\delta^{13}C$) and atmospheric N_2 ($\delta^{15}N$) values.

Four runs of 62 samples each were analysed and each run started with a blank (an empty tin cup) followed by two nasturtium and two Merck gel samples. A nasturtium sample and Merck gel was used as samples after every ten samples to monitor variation in the mass spectrometer readings during the run. Samples were corrected to the degree to which Merck gel samples deviated from what the standard $\delta^{15}N$and $\delta^{13}C$ values for Merck gel are supposed to be.

Nitrogen and carbon isotope values for leaves of plants in the *Proteaceae* and *Restionaceae* families were derived from literature (Spriggs et al., 2004 and Smythe, 1997). Only foliage data for *Leucadendrons* and *Restios* were used for comparison, as these are the large nutted seed producing plants.

Data analysis were done using Microsoft® Excel 2000 and scatter grams in Statistica (6.1)

Results

To include as many species as possible within a range of habitats a number of rodents from various habitats were used in the isotope analysis. To show differences in the $\delta^{15}N$and $\delta^{13}C$ values of species with differing modes of nutrition were used (e.g. granivores, insectivores and foliavores).

Table 1 indicated the locations samples and the species sampled at each location as well as the mode of nutrition.

Location	Species	Number of individuals
Assegaaibos	*Ambylosomes sp*	1
	Cryptomys hottentotus	1
Cape infanta	*Mus minutoides*	1
Cedarberg	*A. subspinosus*	19
	Otomys irroratus	5
De Hoop	*Rhabdomys pumilio*	7
	Acomys subspinosus	3
	Otomys irroratus	2
	Mus minutoides	3
	Tatera afra	4
Doring River	*Proamys*	6
Elgin	*Acomys subspinosus*	12
	Aethomys namaquensis	8
	Rhabdomys pumilio	9
Gaukasberg	*Elephantulus edwardii*	4
Jonkershoek	*Acomys subspinosus*	2
Koeberg	*Rhabdomys pumilio*	4
	Tatera afra	1
Stellenbosch	*Suncus infinitesimus*	

Foliage and seed data

Foliage $\delta^{15}N$ and $\delta^{13}C$ values from the literature indicate that leaves have a range of $\delta^{15}N$ and ^{13}C values that vary both intra-specific and inter-specifically (Table 2).

Table 2. $\delta^{13}C$ and $\delta^{15}N$ values of leaves for various species of *Proteaceae* and *Restionaceae*. (Adapted from Smythe, 1997, unpublished data).
(R.) = *Restionaceae* and (P.) = *Proteaceae*

Species	Mean $\delta^{13}C$ value (‰) and standard deviation		Mean $\delta^{15}N$ value (‰) and standard deviation	
Hypodiscus species (R.)	No data		-0.29	(±0.36)
L. concarpodendron (P.)	-28	(±0.175)	0.333	(±0.1925)
L. coniferum (P.)	-27	(±1)	1.5	(±0.5)
L. laureolum (P.)	-26	(±0.125)	1.25	(±0.5)
L. meridianum(P.)	-25.5	(±0.75)	0.2	(±0.05)
L. xanthoconus (P.)	-26.25	(±0.4583)	1.25	(±0.25)
P. compacta (P.)	-26	(±0.833)	2.25	(±0.333)
P. coronata (P.)	-28	(±0.15)	3	(±0.15)
P. lepidocarpodenron (P.)	- 27	(0.225)	3.25	(±0.2)
P. neriifolia (P.)	-28	(±0.3)	2.5	(±0.15)
P. obtusiflolia (P.)	-28.25	(±0.5)	1.4	(±0.025)
P. repens (P.)	-24.5	(±0.4)	1.75	(±0.1)
P. susannae (P.)	-27.5	(±0.75)	4.25	(±0.025)
P.burchellii (P.)	-28.25	(±0.214)	1.75	(±0.156)
Thamnocortus punctatus(R.)	No data		0.59	(±0.42)
Restio sp. (R.)	No data		0.195	(±0.04)
Restio 2 sp (R.)	No data		1.36	(±1.92)

From the data collected from the mass spectrometer samples it seems that seeds are enriched relative to leaves. There are also differences between different species as some species have much higher nitrogen signals than others.

Data from the literature indicates that $\delta^{15}N$ content of leaves are generally low. The *Leucadendron* samples from the literature have really low $\delta^{15}N$ values with a

low range, 0.2-3 (Figure 1). The $\delta^{13}C$ values were low and varied from –28 to –25 (Figure 1).

For the *Proteoids* the $\delta^{15}N$ and $\delta^{13}C$ values were also low but higher than that of the *Leucadendrons*. Here the ranges of the leaves are from $\delta^{15}N$ (1.3 to 4.5) and $\delta^{13}C$ (-28.5 to –23.5) (Figure 2).

