Intrapopulation Variation in Ecological Energetics of the Garter Snake *Thamnophis sirtalis*, with Analysis of the Precision of Doubly Labeled Water Measurements

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ABSTRACT

The evolution of energetics must begin with variation within populations in ecologically realized rates of energy acquisition and expenditure. We measured aspects of field energy budgets (including metabolic rates, feeding rates, and growth rates) in a large sample of free-living garter snakes (*Thamnophis sirtalis*) from a single temperate/mesic population in northwestern California during their summer active season. We then analyzed interindividual variation for correlations among variables and patterns attributable to body size and sex. Field metabolic rates (measured with use of doubly labeled water) scaled in direct proportion to body mass. These rates of field energy expenditure were higher (both in absolute terms and in relation to resting metabolic rates) than those previously measured in snakes and iguanian lizards and were similar to those reported for highly active, widely foraging scincomorphan lizards. Feeding rates (as indexed by water influx rates) and growth rates were correspondingly high compared to those of other squamate reptiles. We found considerable residual variation in all measured variables not attributable to body size. Effects of sex were detected for water influx and growth rates (females $>$ males), but not for field metabolic rate. Individual field metabolic rate was apparently consistent (repeatable) over time, water influx rate was not, and individual growth rates were strongly negatively correlated over two sequential time periods. We were unable to detect convincing correlations between any individual measures of field energetics and any commonly measured, standard laboratory measurements of oxygen consumption (standard metabolic rate at two body temperatures and maximal oxygen consumption for exercise) made on the same individuals. However, body-size-independent field rates of energy expenditure, energy intake, and growth were strongly and positively intercorrelated among individuals. We attribute these patterns to an overriding effect of costs associated with digestion and growth on field energetics, such that individual snakes that were effective foragers achieved high feeding rates and, hence, high growth rates, but also incurred high costs of growth and digestion that largely determined field metabolic rate.

Introduction

Much of contemporary ecological theory relies on energy allocation arguments, with use of chemical potential energy as a common currency for comparing the costs and benefits (fitness correlates) of alternative behavioral or life-history phenotypes (Townsend and Calow 1981; Congdon et al. 1982; Sibly and Calow 1986; Stephens and Krebs 1986; Dunham et al. 1989; Ricklefs 1991; Stearns 1992). Evaluation and refinement of this burgeoning body of theory requires direct measurement of the rates of energy intake and energy expenditure realized by populations under natural ecological conditions. Further, understanding of the evolution of energy-allocation phenotypes by natural selection will require knowledge of the fitness correlates of variation in ecological energetics among individuals within populations (Congdon et al. 1982; Spotila and Standora 1985; Bennett 1987; Bryant and Tatner 1991; Stearns 1992; Tinbergen and Dietz 1994). However, empirical corroboration of predictions and assumptions has lagged behind theory, and our present knowledge of natural variation in ecological energetics is limited.

Several decades of measurements of energy expenditure (metabolic rates) under standardized laboratory conditions have resulted in an impressive accumulation of comparative data (e.g., squamate reptiles: Bennett and Dawson 1976; Andrews and Pough 1985). The relevance and applicability of such standardized laboratory measurements to ecological questions have, however, been questioned (e.g., Kendeigh 1974; Waldschmidt et al. 1987; Walton 1988; Koteja 1991; Niewiarowski and Waldschmidt 1992; Ricklefs et al. 1996).

In contrast, the doubly labeled water (DLW) technique

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allows measurement of the total rates of energy metabolism and energy intake actually experienced by animals behaving normally in their natural habitat (Nagy 1975, 1983*a*, 1989*a*). Field metabolic rates (FMRs) have now been measured in enough species of vertebrates to permit comparative analyses of patterns of interspecific variation (Nagy 1982, 1987, 1994; Daan et al. 1990; Peterson et al. 1990; Koteja 1991; Nagy and Obst 1991; Tatner and Bryant 1993; Ricklefs et al. 1996), but there remain many gaps in the comparative database for FMR. In particular, the available data for ectotherms are predominantly for lizards (mostly iguanian species) occupying arid and/or low-latitude habitats; little is known of the consequences for FMR of other environmental conditions (Nagy 1982; Benabib and Congdon 1992; Christian and Green 1994; Plummer and Congdon 1996). Ectotherms that range into temperate latitudes are constrained by shorter activity seasons, with consequences for demographic and life-history traits (e.g., Adolph and Porter 1993), which are presumably mediated through energetics. Populations occupying mesic habitats may be less constrained by absolute food abundance, which is characteristically limited in deserts (Dunham 1978; Congdon 1989). A fuller understanding of the ecological energetics of ectothermic vertebrates will require a broader comparative database, including measurements of populations representing various habitats and taxonomic groups.

Moreover, despite assertions that DLW analysis is now precise enough to detect differences in FMR among individuals (Nagy 1989*a*, 1989*b*; Weathers et al. 1990; Speakman et al. 1994; see Appendix), knowledge of intrapopulation variation in FMR is very limited (but see Bryant and Tatner 1991; Tinbergen and Dietz 1994; Berteaux et al. 1996; Peterson 1996; Meerlo et al. 1997). Knowledge of relationships among simultaneously measured rates of metabolism, energy intake, growth, and reproduction in large samples of individuals would be especially relevant to evolutionary questions relying on allocation theory (Stearns 1992; Sinervo and Adolph 1994).

We used DLW to measure FMRs and field feeding rates of a large sample of rapidly growing individuals from a single population. In view of the issues raised above, our objectives included the following: (1) measurement of field energetics in a temperate, mesic dwelling ectotherm, the garter snake *Thamnophis sirtalis;* (2) characterization of intrapopulation variation in FMR and feeding rate, including effects of body size and sex, and preliminary examination of the repeatability of field energetics among individuals; (3) analysis of the intercorrelations among energy expenditure, energy intake, and growth under natural conditions; and (4) comparison of measurements of field energetics with standardized laboratory metabolic rate measurements for the same individuals.

Material and Methods

Study Site

We studied an isolated population of common garter snakes (*Thamnophis sirtalis fitchi*) resident at a single pond and meadow near Eagle Lake in Lassen County, California. The meadow is in a pine-sage ecotone area at about 1,800 m elevation. Aspects of the ecology of this snake population (e.g., food habits, body temperatures, survivorship) have previously been studied (site 12 of Kephart 1982; Kephart and Arnold 1982; Jayne and Bennett 1990*b*), and the site is described in detail by Jayne and Bennett (1990*b*). Snakes have been individually marked (notched ventral scutes) and studied at this site intermittently since 1979. We resumed a mark-recapture study of the population in June 1991; many previously marked snakes were still present.

DLW Study

Between June 14 and July 3, 1992, we collected 97 snakes at the site and maintained them individually with access to water, but not food, until July 5, 1992. On July 5 and 6, 1992, we injected each snake intraperitoneally with 0.03–0.2 mL (depending on body mass) of water containing 0.35 mCi ^{3}H and 0.75 mL 95% $H_2{}^{18}O$ mL^{-1} injection solution. After waiting at least 3 h for isotopes to equilibrate with body water, we took a small ($\leq$0.1 mL) blood sample from a caudal vein, weighed each snake (to 0.01 g, Hainsworth electronic balance), applied a small dorsal paint mark to facilitate later recapture, and released each snake at the pond. We also sampled blood from four uninjected snakes to measure background isotope levels.

Between July 14 and 23, 1992, we recaptured 78 of the labeled snakes (mean measurement period = 12.5 d). Ten snakes were recaptured twice; length of the two measurement periods varied from 8 to 13 d (mean = 9.5 d) for the first period and from 5 to 9 d (mean = 7.2 d) for the second. No snakes contained palpable food in the gut on recapture. Recaptured snakes were weighed and bled as described above and then either released immediately or retained for respirometry measurements and released later.

All blood samples were immediately flame sealed in heparinized microhematocrit tubes and refrigerated pending analysis. Upon return to the University of California, Irvine, blood samples were microdistilled under vacuum to produce pure water (Wood et al. 1975). Tritium activity in duplicate 5-μL water samples was determined by liquid scintillation counting. A third sample was counted if the coefficient of variation of the duplicates was greater than 1%. Remaining water samples were sent to the University of California, Los Angeles, Laboratory of Biomedical and Environmental Sciences for determination of ^{18}O content by proton activation analysis (Wood et al. 1975). Initial samples were analyzed in triplicate following standard procedure (Nagy 1983*a*), but in order to maximize the accuracy of individual FMRs, recapture samples were analyzed in sextuplicate (see Appendix). Mean values of replicates with a coefficient of variation of less than 1% were used in calculations.

