Validation and use of ^{22}Na turnover to measure food intake in free-ranging lizards

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Summary. As the food intake of free-ranging animals has proved to be difficult to measure by traditional means, the feasibility of using radioactive Na to measure food consumption in a small scincid lizard (*Lampropholis guichenoti*) was assessed. This technique has previously been used only for several species of mammal. A significant relationship between food intake and Na turnover was found in the laboratory, with Na turnover underestimating intake by 7.6%. The food intake of free-ranging members of a field population was estimated by ^{22}Na turnover to be 9.55, 0.65, 9.39 and 13.75 mg dry weight $(day)^{-1}$ during autumn, winter, spring and summer respectively. Estimates of assimilated and expended energy from these food intake values agree closely with data reported for other lizards using alternative techniques. This study also describes the technical innovations which were necessary to study lizards weighing less than 1 g; and it suggests that ^{22}Na can provide an easy, reliable and inexpensive means of studying the energetics of many free-living animals.

Introduction

In this paper, we present (i) a laboratory assessment of the feasibility of using ^{22}Na to measure food consumption in a small scincid lizard, and (ii) the results of a field study of a free-ranging population over 1 yr using this technique.

Direct estimates of food intake for free-living reptiles have been made previously by establishing a laboratory relationship between food intake and output of faecal or nitrogenous wastes, and then measuring the output of field-captured animals (Avery 1971, 1978, 1981; Andrews and Asato 1977); or from a knowledge of the dietary water input and the water content of the food (Minnich and Shoemaker 1970; Nagy 1975; Nagy and Shoemaker 1975; Congdon et al. 1982). However, the first of these methods is undesirable because the animals must be removed from the field for several days at each sampling; and the latter method is possible only for non-drinking animals.

More commonly, and probably less reliably, food consumption has been estimated by indirect methods. These include: analysing the composition and rate of production of the faeces (Harris 1964); analysing gut contents and making assumptions about stomach capacity and its rate of fill (Johnson 1966; Congdon et al. 1982); combining laboratory feeding regimes with estimates of field behaviour (Mueller 1970; Dutton et al. 1975); and combining data on field energy expenditure with estimates of assimilation efficiency and secondary production (see Avery 1971, 1978, 1981; Nagy 1975; Turner et al. 1976; Bennett and Gorman 1979).

However, a direct measure of food intake can be made if there is a close positive correlation between food intake and the rate of Na turnover (NaTO) in the species under consideration. NaTO, in turn, can be accurately measured using the turnover rate of ^{22}Na (Buscarlet 1974), by following the exponential decline in the specific activity of the isotope, in a manner similar to the use of labelled water to measure water turnover (e.g. Lifson and McClintock 1966; Nagy and Costa 1980). In those animals whose major source of Na is dietary, reliable estimates of food intake can be established using ^{22}Na turnover if the Na content of the diet is known. Furthermore, food, and hence energy, intake can then be converted to assimilated energy by determining the digestive efficiency of the species. If the animal is in a steady state with respect to energy, assimilated energy is also a good approximation to metabolised, or expended, energy.

In captivity, a strong correlation between NaTO and food intake has been found for five species of mammal: rabbits, *Oryctolagus cuniculus* (Green and Dunsmore 1978), dingoes, *Canis familiaris dingo* (Green 1978), Tasmanian devils, *Sarcophilis harrisii* (Green and Eberhard 1979), Australian native cats, *Dasyurus viverrinus* (Green and Eberhard 1979), and swamp buffaloes, *Bubalus bubalis* (Williams and Green 1982). These results suggest that the method could successfully estimate food intake in a wide variety of animals. The only reports of the use of ^{22}Na to measure the food consumption of free-ranging animals are by Green et al. (1978), who studied a number of populations of *O. cuniculus* for periods of 1-3 weeks to compare NaTO with the Na status of vegetation; and by Williams and Ridpath (1982), who studied a small group of *B. bubalis* for 6-15 days to examine the effect of their grazing on a swampland.