The $\delta^{15}N$ values for seeds obtained from mass spectrometry data the values for the *Leucadendron* seeds are high compared to leaves. The range of $\delta^{15}N$ is much higher (1-9) while $\delta^{13}C$ values are similar to that of the leaves (-28.5 to –24) (Figure 4). While the $\delta^{15}N$ values for the restios are the lowest of all (Figure 3)

For the *Restionaceae* the $\delta^{15}N$ values are as high in the seeds as in the *Leucadendrons*. Here, quite a few species (*C. paviflora, C arrista* and *C. vigita*) are in the high $\delta^{15}N$ and high $\delta^{13}C$ range (Figure 5). In summary the seeds have higher $\delta^{15}N$ and $\delta^{13}C$ values than leaves. The standard deviations for the seeds are low (table 3).

Table 3: $\delta^{13}C$ and $\delta^{15}N$ means and standard deviations of seeds for various Proteaceae and Restionaceae species(R.) = *Restionaceae* and (P.) = *Proteaceae*

	Mean $\delta^{15}N$(‰)	$\delta^{15}N$ Standard deviation	Mean $\delta^{13}C$ (‰)	$\delta^{13}C$ standard deviation
C. aristata (R.)	7.6	0.437	-23.88	0.41
C. argenteum(R.)	2.10	1.206	-29.01	0.64
C.parviflora (R.)	7.38	0.21	-23.07	0.32
C.virgata (R.)	7.49	1.22	-24.30	0.13
L.concum (P.)	3.33	1.34	-27.76	2.76
L. elimise (P.)	6.66	0.97	-24.88	1.87
L. sericeum (P.)	8.03	0.16	-25.66	1.57
L. rubrum (P.)	3.46	0.86	-27.31	1.65
L.salicifolium (P.)	1.05	0.33	-28.64	0.19
L.sessile (P.)	4.01	4.18	-26.5	0.36
P. compactor (P.)	5.27	0.45	-25.11	0.62
P. neriifolia(P.)	6.80	1.14	-26.34	0.66
P. repens(P.)	6.46	0.20	-24.99	0.42
P.eximea(P.)	5.51	0.65	-24.49	0.64
P.sussannas	5.14	0.64	-25.66	0.81

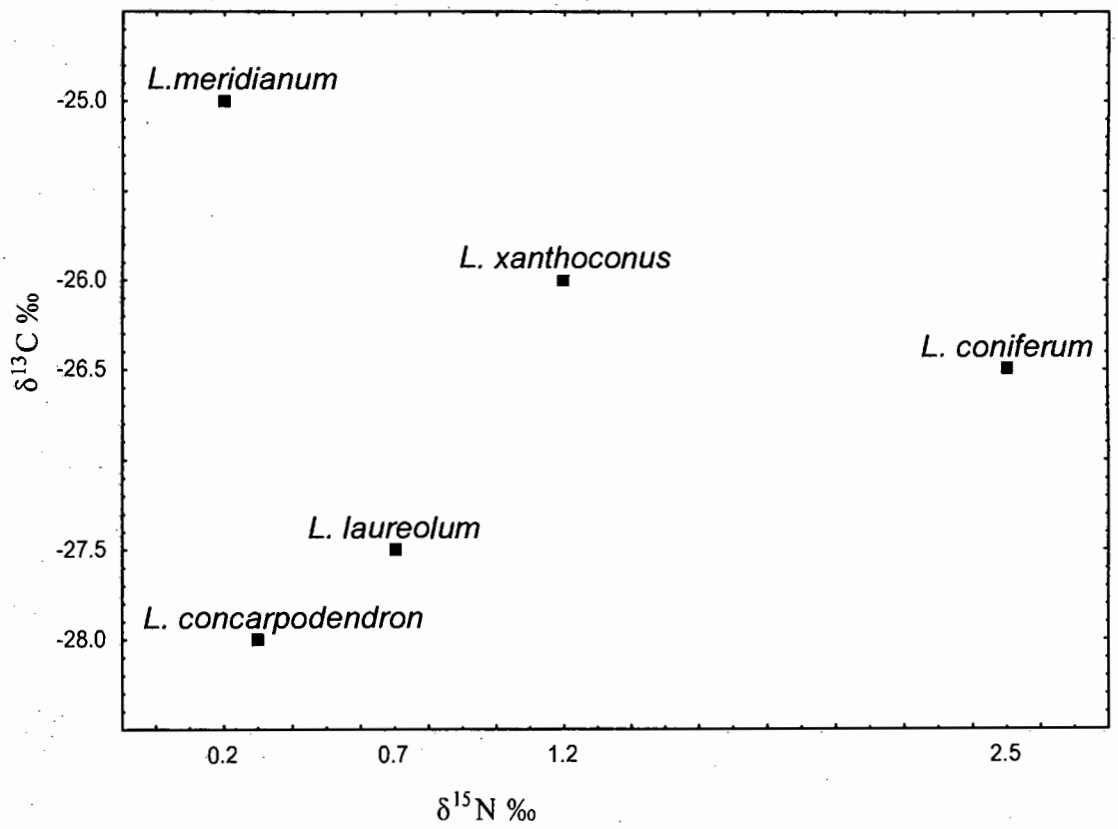

Figure 1: Two-way scatter plot showing the relationship between $\delta^{15}N$ and $\delta^{13}C$ of leaves for five species of *Leucadendron*.

