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# The importance of physiological ecology in conservation biology

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**Synopsis** Many of the threats to the persistence of populations of sensitive species have physiological or pathological mechanisms, and those mechanisms are best understood through the inherently integrative discipline of physiological ecology. The desert tortoise was listed under the Endangered Species Act largely due to a newly recognized upper respiratory disease thought to cause mortality in individuals and severe declines in populations. Numerous hypotheses about the threats to the persistence of desert tortoise populations involve acquisition of nutrients, and its connection to stress and disease. The nutritional wisdom hypothesis posits that animals should forage not for particular food items, but instead, for particular nutrients such as calcium and phosphorus used in building bones. The optimal foraging hypothesis suggests that, in circumstances of resource abundance, tortoises should forage as dietary specialists as a means of maximizing intake of resources. The optimal digestion hypothesis suggests that tortoises should process ingesta in ways that regulate assimilation rate. Finally, the cost-of-switching hypothesis suggests that herbivores, like the desert tortoise, should avoid switching food types to avoid negatively affecting the microbe community responsible for fermenting plants into energy and nutrients. Combining hypotheses into a resource acquisition theory leads to novel predictions that are generally supported by data presented here. Testing hypotheses, and synthesizing test results into a theory, provides a robust scientific alternative to the popular use of untested hypotheses and unanalyzed data to assert the needs of species. The scientific approach should focus on hypotheses concerning anthropogenic modifications of the environment that impact physiological processes ultimately important to population phenomena. We show how measurements of such impacts as nutrient starvation, can cause physiological stress, and that the endocrine mechanisms involved with stress can result in disease. Finally, our new syntheses evince a new hypothesis. Free molecules of the stress hormone corticosterone can inhibit immunity, and the abundance of “free corticosterone” in the blood (thought to be the active form of the hormone) is regulated when the corticosterone molecules combine with binding globulins. The sex hormone, testosterone, combines with the same binding globulin. High levels of testosterone, naturally occurring in the breeding season, may be further enhanced in populations at high densities, and the resulting excess testosterone may compete with binding globulins, thereby releasing corticosterone and reducing immunity to disease. This sequence could result in physiological and pathological phenomena leading to population cycles with a period that would be essentially impossible to observe in desert tortoise. Such cycles could obscure population fluctuations of anthropogenic origin.

## Historical roots of physiology in ecology and conservation biology

All of biology emanated originally from scholarship in the philosophy of science and in the study of natural

history. These areas gave birth to biology and separately to ecology, albeit ecology certainly devolved from biology as well as natural history in the latter half of the nineteenth century. Physiology is one of the

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principal disciplines within biology, and particularly important historical developments in environmental physiology of animals arose in the early part of the twentieth century with the works of Irving and Scholander (Scholander 1990) who studied homeostasis in endothermic vertebrates. In 1944, a revolutionary paper (Cowles and Bogert 1944) revealed that ectothermic vertebrates frequently regulate physiological systems with comparable precision as that seen in endotherms. That paper ushered in the field of animal physiological ecology and decades of exploration about the nature and extent of homeostatic mechanisms in animals (Dawson 2005). The exploratory era of animal physiological ecology continues to be important today, but 2 decades ago new syntheses became necessary (Feder and others 1987). These stimulated some physiological ecologists to embrace questions about the evolution of physiological systems. That new line of inquiry in physiology led to reductionistic exploration into the molecular basis of physiological phenomena, the selective forces leading to physiological adaptation, and the evolutionary consequences of evolving in particular physical environments; for example, macroecological patterns in physiological processes that could contribute to physiological, morphological and ecological pattern at levels larger than the landscape scale (Brown and Maurer 1989; Chown and others 2004).

In the 1980s, conservation biology emerged as an important discipline (Wilson 1988), and the importance of physiological ecology in understanding sensitive species has caused this field to become an important dimension in conservation biology (Meffe and Carroll 1994, 1997; Groom and others 2005). Indeed, many of the threats to the persistence of populations of sensitive species have physiological or pathological mechanisms. For example, invasions of pathogenic species such as the chytrid fungus (*Batrachochytrium dendrobatidis*) have had disastrous consequences for amphibian populations worldwide, and understanding the thermal sensitivity of the pathogen, and the interplay of physiological costs and benefits at different temperatures for both pathogen and host has been key to conservational prescriptions (Blaustein and others 2005). Similarly, changes in habitat that modify nutritive resources for individuals can cause suboptimal physiological states that have damaging demographic consequences for sensitive populations (Owen-Smith and Mills 2005).