Materials and methods

The species

Lampropholis guichenoti (Dumeril & Bibron) is an insectivorous skink with an average body weight of 1 g and snout-vent length of 4 cm. These oviparous lizards are common in south-eastern Australia, where they occur in heathlands, woodlands and forest clearings. They are active diurnally (Heatwole 1976); and are most abundant in suburban gardens, where they inhabit leaf litter, grass, rock piles and logs. All animals used were collected in Sydney, New South Wales.

Laboratory validation

The assessment of NaTO by ^{22}Na depends upon the relationship:

$$NaTO = ExNa \times k \quad (1)$$

where ExNa is the size of the animal's exchangeable Na pool and k is the fractional rate of turnover of ^{22}Na. If ExNa remains constant, then k also represents the fractional turnover rate of body Na. ExNa and k are usually determined by repeat blood sampling, but the small size of *L. guichenoti* prohibits this. Accordingly, suitable alternatives had to be found.

Estimation of exchangeable Na. Much of the body Na of vertebrates is non-exchangeable, being mainly located in the bone matrix, and this is not labelled when an animal is injected with ^{22}Na (Green 1978). ExNa in the experimental lizards could not be determined directly by isotope dilution, as it is difficult to remove blood samples from such small animals without killing them. Instead, the proportion of total body Na (TBNa) which was exchangeable was determined for a subsample of the animals; and then a relationship was established between TBNa and body weight. Hence, ExNa could be estimated for an animal of a given body weight.

To determine ExNa as a proportion of body weight, three lizards were labelled by a single intraperitoneal injection of 2.5 µl of $^{22}NaCl$ at 20 [µCi ml^{-1} (1.85 kBq), and then decapitated after an equilibration period of 3 h. Blood samples were collected into microcapillary tubes and centrifuged for 5 min at 3,000 rpm; and three replicate 1.0 µl samples of the plasma from each animal were removed for liquid scintillation counting. Three replicate samples of dilution standards, prepared by adding 5.0 µl of $^{22}NaCI$ to 1.0 ml of distilled water, were counted also. The remaining plasma from each animal was analysed for Na by flame photometry. The amount of body Na which was labelled was then calculated as:

$$ExNa = A_s/A_p \times 1 \text{ ml} \times Na_p \quad (2)$$

where A_s is the specific activity of the standard in counts min^{-1} μl^{-1}, A_P is the specific activity of the plasma in counts min^{-1} μl^{-1}, and Na_P is the Na content of the plasma in µmoles ml^{-1}.

The relationship between TBNa and body weight was determined in a series of 13 lizards, each of which was weighed, killed by freezing, and analysed for total Na by acid digestion as described for the fruitfly pupae below.

Calculation of NaTO using Eq. 1 depends upon ExNa remaining constant for each lizard during the measurement period; and this is normally assessed by comparing values of ExNa determined by isotope dilution before and after the experiment. For *L. guichenoti,* individual values of ExNa were not determined. Instead, all experimental lizards were weighed daily, assuming that changes in the Na pool would be reflected by changes in body weight (cf Green and Eberhard 1979).

Measurement of fractional turnover rate. As k could not be determined by monitoring the specific activity of blood samples, techniques for whole body counting of ^{22}Na activity were developed. Either an Australian Atomic Energy Commission Type 238 portable scintillation counter or a Packard Tri-carb Liquid Scintillation Spectrometer Model 3375 was used, depending upon which was available. A1though the counting efficiency of these two types of counters differ (i.e. they give different absolute counts), they are equally capable of monitoring changes in ^{22}Na activity (i.e. they give similar relative counts).

When using the portable counter, each lizard was placed in a plastic scintillation vial, allowed to curl up in the bottom, and was held in this position by a soft foam plug to keep it in a reproducible position relative to the detector below the base of the vial. Each animal was counted in this way for 10 min. For counting in the other system, with a lateral detector, each lizard was placed in a similar vial, which was half-filled with distilled water to keep the lizard in a vertical position with its head out of the water. As the counts recorded are dependent on

the orientation of the animal, each lizard was counted for 2 min in each of six random directions, and its activity estimated from the mean of these counts.