A pilot field study in the summer of 1991 suggested that water flux rates in this population were high, but not so high as to preclude use of DLW (as in aquatic turtles; Congdon et

al. 1982). We used our pilot data to guide our recapture efforts in 1992, such that all DLW measurements reported herein, with only four exceptions, span more than two biological half-lives of ^{18}O abundance. We discarded FMR data for six of 78 recaptured snakes (and six of 10 second recaptures) because the ^{18}O abundance of their body water was too close to background levels (^{3}H activity in those individuals was still well above background, so we were able to use their water flux data).

Total body water volumes at the time of injection were determined from ^{18}O dilution for each labeled snake. Total body water averaged 0.76 mL g^{-1} and was not related to body size; we assumed that the total body water proportion of each individual remained constant over the measurement period.

Respirometry

We measured standard metabolic rate (SMR) at 15° and 30°C and maximal oxygen consumption for exercise ($\dot{V}O_{2max}$) at 30°C for each snake within 10 d of FMR measurements. The field active body temperature of these snakes is 30°C (Jayne and Bennett 1990*b*), and 15°C is the approximate temperature of soil near the pond at night during the summer.

These respirometry measurements are a subset of a larger data set that we will report and analyze in detail elsewhere. Briefly, SMR was measured by closed-system respirometry. Postabsorptive snakes (at least 5 d without food) were sealed in airtight containers in a completely dark incubator for about 2 h (15°C) on one night, then for 1 h (30°C) on the next night (all metabolic rate measurements were made between 2000 and 0100 PST and were preceded by adjustment periods of 24 h at 15°C and 8–12 h at 30°C). Initial and final air samples were injected through water and CO_2 absorbants into an Ametek S3-A oxygen analyzer, and output (as percent oxygen) was stored and analyzed by computer (DataCan, Sable Systems). Standard oxygen consumption was calculated according to Vleck (1987).

$\dot{V}O_{2max}$ was measured by flow-through respirometry on the day following the 30°C SMR measurements. Each snake was fitted with a loose-fitting plastic mask and induced to exercise as vigorously as possible for 3–7 min on a moving treadmill maintained at 30°C. Air was drawn sequentially through the mask, water and CO_2 absorbants, and the oxygen analyzer at 50–100 mL min^{-1}. Data were stored and analyzed by computer; oxygen consumption was calculated according to Withers (1977) for the highest continuous minute for each snake.

Calculations

Rates of field water influx (mL d^{-1}) and FMRs (mL CO_2 d^{-1}) were calculated with appropriate equations (Nagy 1980; Nagy and Costa 1980), assuming a linear change in body water volume over time. Energy equivalents of CO_2 production were calculated assuming 25.8 J mL^{-1} CO_2 (this conversion factor, from Nagy [1983*a*], is for a diet made up of vertebrates; during the measurement period the snakes' diet consisted entirely of small hylid frogs and tadpoles).

Feeding rates (g prey d^{-1}) and energy intake rates (kJ d^{-1}) were calculated from DLW results in two ways (Nagy 1975, 1989*a*). First, we assumed that all field water influx resulted from metabolic water production and preformed water in food (i.e., not from drinking, vapor, or osmotic water influx). Metabolic water production was calculated from FMR assuming 0.617 mL H_2O mL^{-1} CO_2 (Nagy 1983*a*) and subtracted from total water influx to yield preformed water influx. Feeding and energy intake rates were then calculated from this value together with the measured water and energy contents of prey (*Pseudacris regilla,* 0.83 mL H_2O g^{-1} and 20.17 J g^{-1} dry weight) and the metabolizable energy assimilation efficiency of *T. sirtalis* eating *P. regilla* (80%; data from Bear [1994]). Second, we calculated the energy intake rate required to fuel the measured FMR plus the energetic equivalent of the measured change in body mass over the period (assuming 22.8 J g^{-1} dry snake tissue; Reichenbach and Dalrymple 1986). We also estimated gross production efficiency as energy content of the increase in snake mass divided by the energy content of food consumed during the growth period (calculated from water influx rate as above).

Statistics

All statistics (described below) were performed with SigmaStat or SYSTAT software. To remove confounding effects of body mass in comparisons of rate variables, we compared residuals from regressions on body mass (mass residuals; see Bennett 1987). Repeatability of field rate variables was assessed by Pearson correlation of mass residuals for repeated measures of individuals in two sequential time periods (Hayes and Jenkins 1997).

Results

FMRs

FMR scaled directly with body mass in this population (Table 1; Fig. 1*a*). The scaling exponent of 0.91 was not significantly different from 1.0 (95% confidence limits = 0.78–1.04); hence, average FMR was 9.18 mL CO_2 g^{-1} d^{-1}, equivalent to 237 J g^{-1} d^{-1}. Sexes did not differ significantly in FMR (ANCOVA with body mass covariate, $F_{1, 69} = 1.17$, $P = 0.28$). A multiple regression analysis corroborated the above conclusions and gave no evidence for effects of length of measurement period or time in captivity on FMR (Table 1).

Four of 10 twice-recaptured individuals yielded FMRs for two serial measurement periods. There was no significant difference in scaling of FMR between the two periods (ANCOVA, $F_{1, 9} = 0.115$, $P = 0.742$). Although the small sample size precluded statistical significance of repeatability (correlation of body mass

Table 1: Allometric and multiple regression coefficients for field-measured rate variables

Dependent Variable	Constant	Independent Variables: Body Mass (g)	Sex	Days Measured	Days Captive	r^2 or R^2 (n, P)
log FMR (L CO_2 d^{-1})	−1.946	.910 (log)	NS	NS	NS	.734 (72, <.001)
log water influx rate (mL H_2O d^{-1})	−.725	.847 (log)	. . .	. . .	. . .	.634 (78, <.001)
log water influx rate (mL H_2O d^{-1})	−.632	.846 (log) (<.001)	.117 (.003)	−.013 (.035)	NS	.703 (78, <.001)
log water efflux rate (mL H_2O d^{-1})	−.872	.894 (log)	. . .	. . .	. . .	.668 (78, <.001)
log water efflux rate (mL H_2O d^{-1})	−.957	.907 (log) (<.001)	.112 (.005)	NS	NS	.702 (78, <.001)
log growth rate (g d^{-1})[a]	−1.314	.818 (log)	. . .	. . .	. . .	.526 (76, <.001)
Growth rate (g d^{-1})[a]	−.031	.033	. . .	. . .	. . .	.618 (76, <.001)
Growth rate (g d^{-1})[a]	.504	.029 (<.001)	.199 (.002)	−.037 (<.001)	NS	.788 (76, <.001)
Growth rate (g d^{-1})	−.030	.031	. . .	. . .	. . .	.522 (78, <.001)
Growth rate (g d^{-1})	.241	.027 (<.001)	.208 (.006)	−.052 (<.001)	.025 (.002)	.691 (78, <.001)

Note. For allometric equations (body mass as single predictor variable), statistics are shown in the rightmost column. For multiple regressions, P values are given in parentheses below each significant coefficient, and multiple R^2 is shown in the rightmost column. Sex was coded as male = 0, female = 1. NS = not a significant predictor for this dependent variable ($P > 0.05$).

[a] Data for two individuals that lost body mass are excluded.

residuals for individual FMR in the two periods, $r = 0.614$, $n = 4$, $P = 0.386$), the data are strongly suggestive (Fig. 1*b*).

Water Flux Rates

Water influx rate scaled exponentially with body mass in this population (Table 1; Fig. 2*a*); the scaling exponent of 0.85 was significantly different from 1.0 (95% confidence limits = 0.70–0.99). Rate of water efflux scaled similarly (Table 1). Growing snakes were in strongly positive water balance (influx > efflux). Sex was a significant factor (ANCOVA) for both water influx rate ($F_{1, 75} = 11.85$, $P = 0.001$) and water efflux rate ($F_{1, 75} = 8.463$, $P = 0.005$), with females tending to have higher rates than males (Fig. 2*a*). Multiple regression analysis confirmed these patterns and indicated an additional, negative effect of length of measurement period on water influx rate, but not on water efflux rate (Table 1).

Ten individuals yielded two successive water flux rates. Rates were significantly higher in the first measurement period than in the second for water influx rate (Fig. 2*b;* ANCOVA, $F_{1, 17} = 13.59$, $P = 0.002$) and, marginally significantly, for water efflux rate ($F_{1, 17} = 4.31$, $P = 0.053$). Neither rate was significantly repeatable within individuals (mass-residual correlations: water influx rate, $r = -0.18$, $P = 0.612$; water efflux rate, $r = 0.445$, $P = 0.198$).