Physiological ecology is an inherently integrative discipline that has proven essential to an understanding of evolution and conservation (Carey 2005). Thus, the importance of physiological ecology, as well as its depth and breadth, have increased dramatically in

recent decades insofar as the field is not studied just to understand the physiological ecology of organisms, but increasingly to provide important mechanistic explanations for phenomena in other disciplines. In this paper, we will use the desert tortoise (*Gopherus agassizi*) as an exemplary species demonstrating how an understanding of physiological ecology is of keystone importance in conservation. Indeed, we will show that understanding the physiological ecology of the desert tortoise is critically important for the conservation of this species. Furthermore, we argue that using rigorous scientific analyses and modeling will allow the maximum benefit to be obtained from physiological and ecological data as conservation alternatives are considered.

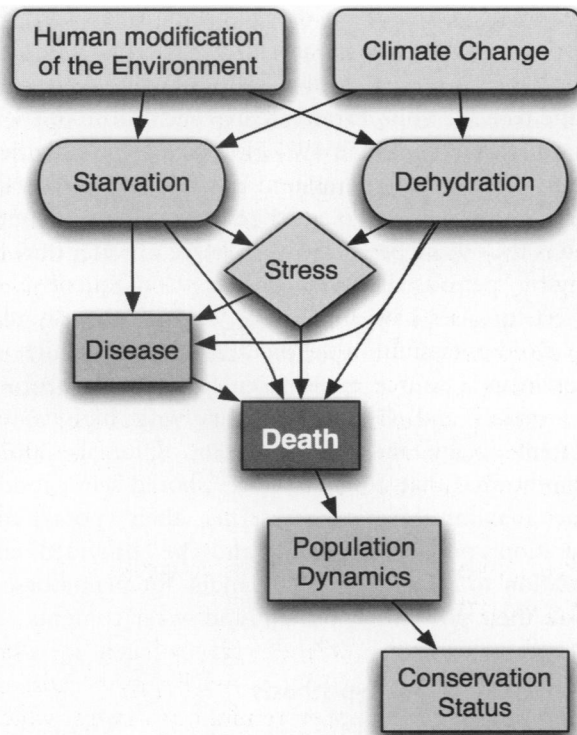
## The desert tortoise as a model organism

The desert tortoise was emergency-listed in 1989 under the Endangered Species Act largely because of a newly recognized upper respiratory disease, which was thought to cause individual mortality and severe population decline. The recovery plan for the species (U.S. Fish and Wildlife Service 1994) and a more recent assessment of that recovery plan (Tracy and others 2004) determined that a huge number of factors threaten the persistence of this species. Several of the threats are physiological. Furthermore, interactions among physiological and pathological processes are thought to affect population dynamics in ways that are important for conservation and management of this species (Fig. 1). Changes in the abiotic and biotic environment can affect processes that determine the extent to which individuals obtain nutrients, and affect their ability to obtain water. Thus, changes in abiotic and biotic environments can cause starvation and/or dehydration and/or result in physiological stress and/or disease. Both stress and disease can affect mortality, which is important in population dynamics and as a key predictor of the persistence of populations (Fig. 1). Here, we review hypotheses about the physiology of acquisition of resources and show how these hypotheses can be linked to hypotheses about the immune system and to population phenomena important for the conservation of desert tortoise populations.

## Resource acquisition hypotheses

### Overview

Resource acquisition for any species is of key importance at all levels of ecological integration. Additionally, resource acquisition is critical to



**Fig. 1** The relationships among changes in environment (human-induced changes and natural changes), physiological processes (for example, starvation and dehydration), consequences to individuals (for example, stress and/or disease), and resulting demographic phenomena (for example, mortality) and the population consequences of demographic phenomena (for example, changes in population dynamics), and consequent conservation status of the species.

conservation status insofar as abundant resources will typically result in the potential for populations to increase, and limited resources can cause populations to decline and/or go extinct. In the case of the desert tortoise, the conservation hypotheses associated with food and water include the idea that threats from domestic grazers, fires and invasion of weedy plants have reduced availability of nutrients to desert tortoises (U.S. Fish and Wildlife Service 1994). These are generally untested hypotheses, which meet the test of logic, but their importance can only be determined by direct research. These are generally untested hypotheses that meet the test of logic, but their importance can only be determined using principles of epistemology. Additionally, all such hypotheses benefit from going beyond physiological ecology alone to incorporate statistical modeling and ecological theory to elucidate complex ecological processes such as resource acquisition.