Values of k in day^{-1} were calculated from the slope of the least squares linear regression in ln whole body activity versus time for each lizard.

Comparison of Na intake and turnover. Twelve lizards were kept in separate plastic containers (30 x 20 x 15 cm) in a laboratory maintained at a constant temperature of 25° C. Na-free drinking water was supplied at libitum, both during and for 4 weeks prior to the experiment. The animals were randomly assigned to four groups of three lizards each, which were then fed *Dacus tryoni* (Queensland fruitfly) pupae at rates of 1, 2, 3, or 4 per day. All pupae were of equivalent weight, and all food presented was consumed.

To determine the Na intake of the lizards, ten, replicate samples of three *D. tryoni* pupae each were dried at 105° C, and digested on a hotplate for 3 days in 5 ml of $HN0_3$ and 1 ml of HCI. Each solution was diluted and analysed for total Na by flame photometry. To determine NaTO, each lizard was labelled with ^{22}Na as above, and the ^{22}Na activity measured using the portable scintillation counter. Counting was carried out every alternate day for 30 days.

Field study

Study site. This study was carried out on a free-ranging population of *L. guichenoti* within an area of 50 m^2 in a private garden in a southern suburb of Sydney. The study site consisted of a sloping concrete driveway, bounded on one side by a narrow garden bordering a low brick fence, and on the other by a large rocky garden. Beyond the rocky garden was a large expanse of lawn onto which the lizards seldom ventured. The lizards were observed to cross freely from one side of the driveway to the other, this movement apparently depending on the position of the sun. The lizards were predominantly found in cracks at the base of the brick fence, and amongst rock piles and under leaf litter and low bushy plants in the gardens.

Rates of Na turnover. In early April (mid-autumn) 1981, 25 lizards were collected and transferred to the laboratory. Each animal was weighed, injected with 5.0 µl of $^{22}NaCI$ at 20 µCi $m1^{-1}$ (3.7 kBq), and the ^{22}Na activity measured using the Packard liquid scintillation counter. Each animal was then uniquely toe-clipped and marked with white paint, and released at the point of capture.

Attempts to recapture labelled individuals were made at frequent but irregular intervals throughout the next 6 months. Within 24 h of recapture, each animal was reweighed, its ^{22}Na activity determined in the laboratory, and then released.

As the levels of ^{22}Na in the lizards were low by the end of spring (about twice background level), and marked individuals were becoming difficult to locate, a second sample of ten lizards was collected in November for the assessment of NaTO during summer. None of these individuals had been used in the earlier study. Each animal was treated as before, except that the portable scintillation counter was again available to determine ^{22}Na activity. Recaptures were made at irregular intervals over the next 3 months.

Rates of NaTO were calculated using Eq. 1, with ExNa being calculated for each individual using the previously determined relationship with body weight (Eq. 4). Since no changes in body weight greater than 1 % occurred for any lizard throughout the study periods, it was assumed that ExNa remained constant.

For summer, k values were calculated as before. However, for the autumn-winter-spring study the graph of ln whole body activity vs. time was divided into three seasons on the time axis: autumn (30 days), winter (120 (lays) and spring (30 days) ; and values of k were calculated for each animal within each of these seasons. In all cases, the activity of the isotope in each lizard at each sample time was first corrected for the radioactive decay rate of ^{22}Na (cf. Nagy and Costa 1980).

Na content of food. Na turnover can be converted to food intake if the Na content of the diet is known. *L. guichenoti* are opportunistic arthropod feeders, and their diet therefore varies geographically and seasonally with the activity and abundance of the various arthropod taxa (Crome 1981). As an estimate of the Na content of the diet, three species of arthropod which were abundant locally, and which were observed to constitute a large part of the diet of the lizards, were analysed for Na. These species were from the orders Diptera, Hymenoptera and Isopoda (*Porcellio laevis*); and the Na content of a known mass of each species was determined by acid digestion and flame photometry as above.