Growth Rate

Almost all labeled snakes grew rapidly during the measurement period, averaging a daily increase of 3.1% of mean body mass (range, −1.2%–6.9% d^{-1}). Absolute rate of change in body mass (growth rate, g d^{-1}) was significantly related to body mass (Table 1; Fig. 3*a*). Both sex (females > males) and length of measurement period (negative effect) had significant influences on growth rates (Table 1).

For the 10 individuals recaptured twice, patterns of growth rates were very different for the two measurement periods. For the first period, growth rate scaled to body mass in a manner similar to the overall data set, but in the second period growth

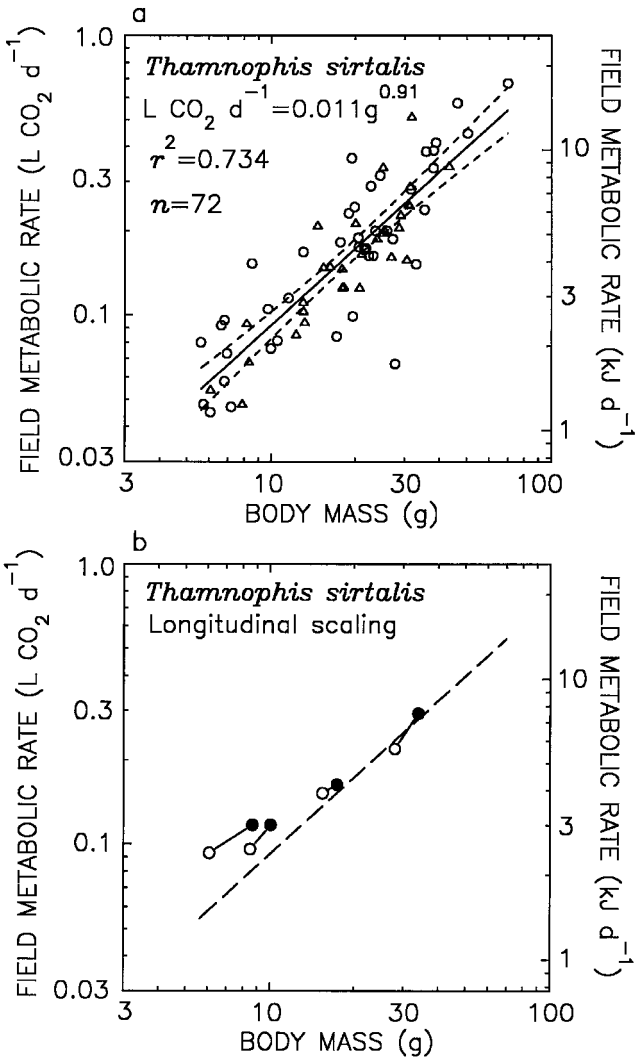

Figure 1. Allometric scaling of FMR, measured with DLW. *a,* Cross-sectional scaling, showing results for the entire sample. Circles represent data for females, triangles males. The line is the least-squares regression of log-transformed data, shown with 95% confidence limits of the line. *b,* Longitudinal scaling of repeated measures of individuals. The dashed line is the regression line from part *a.* Open circles represent the first measurement, and filled circles the subsequent measurement; lines connect the two measurements for each individual.

in mass was not related to body mass (Fig. 3*b*). Body-mass-independent growth-rate residuals for the two periods were strongly negatively correlated (Fig. 3*c*), such that snakes with relatively high growth rates for their body mass during the first period tended to grow relatively slowly in the subsequent period, and vice versa.

Feeding Rates

The results reported above were used to calculate energy intake rates by two different means (see Material and Methods). The two methods yielded similar results (Fig. 4*a; r* = 0.913, *n* = 72). Mass-independent rates of energy intake (mass residuals) calculated by the two methods were correlated (*r* = 0.744; Fig. 4*b*). Further, using Wilcoxon signed ranks tests (for nonnormally distributed, paired data), we were unable to detect significant differences between results of the two methods for whole-animal data (*P* = 0.114) or mass residuals (*P* = 0.220).

Respirometry

For respirometry variables (Table 2), allometric scaling exponents varied from 0.55 (SMR at 15°C) to 0.88 ($\dot{V}O_{2max}$ at 30°C).

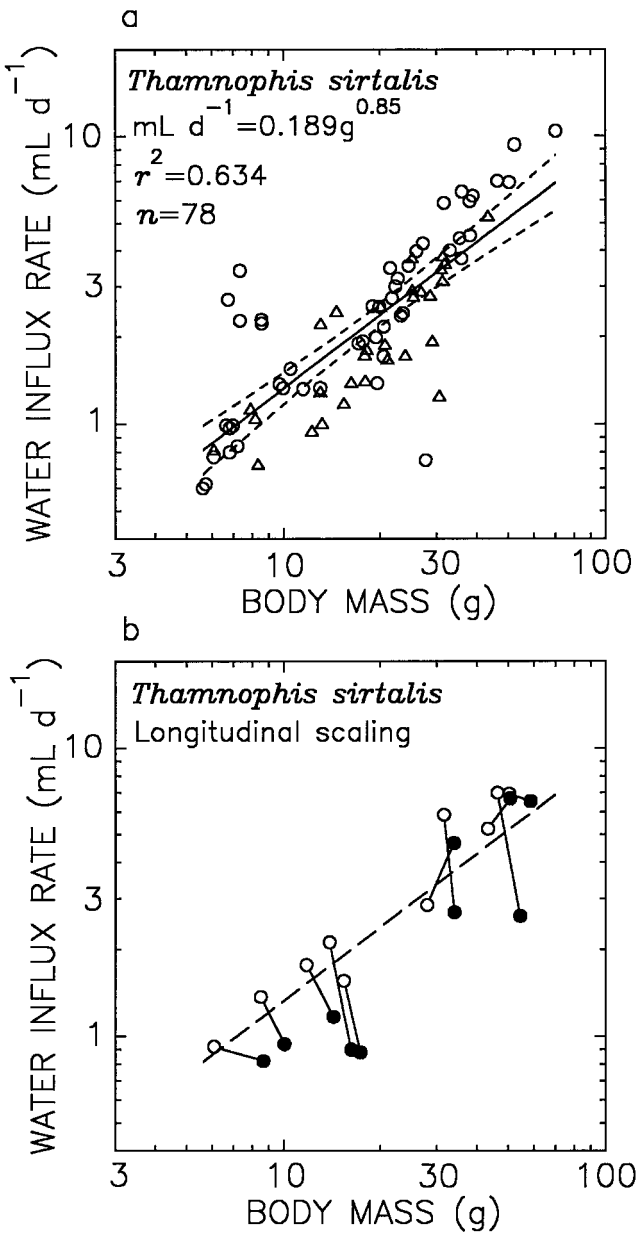

Figure 2. Allometric scaling of field water influx rate, measured with isotopically labeled water. *a,* Cross-sectional scaling; symbols and format as in Figure 1. *b,* Longitudinal scaling of repeated measured of individuals; symbols and format as in Figure 1.

