Reproductive Effort and Reproductive Nutrition of Female Desert Tortoises: Essential Field Methods

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SYNOPSIS. I used three innovative, nondestructive field methods (gas dilution, doubly labeled water and radiography) to measure individual energy and water budgets of wild, female desert tortoises (Gopherus agassizii). With these budgets, I evaluated whether body reserves help females produce eggs independent of rainfall and food availability. Female desert tortoises used large seasonal and annual changes in metabolism and body water, protein and energy reserves to survive and produce eggs. Although lipid reserves are important to female desert tortoises, nitrogen or crude protein appears to be the primary limiting resource for producing eggs. By reducing metabolic rates 90%, females conserved enough body reserves to produce eggs during extreme drought conditions; this is an effective bet-hedging reproductive pattern in an extreme and unpredictable environment.

INTRODUCTION

Physiological ecologists often pursue field studies in harsh environments in search of extreme physiological tolerances, adaptations or exaptations enabling organisms to endure in these environments. Consequently, the extremely low, variable and unpredictable rainfall and primary productivity of deserts (Pianka, 1978; Louw and Seely, 1982; Henen, 1997; Henen et al., 1998) has intrigued many physiological ecologists (Louw and Seely, 1982; Schmidt-Nielsen, 1997). Rainfall and primary or secondary productivity are strong cues for reproduction in desert organisms, yet reproductive strategies of desert animals are diverse (e.g., Kenagy and Bartholomew, 1985; Turner et al., 1984a, b). Despite experiencing a 10-fold variation in their food availability, desert tortoises (Gopherus agassizii) did not vary their annual egg production in 1983–85 (Henen, 1997). How did female desert tortoises reproduce seemingly independent of rainfall and food availability?

To produce eggs independent of rainfall and food availability, female desert tortoises may store energy before winter and use these reserves the ensuing spring to produce eggs. To evaluate this possibility, I measured annual energy budgets of female G. agassizii. These budgets were also designed to quantify reproductive effort (Congdon et al., 1982; Nagy, 1983) and identify periods when nutrient limitations influence female activity and reproduction. I used three nondestructive methods to measure energy allocations to field metabolism (doubly labeled water), eggs (X-ray radiography) and body lipid and non-lipid energy content (gas dilution). Gas dilution requires a long equilibration and measuring many equilibration parameters.

However, these measurements enable accurate, nondestructive estimation of lipid mass (Lesser et al., 1952; Henen, 1991, 2001; Gessaman et al., 1998) and nonlipid mass (Henen, 1991, 2001). Gas dilution is accurate for animals with low, or slowly changing, lipid reserves, and variable body temperatures and hydration states (e.g., desert tortoises).

This analysis focuses on field evaluations of the body composition of wild, female desert tortoises. In doing so, it demonstrates physiological, ecological and evolutionary features enabling desert tortoises to survive and reproduce for decades in the harsh, variable and unpredictable desert clime.

MATERIALS AND METHODS

Study design

The study site was in remote creosote bush (Larrea tridentata)—burrobush (Ambrosia dumosa) scrub habitat of the eastern Mojave Desert (34°51’N, 115°09’E, 680 m elevation). Female G. agassizii become active in early spring (ca. mid-March to mid-April) and produce eggs in spring (ca. mid-April to mid-July). The tortoises use burrows to avoid the hot midday hours in summer (July to September) and enter winter burrows in autumn (ca. mid-October), remaining dormant until March. To evaluate budgets for two reproductive cycles, I recaptured nine female G. agassizii, fitted with radiotransmitters, from July 1987 to July 1989. Females were radiographed every two weeks during each spring to quantify egg production. Body composition and doubly labeled water data were measured at least once each season (Fig. 1).

Doubly labeled water and X-ray radiography

The doubly labeled water and X-ray radiography methods were detailed in Henen (1997) and will only be briefly outlined. At the beginning of the study, a 100 μl brachial or supraorbital blood sample was collected from each female. Females were then injected intramuscularly with a calibrated volume of doubly labeled water (oxygen-18 and tritium or deuterium). After ≥14 hour of equilibration, another small blood
sample was collected for determining equilibrium isotope concentration and total body water (in g) by isotopic dilution. I then released the animals to range freely until recaptured.