Results

Laboratory validation

Total and exchangeable Na pools. ExNa accounted for a mean of 92% (s.e. =1.2, *n* = 3) of TBNa in the lizards tested. TBNa in μmoles was, in turn, found by least squares linear regression of the log-log data (Fig. 1) to be related to body weight (BW) in g by the equation:

$$TBNa=72.2\ BW^{0.63(s.e.=0.06)} \quad (3)$$

An analysis of variance indicates that the slope of this regression line is significant (F=94.5, df=1,11, P<0.001). Thus, ExNa in turnover estimates was calculated as:

$$ExNa=66.4\ BW^{0.63} \quad (4)$$

None of the lizards showed a change in body weight greater than 1 % during the NaTO experiment. Therefore, it was assumed that ExNa had remained constant for each lizard, indicating that the fractional rate of turnover of Z2Na represents the fractional turnover rate of body Na.

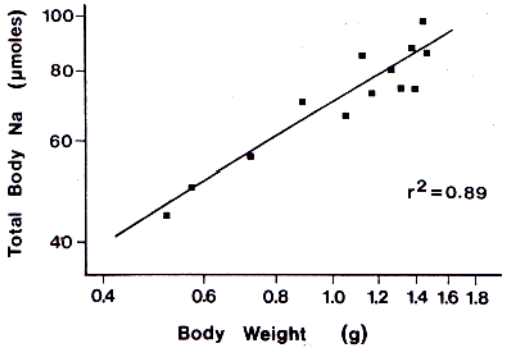

Fig. 1. Logarithmic relationship between total body Na and body weight in *L. guichenoti.* Line fitted by linear regression

Na turnover and food intake. NaTO and food intake were found by least squares linear regression (Fig. 2) to be related by:

$$NaT0=0.304\ FI+0.093$$

where FI is the food intake in pupae day^{-1}. An analysis of variance indicates that the slope of the regression line is significant (F=142.9, df=1,10, P < 0.001). *Dacus* pupae contained 0.380 μmoles Na per pupa (s.e.=0.023, *n=10);* and NaTO underestimated this Na intake by a mean of 7.6% (s.e.=3.1) (Table 1).

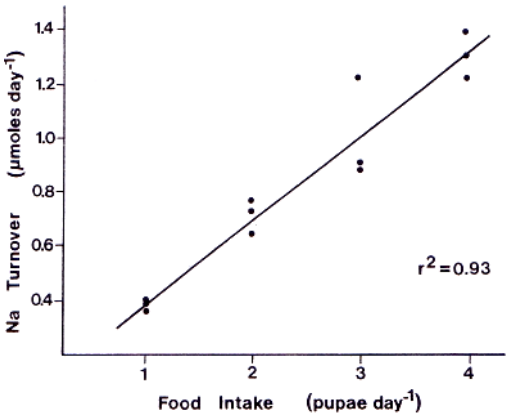

Fig. 2. Relationship between Na turnover and food intake in *L. guichenoti.* Line fitted by linear regression

Table 1. Comparison of Na turnover rate (from whole body activity of ^{22}Na) and Na intake (from Na content of diet) in 12 *L. guichenoti* measured under different feeding regimes

Food intake (pupae day^{-1})	Weight (g)	Na turnover (μmoles day^{-1})	Na intake (μmoles day^{-1})	Deviation of turnover from intake (%)
1	0.93	0.387	0.380	+ 1.8
	1.17	0.405	0.380	+ 6.6
	1.59	0.365	0.380	- 3.9
2	0.93	0.729	0.760	- 4.1
	1.19	0.641	0.760	-15.7
	1.08	0.773	0.760	+ 1.7
3	1.53	0.899	1.140	-21.1
	1.34	0.908	1.140	-20.4
	1.24	1.229	1.140	+ 7.8
4	1.37	1.390	1.520	- 8.6
	0.93	1.296	1.520	- 1.7
	0.99	1.215	1.520	-20.1