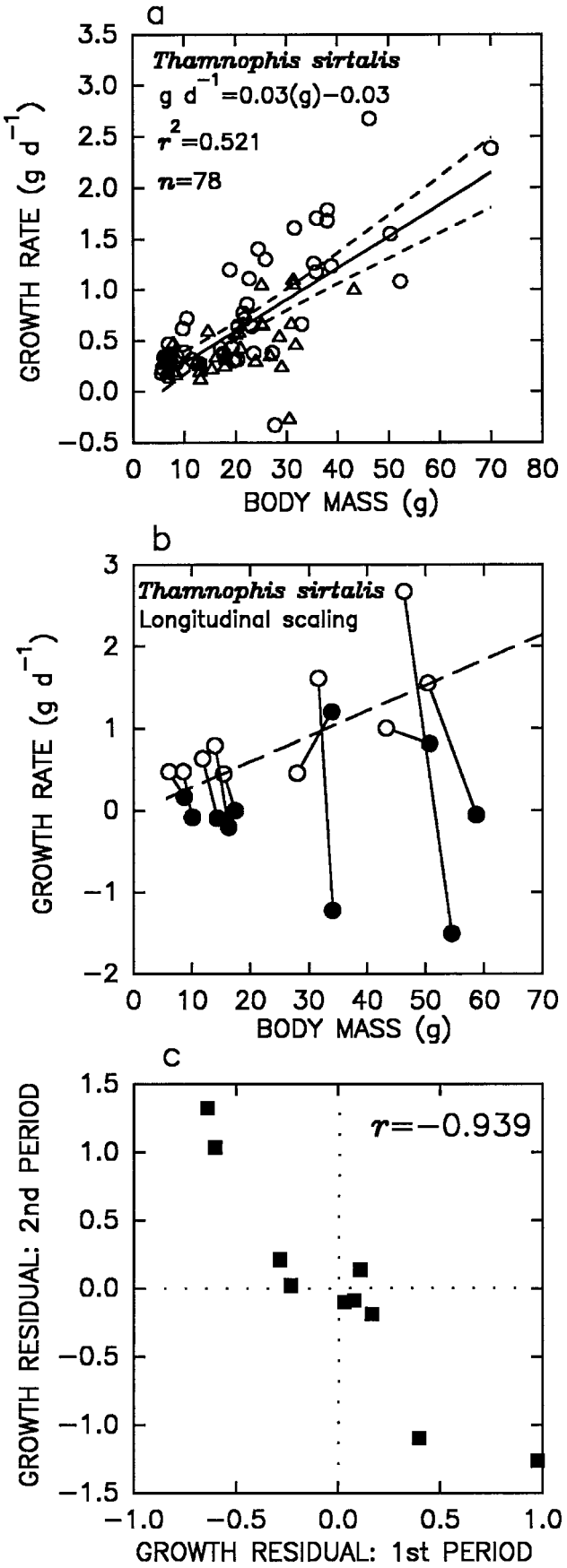

Figure 3. Scaling of field growth rate (in mass) to body mass. *a,* Cross-sectional scaling; symbols as in Figure 1 (note arithmetic coordinates). *b,* Longitudinal scaling of repeated measures of individuals; symbols as in Figure 1. *c,* Correlation of mass residuals (measured minus predicted) of growth rate in the first measurement period with growth rate in the second period for each individual, calculated from data in part *b.*

At mean body mass (23 g), Q_{10} for SMR was 2.45 and factorial scope for activity was 3.6. Because of the differences in scaling, both parameters increased with body mass.

Relationships among Variables

Because all variables scaled to body mass, residuals of allometric equations were used to examine the data for correlations among rates for individuals (Table 3). Among respirometry variables, SMR at 15°C was correlated significantly with SMR at 30°C, but neither SMR was significantly correlated with $\dot{V}O_{2max}$. All four field-measured variables (FMR, water influx rate, water efflux rate, and growth rate) were strongly and positively intercorrelated. However, except for relatively weak correlations between SMR at 30°C and water influx and efflux rates, field variables did not correlate significantly with respirometry variables.

Discussion

The data reported here constitute the largest simultaneous sample of field energetics in a single population of animals measured to date. As such, this data set offers the highest statistical power available for assessment of patterns of variation in ecological energetics among members of a population ostensibly exposed to the same environmental conditions. We looked for patterns attributable to effects of body size (over more than an order of magnitude of body mass: 5.6–70 g), sex, and recent history of individual garter snakes.

FMRs

Body size was the only significant predictor of FMR; FMRs of males and females were indistinguishable, and we detected no effect of the length of the measurement period. FMR scaled directly (exponent ≈ 1.0) with body mass in this population of garter snakes in July 1992. Previously reported intraspecific scaling exponents for FMR in lizards (all based on smaller samples and, usually, smaller ranges of body mass) range from 0.53 (*Iguana iguana;* van Marken Lichtenbelt et al. 1993) to 1.35 (*Aporosaura anchietae;* Robinson 1990); most are not significantly different from 1.0 (e.g., *Amblyrhynchus cristatus,* 0.97 [Nagy and Shoemaker 1984]; *Angolosaurus skoogi,* 1.06 [Nagy et al. 1991]; *Podarcis lilfordi,* 0.93 [Brown and Perez-Mellado 1994]). Direct proportionality of FMR to body mass may be a common feature of the ecological energetics of squamates

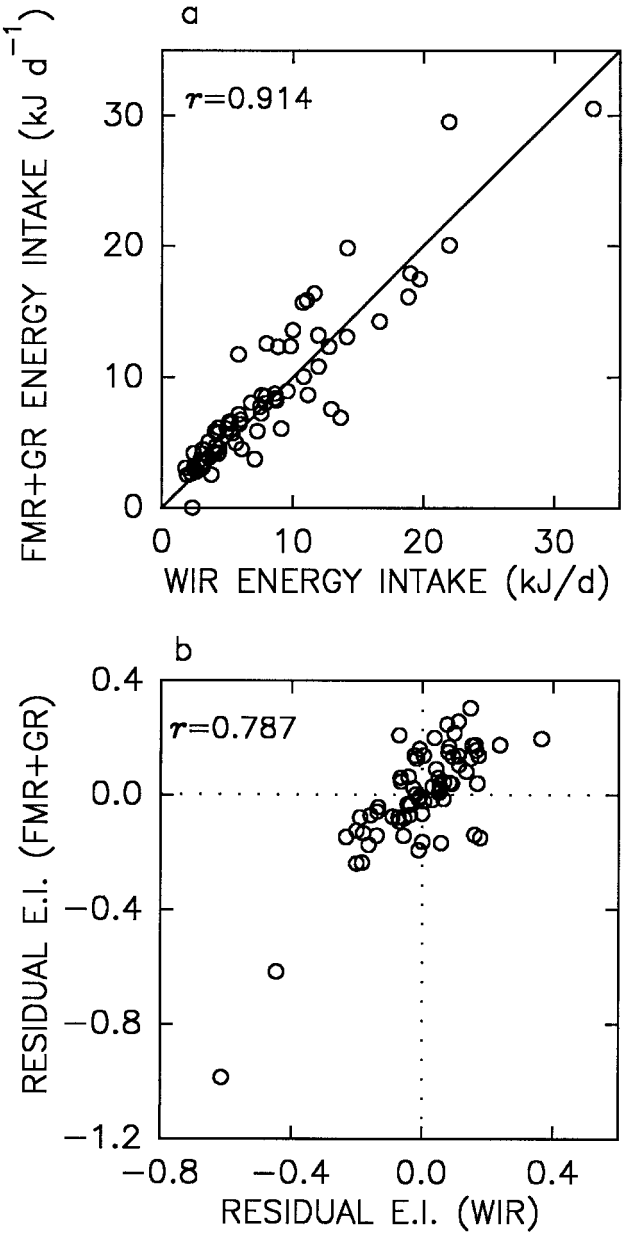

Figure 4. Comparison of energy intake rates calculated by two methods: from water influx rate (*WIR*) and from FMR plus growth rate (*GR;* see text). *a,* Correlation of intake rates calculated by the two methods. The line represents equivalence (not a calculated regression). *b,* Correlation of mass residuals (actual minus predicted) of intake rates calculated by the two methods.

(an exception is *Dipsosaurus dorsalis,* with a scaling exponent of 0.75 ± 0.03 SE; Mautz and Nagy 1987).

Because FMR scaled to body mass with an exponent of 1.0, but SMRs of the same individuals scaled with exponents significantly less than 1.0 (Table 2), larger snakes were apparently working harder, relative to resting energy expenditures, than smaller individuals. To quantify this effect of body size, we calculated ratios of FMR to SMR integrated over 24 h. For ectotherms, this ratio's denominator (equivalent to "daily resting energy" of Congdon et al. [1982] and "total resting metabolism" of Benabib and Congdon [1992]) depends on the daily pattern of body temperature, and we lack such data for the individual snakes in our study. However, published measurements and analyses suggest that these garter snakes can regulate their body temperatures near 30°C for 6–20 h d^{-1} in summer, depending on climatic conditions and characteristics of available retreat sites (Peterson 1987; Huey et al. 1989; Peterson et al. 1993). We therefore calculated a range of ratios for each snake, using our individual measurements of FMR and SMR and assuming a body temperature of 30°C for 6, 12, 18, and 24 h d^{-1}, with the remainder of time assumed to be spent at 15°C (Table 4). Ratio values scaled with body mass (e.g., 12 h at 30°C: log − log $r = 0.32$, $P < 0.001$; 24 h at 30°C: log − log $r = 0.27$, $P < 0.05$), such that the largest snake in the sample (70 g) was predicted to have a ratio value at least (denominator for 24 h at 30°C) 1.76 times that of the smallest snake (5.6 g). This body-size effect may reflect an ontogenetic increase in capacity for aerobic activity and metabolism in *Thamnophis sirtalis* (Pough 1977; note also the relatively high scaling exponent of $\dot{V}O_{2max}$ in Table 2).

The ratios reported in Table 4, which average at least 4.7, are high relative to similar estimates for other populations of ectotherms in their active seasons. Published values for lizards range from 1.0 to 5.6, with only a very few above 4.0 (references in Peterson et al. [1990]; Benabib and Congdon [1992]; Christian et al. [1996]). The limited data for snakes indicate ratios of 3.2 (*Crotalus cerastes*), 4.3 (*Masticophis flagellum*), 4.6 (*Coluber constrictor*), and 2.9–4.4 (two populations of *Crotalus lepidus;* Secor and Nagy 1994; Beaupre 1996; Plummer and Congdon 1996).