 Upon recapture of each animal, I collected voided urine or 100 µl of blood, injected 0.9 ml of singly labeled water (tritiated or deuterated water) or doubly labeled water, collected blood after isotopic equilibration, and released the animal. Reinjections maintained isotope levels for accurate analysis and helped measure total body water for body composition calculations. Sample isotope concentrations were used to calculate field metabolic rates (liter CO₂ produced/day, kJ/day and MJ/yr).

 Females were radiographed using a portable X-ray machine (MinXray 300) and a technique of 70 kVP and 5 mA·sec. Eggshells become radio-opaque within days after ovulation (unpublished data, B.T.H.), allowing quantification of clutch size, egg size and annual fecundity (Turner et al., 1986; Henen, 1994, 1997; Wallis et al., 1999). Using fecundity and the energy content of captive-tortoise eggs, I calculated the chemical potential energy each female invested in eggs. Ultrasonography indicates that females can reabsorb follicles before ovulation (Rostal et al., 1994; B. T. Henen and O. T. Ofstead, unpublished data). After ovulation however, females add albumen and shell to complete each egg and proceed to lay their eggs. Because of this commitment at ovulation, I assigned reproductive allocations to spring periods when females ovulated.

**Body composition**

The body composition of each female was measured 10 times (with two exceptions; Fig. 1) using the dilution of cyclopropane gas; consult Henen (1991, 1997, 2001) for details. An organism inside a glass chamber with a lipid soluble gas (e.g., cyclopropane) will absorb much of the gas in its lipids. For cyclopropane dilutions on female desert tortoises, I measured the cyclopropane concentration of chamber gas samples in the field on a portable gas chromatograph (Foxboro OVA128BIE-GC Gas Analyzer). The moles of cyclopropane absorbed in each female’s lipids were calculated using the 1) concentration of cyclopropane in chamber gas samples, 2) solubilities of cyclopropane for nonlipid matter, 3) temperature, pressure and gas pressure inside the chamber, and 4) animal’s body temperature, approximate volume, and total body water. I then used the moles of cyclopropane in the lipids and the lipid solubility coefficient to calculate body lipid mass (in g).

I calculated body dry mass (=body mass – total body water) and nonlipid dry mass (=dry mass – lipid mass) by difference. Lipid and nonlipid dry masses were converted to energy units (kJ or MJ) using energy densities (37.5 and 9.80 kJ/g, respectively) measured with bomb calorimetry on lipid and nonlipid extracts of roadkill carcases. For each period I calculated individual anabolic and catabolic rates (positive and negative values, respectively; kJ/day or MJ/yr) from serial measurements of body lipid and nonlipid energy. Metabolizable energy intake (kJ/day or MJ/yr) equaled the sum of field metabolic rate, chemical potential energy of eggs produced, and the change in body energy content (=lipid + nonlipid energy).

**Statistics**

Body composition measures were summarized as means ±95% CI and compared among periods using one-way repeated measures ANOVA (RMANOVA, all df = 9 and 86). RMANOVA were followed by Student-Newman-Keuls multiple comparisons (SigmaStat 2.0 for Windows, Jandel Scientific) to determine which means differed. Results were significant at P < 0.05.

**RESULTS AND DISCUSSION**

Egg production was lower in 1988 and 1989 than in 1983–1985 (Henen, 1993, 1997), so G. agassizii reproduction is affected by rainfall and food availability (see also Turner et al., 1987; Wallis et al., 1999). The relationship of egg production to food abundance is not simple, egg production appears to approach an asymptote at moderate to high food abundances, like those in 1983–1985 (Henen, 1993; Wallis et al., 1999).
However, females were able to produce a few eggs in 1988 and 1989 by drawing upon body nutrient reserves (Henen, 1994, 1997).

**Body mass, water, and dry matter**

Body mass and body water changed significantly through time (Fig. 1) and showed more extreme changes than did dry matter and percent body water. Body mass and body water were highly correlated \((r = 0.99)\) and increased substantially when females drank rainwater (both summers and spring 1988) or ate fresh annual plants or succulent perennial plants (early spring, both years; Henen, 1994). Body mass and body water decreased in spring 1988 and 1989 primarily because egg production was demanding (Henen, 1994, 1997; Wallis et al., 1999).

Body mass and body water increased 7 and 12%, respectively, during the first year, which included many opportunities for females to drink rainwater and consume fresh annual plants. However, the second year included a very dry winter followed by very low plant productivity. Consequently, three small females forfeited egg production in 1989, posting modest gains in body mass and water (9 and 5%, respectively) and an 18% increase in dry matter. Conversely, the other six females produced about four eggs each, and suffered 12, 15 and 6% losses in body mass, water and dry matter, respectively (Henen, 1994), emphasizing the importance of body reserves to egg production.