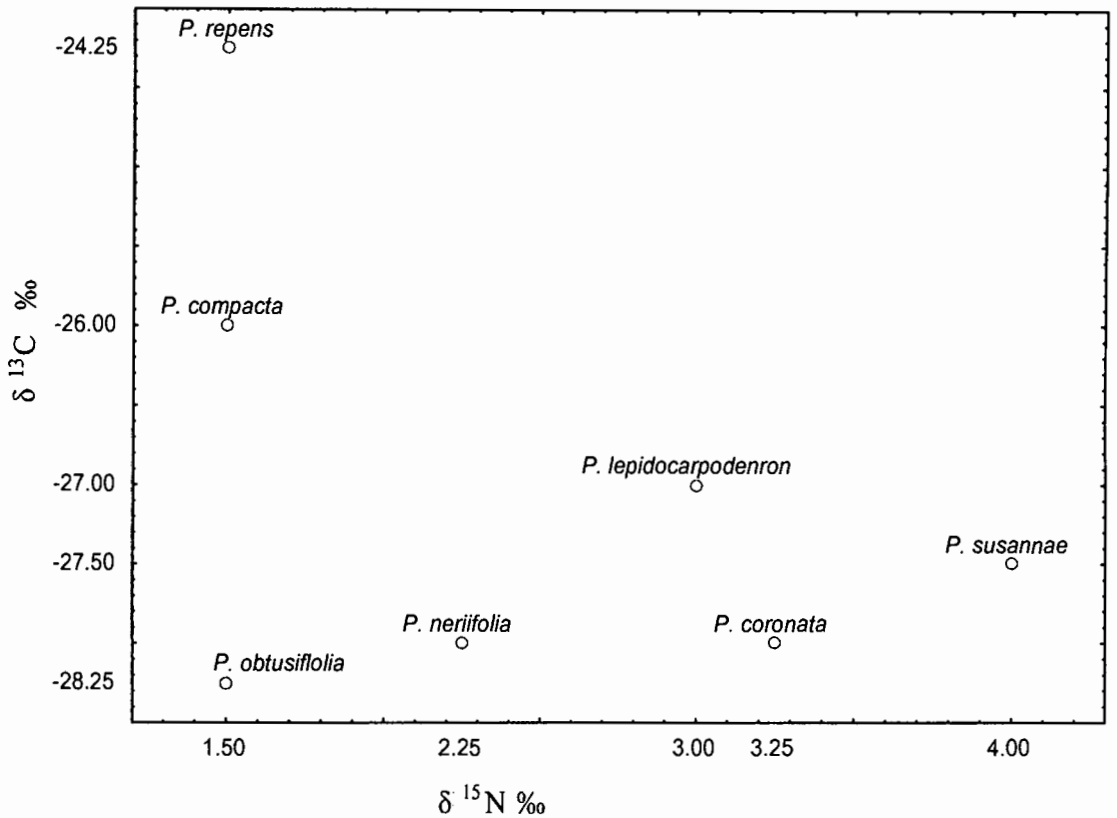

Figure 2: Two-way scatter plot showing the relationship between $\delta^{15}N$ and $\delta^{13}C$ of leaves for seven species of *Protea*.

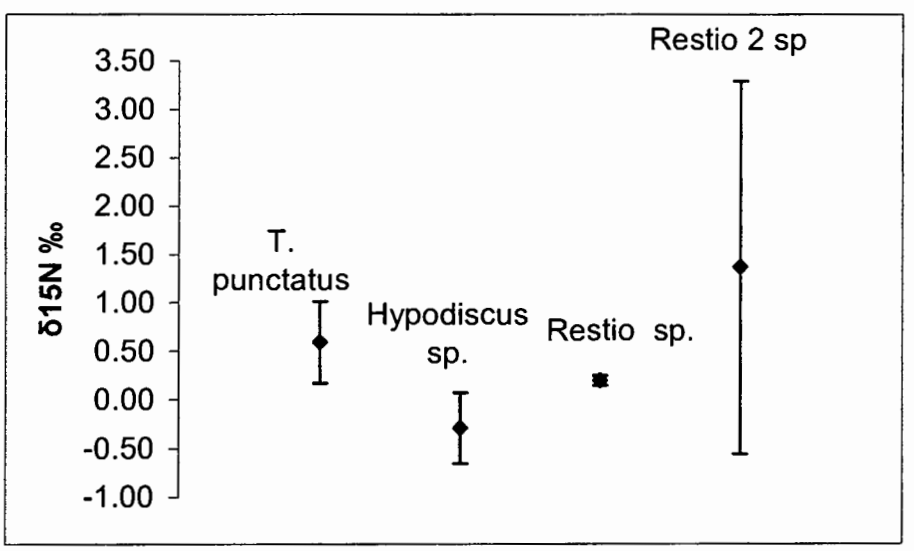

Figure 3: Scatter plot showing the $\delta^{15}N$ signal of leaves for four Restios. Error bars indicate standard deviation.