Table 2: Allometry of respirometry variables for snakes used in the field study

	r^2	n	P
log SMR15 = (.547 × log BM) − .895	.453	78	<.001
log SMR15 = (.555 × log BM) − .906	.422	72	<.001
log SMR30 = (.677 × log BM) − .486	.716	78	<.001
log SMR30 = (.712 × log BM) − .534	.722	72	<.001
log $\dot{V}O_{2max}$ = (.876 × log BM) − .205	.839	78	<.001
log $\dot{V}O_{2max}$ (.881 × log BM) − .211	.820	72	<.001

Note. Two equations are given for each variable, one for all 78 recaptured snakes and one for the subset of 72 snakes for which FMR was measured. Abbreviations: SMR15, SMR measured at 15°C; SMR30, SMR measured at 30°C; $\dot{V}O_{2max}$ measured at 30°C (all as mL O_2 h^{-1} STPD); BM, body mass (g).

Table 3: Matrix of correlations among body mass residuals for respirometry and field variables

	FMR	WIR	WER	GR	SMR15	SMR30	$\dot{V}_{O_{2max}}$
FMR	. . .	.526*	.475*	.432*	.015	−.099	.112
WIR	72	. . .	.957*	.556*	.096	.283	−.058
	<.001						
WER	72	78	. . .	.336	.105	.300	−.042
	<.001	<.001					
GR	72	78	78	. . .	.006	.041	−.038
	<.001	<.001	.003				
SMR15	72	78	78	78	. . .	.570*	−.165
	.898	.404	.358	.959			
SMR30	72	78	78	78	78	. . .	−.184
	.407	.012	.008	.720	<.001		
$\dot{V}_{O_{2max}}$	72	78	78	78	78	78	. . .
	.347	.616	.714	.738	.148	.108	

Note. Above the diagonal: Pearson correlation coefficients (r); below the diagonal: number of data pairs (n) and P value for each correlation. Abbreviations: WIR, water influx rate; WER, water efflux rate; GR, rate of change in body mass (growth rate); SMR15, SMR measured at 15°C; SMR30, SMR measured at 30°C; $\dot{V}_{O_{2max}}$ measured at 30°C.

* Significant at the 0.001 level.

The relatively high FMR : SMR ratios of garter snakes primarily reflect their relatively high FMRs. FMRs averaged 1.9 times higher than predicted from Nagy's (1982) allometric equation for iguanian lizards and were similar to FMRs reported for widely foraging teiid and lacertid lizards (*Cnemidophorus tigris* [Anderson and Karasov 1981, 1988]; *Cnemidophorus hyperythrus* [Karasov and Anderson 1984]; *Lacerta viridis* [Bradshaw et al. 1991]; *P. lilfordi* [Brown and Perez-Mellado 1994]) that are very active and regulate relatively high body temperatures when active. FMR data for snakes are very few, ranging from 1.51 mL CO_2 g^{-1} d^{-1} in *Crotalus cerastes* (Secor and Nagy 1994) to 3.77 mL CO_2 g^{-1} d^{-1} in *Coluber constrictor* (Plummer and Congdon 1996), compared to the mass-specific value of 9.18 mL CO_2 g^{-1} d^{-1} we measured in *T. sirtalis*. Differences in body size among these species (*Thamnophis* is smallest) only partially explain the differences in mass-specific rates of CO_2 production; a 50-g garter snake is predicted to expend about the same amount of energy per day as a 125-g *Coluber*. We suggest an explanation below ("Components of FMR") for the surprisingly high rates of energy expenditure we report for *T. sirtalis*.

Table 4: Calculated ratios of FMR to integrated 24-h SMR for garter snakes under four hypothetical daily body-temperature patterns

	Daily Body-Temperature Pattern			
	6 : 18	12 : 12	18 : 6	24 : 0
Mean ratio	10.17	7.26	5.66	4.65
SD	±5.06	±3.48	±2.67	±2.18
Minimum	2.16	1.58	1.25	1.03
Maximum	23.61	16.68	12.95	10.59

Note. Ratios were calculated for four hypothetical patterns of daily body temperatures, which differed in number of hours at 30°C and 15°C (shown as hours at 30°C : hours at 15°C). Ratios were calculated for each temperature pattern for each individual snake with use of individual measurements of SMR at the two body temperatures and FMR.

Water Flux, Feeding, and Growth Rates

Like FMR, water influx rate was high in *T. sirtalis* relative to most previously measured terrestrial ectotherms. Water influx rates of garter snakes in our study averaged 2.9-fold higher than predicted from Nagy and Peterson's (1988) allometric equation for free-living nondesert reptiles. Because the snakes we studied were semiaquatic, foraging for frogs in and on the shore of a freshwater pond, a high rate of water influx was not unexpected.

However, we contend that the high water influx rates we observed were attributable not to drinking and/or integumentary water influx, but to high feeding rates. Feeding rates calculated from water influx rates were similar to those calculated from metabolic and growth rates (Fig. 4*a*); if drinking or some other nonprandial route of water influx were important, then water influx rates would have overestimated feeding rates, and the data shown in Figure 4*a* would be shifted to the right (and significant differences would be shown by Wilcoxon tests). This comparison is in part contingent on an assumed value for the energy content of snake tissue added in growth (22.8 kJ g^{-1} dry weight; Reichenbach and Dalrymple 1986), which in turn assumes particular proportions of lean

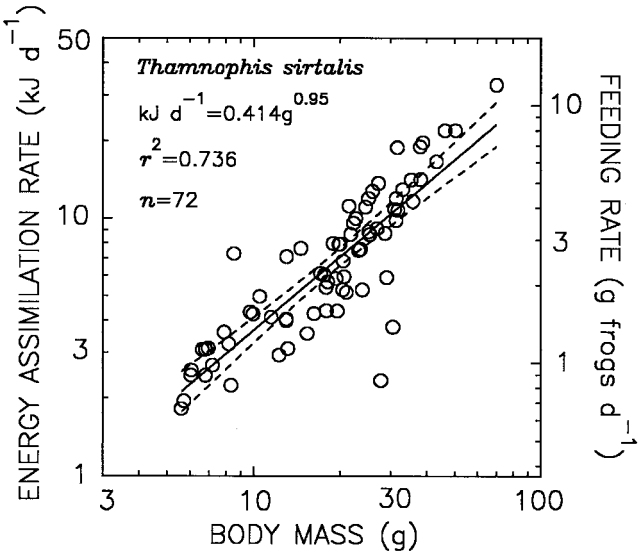

Figure 5. Allometric scaling of field feeding and energy assimilation rates (related by a constant multiplier that accounts for energy content of frogs and assimilation efficiency of snakes digesting frogs), calculated from field water influx rates (see text).

tissue and fat. Large increases in body mass coupled with strongly positive water balance suggest lean tissue growth as opposed to fat storage. Substitution of a lower energy content more typical of lean snake tissue (20 kJ g^{-1}; Smith 1976; Hailey and Davies 1987) changes calculated feeding rates by only 5% on average and in fact improves the match to water influx-based rates (Wilcoxon test, $P = 0.854$). Therefore, the close match between feeding rates calculated by the two methods suggests strongly that drinking, vapor, and osmosis across integument or epithelia were not important routes of water influx, so water influx rates index feeding rates.

On the strength of this evidence, we used water influx rate corrected for metabolic water production (calculated from FMR) and measurements of the water and energy contents of *Pseudacris regilla* (see Material and Methods, *Calculations*) to calculate feeding and energy intake rates for each individual snake (Fig. 5). Calculated rates of metabolizable energy assimilation scaled in direct proportion to body mass and averaged 1.65 times FMR. Snakes apparently ingested, on average, 14% of their body mass in frogs per day (range 3%–22% d^{-1}, with one outlier at 31% d^{-1}). These feeding rates were much higher than those reported for desert snakes during their activity season (0.9% and 2.4% of body mass per day for *Crotalus cerastes* and *Masticophis flagellum,* respectively; Secor and Nagy 1994) and for grass snakes (*Natrix natrix*) feeding on toads (1.6%–2.3% d^{-1}; Reading and Davies 1996). They were also higher than similar values calculated for widely foraging scincomorphan lizards (*Cnemidophorus tigris,* 4.4% d^{-1} [Anderson and Karasov 1981]; *Eremias lugubris,* 9.2% d^{-1} [Nagy et al. 1984]). The relatively high feeding rates of garter snakes presumably reflect both the general propensity of snakes to take large meals (Greene 1983; Pough 1983) and the extremely dense food resources available during our study.