Several hypotheses have been posited to explain how resources are obtained by individuals. Each of

the hypotheses predicts the evolutionary trait or strategy that will result from natural selection maximizing acquisition of resources. These hypotheses include the nutritional wisdom hypothesis, optimal foraging hypothesis, optimal digestion hypothesis and the cost of switching hypothesis.

### Nutritional wisdom hypothesis

The nutritional wisdom hypothesis posits that animals should forage for nutrients rather than for particular food items. In the case of an herbivore, such as the desert tortoise, individuals are predicted to forage for nutrients, such as protein or calcium, rather than for particular plants. If desert tortoises were to forage for nutrients rather than for particular plants, then tortoises would be predicted to obtain nutrients in proportions different from that obtainable via random foraging. In our tests of this hypothesis, a random diet was defined to be one in which food items are eaten in proportion to their availability in the field. This model posits that animals learn to avoid plants that could be toxic, such as plants containing secondary compounds, or plants deficient or excessive in certain nutrients. Whereas nutrient aversions have been demonstrated in rats, coyotes and several carnivorous vertebrates, the mechanisms underlying such a trait may not apply to herbivores. Many herbivores, including tortoises, can consume a variety of plant species over the course of a day, or during a single feeding bout (Rand 1978). Because the rates at which food passes through the gut in herbivores can span several days (and even several weeks), herbivores are unlikely to discriminate which of the food items eaten caused their illness, and therefore they are less likely to develop an aversion to any particular food item (Zahorik 1977). Additionally, learned food aversions may not be important to desert tortoises because their diet is dominated by annual plant species (Esque 1994), which generally do not have high contents of secondary compounds, such as tannins or resins, that reduce digestive efficiency (Rhoades and Gates 1976).

Experience, or learned preference, has also been proposed to explain how some animals achieve diets rich in nutrients. However, studies have failed to demonstrate that animals learn or acquire diet preferences for particular nutrients (Zahorik and Haupt 1977, 1981). Diets of desert tortoises, which typically consist of a few “favored” plant species (C. Richard Tracy, unpublished data), differ greatly among years (Esque 1994), and are, therefore, not consistent with the learned preferences hypothesis. “Nutritional wisdom” was proposed as a mechanism for the evolution of a strategy whereby animals can obtain

a nutritionally balanced diet Richter (1943). This hypothesis proposes that animals have “specific hungers” that allow them to sense particular nutrient deficiencies, and to detect the presence of each element they require in potential food items (Richter 1943). Many studies, however, have failed to demonstrate these abilities except for sodium and water appetites in rats and moose (Rozin 1977; Zahorik and Haupt 1977; Belovsky 1984; Stephens and Krebs 1986). Another study on green iguanas (*Iguana iguana*) found modest preference for foods high in protein (van Marken Lichtenbelt and others 1993). Despite a lack of understanding of how herbivores make foraging decisions and gain their nutritional requirements, “nutritional wisdom” is frequently cited as a possible mechanism underlying diet selection by desert tortoises (Jarchow 1984; Nagy and Medica 1986; Esque 1994; McArthur and others 1994). Research on desert tortoises has focused on 2 nutrient wisdom hypotheses (Minnich 1977, 1979; Nagy and Medica 1977, 1986; Jarchow 1984; Oftedal and Allen 1996; Oftedal 2002; Oftedal and others 2002). The first hypothesis centers on balancing the need for calcium, phosphorus and magnesium for bones, shell and eggs (Esque and Peters 1994). Additionally, calcium is required for maintenance of osmotic balance in cells, blood coagulation, nerve transmission and muscular activity (Despopoulos and Silbernagl 1991). Phosphorus is involved in many enzyme systems, particularly in the storage and transfer of energy (Despopoulos and Silbernagl 1991). Calcium and phosphorus are closely interrelated nutritionally, and are generally recommended at a 2:1 or greater C:P ratio in reptilian diets (Jarchow 1984; Donoghue 2006). Imbalances of these nutrients have been shown to cause illness in reptiles generally, such as metabolic bone disease (Jarchow 1984; Donoghue 2006). Thus, the needs for these elements should compel desert tortoises to forage differentially for foods rich in calcium, phosphorus and magnesium.