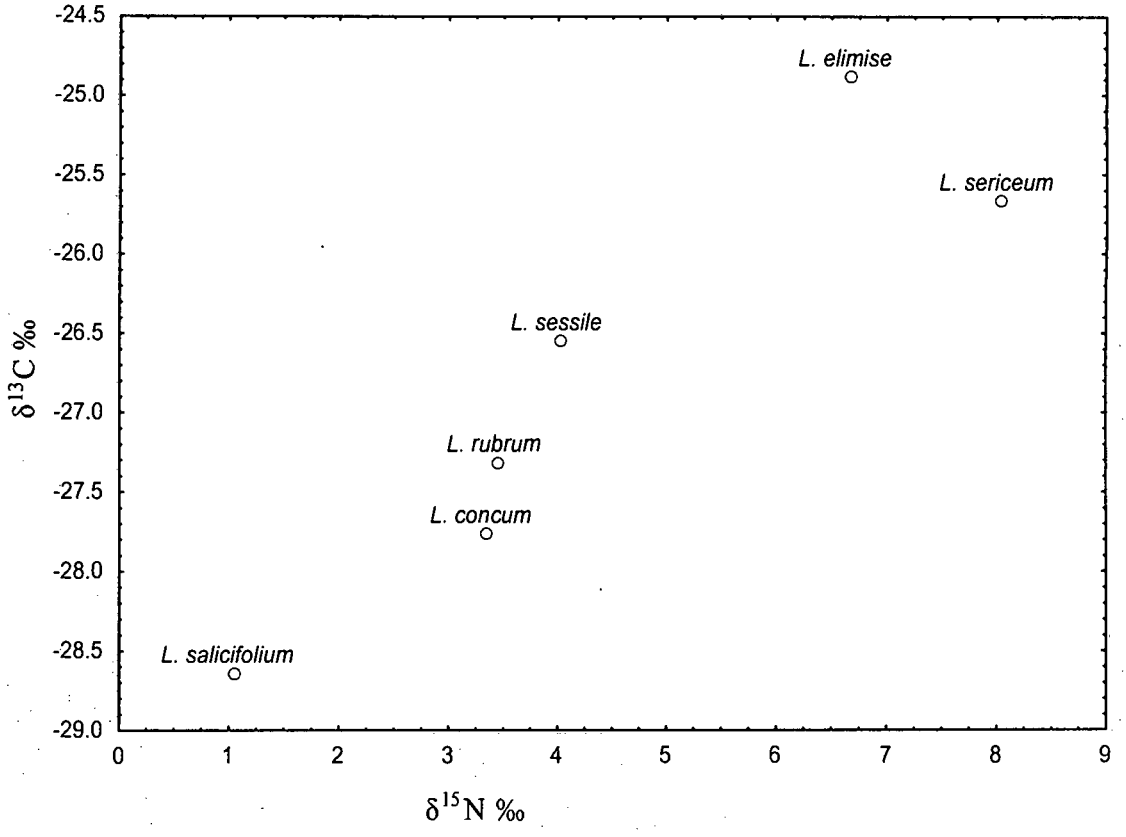

Figure 4: Two-way scatter plot showing the relationship between $\delta^{15}N$ and $\delta^{13}C$ of seeds for six species of *Leucadendron*.