Garter snakes used the energy profits from their high feeding rates to support high growth rates. Growth rates (g d^{-1}) measured in our short-term study averaged 2.4–5.5 times higher than predicted from Figure 7 of Andrews (1982), which summarizes field growth rates of a variety of reptiles. Similarly, if the regression line shown in our Figure 3*a* is projected onto Figure 1 of Case (1978), it lies well above all data for squamates (including a point for *T. sirtalis*) and is bracketed by lines describing growth rates of altricial and precocial land birds. It is very doubtful that snakes could maintain such high rates of growth throughout the active season; in fact, below (*Longitudinal Patterns*) we discuss evidence that growth rates declined through the course of our study.

Gross efficiency of production (energy invested in tissue divided by metabolizable energy from food) averaged 45% in *T. sirtalis* feeding on frogs. This figure, calculated from field-measured growth rates and feeding rates, is similar to previously reported production efficiencies of free-ranging snakes (which incorporate field-measured growth rates but use assimilation rates calculated with use of laboratory data and a variety of assumptions): 49% for *Vipera berus* feeding on rodents (Pomianowska-Pilipiuk 1974) and 48% for *Natrix maura* feeding on fish (Hailey and Davies 1987).

In contrast to FMR, we detected sex differences in feeding and growth rates; females tended to have higher feeding rates than males of similar body size (Fig. 2*a*) and to grow faster as well (Fig. 3*a*). Further, females apparently grew more efficiently, on average, than did males; mean production efficiency of females (calculated as above) was significantly higher than that of males ($t = 3.15$, df = 68, $P = 0.002$). Thus, a likely physiological mechanism underlying sexual size dimorphism in this population (females larger than males) was detectable even over a short-term measurement period.

Longitudinal Patterns

Although we were able to recapture only a few snakes twice, analysis of these repeated measures of field energetics revealed some interesting patterns. We found that FMR was consistent within individuals (repeatable; Fig. 1*b*); that is, longitudinal scaling of FMR was not apparently different from cross-sectional patterns (sensu Jayne and Bennett 1990*a*). A recent study of voles (*Microtus pennsylvanicus*) found low repeatability of FMR over successive 24-h periods (Berteaux et al. 1996). Although our data are preliminary, we suggest that because the DLW technique can integrate longer periods of energy expenditure in ectotherms than in endotherms (Nagy 1983*a*), stochastic fluctuations in energy balance are less likely to affect results, and repeatable differences in FMR among individuals should be more easily demonstrable in reptiles than in small mammals or birds.

In contrast to FMR, both feeding rates (as indexed by water influx rates, Fig. 2*b*) and growth rates (Fig. 3*b*) were, almost uniformly, strikingly lower in the second measurement period

than in the first. Among snakes recaptured only once, this same pattern is illustrated by the significant negative effect of measurement-period length on day-averaged rates of growth and water influx (Table 1).

Apparently, snakes fed more frequently, and consequently grew faster, during the early portion of our measurement period than they did several days later. This could have happened for two (nonmutually exclusive) reasons: a decline in food availability or a behavioral reduction in foraging effort. The habitat dried considerably during our DLW study, which may have reduced food availability to snakes in several ways. As the pond decreased in area, newly metamorphosed froglets, which were formerly concentrated along the shore, took refuge in crevices in the newly exposed mud and thereby became both distributed over a larger area and better concealed. Further, as more frogs completed metamorphosis, they probably became better able to escape capture than tadpoles or metamorphs (Wassersug and Sperry 1977; Arnold and Wassersug 1978). At the same time, as July continued and the habitat became more open, biophysical constraints may have progressively limited the time available to snakes for foraging (Porter and Tracy 1974; Scott et al. 1982; Peterson et al. 1993). However, the lack of any effect of measurement period on FMR and water efflux rate (Table 1) suggests a decrease in foraging efficiency rather than effort.

Alternative explanations are also possible. Snakes were captured, and subsequently held without food, for 3–21 d before they were injected with DLW and released. Therefore, the high growth rates we observed early in the measurement period could have represented a form of catch-up growth (references in Sibly and Calow [1986]), or reestablishment of homeostatic body mass after a period of fasting, rather than true, normal growth in mass. We tested for an effect of days held captive (therefore days fasted) on rate variables and found a significant positive effect for growth rates; however, this effect was entirely due to two individuals that lost body mass over the measurement period (Table 1), both of which were in captivity for the minimum of 3 d before release. We also compared growth rates of snakes in the upper quartile of captivity duration ($>$17 d) with those in the lower quartile ($<$12 d) and found no significant difference ($P = 0.30$; ANCOVA on quartile and sex with mass covariate). We conclude that length of captivity did not affect subsequent growth rates in our study.

Further evidence that we observed bona fide growth (rather than a homeostatic adjustment in body mass) comes from limited data on growth in length. We measured snout-vent lengths of nine animals both before and after the DLW measurement period. Among these individuals (range of initial body mass, 4.6–24.8 g; range of initial snout-vent length, 23.1–39.2 cm), there was a significant rank correlation between mass-adjusted (residual) rate of increase in mass and length-adjusted rate of increase in length (Spearman $r_s = 0.73$, $P < 0.005$). Hence, snakes that increased most in mass also tended to increase most in length, that is, grew most.

The strong negative correlation between serial size-independent growth rates in snakes recaptured twice (Fig. 3*c*) implies that snakes that grew relatively fast on initial release subsequently slowed, while those that grew relatively slowly at first continued to grow later. Such a pattern is inconsistent with the idea that individuals are selected to maximize growth rates constantly; instead it suggests that individuals may converge on some long-term average submaximal (optimal?) growth rate (see Case 1978; Calow 1989; Arendt 1997). It is tempting to interpret this finding in light of the stabilizing selection for mass-on-length residual (body condition or "stoutness") detected in juveniles of the same population by Jayne and Bennett (1990*b;* see also Forsman and Lindell 1991).

Relationships among Variables

Our measurements of common, standard, laboratory-measured values of oxygen consumption were not predictive of individual variation in field energetics. Similarly, a recent study of field voles (*Microtus agrestis*) found no correlation between mass-corrected basal metabolic rates and FMRs among individuals (Meerlo et al. 1997).

All of our field measurements, however, including FMR, water influx rate, and growth rate, were strongly and positively intercorrelated (Table 3). Thus, among individuals of this population, realized field rates of energy expenditure were positively related to both feeding rates and growth rates. This pattern is opposite of that predicted from a trade-off paradigm in which energy intake is allocated competitively to respiration or production (i.e., a negative relationship between mass-independent FMR and growth rates; see also Konarzewski 1995). In search of an alternative explanation, we attempted to partition FMR into additive components (Congdon et al. 1982; Nagy 1983*b;* Secor and Nagy 1984).

Components of FMR

Our calculations of FMR : SMR ratios (Table 4) indicated that SMR could at most account for 22% of FMR (because, unrealistically assuming 24 h at 30°C, FMR averaged 4.65 times SMR). The remaining 78% or more of average daily energy expenditure in our nonreproductive snakes must have been due to additional costs of alertness, physical activity (presumably foraging effort), digestion, and growth.

Although we tried, we were unable to follow individual focal animals for periods long enough to estimate daily time budgets of foraging snakes, a necessary first step in accurately estimating energy expenditures for locomotor activity (Congdon et al. 1982; Goldstein 1988; Karasov 1992; Secor and Nagy 1994). To establish an upper bound on possible field energy expenditures for physical activity, we converted our FMR measurements and laboratory measurements of $\dot{V}O_{2max}$ to comparable units and were astonished to discover that the two rates were

virtually indistinguishable (Fig. 6). (This coincidence reflects both the relatively high FMRs of garter snakes and their relatively low $\dot{V}O_{2max}$ for exercise [cf. Bennett 1982; Thompson and Withers 1997]. Note that the similarity in rates is an emergent property of the whole data sets; among individual snakes, mass-independent $\dot{V}O_{2max}$ and FMR were not significantly correlated.) How can this observation be explained? Obviously, snakes could not have been exercising at their maximal rates at 30°C for 24 h d^{-1} for 2 wk. Instead, we interpret these data as suggesting that costs of physical exercise alone could not account for the high FMRs of these garter snakes. This interpretation is further supported by the lower FMRs of much more active snakes such as *Coluber* (Plummer and Congdon 1996) and *Masticophis* (Secor and Nagy 1994). If activity did not determine FMR in *Thamnophis*, some other energy expenditure must have been more important.