Field study

Na turnover. Of the 25 lizards labelled in autumn, 11 were never recaptured. Three lizards recaptured only in the next 14 days were not used, as they may not give accurate turnover estimates. The hypothesis that the remaining 11 lizards were all caught with equal frequency throughout the study period (i.e. that no lizards were particularly prone to recapture, and therefore biassing the data) was tested using x^2 goodness-of-fit. From these data, x^2= 6.858 (df=10), which is not significant at P=0.05. Similarly, for the ten lizards labelled in summer, six were never recaptured; and the data for the remaining four lizards yield x^2 = 0.40 (df= 3), which is also not significant at P=0.05. Thus, no one lizard was recaptured significantly more often than any other. Changes with time in the activity of ^{22}Na can be expressed as a proportion of the initial activity, so that every animal has a common y-intercept, enabling data from all lizards to be pooled (Fig. 3). An analysis of variance on random subsets of four lizards (only three from spring) from each season (Cochran's C=0.5104, ns at P=0.05; F=180.0, df = 3,11, P<0.001), followed by Student-Newman-Keuls tests (at P=0.05), reveals the following relationship between the rates of NaTO (and hence food consumption) for each season (Table 2):

winter < autumn = spring < summer.

No significant weight-related differences in rates of NaTO were found in any of the seasons, as determined by analyses of variance on the least squares linear regressions of the log-log data (autumn: F=4.70, df=1,9; winter: F=0.07, df=1,8; spring: F=0.59, df=1,1; summer: F= 11.82, df=1,2; P>0.05). However, this result may reflect only the small number of lizards recaptured, or the small range of body weights of these lizards.

Food intake. The mean Na content of the three species of arthropod was 0.1476 μmoles (mg dry weight)$^{-1}$ (s.e.= 0.0091, n=3). This mean was used to calculate the food intake for each lizard (Table 2), and corrections were made for the 7.6% underestimation found in the laboratory study.

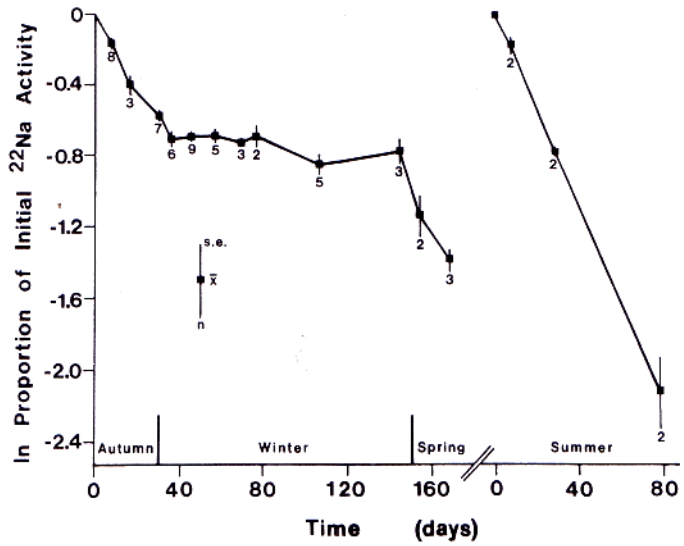

Fig. 3. Proportional changes m the activity of ^{22}Na in a free-ranging population of *L. guichenoti* during four seasons

Table 2. Measured Na turnover and calculated food intake in a free-ranging population of *L. guichenoti* during four seasons

Lizard	Weight (g)	Na Turnover' (μmoles/day)				Food intake' (mg dry weight/day)			
		Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer
1	1.06	1.10	0.26	-	-	8.15	1.93	-	-
2	0.68	1.06	0.01	-	-	7.86	0.07	-	-
3	0.92	1.48	0.15	-	-	10.97	1.11	-	-
4	1.05	1.43	0.07	1.32	-	10.60	0.52	9.78	-
5	1.03	1.26	0.02	-	-	9.34	0.15	-	-
6	0.89	1.22	0.20	-	-	9.04	1.48	-	-
7	0.99	1.32	0.05	1.21	-	9.78	0.37	8.97	-
8	1.10	1.34	0.06	1.27	-	9.93	0.44	9.41	-
9	1.04	1.06	0.01	-	-	7.86	0.07	-	-
10	1.49	1.61	-	-	-	11.93	-	-	-
11	1.16	1.29	0.05	-	-	9.56	0.37	-	-
12	1.08	-	-	-	2.01	-	-	-	14.90
13	1.15	-	-	-	1.91	-	-	-	14.16
14	0.68	-	-	-	1.65	-	-	-	12.23
15	0.88	-	-	-	1.85	-	-	-	13.71
Mean	1.01	1.29	0.09	1.27	1.86	9.55	0.65	9.39	13.75
(s.e.)	(0.05)	(0.05)	(0.03)	(0.03)	(0.08)	(0.39)	(0.20)	(0.23)	(0.56)