The second nutritional hypothesis relates to the fact that tortoises have fewer options than do other reptiles of excreting excess dietary potassium. Potassium ion levels in urine increase during dry periods in desert tortoises (Nagy and Medica 1986) because these reptiles lack extra-renal salt glands (Minnich 1977; Nagy and Medica 1986). Excess potassium is harmful to desert reptiles (Minnich 1977) and may contribute to the interruption of general cellular and nervous system functions. Desert tortoises sequester potassium in the bladder, where potassium combines with nitrogen to produce potassium urate crystals. Desert tortoises also store urine in their bladders because water availability is stochastic and access to standing

water can be separated by months, or even years (Peterson 1996; Henen and others 1998). Tortoises typically stop eating and become dormant when their urine osmotic concentrations approach those of their blood plasma (Minnich 1977, 1979; Nagy and Medica 1986). Because the potassium in the bladder crystallizes, the water in the bladder remains relatively dilute and is thus more beneficial as a source of water during lengthy periods between drinks. Consequently, if desert tortoises have nutritional wisdom, they should (1) avoid potassium in the diet, (2) seek plants high in protein as a source of nitrogen for potassium urate production and (3) select plants with high water content (Nagy and Medica 1986). Oftedal (2002) hypothesized that desert tortoises should select foods that maximize what he terms their potassium excretion potential (PEP), and he provided an equation to calculate the PEP index for plants based upon their potassium, protein and water contents.

### Optimal foraging hypothesis

The optimal foraging hypothesis has a rich literature dating back to the 1960s (Emlen 1966; MacArthur and Pianka 1966), and it predicts the circumstances under which an animal should take a prey item in relationship to all other prey items available. The hypothesis is formalized in the equation (Schoener 1971):

$$\frac{\sum_{i=1}^n p_i e_i + p_x e_x - C_s T_s}{\sum_{i=1}^n p_i t_i + p_x t_x \bullet T_s} > \frac{\sum_{i=1}^n p_i e_i - C_s T_s}{\sum_{i=1}^n p_i t_i \bullet T_s} \quad (1)$$

where:  $p_i$  = the frequency of any food item  $I$ ;  $e_i$  = the energy contained in any food item  $I$ ;  $t_i$  = the time to pursue any food item  $I$ ;  $p_x$  = the frequency of a considered food item  $x$ ;  $e_x$  = the energy contained in a considered food item  $x$ ;  $t_x$  = the time to pursue a considered food item  $x$ ;  $C_s$  = the cost of search for an average item per unit time  $T_s$ ;  $T_s$  = the mean search time.

In general, the hypothesis predicts that an individual should search and pursue a particular food item ( $x$ ), if and generally only if, including that particular food item will provide more energy per unit time than searching for and pursuing an average item in the environment. When an individual forages in ways that optimize acquisition of energy per unit time as suggested in Equation 1, several predictions can be derived (Schoener 1971; Glander 1981). Two general predictions are especially important. First, when a forager is in an energy-rich environment, the time required to search for a new food item will be short,

and the equation will be dominated by the time and energy related to pursuit of the item. When that is the case, minimizing pursuit time and maximizing energy retrieved from each food item can be accomplished best by picking the “best” food items available (for example, highest in energy) from the abundance of items in the environment. In other words, the forager should become a diet specialist. On the other hand, in an energy-depauperate environment, the search time dominates the equation, and passing up the rare food items in such an environment would result in another long search by the forager. Thus, when search times dominate the equation, the forager should pursue and take essentially every item discovered, and the predator should become a diet generalist.