Changes in body mass and body water exceeded those for body dry matter on an absolute scale (Fig. 1), but less so on a relative scale (Table 1). Dry matter peaked when tortoises ate fresh food (Summer 1988; early Spring 1988 and 1989; Henen, 1997) but declined each spring (1988: 72 g, 1989: 64 g), in part due to substantial allocations of dry matter to eggs (33 and 32 g, respectively). Dry matter did not change annually except during the dry year, declining 30 g for egg producing females, which invested 47 g of dry matter in eggs, and increasing 63 g for females foregoing reproduction (Henen, 1994).

The large changes in body composition are consistent with *G. agassizii*’s behavioral (Medica et al., 1980; Nagy and Medica, 1986; Henen, 1994), morphological (Schmidt-Nielsen, 1997) and physiological features (Minnich, 1982; Nagy and Medica, 1986; Henen, 1994, 1997; Peterson, 1996; Henen et al., 1998; Christopher et al., 1999) for acquiring water and reducing water losses. Desert tortoises can drink as much as 40 or 50% of their body mass in water within 15 min (Henen, 1994; unpublished data, B.T.H.) ranking them with camels (Schmidt-Nielsen, 1997) and amphibians (Pough et al., 1998) in rehydration abilities. The dehydration tolerances of desert tortoises are also exceptional (Nagy and Medica, 1986; Henen, 1994; Peterson, 1996).

**Lipid metabolism**

Changes in dry matter reflected primarily the changes in nonlipid dry matter (Fig. 2). A simple comparison of RMANOVA F values, \(P\) values and the number of significant differences among means (Figs. 1 and 2), suggests that dry matter varied less in time than did lipid mass and nonlipid dry mass. Similar analyses suggest that lipid mass, and lipid mass relative to body mass (Fig. 2) or lipid mass relative to nonlipid dry mass (\(F_{3,38} = 43.5, P = 5.50 \times 10^{-23}\)), varied more in time than did nonlipid dry matter. This is consistent with perceptions that lipids are more labile than nonlips (Pond, 1978; Henen, 1991, 1994, 1997).

Body nonlipid energy and lipid energy were simple products of nonlipid and lipid masses, respectively, and thus had the same statistical results as did nonlipid and lipid masses (Figs. 2 and 3). Since lipid holds more energy per gram than does nonlipid dry matter (e.g., 38 and 9.8 kJ/g, respectively), small errors in lipid mass estimates (Fig. 2) can affect the accuracy of energy budgets (Fig. 3). Thus the gas dilution method may have been the only nondestructive, body composition method accurate enough for this study (Henen, 1991, 2001).

Lipid catabolism helped females survive winter dormancy but lipids remained unchanged during each spring (Figs. 2, 3). Body lipid mass and energy were higher at the end of both years compared to values at the beginning of the study. These results, plus the increase in total body energy during the first year (Fig. 3), suggest that lipid and energy availability did not limit egg production in either year (Henen, 1997).

Chelonia tend to have very little adipose tissue (Pond, 1978). Desert tortoises appear to have little if any adipose tissue, but vitellogenic follicles may contain much of the lipids in female *G. agassizii* (unpublished observations, B.T.H.), so lipids are important to egg production. However, some chelonia may depend heavily upon body lipid reserves to complete vitellogenesis and egg production (Congdon and Tinkle, 1982; Long, 1985). Yolk lipids can also represent important maternal investments in neonatal survival (Congdon and Tinkle, 1982), revealing the nutritional and evolutionary complexity of life histories. Regardless, the availability of lipids or energy, relative to protein (nitrogen) and water availability, may rarely limit egg production in desert tortoises (Henen, 1994, 1997).