[a] Missing data indicate that the lizard was not caught at least twice during that season

Discussion

This study provides the first validation of the use of ^{22}Na to measure food intake in a non-mammalian species. The close positive correlation of Na turnover rate with food intake indicates that, despite some technical difficulties, this method can provide a reliable estimate of food consumption in small animals such as *L. guichenoti*. Although difficulties were encountered in administering the isotope, most of the technical difficulties arose from the inability to collect plasma samples from live animals due to their small size. Hence, ^{22}Na activity was monitored by counting the whole body instead of the usual plasma samples; ExNa was measured indirectly; and constancy of ExNa was assessed by changes in body weight.

The use of whole body counting allows an important advantage over other reported studies, in which isotope turnover rates have been calculated on the basis of only two samples: one at the beginning and one at the end of the experiment. Any uncertainty in either of these measurements could have a profound effect on the results obtained. However, whole body counting is more convenient and less time-consuming than the analysis of blood samples, and has the advantage of being less disturbing to the animals. As a result, frequent assessment of

^{22}Na activity can be made; and this provides a more reliable estimate of Na turnover rates, as well as allowing temporal changes in feeding rate to be detected. Whole body counting of ^{22}Na has been reported previously only for flies (Fairbanks and Burch 1968) and crocodiles (Taplin 1982).

Laboratory validation

The 7.6% deviation of NaTO from measured Na intake compares favourably with the underestimates observed in previous studies: 6.6% for Tasmanian devils (Green and Eberhard 1979), 12.5% for dingoes (Green 1978), 15.7% for native cats (Green and Eberhard 1979) and 22.7% for rabbits (Green and Dunsmore 1978); although an exact correlation between NaTO and Na intake was found for swamp buffaloes (Williams and Green 1982). The underestimates could be the result of: (i) errors in the estimation of ExNa; (ii) violation of the assumptions inherent in the technique; or (iii) some Na passing through the digestive system without being absorbed.

ExNa in *L. guichenoti* could only be estimated indirectly, due to the small body size of these lizards; and only 5 µl of plasma could be obtained from each animal, so that analysis of these samples by flame photometry was difficult. Consequently, errors in ExNa determinations may be possible. However, the value of 92% found for *L. guihenoti* is consistent with the 95-100% found for crocodiles and turtles (Taplin 1982), and there was only a low variability around this value.

As well, the use of ^{22}Na to measure NaTO is subject to the following assumptions (Lifson and McClintock 1966; Green 1978): (i) the exchangeable Na pool remains constant throughout the measurement period; (ii) the rates of Na influx and efflux are constant; (iii) the isotope is not chemically bound in the body, and therefore removed from the pool; and (iv) the specific activity of the isotope in Na lost from the body is the same as that of body Na. The magnitude of errors due to violation of these assumptions depends on the species under consideration and the conditions under which the study is carried out. Perhaps the most important of these assumptions for this study are (i) and (iv). Since ExNa could not be measured both before and after the experiment by isotope dilution, changes in ExNa were assumed to be represented by changes in body weight; however, it is recognised that the Na content of the body may change irrespective of body weight (e.g. replacement of muscle by fat). Assumption (iv) is violated if, for example, the body is preferentially retaining labelled molecules, and an underestimation of Na flux rates would result. Green and Dunsmore (1978) suggested this latter case as a possible explanation for the 23% underestimation they reported for rabbits. Nevertheless, the comparatively small underestimation in *L. guichenoti* suggests that no major violations have occurred.