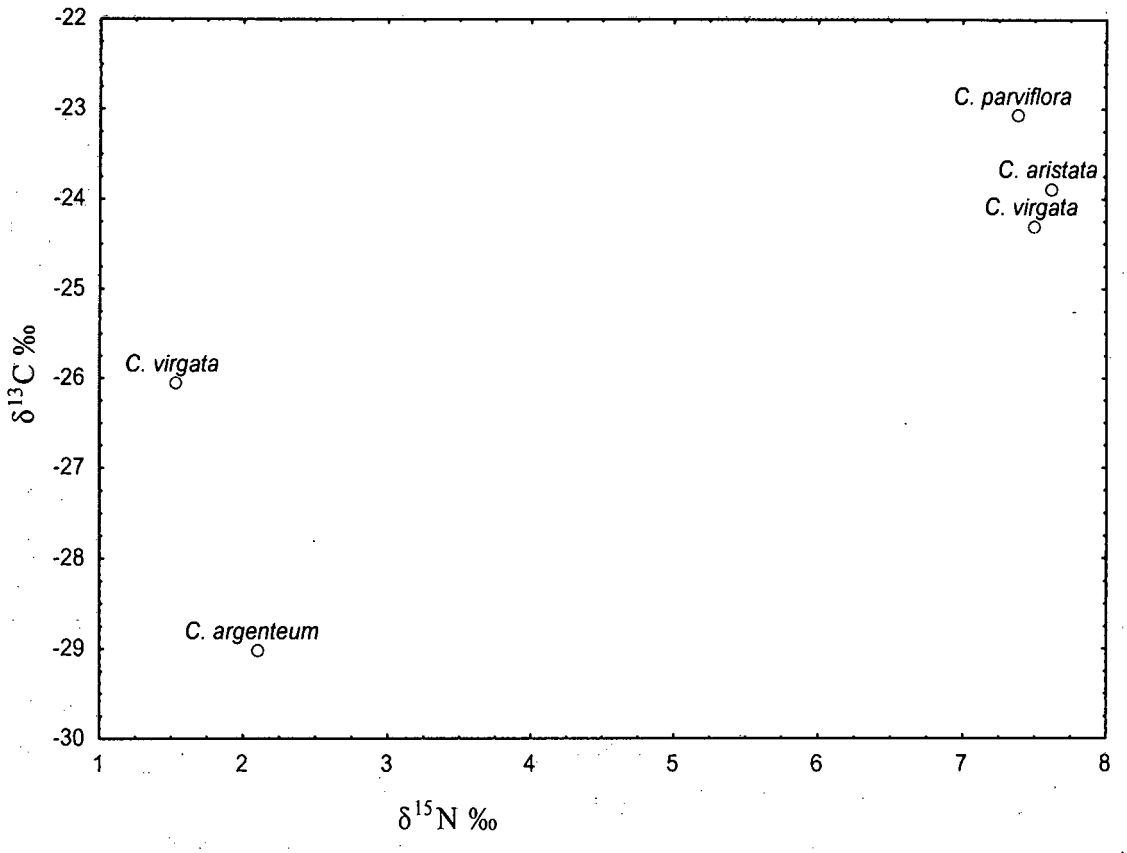

Figure 5: Two-way scatter plot showing the relationship between $\delta^{15}N$ and $\delta^{13}C$ of seeds for four species of *Cannamois* and *Ceratocaryam argenteum* (*Restionaceae*).

Hair Isotopes

The $\delta^{13}C$ and $\delta^{15}N$ values for the rodents are, on average, more positive than leaves and seeds. The difference in signals between generalist feeders, vegetarians and insectivorous can be seen in the $\delta^{15}N$ and $\delta^{13}C$ values (Figure 6).

There is no significant difference between the $\delta^{15}N$ signals of the inner and outer values of *A. subspinosus* hairs ($t=1.1084$, $p<0.2808$); the $\delta^{13}C$ values for the inner hair are not significantly different from the outer hairs either ($t=0.00627$, $p<0.06270$). The $\delta^{15}N$ value for *A. subspinosus* from the Cedarberg is higher than that of *A. subspinosus* from Elgin ($t=2.1788$, $p<0.005$). There was no significant difference in the $\delta^{13}C$ values for *A. subspinosus* from Elgin and the Cedarberg ($t=2.0453$, $p<0.823$). For *R. pumilio,* there is no significant difference between the $\delta^{15}N$ signal for rodents from De hoop and those from Elgin ($t=2.2281$, $p<0.174$) but the $\delta^{13}C$ was higher for rodents from De hoop than from Elgin ($t=2.1147$, $p<0.0001$).

Table 4: $\delta^{13}C$ and $\delta^{15}N$ means and standard deviations of various small mammals. Standard deviations not applicable (n/a) are for species where only one hair sample was used for mass spectrometry

Species	Mean $\delta^{15}N$ (‰)	$\delta^{15}N$ standard deviation	Mean $\delta^{13}C$ (‰)	$\delta^{13}C$standard deviation
Acomys subspinosus	3.85	1.70	-21.97	1.17
Aethomys namaquensis	2.35	1.85	-22.13	1.72
Elephantulus edwardii	6.94	1.88	-19.10	0.92
Mus minutoides	6.21	0.12	-21.98	0.17
Rhabdomys pumilio	3.37	1.68	-22.45	1.10
Otomys irroratus	5.31	1.52	-22.78	1.90
Tatera afra	5.51	2.53	-20.86	0.65
Amblysomus sp	14.76	n/a	-11.35	n/a
Chrysochloris asiatica	8.80	n/a	-21.70	n/a
Cryptomys hottentotus	5.47	n/a	-23.44	n/a
Georychus capensis	12.11	n/a	-23.47	0.90
Graphiurus sp.	6.55	0.06	-19.88	1.01
Proamys	3.99	2.37	-21.78	n/a