In the case of herbivorous desert tortoises, the food items are sedentary plants, and the abundance of food items depends upon rainfall. When rainfall is average or plentiful, there will be an abundance of edible plants (annual forbs and grasses) available for a brief time before the plants dry in the desert heat and eventually blow away. During this time of plant abundance, the search time for food items is short, so the optimal foraging hypothesis predicts that desert tortoises should specialize on the plants with the most assimilable energy.

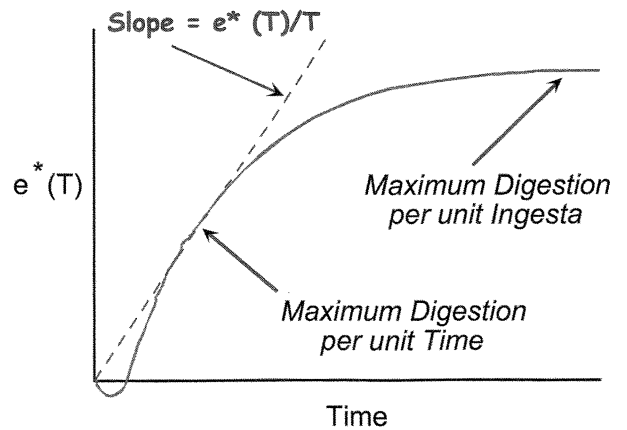
### Optimal digestion hypothesis

Sibley (1981) shaped the concept of predicting the ways in which natural selection should mold digestive processes, digestive morphology and ecological choice of food items in relation to maximization of resource acquisition. Energy extracted by digestion per unit time can be calculated from the equation (Sibley 1981):

$$E/T = (m/T) * e^*(T) \quad (2)$$

Here,  $E$  = energy extracted from food,  $T$  = time food is in the gut,  $m$  = mass of food ingested and  $e^*(T)$  = function of energy extracted as a function of time in gut.

Sibley's ideas stem from the relationship between percent of energy in the ingesta that is digested/assimilated in relation to the time that ingesta spends in the gut (Fig. 2). In the first moments, minutes, hours, or days after food is ingested (depending on the kind and size of the animal), more metabolic energy is expended by the digestive process than is derived from the digestion of the food. Next, the energy derived from the digestive process monotonically increases in relation to the time food is present in the gut until the maximum amount



**Fig. 2** Theoretical relationship between energy extracted during digestion as a function of time food is in the gut. The maximum rate of digestion occurs at the tangent to the monotonically increasing line of energy extraction as a function of time food is in the gut. The maximum amount of energy digested from the food occurs when the food is retained in the gut at least as long as it takes to extract all the energy possible from the food.

of energy that can be extracted is assimilated (Penry and Jumars 1986, 1987). Thereafter, food remaining in the gut will produce little, or no, additional assimilated energy (Fig. 2). This relationship leads to some predictions about optimizing digestion. First, the maximum rate of digestion per unit time will occur when food is retained the length of time calculated as the tangent to the monotonically increasing energy extraction of food as a function of time food is in the gut (Fig. 2). Second, the maximum amount of energy extractable per unit ingesta will occur when the food is retained in the gut for a long time (Fig. 2). Thus, it can be inferred that optimal digestion will occur in food-limited environments when food is retained in the gut until the digestion process results in the maximum amount of energy assimilated per unit food ingested. Conversely, optimal digestion in energy-rich environments will occur when food is not held until the maximum amount of energy is extracted from each unit of ingesta, but rather when food is rushed through the gut at a rate that maximizes the rate of digestion per unit time (Fig. 2). These general predictions are applicable to desert tortoises and both situations concerning food abundance can occur for these reptiles in different places and at different times of the year.

### Cost of switching hypothesis

Desert tortoises are herbivores that have the capacity to ferment the fiber portion of plants, resulting in production of volatile fatty acids that can be absorbed