Specific dynamic action (SDA, the increase in metabolic rate following feeding) has recently been shown to be an important component of the energy budget of some snake species (Hailey and Davies 1987; Secor et al. 1994; Secor and Diamond 1995). In fact, peak oxygen consumption during digestion of a large meal can exceed $\dot{V}O_{2max}$ for exercise in snakes (Andrade et al. 1997; Secor and Diamond 1997). Measurements of SDA in *T. sirtalis* suggest that SDA was probably the major component of field energy expenditures in the snakes we studied (Bear 1994). The metabolic response to a typical, single meal of treefrogs at 30°C overlaps the range of measured FMRs for over 24 h (Fig. 7). Further, a computer-simulation model of time-cumulative energy expenditures (which integrated realistic parameters for feeding frequencies, meal sizes, daily body temperature profiles, temperature-dependent resting metabolism, and temporal patterns of SDA for snakes digesting frog meals of various sizes at various body temperatures) predicted that SDA alone (over and above SMR) could account for about 40% of the mean FMR we measured (Bear 1994). SDA was estimated to account for 43% and 20% of total FMR in two species of desert snakes, which fed less frequently and grew more slowly than *T. sirtalis* (Secor and Nagy 1994).

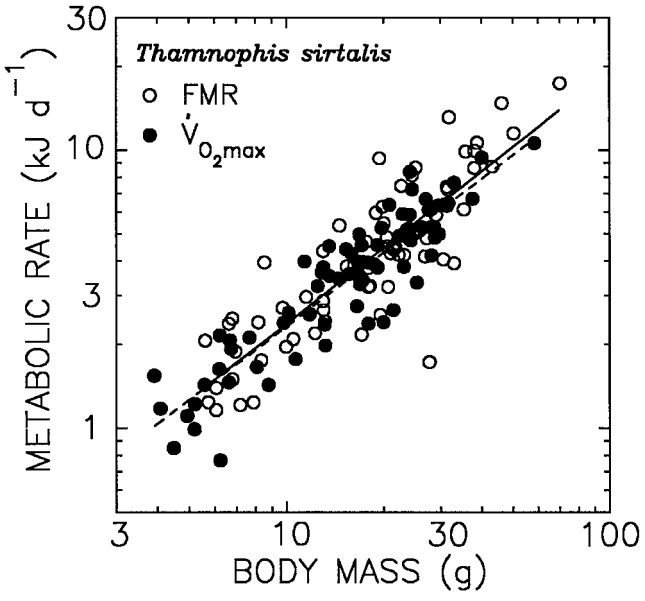

Figure 6. Comparison of FMR and $\dot{V}O_{2max}$ measured in the same individual snakes.

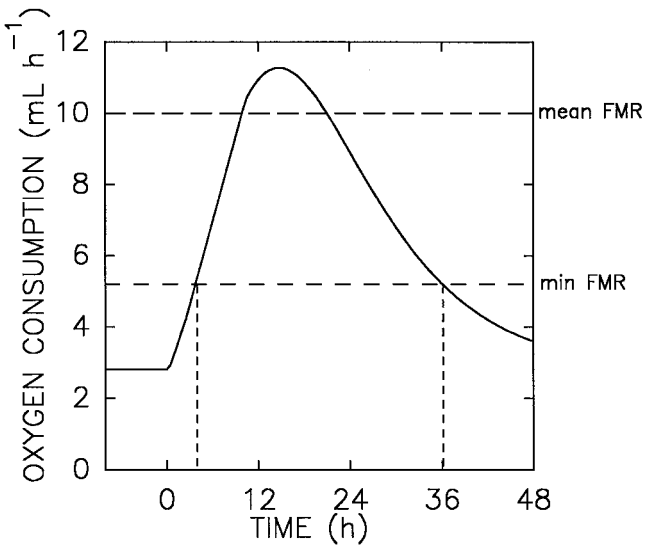

Figure 7. Diagrammatic representation of SDA in *Thamnophis sirtalis*. The solid line represents the time course of oxygen consumption in a 24-g garter snake that eats an 8-g treefrog meal at time zero and digests it at 30°C. Horizontal dashed lines show the mean and minimum FMR measured in snakes of that size class (see Fig. 1). Vertical dashed lines illustrate the temporal extent of overlap between SDA from a single meal and field energy expenditure. SDA curve was calculated according to Bear (1994) and R. Bear (personal communication).

Had we not considered SDA, but rather attributed the portion of FMR above resting expenditures to activity, as is sometimes done, we would have considerably overestimated activity rates in these animals (see also the caveats of Waldschmidt et al. [1987] and Niewiarowski and Waldschmidt [1992]). For similar reasons, previously reported energy budgets of snakes (based on laboratory measurements and a variety of assumptions) are probably underestimates (e.g., Porter and Tracy 1974; Reichenbach and Dalrymple 1986; Bozinovic and Rosenmann 1988).

If costs associated with digestion and growth (including SDA) are a major component of FMR in garter snakes, the intercorrelations we observed among field rates of energy expenditure, energy intake, and growth can be explained as follows. Suppose that some individual snakes are (for any reasons) more effective foragers than others. These effective foragers would achieve relatively high rates of energy intake and, consequently, production, but would also incur relatively high energy costs associated with their larger and/or more frequent meals. Such costs may include foraging effort (as time and/or intensity), costs of ingestion, enzymatic digestion, and intesti-

nal transport, and costs of anabolism and biosynthesis (Secor and Diamond 1997), as well as increased costs of thermoregulation and maintenance resulting from postprandial thermophily (Peterson et al. 1993). Under this scenario, high FMRs would result from costs of digestion and growth resulting in turn from high feeding rates, and FMR, water influx rate, and growth rate would be positively correlated. Interindividual variation in foraging effectiveness is probable; captive *T. sirtalis* offered food ad lib. showed consistent, repeatable differences among individuals in feeding frequency and relative meal sizes and therefore must have incurred different sustained SDA costs (Bear 1994).

This "foraging effectiveness" hypothesis plausibly explains several features of our data in addition to the intercorrelations discussed above. The high FMRs of garter snakes (compared to their own SMRs as well as to those of other squamates) are interpreted as resulting from high feeding rates, which were due in turn to high food availability in this restricted, seasonally productive temperate habitat. Differences in energy budgets between conspecific populations of rattlesnakes have been attributed to differences in resource abundance (Beaupre 1996). Similarly, the higher FMR : SMR ratios of larger garter snakes (and the resulting direct mass proportionality of FMR) may reflect higher SDA costs imposed on larger snakes by taking larger meals and/or diet items (Arnold 1993). The metabolic scaling exponent of 1.0 may also reflect the importance of metabolic costs of growth (an important component of SDA) in these rapidly growing animals (Jorgensen 1988; Wieser 1994).

Our results and interpretations beg further study of the importance of costs of digestion and production to the field energy budgets of ectotherms (see also Parry 1983; Clarke 1993; Secor and Nagy 1994; Wieser 1994). Moreover, costs of growth and digestion should be explicitly considered in future discussions of the evolution of food habits, activity, energetics, and body size in snakes. Finally, we suggest that interindividual variation in foraging effectiveness may be as important in the evolution of life histories as is variation in the allocation of energy acquired.

Acknowledgments

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Appendix

Precision of DLW Measurements

Metabolic rates of free-living animals in nature are preferentially measured with use of the isotopic DLW method (Nagy 1975, 1983*a,* 1989*a*). The theory behind the DLW method was elaborated by Lifson and McClintock (1966); Nagy (1980) discussed its assumptions and limitations with reference to field studies and summarized results of validation studies (see also Nagy 1989*b;* Speakman 1997). A general conclusion from these analyses was that the method is robust under conditions that pertain to most field studies. Nagy (1989*b,* p. 241) estimated that DLW measurements "in free-living endotherms are accurate to ±8%; and, in reptiles during their activity seasons, a reasonable error range would average ±11%." However, under some circumstances, errors can be much higher: in particular, when there is minimal decline in isotope concentration over the sampling period, when isotope abundance has approached background levels, or when rates of water flux are high relative to CO_2 production rates (Nagy 1980; Congdon et al. 1982).

Measurements of field metabolism by DLW have generally been used to characterize mean values for populations or species and in interspecies comparisons. The technique also has great potential to test ecological theory through the analysis of interindividual differences (Bennett 1987; Hayes and Jenkins 1997) in energy allocation and utilization within a single population. We report herein such a study examining correlations among FMRs, growth, and water flux rates, and laboratory-measured metabolic rates in a large sample of individual garter snakes from a single population. However, the robustness of patterns and correlations deduced from intrapopulation variation in FMR depends greatly on the precision of individual DLW measurements. Because our study required the analysis of FMRs for each individual, not just mean rates for the population, we made every effort to maximize the precision of our DLW measurements.