Field study

No previous studies on reptiles have been conducted in the field throughout all four seasons. In *L. guichenoti,* the pattern of ^{22}Na loss was consistent for all of the animals, the rate of NaTO (and hence food intake) being significantly higher in spring, summer and autumn than in winter. This result is consistent with observations of the behaviour of the animals: during the warmer seasons they were conspicuous and actively feeding, whereas in winter they were usually found dormant under rocks. Temperature has been shown to influence the level of activity of members of this species, while light seems to be an important factor controlling the pattern of the daily activity cycle (Firth 1968). The lizards only live for 2-3 yr, reaching sexual maturity after 1 yr (Clarke 1965). Ovulation begins in October-November, with shelled eggs appearing in the oviduct in December (Heatwole and Vernon unpubl., in Heatwole 1976); and the young hatch in summer from January to March (Clarke 1965). This pattern is also consistent with our data, as higher energy requirements would be expected for reproduction in spring and summer.

Comparison of our results with previous studies is difficult, as food consumption depends largely on the species being examined and the prevailing physical conditions. For example, Avery (1971, 1978) used analysis of faecal output and/or nitrogenous wastes to measure food intake in three other small diurnal insectivorous lizards: *Lacerta vivipara* (1-5 g) in England, and *Podarcis muralis* and *P. sicula* (2-10 g) in Italy. He derived equations which predict food intakes for an animal the weight of *L. guichenoti* (mean 1.01 g, Table 2) which are between 31% and 153% higher than the values found under equivalent physical conditions using ^{22}Na turnover. These deviations may be an artifact of the differences in techniques used to measure food intake (the faecal output/nitrogeneous wastes methods are reported to give high values (Avery 1971, 1978)), or they may reflect differences in factors such as the activity of the animals, the availability of food, or differences in digestive physiology.

Comparison with alternative techniques

Turner et al. (1976) have suggested, on the basis of results from other authors, that the daily food consumption of lizards is probably about 5% of their body weight, except that smaller lizards may have higher consumptions than larger ones, because assimilated energy and body weight are related by a power function with an exponent of less than one (see below). If the insects eaten by free-ranging *L. guichenoti* are assumed to contain 70% water (cf Edney 1977), then their food intake was 3.2%, 0.2%, 3.1% and 4.6% of their body weight per day

during autumn; winter, spring and summer respectively. These data suggest that smaller lizards may have similar food intakes to larger lizards relative to body weight, despite what would be expected from the relationship between assimilated energy and body weight. This discrepancy could be explained in terms of energy assimilation efficiencies. Even though the food intake of the smaller lizards is low, the assimilated energy could still be high if the assimilation efficiencies of smaller lizards are higher than those of larger lizards. Unfortunately, there are insufficient data in the literature to test this proposal. However, Pough (1973) has suggested that, energetically, larger lizards should be herbivores while smaller ones should be carnivores; and the data to date indicate that herbivorous lizards do have lower assimilation efficiencies than carnivorous ones (Pough 1973; Bennett and Dawson 1976).

By pooling data from the literature on energy assimilation by 14 species of active lizards, both in the laboratory and in the field, Turner et al. (1976) derived the following relationship between energy assimilation (A) in kJ day^{-1} and body weight:

$$A=0.317\ BW^{0.81}$$

This equation predicts an energy assimilation of 0.32 kJ day^{-1} (s.e.=0.08) for a 1.01 g *L. guichenoti. To* compare this value with our own data, the assimilation efficiency of *L. guichenoti* needs to be determined. This was not done in this study. However, using the value of 0.89 found by Avery (1971) for *L. vivipara* (similar values have been reported for other insectivorous lizards (Pough 1973; Bennett and Dawson 1976)), and assuming an energy content of insects of 23.0 J (mg dry weight)$^{-1}$ (Golley 1961), reasonable estimates can be made. This yields values of 0.20, 0.01, 0.19 and 0.28 kJ day^{-1} for autumn, winter, spring and summer respectively: The values for the three active seasons are not significantly different from the predicted value (t-tests; autumn: t=1.43, spring: t-1.55, summer: t=0.47; df=13, P>0.05); but the value for the nonactive winter lizards is significantly lower (t= 3.69, df=13, $0.002 < P < 0.005$).