as an energy source (Bjorndal 1997). The fermentation process is facilitated by a community of endosymbiotic microbes in the gut (Bjorndal 1997). The composition of the endosymbiotic community depends upon the substrate (that is, the digesta), and each time the substrate changes, the composition of the community of endosymbionts also changes (Van Soest 1982; DL Andres, RE Espinoza, P Wilson, SM Secor, and RI Mackie unpublished data). When the community is changing in composition, it is not maximally efficient and the amount of energy or nutrients derived from fermentation by the community will be less than it would be when the community is in equilibrium with the food ingested (Van Soest 1982, 1994). For example, the endosymbiont community of herbivorous desert iguanas (*Dipsosaurus dorsalis*), whose transit time is just 2 days, took up to 2 weeks to stabilize following a switch in diet (DL Andres, RE Espinoza, P Wilson, SM Secor, and RI Mackie unpublished data). The time it takes for the community to equilibrate to the new food is unknown for desert tortoises, but it likely takes several weeks as the transit time for digesta in adult desert tortoises is nearly 2 weeks (Dean-Bradley 1995; Tracy and others 2006). Because the length of time during which green forbs and grasses are available as food for tortoises in any year is usually less than 2 months, any periods of inefficient digestion could significantly reduce the amount of energy that a tortoise could obtain from the plants it eats during the activity season. We term this reduction in efficiency, “the cost of switching” to imply that every time a tortoise switches the kinds of plants it eats, there is a cost in efficiency of digestion.

## Tests of the resource acquisition hypotheses

Tests of resource acquisition hypotheses require both field observations of tortoises, laboratory analyses of the composition of plants and laboratory experiments of digestion by tortoises.

### Plant production in the field

Data on plant productivity were collected from a field site immediately north of St George, Utah, in the northeastern Mojave Desert, from May to June 1991. Plant production was estimated from 1 m<sup>2</sup> quadrats sampled at 10 m intervals along random transects that were 50 m in length. Above-ground biomass production was measured for each species of nonwoody plant, and each quadrat was treated as a random sample point. One hundred and eight quadrats were sampled during the study. Adequacy of sampling for

annual species was determined by graphing the cumulative variance in the estimated mean biomass versus the total mean biomass for all species on 1 m<sup>2</sup> quadrats. Sampling was considered adequate if the slope was not different from 0 for 5 consecutive transect points. Vegetation transects were completed within 3 weeks during peak plant production. Plant samples were dried at 45°C to a constant mass and weighed to estimate annual plant biomass. Biomass production of annuals was estimated for individual plant species and as total mean plant biomass.

### Nutrient content of plants

Dried plant samples of annual forbs and grasses at the St George site were analyzed for nutrient content according to the Hach Digestahl method, using a DR2000 Spectrophotometer (Hach, Loveland, CO). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed using a modified Van Soest (1982) procedure (Hammond 1989). To test the nutrient wisdom hypothesis that tortoises forage for nutrients to deal with excess potassium, we computed the PEP index of nutrients developed by Oftedal (2002). PEP indices were computed for both actual and random diets for comparisons. Because we did not know the water content of the plants in either the actual or random diets, we computed the PEP indices for 2 possible water contents of plants that likely bracketed actual values: 50 and 85% water content.

### Actual tortoise diets

Thirteen adult tortoises (6 males and 7 females) from the St George site were observed with binoculars at a distance of at least 10 m, and each bite of every plant eaten by the tortoises was recorded. A total of 22 140 bites were observed being taken by 13 tortoises in June of 1991. The amount of each nutrient taken by the tortoises was calculated to be the sum of the amount of each nutrient contributed by each plant species eaten (assuming that each bite produced an equal mass of food intake). Thus, the amount of nutrient consumed by each tortoise was calculated as the proportion of the nutrient in each plant species eaten weighted by the proportion of all bites of each plant species in the sample of bites for each individual tortoise. This is equivalent to summing the amount of nutrient from each bite, for each plant species, across all bites and expressing that amount as a percent of the total amount of food eaten by the tortoise.

### Random tortoise diets

A random diet was calculated from data on plant “availability” using a random bite generation model

written in True Basic. Plant availability was defined to be the estimated percent above-ground plant biomass for each species in the habitat for the given calendar month. The model drew a random number between 0 and 1 and matched that value with the plant species that comprised that relative fraction of plant species availability. For example, if the plant species *Bromus madritensis* comprised 20% of the above-ground biomass in the habitat, then all random numbers drawn between 0.80 and 1.00 would be assigned as a bite of that species. This process was repeated until the number of simulated bites for each tortoise was equal to number of actual bites observed for each tortoise during the field observations. Based on the plant nutrient percentages derived from laboratory analyses for each plant in each month, the model then calculated the percentage of each nutrient constituent that would have been acquired in a random diet of the same number of bites eaten by each tortoise. The model was iterated 1000 times to give a distribution and estimate of the mean and variance of the random diet prediction for each tortoise in each month. This yielded a random diet (null model) for comparison with the actual diet of each animal, using the same number of bites taken by each tortoise. Differences between actual and random diets were considered significant, if the mean actual tortoise diet value fell outside of the central 95% of the distribution of random diets.