Drinking in August 1987 enabled females to eat dry grass and store lipids at rates more than 10-fold that of pre-drinking rates (Fig. 3). However, converting dry grass to body lipids was costly in terms of body water and nonlipids (e.g., digestive mucosa and enzymes; Fig. 2; Henen, 1994; Nagy et al., 1998). Using body water and nonlipids to accrue lipids in summer may be an inherent metabolic preparation for winter that conserves body nonlipids during winter (Henen, 1997). Nonetheless, the large loss of nonlipid dry matter (probably protein) in summer 1987 probably reduced reproductive output in 1988. Other animals also anabolize and catabolize protein in response to changes in dietary nitrogen availability (Henen, 1997). This mechanism is consistent with *G. agassizii*’s propensity to relax homeostasis (Nagy and Medica, 1986; Henen, 1994, 1997).
**Table 1.** Maximum increase and decrease (percent change and percent change/d) of mean composition values of female *Gopherus agassizii* and *Uta stansburiana* during the reproductive season (Rep) and the rest of the year (Non).*

<table>
<thead>
<tr>
<th></th>
<th>Desert tortoise</th>
<th>Size-blotched lizard</th>
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<tbody>
<tr>
<td></td>
<td>Increase change</td>
<td>Rate % per day</td>
</tr>
<tr>
<td></td>
<td>Rep Non Rep Non Rep Non Rep Non Rep Non Rep Non Rep Non</td>
<td></td>
</tr>
<tr>
<td>Body mass</td>
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<tr>
<td>NL dry mass(d)</td>
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<td>Body lipid, %(a)</td>
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<tr>
<td>Body energy</td>
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</tr>
<tr>
<td>NL energy</td>
<td>18.4</td>
<td>0.32</td>
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<tr>
<td>Lipid energy</td>
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</tr>
<tr>
<td>Lipid energy, %</td>
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<tr>
<td>L/NL, energy</td>
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* The final column indicates percent of significant RMANOVA multiple comparisons for *G. agassizii* that were detected via standard ANOVA multiple comparisons.

\(a\) Reproductive season—mid-April to mid-July; percent lipids increased due to large declines in nonlipids.

\(b\) Calculated from female lizard data in Figure 2.2, Table 2.1 and body energy values, of Nagy (1983). Reproductive data: late February to late June. December values were calculated averages of November and January values. Percent body lipid equaled the sum of constituent lipids calculated from constituent masses and lipid contents (i.e., extractable somatic lipid, 100% lipid; reproductive tissues, 40.2% lipid; fat bodies, 74.4% lipid between May and September, 83.2% lipid otherwise). Lipid percentages for fat bodies and reproductive tissues interpolated from tissue energy densities and measured energy densities of lipids (38.1 kJ/g) and nonlipids (17.7 kJ/g for a 50:50 carbohydrate-protein mix; Nagy, 1983).

\(c\) Figures 1–3 indicate the number of significant comparisons for the RMANOVA.

\(d\) L = lipid, NL = nonlipid, L/NL = percent change in ratio of lipid energy to NL energy; percent categories are value as percent of body mass (Body water, %; Body lipid, %) or total body energy (Lipid energy, %).
Metabolism of nonlipid dry matter

Nonlipid dry matter supported winter 1987–88 metabolism and was forfeited each spring (1988: 65 g; 1989: 51 g), with 25.2 and 24.5 g, respectively, being invested in eggs. The remainder of these spring declines was probably realized through the turnover of digestive enzymes and mucosa (Nagy et al., 1998) and urate excretion (Nagy and Medica, 1986; Oftedal and Allen, 1996; Nagy et al., 1998).

Nonlipid dry matter decreased 11% during the first year (Figs. 2 and 3). Additionally, females laying eggs in 1989 forfeited nonlipid dry matter (22 g or 0.21 MJ) during the second year while nonreproductive females increased nonlipid dry matter (74 g or 0.73 MJ; Henen, 1994, 1997). Limited nitrogen consumption before hibernation constrains egg production, apparently by limiting prehibernatory development of ovarian follicles (Henen and Oftedal, 1999). Thus, prehibernatory nitrogen consumption in 1982–1984 may have helped the 1983–1985 egg production appear independent of rainfall and food availability. Biologists have long suspected that protein (nitrogen) availability limits avian egg production (Drent and Daan, 1980).

Homeostasis, reproductive effort and bet-hedging

Body energy fluxes (total) were substantial portions of energy budgets. The fluxes represented up to 85% of positive, and 400% of negative, rates of metabolizable energy intake. These fluxes also exceeded field metabolic rates (FMR) in four intervals, and exceeded reproductive allocations in spring of both years (Henen, 1997). The 10-fold seasonal differences in FMR were reasonable for an ectothermic poikilotherm. However, early spring FMR differed 10-fold between years (1989 < 1988; Henen, 1997) as females restricted surface activity to avoid the drought and poor forage conditions in 1989. This helped some females conserve enough nutrients to produce eggs (Henen, 1997). Females rarely achieved energy balance, which they did in spring of both years, illustrating their relaxed homeostasis towards energy.