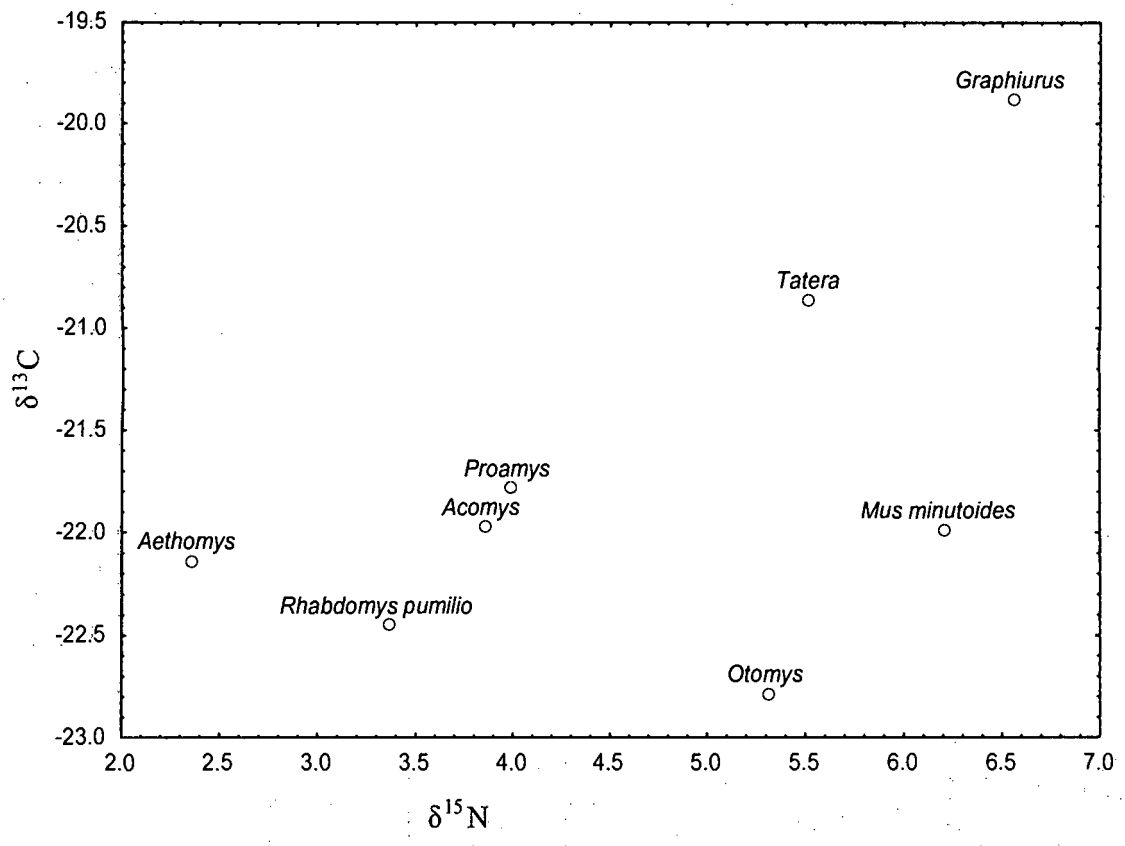

Figure 6: Two-way scatter plot showing the relationship between $\delta^{15}N$ and $\delta^{13}C$ of hairs from rodents.

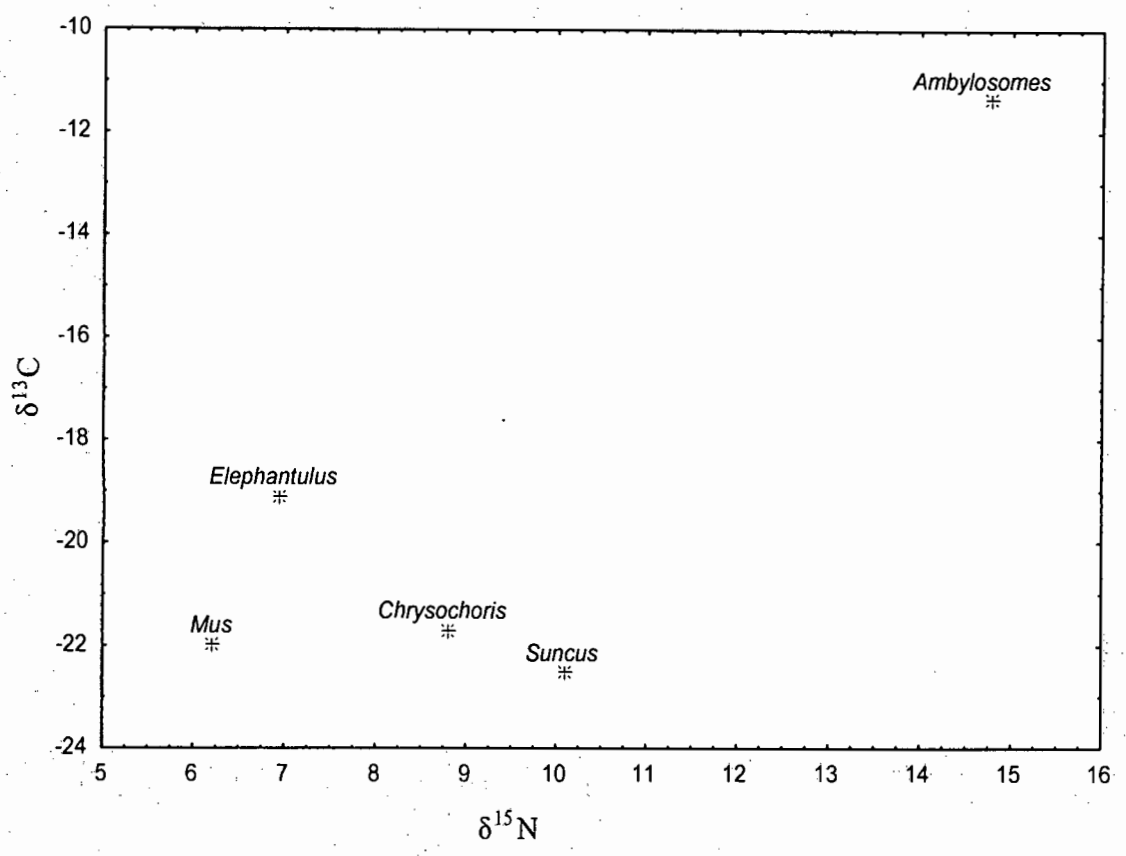

Figure 7: Two-way scatter plot showing the relationship between $\delta^{15}N$ and $\delta^{13}C$ of hairs from rodents. All these rodents are insectivores.