### Switching experiment

Two groups of 10 captive-born juvenile tortoises (mean initial carapace length = 70 mm) were fed diets of a relative indigestible grass (*B. madritensis*) and a more digestible forb (*Erodium cicutarium*), and then the diets were switched for both groups. The diets were made up by grinding dried plants to the same size using a Wiley Mill, and hydrated to 80% water content just prior to feeding. Tortoises were kept in individual containers (30 × 30 × 10 cm) with plastic grating on the floor to reduce coprophagy. These containers were randomly rotated each day through an environmental chamber with a 14L:10D photoperiod and a thermal cycle of 34°C during photophase and 28°C during scotophase. Tortoises were soaked in water weekly. Each experimental group was acclimated to their initial *ad libitum* diet for 14 days after which we measured food intake and feces production for 28 days. After data were collected on the initial diets, the groups were switched to the other diet and intake and feces production were measured for an additional 28 days. Apparent dry-matter digestibility (DMD) was calculated as the dry matter intake on any

day (X) minus the dry feces production on day (X+12) divided by the dry matter intake. Apparent DMD generally underestimates digestive efficiency because some host tissue (for example, blood, epithelial lining) may be eliminated with feces (Van Soest 1982). DMD is the percent of food intake in that is digested. There are 2 assumptions: (1) the feces appearing 12 days after food intake on day X is egesta for food taken on day X [mean transit time for the dry portion of food fed to juvenile desert tortoises was ~12 days (Tracy and others 2006)], and (2) the percent differences between the apparent digestibilities and the actual digestibilities are small and not related to the food eaten.

## Results

### What did tortoises eat?

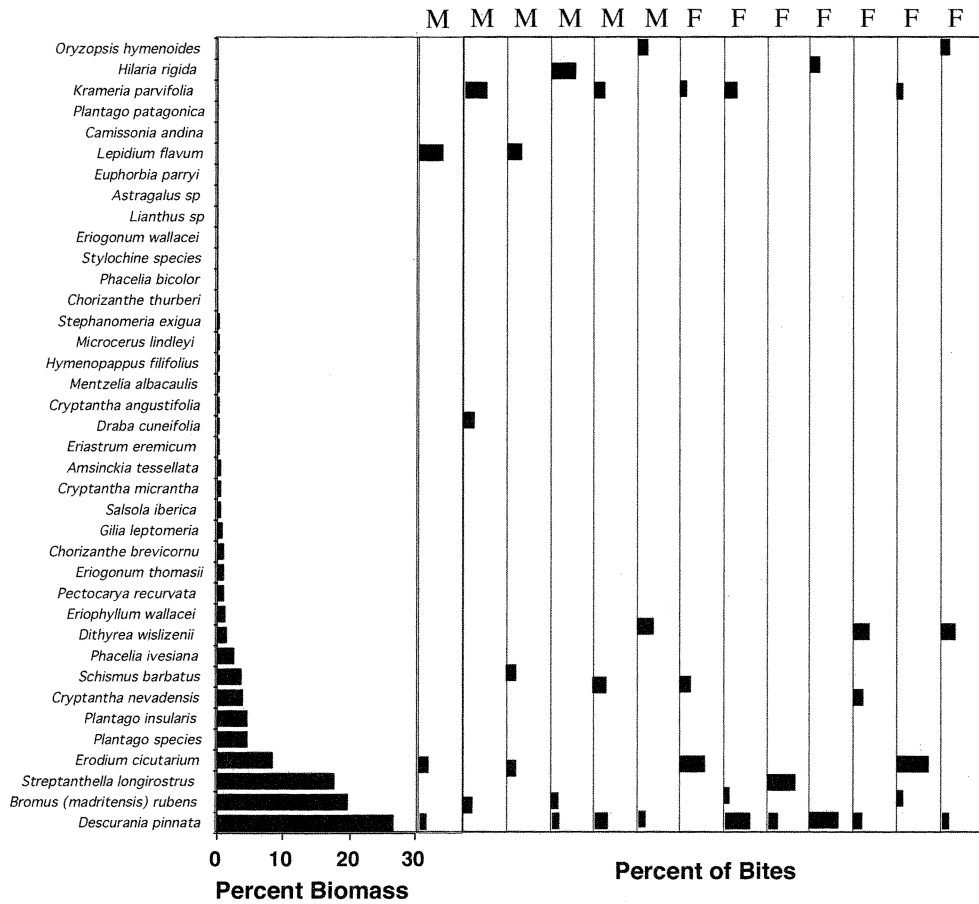
Approximately 100 species of plants were identified at the study site, and only 8 plants comprised ~90% of the plant biomass (Fig. 3). Of those 8 abundant plants, half are nonnative species. Tortoises clearly did not simply eat the most abundant species available to them. Indeed, tortoises frequently included very rare species in the forage plants comprising most of their diets (Fig. 3).