Many different techniques have been used to assess the energy expenditure of free-ranging lizards; but the use of doubly labelled water is the most reliable of these, as it is the only one which can directly measure energy expenditure in unrestrained animals (Mullen 1973). For a steady state animal, assimilated energy is a good approximation to expended energy; and so it is possible to make comparisons with studies which have used this technique from the above estimates of assimilated energy. After adjusting all values to J $g^{-0.80}$ day^{-1}, to correct for the relationship between body size and energy use per unit body weight at 20° C (Bennett and Dawson 1976), the calculated values for *L. guichenoti* compare closely with those obtained by previous workers (Table 3). This is significant, because the use of doubly labelled water is a complicated and expensive technique, and facilities are often not available. Furthermore, this technique does not allow the advantages conferred by whole body counting, and hence the use of ^{22}Na may be preferable in many situations.

Table 3. Comparison of ^{22}Na estimates of energy expenditure for *L. guichenoti* with doubly labelled water estimates for other free-ranging lizards

Species	Weight (g)	Energy expenditure ($Jg^{-0.80}day^{-1}$)				Source
		Autumn	Winter	Spring	Summer	
Lampropholis guichenoti	1	*194*	*13*	*191*	*279*	Present study
Cnemidophorus draconoides	9	-	-	-	*209*	Anderson and Karasov *(1981)*
Sceloporus jarrovi	12	-	*35*	-	-	Congdon et al. *(1979)*
Sceloporus occidentalis	12	*209*	-	*232*	-	Bennett and Nagy *(1977)*
S. occidentalis	14	-	*6*	-	-	Bartlett *(1976)*
Cnemidophorus tigris	16	-	-	-	*364*	Anderson and Karasov *(1981)*
Sauromalus obesus	167	-	*48*	-	*293*	Nagy and Shoemaker *(1975)*

Concluding remarks

The accuracy and reliability of the ^{22}Na technique in field studies could be improved in a number of ways. Firstly, the relationship between NaTO and food intake was validated only at 25° C, and the possibility that this relationship is dependent on the prevailing physical conditions cannot be ignored. As well, recapture of labelled lizards was unreliable (only 40-45% of the labelled lizards were recaptured sufficiently often), and the use of radiotelemetry equipment (difficult for small animals) or even a radioactivity detector (cf Turner et al. 1976) would ensure more reliable relocation of animals. Also, intensive seasonal sampling of the dietary components, in co-ordination with a detailed study of dietary preference, would yield a more accurate seasonal estimate of the Na content of the diet. Finally, regular Na analysis of available drinking water, in conjunction with the use

of tritiated water to determine the volume consumed (cf Gallagher and Taplin 1983), would allow this source of Na intake to be accounted for.

Nevertheless, this study shows that the use of ^{22}Na provides a reliable, yet simple and inexpensive means of studying the energetics of animals in their natural environments. Seasonal changes in food intake could be detected with the relatively small sample sizes used in our study; and, with larger sample sizes and the more precise measurement techniques discussed above, there is the likelihood of being able to detect changes in food intake attributable to other ecological parameters. These include individual energetic costs such as defence, territoriality, reproduction, foraging, thermoregulation and maintenance; as well as larger-scale energetic interactions such as predator/prey relationships and the impact of a species on its environment (e.g. carrying capacity). To date, studies using ^{22}Na have concentrated on the effect of herbivores on their environment (e.g. Green et al. 1978; Williams and Dudzinski 1982; Williams and Ridpath 1982); but predators of vertebrates would be ideal subjects for study using this technique, as the Na content of all vertebrates examined to date is very similar (Green 1978), and this would reduce the need to analyse prey consumption. Problems may be encountered, however, in applying this technique to herbivores which feed on a variety of plant species, as the Na content of plants is very variable (Allen et al. 1974).

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