Discussion

Isotope signals from *A. subspinosus*

Comparisons between $\delta^{15}N$ signals from hair samples from *A. subspinosus* in the Cedarberg and Elgin showed that *A. subspinosus* from the Cedarberg had a significantly higher $\delta^{15}N$ signal. The difference between the two could be due a difference in locations, as there may have been more seeds available in the Cedarberg and thus *A. subspinosus'* $\delta^{15}N$ value is higher. However, the difference between the two sites could also be because hair samples from the Cedarberg were collected in the summer and those in Elgin were collected in spring. Seeds are often released in summer and thus *A. subspinosus* may have a higher $\delta^{15}N$ signal

in the Cedarberg, because of differences in seasonally available food sources. Thus some degree of seasonality may play a role in the $\delta^{15}N$ signals for *A. subspinosus*. The assumption that this proves that there is a seasonal difference in $\delta^{15}N$ signals cannot be conclusively drawn from this reason. For one to be able to say for sure that the differences between the Elgin and Cedarberg δ15N and δ13C signals are due to seasonality, hairs from *A. subspinosus* in both locations from trapping in summer and some other time in the year.

The above is contradicted by the result that inner there is no significant difference between the $\delta^{15}N$ and $\delta^{13}C$ values of the inner and outer hairs of *A. subspinosus*. This data suggests that seasonality does not play a role in the $\delta^{15}N$ and $\delta^{13}C$ values for *A. subspinosus*. West et al (2004), however, have demonstrated that isotope analysis has the potential to be of high resolution because changes in diet as short as one day can be observed using isotope analysis on finely scaled hair samples. Therefore the lack of a significant difference between the inner and outer hair could be due to the randomness of the cut off point for the inner and outer hairs. Theoretically, I should have been able to find a significant difference between inner and outer hairs. Although, some idea of the growth rate of hairs is also needed to determine how much hairs grow (in terms of length) seasonally. An indication of age of hair would also be indispensable knowledge in determining seasonal variability.

Changes in isotope signals also changes with age and thus rodents of various age classes can have different $\delta^{15}N$ and $\delta^{13}C$ signals. This means that the age of the individual could affect the isotope values.

Dietary signals

The method of using $\delta^{15}N$ and $\delta^{13}C$ isotope fractionation as a surrogate indicator of dietary composition and trophic status for small mammals in the Cape is

feasible. The $\delta^{15}N$ and $\delta^{13}C$ values do indicate a degree of hierarchy of trophic level structure. It is most clearly illustrated in the $\delta^{15}N$ and $\delta^{13}C$ values of those species that are insectivorous. This is because the insectivorous animals have the higher $\delta^{15}N$ values than that of generalists or grazers (Figure 6). This suggests that this method of obtaining useful dietary information may be used on many unstudied species in the Cape. This method would thus also allow unbiased dietary information in a time and cost-effective manner. The $\delta^{15}N$ and $\delta^{13}C$ values of *A. subspinosus, A. namaquensis, R. pumilio* and *Proamys* are low, this suggests that their diets are mainly vegetarian. *A. namaquensis* has the lowest $\delta^{15}N$ and $\delta^{13}C$ values as expected because *A. namaquensis* lives on a purely vegetarian diet, with about 90% of its diet is being green vegetation (Apps,1986). *R. pumilio* is characterised as a generalist feeder, which explains its low nitrogen and carbon values. This is because a generalist feeder is able to live on a variety of different food types, which makes it able to live in environments where there are not nutrient rich food sources. The low $\delta^{15}N$ and $\delta^{13}C$ values are from Elgin indicates that *R. pumilio* is living on a low quality diet in that area. This statement is supported by the fact that the De hoop *R. pumilio* δ $\delta^{13}C$ values are higher than the Elgin ones. The fact that the Elgin area seems to be a low quality environment is also supported by the low $\delta^{15}N$ and $\delta^{13}C$ values for the *A. subspinosus* found here.

The $\delta^{15}N$ and $\delta^{13}C$ values of *A. subspinosus* is not that much higher than that of the average of seeds and this may be due to the low $\delta^{15}N$ and $\delta^{13}C$ values from Elgin; which is related to the diet it has in Elgin. Thus I assume that the diet of *A. subspinosus* consists mainly of low $\delta^{15}N$ foliage. The average $\delta^{15}N$ and $\delta^{13}C$ values for *A. subspinosus* in the Cedarberg is higher than that of *A. subspinosus* in Elgin. This could be because in the Cedarberg *A. subspinosus* trapping was in *L.concarvum* stands while in Elgin there was no nut-fruited vegetation in the trapping location (pers. obs.).

In general, the results seem to indicate that *A. subspinosus* is more of a foliage feeder than a granivore because its low $\delta^{15}N$ and $\delta^{13}C$ signals suggest that it doesn't rely to heavily on stored seeds in the non seed releasing seasons. In Australia, Smith et al (2002) found that temporal patterns of ^{13}C values in rodents reflects the differences in C_3 and C_4 plants in their diet, due to differential reliance of seasonal seed groups and seed stores in kangaroo rats and other granivorous animals. This once again seems to indicate that if *A. subspinosus* were dependant on caches seeds all year round this would've been picked up in the hair isotopes. But the reason there was no significant difference between the $\delta\ ^{15}N$ and $\delta^{13}C$ content of the inner and outer hairs could just be due to the technique used to separate inner from outer hairs.