There are two sources of variation that lead to errors in estimating the "true" value for any trait of an individual organism: those inherent in the precision of each of the measurement procedures and those due to sampling error per se. The latter can be reduced by increasing the number of replicates measured and thereby more closely approximating the "true" mean value. However, the increased accuracy may be offset by limitations on the amount of material available or by the cost of analysis. Here, we detail the results of simulation studies undertaken to estimate the precision of any single measurement estimating the FMR of an individual animal, the optimal replicate sampling protocol to minimize estimate error, and the effect of residual variability on correlations between FMR estimates and other study variables of interest.

In our study, we were particularly concerned about effects of the high water flux rates of these snakes on our calculations of FMR (Congdon et al. 1982). DLW measures CO_2 production as the difference between the washout rates of an oxygen isotope (which traces both H_2O and CO_2) and a hydrogen isotope (water only). When hydrogen turnover is 90% or more of oxygen turnover (as pertains when water flux is very high relative to CO_2 production), small errors in measurement of isotope activities can cascade into very large errors in calculated FMR (see Fig. 4 of Nagy [1980]). In part because isotope measurements enter the FMR equation as the natural logarithm of a ratio, the effects of variation in any one isotope measurement on calculated FMR are complex and nonintuitive. For the garter snakes

in our study, hydrogen turnover averaged 88% of oxygen turnover (range 77%–94%), so it was especially important to maximize the accuracy of the isotope measurements.

A standard DLW procedure for small animals involves liquid scintillation counting for tritium and proton activation analysis for high-enrichment ^{18}O (Nagy 1983*a,* 1989*a;* in an alternative methodology deuterium and low-enrichment ^{18}O are assayed by isotope ratio mass spectrometry). The precision of liquid scintillation is dependent primarily on the pipeting skills of the user (Nagy 1983*a*); for duplicate 5-μL subsamples, a coefficient of variation (CV, also known as relative standard deviation) of less than 0.8% is routinely obtainable for typical DLW enrichments (C. C. Peterson, personal observation). Proton activation analysis of ^{18}O is similarly precise if enrichments are relatively high (Wood et al. 1975; Speakman et al. 1990), but there is an inherent limit to the precision of the technique due to small variations in capillary tube diameter, counting geometry, and so forth (Nagy 1983*a*).

We used computer simulation (BASIC) to assess empirically the effects of these unavoidable precision limits on the accuracy of FMR calculations. (Speakman [1995] presents a similar analysis for mass spectrometer analysis as applied to clinical human studies.) FMR is calculated from six isotope measurements (tritium and ^{18}O in background, initial, and recapture samples, each value of which is a mean of replicate assays), initial and recapture body masses and total body water pools, and elapsed time (eq. [2] of Nagy [1980]). We began by stipulating arbitrary but reasonable "true" values for the six isotope measurements that, when entered into the equation for FMR with typical total body water proportions and growth rates, yielded the allometrically predicted FMR for a garter snake of average body size, 22 g (repeated simulations for 6-g and 50-g snakes obtained essentially identical results). We then stipulated a precision of measurement for each isotope, resulting in a normal distribution of subsample values with a mean equal to the "true" value and standard deviation resulting from the defined precision limits. By trial and error we settled on a predefined standard deviation of 1% of the "true" mean for each isotope sampling distribution; this resulted in a mean CV for duplicate tritium subsamples of 0.46% and a mean CV for replicate ^{18}O samples of 0.64%, which are similar to the precisions of the real isotope measurements we used to calculate FMR in our study (mean CVs of 0.50% and 0.64%, respectively).

For each replicate sampling paradigm (see below), 10,000 iterations of the simulation were run. In each iteration, the computer program randomly sampled the stipulated number of replicate values from normal distributions of each of the six isotope measurements and used the mean estimate for each to calculate a value for FMR. The program rejected any isotope mean and resampled if the CV of the replicate values was greater than 1%. Program output consisted of the mean and standard deviation of 10,000 calculated values for FMR as well as the mean CV of each of the six isotope measurements. This procedure exactly mimics standard DLW methods, in which isotope abundance is measured with some finite precision in replicate water samples for the six isotope measurements and means of replicates with CV less than 1% are used to calculate FMR. However, actual FMR calculations are based on a single replicated assay of each isotope value; the computer simulations allowed us to sample repeatedly from the same precision-defined distributions and statistically assess the effects on the distribution of calculated FMRs.

The tritium/high-level ^{18}O DLW procedure involves liquid scintillation analysis of tritium samples in duplicate and proton activation analysis of ^{18}O in triplicate (Nagy 1983*a*). We symbolize this replicate sampling scheme as 2/2/3/3, referring to the number of replicate subsamples of the initial and recapture ^{3}H and initial and recapture ^{18}O, respectively, with background samples replicated as were recapture samples. From 10,000 iterations using this standard replication scheme, we obtained a normal distribution of calculated FMRs with a CV of 12.83% of the mean (i.e., the "true") FMR. We interpret this result as suggesting that any single FMR calculation based on single replicated measurements of the four isotope values has a 95% probability of being within 25.7% of the true FMR (because in a normal distribution, ca. 95% of observations are within ±2 standard deviations of the mean; in contrast to Speakman's [1995] slightly different simulation, the distribution of simulated FMRs did not differ significantly from a normal distribution of identical mean and CV).

Because FMR calculations are most sensitive to the values of the recapture and background ^{18}O measurements (see, e.g., Table 4 of Wolf et al. [1996]), we repeated our computer simulation with tritium samples again sampled in duplicate and initial ^{18}O in triplicate, but recapture and background ^{18}O sampled in sextuplicate (2/2/3/6). This subsampling regime yielded a 23% improvement in precision of the FMR estimation (CV = 9.86%). Thus, under the 2/2/3/6 replication paradigm, 95% of single FMR calculations would be expected to fall within 19.7% of the true value. Further increments in the number of replicates of ^{18}O samples resulted in only small additional improvements in precision (2/2/3/9: CV = 8.61%; 2/2/6/6: CV = 9.62%); note in particular that doubling the replicate number of the initial ^{18}O measurement resulted in negligible improvement in precision. Likewise, increments in the number of tritium replicates resulted in only modest improvements in precision (2/3/6/6: CV = 9.43%; 3/3/6/6: CV = 9.31%). We concluded that these marginal improvements were insufficient to justify the additional analysis costs and use of limited sample volumes; we therefore used a replicate subsampling scheme of 2/2/3/6 in our study.

Our computer simulations suggest that by doubling the number of replicates of the isotope values to which FMR calculation is most sensitive, the precision of FMR measurements could be improved substantially, from a CV of 12.8% to 9.9%. Our simulations further suggest that this latter value approaches the practical limit of possible precision, given the inherent limitations of the analytical techniques in situations of high water flux relative to metabolic rates.

In regard to our study correlating FMR with other field and laboratory measurements of growth and metabolism, we conclude from the foregoing simulation that each of the individual measurements of FMR in this study has a 95% chance of being within ±19.7% of its true value. Because the sign and magnitude of errors introduced by imprecision of isotope measurements are random, this imprecision in FMRs should not affect estimations of mean values or central tendencies, such as average FMR, analysis of intrapopulation allometric scaling, or any calculations or conclusions regarding these. However, such imprecision could potentially affect analyses of interindividual variation, including correlations between FMR and other traits (e.g., growth rate, laboratory metabolic rates; see Table 3). For two variables that are truly correlated, random errors in measurement of any one variable would be expected to decrease the likelihood of detecting a significant correlation from a sample. To quantitatively assess the effects of random errors in individual FMR measurements on these intraindividual correlations, we performed an additional simulation analysis. One hundred artificial data sets were constructed by changing the FMR of each individual snake by a randomly generated factor between −20%

and +20%. We then calculated allometric equations and mass residuals for each of the artificial data sets and entered the mass residuals into a correlation analysis that also included mass residuals for measured (unaltered) FMR, water flux rates, and respirometry variables. Mass residuals of the 100 sets of artificially varied FMR data were correlated with measured FMR data with correlation coefficients (r) ranging from 0.927 to 0.958 (all $P < 0.001$). The artificially varied FMR data sets were correlated with each other with r's ranging from 0.826 to 0.935 (all $P < 0.001$). Therefore, interindividual variation in FMR was conserved despite random manipulation of individual data within limits of precision. Further, all significant correlations and conclusions drawn from the correlation matrix in Table 3 were upheld for all 100 randomized data sets. We conclude that, despite a possible imprecision of individual FMR measurements of up to 20%, conclusions involving interindividual comparisons are robust.

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