Reproductive effort (RE) is the proportion of energy available to an organism that it allocates to reproduction (see Congdon et al., 1982; Nagy, 1983). For this study, available energy equalled annual metabolizable energy intake (MEI). Reproductive allocations included the chemical potential energy in eggs (Rc) and the portion of FMR, Rr, related to reproduction (e.g., nest digging and egg synthesis; Henen, 1997). Reproductive effort (RE = 100 × [Rc + Rr]/MEI) for the second year, the drought year, was twice that of the first year (26 vs. 13%; Henen, 1997) which had moderate rainfall and food availability. Reproductive effort may increase with high food abundance (Swingland, 1977), especially for homeothermic endotherms, due to increased reproductive allocations and relatively stable metabolizable energy intake. However, the opposite occurred for female G. agassizii, metabolizable energy intake declined 60% in the second year while statistically, reproductive allocations were unchanged between years. Fecundity in desert tortoises peaks when food and water are abundant (e.g., El Niño conditions, Henen, 1997; Henen et al., 1998; Wallis et al., 1999). Reproductive effort may then also peak, revealing a complex relationship between reproductive effort and resource abundance.

Why did most females (n = 6), during an extreme drought, forfeit body water (15%) and nonlipid dry matter (5%; Henen, 1994) to produce just a few eggs? Deserts are unpredictable (Louw and Seely, 1982), so females can not predict whether their eggs will hatch
under favorable conditions. Females forgoing reproduction (n = 3) were guaranteed to have zero eggs hatch regardless of ensuing conditions. However, females producing at least a few eggs had a greater chance than nonreproductive females, of having eggs hatch into favorable conditions. This reproductive pattern is consistent with a bet-hedging life history in an unpredictable environment (see Congdon et al., 1982; Philippi and Seger, 1989; Stearns, 1992). Accordingly, spreading small reproductive investments over several rounds, essentially reducing the variance in reproductive allocations among bouts (years or clutches), can increase average winnings (reproductive success or fitness).

Apparently, three females could not afford to forfeit body condition to produce eggs in 1989, so they forfeited one round of reproductive wagering. Reproductive females had relatively large protein and water reserves and could hedge their bets, producing just a few eggs, with a chance their hatchlings would emerge into favorable conditions. This may explain why Mojave Desert females tend to produce at least a few eggs every year (Henen, 1997; Wallis et al., 1999). However, patterns of rainfall and tortoise reproduction are different in the Sonoran Desert (Murray et al., 1994; Henen et al., 2001).

Maximum changes

The highest percent changes in dry matter and non-lipid dry matter approximated those for body water (Table 1), the most labile component on an absolute scale (Fig. 1). However, the greatest relative changes occurred in lipids. The increase in percent lipids during the reproductive season is misleading, resulting from body water and nonlipid dry mass decreasing more rapidly than did lipid mass. Also, maximum body mass, water and dry matter decreases occurred during the reproductive season while most energy decreases were greatest in the nonreproductive season. These patterns emphasize the importance of 1) body water and nonlipid dry matter, more than lipids, to reproduction, and 2) relaxed homeostasis to desert tortoise survival, relative to reproduction. We might expect this for a long-lived, iteroparous species where each reproductive bout is small compared to lifetime reproduction, and high reproductive effort.

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Gopherus agassizii and Uta stansburiana: Contrasts

We can compare the body composition of female desert tortoises (ca. 1 to 3 kg body mass) and wild, insectivorous, female Uta stansburiana (side-blotched lizards, 2 to 5 g, Nagy, 1983), both species were studied in the Mojave Desert. However, the gas dilution method did not discriminate the tissue location of tortoise lipids, so comparisons required several calculations using the lizard data (Table 1).

The components of lizard body composition changed more during the reproductive season than in the nonreproductive season (Table 1). This was probably linked to the lizards’ small size, short lives (ca. 2 yr) and few reproductive bouts (Turner et al., 1984a; Nagy, 1983) relative to G. agassizii (Turner et al., 1986; Henen, 1997; Wallis et al., 1999). For the reproductive season, and each clutch within the reproductive season, body composition changes for desert tortoises were small compared to those for the lizards (this analysis). Conversely, increases in the nonreproductive season were typically lower for lizards than for tortoises (Table 1), probably due to the importance of body nutrient reserves to tortoise survival in the nonreproductive season. Also, on an absolute scale, the small lizards have low food requirements and may face food shortages less frequently (see Nagy, 1983) than other insectivores or desert tortoises.