O. irroratus and *T. afra*, which are generally considered to be grazers have unexpectedly high $\delta^{15}N$ and $\delta^{13}C$ values . *T. afra* has a diet that should consist of grass, roots, some seeds and bulbs (Apps 1986). Thus the high $\delta^{15}N$ and $\delta^{13}C$ values are somewhat surprising, as it is not documented as a being granivorous or insectivorous. The raw data indicates that the high values for *T. afra* are from the De Hoop area and thus the unexpectedly $\delta^{15}N$ and $\delta^{13}C$ values may be due to the habitat in which it lives and nutrient enriched resources available to it in its habitat. Differences in habitat selection could lead to variation in diet and hence one would get dietary separation (Baugh et al. unpublished). The high $\delta\ ^{15}N$ and $\delta\ ^{13}C$ values for *O. irroratus* can be attributed to it being an occasional insectivore or granivore as well as being coprofagous. According the Smithers' mammals of southern Africa (Apps, 1986), the *O. irroratus* found in the South-Western Cape is usually a mixed feeder and has a diet that consists of seeds and plant material. Furthermore, *O. irroratus* has been known to eat their own faeces to obtain nutrients from hind gut fermentation and young also eat the faeces of adults to innocluate gut with hind gut fermenting micro-organisms (Apps, 1986).

The enriched values of $\delta^{15}N$ and $\delta^{13}C$ (Figure 6) in *M. minutoides, E. edwardii, Amblysomus sp, C. asiatica, Graphiurus sp.*, and *S. infinitesimus* are expected as these are all species that are classified as being insectivorous (Apps, 1986). One expects insectivores to have higher $\delta^{15}N$ and $\delta^{13}C$ values than that of herbivores or granivores because $\delta^{15}N$ and $\delta^{13}C$ is accumulated in a trophic manner (Kelly, 1999).

Seeds and leaves

The $\delta^{15}N$ and $\delta^{13}C$ in the leaves of both the *Proteas ,Leucadendrons* and the *Restios* are much lower than in the seeds. This is because seeds often have a concentration of carbohydrates and proteins as germinating seedlings need stored resources to grow and compete successfully for resource. Leaves are used mainly for photosynthetic mechanisms and do not require much nitrogen in them. Further more the fynbos is a nutrient limited system so nitrogen in leaves is low because nitrogen needs to be conserved for seeds production thus there would be a higher $\delta^{15}N$ signal in seeds because there is more nitrogen.

The seeds used in the isotope analyses and the foliage $\delta^{15}N$ and $\delta^{13}C$ data received from the literature are not always from the same species. Therefore, although seeds do seem to be $\delta^{15}N$ enriched, relative to foliage material this assumption should be tested using the seeds and leaves from the same individuals and species. Then the degree to which seeds are enriched relative to foliage material could be determined.

Conclusions

The use of the isotopes to determine where *A. subspinosus* fits in on a scale from purely vegetarian to insectivore has shown that the reliance of *A. subspinosus* in seeds is not that important. This is based on the assumption that if *A. subspinosus* relied on buried seeds then one would expect the $\delta^{15}N$ value for *A. subspinosus* to be higher than it was. Because the $\delta^{15}N$ for *A. subspinosus* was more similar to that of *R. pumillio* and *A. namaquensis* than to other mainly seed

eating and insectivorous eating rodents. If *A. subspinosus* doesn't relay on seeds throughout the year then it means that it only eats seeds when it is available in summer and the seeds that are cached by it don't last long. Thus, caches seeds are probably eaten soon after being cached. Alternatively, caches are wither pilfered or the locations of seeds are not successfully remembered.

The difference between the $\delta^{15}N$ signals from *A. subspinosus* Cedarberg and Elgin sites indicate that available resources are more important in determining the $\delta^{15}N$ of rodents than temporal changes in diet.

Further research

This project has shown that the use of mass spectrometry does seem to be valid tool for inferring diets of various rodents. Thus one can use this technique to determine the relative contribution of various resources to the diets of different species. To determine this more rodent trapping in a variety of different habitats should be done to increase the sample size and include a wider range of small mammals. To determine the relative contribution of various plants, seeds or insects to the diet of the rodents, insects and the foliage material and seeds from plants in the area in which the trapping is was done should be analysed using the mass spectrometer. This way one would, theoretically, be able to infer the seeds, foliage material or insects that form part of the diet of the species trapped at a particular location. Also, to get a real idea of whether $\delta^{15}N$ and $\delta^{13}C$ signals change seasonally because of the availability of seeds in summer, isotope signatures of the same species from the same locations at different times of the year should be done. In this study we weren't able to go back and trap a t the Cedarberg, so we couldn't clearly determine the effect of the availability of seeds in summer only.

Acknowledgements

I would like to thank Prof. Jeremy Midgley for his assistance in the field during data collection and for providing additional information. Furthermore I would like to thank Dr. John Lanham for his assistance in analysing data in the lab with running of the mass spectrometer. I would also like to thank the university of Cape Town and the NRF for financial assistance.

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