Despite the extreme water lability of desert tortoises, their maximum percent change in body water did not always exceed those of U. stansburiana. The large changes seen in U. stansburiana were associated mostly with reproduction (see also Hahn and Tinkle, 1965), whereas G. agassizii changes can be related to reproduction (Henen, 1994), digestion (Henen, 1994, 1997; Nagy et al., 1998), or dehydration (Nagy and Medica, 1986; Henen, 1994; Peterson, 1996).

Body size may underlie most of these species differences. The lizards are small, warm quickly, become active in winter, and subsist on small insects or, if necessary, annual plants (Nagy, 1983). The relatively large mass and thermal inertia of female desert tortoises usually prevents winter activity but facilitates their relaxed homeostasis. Chelonian reproductive patterns seem affected by body size (Swingland, 1977; Congdon et al., 1982; Henen, 1997), with small species having short maturation periods and lives, few reproductive bouts, and high reproductive effort.

Early hatching, rapid maturation and early production of clutches are highly favored in U. stansburiana (Hahn and Tinkle, 1965), selecting for early, rapid and extensive conversion of fat body lipids into yolk material (Hahn and Tinkle, 1965; Nagy, 1983). This explains much of the large body composition changes in the reproductive season and high reproductive effort (45 to 84%; Nagy, 1983) of female U. stansburiana. In contrast, female desert tortoises may reproduce nearly every year for 20 to 50 yr. Although U. stansburiana rely heavily upon body reserves to produce their first clutch of eggs, clutch frequency depends on the current year’s food abundance (Turner et al., 1984a).

Capital and income breeders

The capital, income and mixed reproductive strategies, originally described for birds (Drent and Daan, 1980), have been applied to other organisms including chelonians (Kuchling, 1999). Biologists may consider
desert tortoises “capital” breeders because female tortoises 1) forgo egg production and reabsorb follicles if food availability is poor and body reserves are small (Henen, 1993, 1994, 1997; Rostal et al., 1994), 2) accumulate water and protein reserves prior to winter and use these reserves to help produce eggs (Henen, 1994, 1997; Henen and Oftedal, 1999), 3) have full-sized (“preovulatory”) follicles before entering hibernation (Rostal et al., 1994; Henen and Oftedal, 1999), and 4) can emerge from winter dormancy and ovulate prior to eating (Henen and Oftedal, 1999). However, females can also produce more than one clutch, and larger clutches, if 1) spring forage is abundant (Turner et al., 1986, 1987; Henen, 1993; Wallis et al., 1999) and 2) females lay their first clutch early in the reproductive season (Wallis et al., 1999). This suggests that food (or nutrient) income also influences egg production, and that desert tortoises use a mixed reproductive strategy. Yet, further research is necessary to determine whether females must accrue enough “capital” to meet new or different thresholds for producing additional clutches or larger clutches. Similarly, only further research will quantify the body condition threshold necessary to initiate vitellogenesis (see Kuchling, 1999).

Value of accurate repeated measures

The three nondestructive field methods were critical for 1) ecologically relevant results (Nagy, 1980, 1983; Henen, 1991, 1994, 1997), 2) obviating massive chemical extractions on numerous carcasses (ca. 90 females = 9 females per sample × 10 samples), 3) conserving members of this Threatened Species, and 4) enabling the use of repeated measures statistics. I also analyzed the desert tortoise data by standard one-way ANOVA (all df = 9, 86) followed by Student-Newman-Keuls multiple comparisons. If the data were not normal, I used a one-way analysis of variance on ranks (df = 9), followed by Dunn’s multiple comparisons. The repeated measures ANOVA was much more powerful than standard ANOVA in detecting body composition changes through time (Table 1).

The chosen methods were essential for assessing physiologically (e.g., relaxed homeostasis, nutrition and body composition), ecological (e.g., reproductive effort, resource abundance, principle of allocation) and evolutionary (e.g., life history) features of female desert tortoises. The results of Henen (1994, 1997) have stimulated the use of other cutting-edge technologies to better understand the dietary constraints upon the reproductive cycles of female desert tortoises. These technologies include ultrasonography (Henen and Oftedal, 1999; Henen et al., 2001; see also Rostal et al., 1994 and review by Kuchling, 1999) and stable isotope analyses (unpublished data, B. T. Henen and O. T. Oftedal; see Godley et al., 1998). This illustrates how science and technology advance synergistically in an alternating, iterative fashion.