The species of plants eaten were ranked by preference for each individual tortoise based entirely upon the relative number of bites of each plant species eaten. On average, ~90% of each tortoise's diet was composed of the first 5 ranked plant species (Fig. 4). Thus, tortoises tended to consume only about 5 species, out of the more than 100 species of annual forbs and grasses available at the study site (Esque 1994). The species ranked among the top 5, however, were not the same for all individuals. The total number of plant species that were counted as being within the top 5 for all 13 tortoises included more than 30 species (Fig. 5).

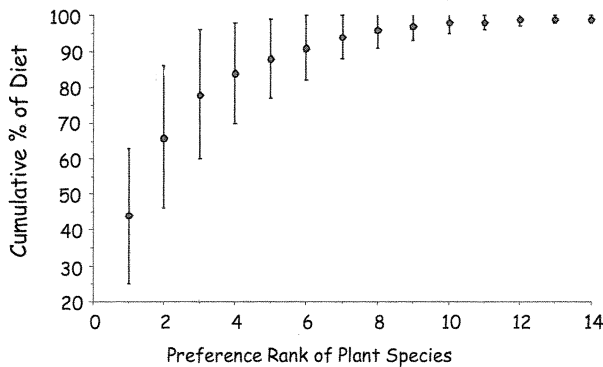
### Nutritional wisdom hypothesis

The actual diets of tortoises were compared to random diets using a resampling technique. The approach can be visualized in Fig. 6 in which the actual diets are portrayed as histogram bars and the 95% confidence range of random diets calculated by resampling is portrayed as a gray zone. Each histogram bar located outside the 95% confidence range of random diets is significantly different from random foraging. When actual diets are more frequently greater than the distribution of random diets (as was the case for Protein and Mg<sup>++</sup>), then tortoises, on average, significantly include more of those components than would be expected by random foraging. Similarly, when actual diets are more frequently less than the

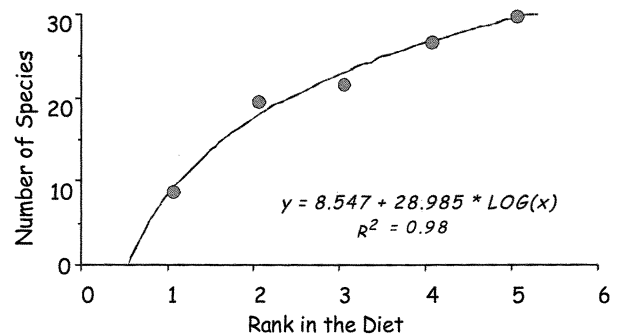




**Fig. 3** Percent biomass of forage plants at the St George site arranged in order of relative biomass at the site. The boxes to the right of the plant biomasses have histogram bars that are depictions of the relative number of bites, of particular plant species, taken by 6 male (M) and 7 female (F) tortoises. Thus, a bar, for any particular plant species, filling half of the width of the box represents 50% of the bites taken by that tortoise. In most cases, only 2 or 3 plant species per individual tortoise are represented, but that number of species summed to 75–100% of all bites by each tortoise.

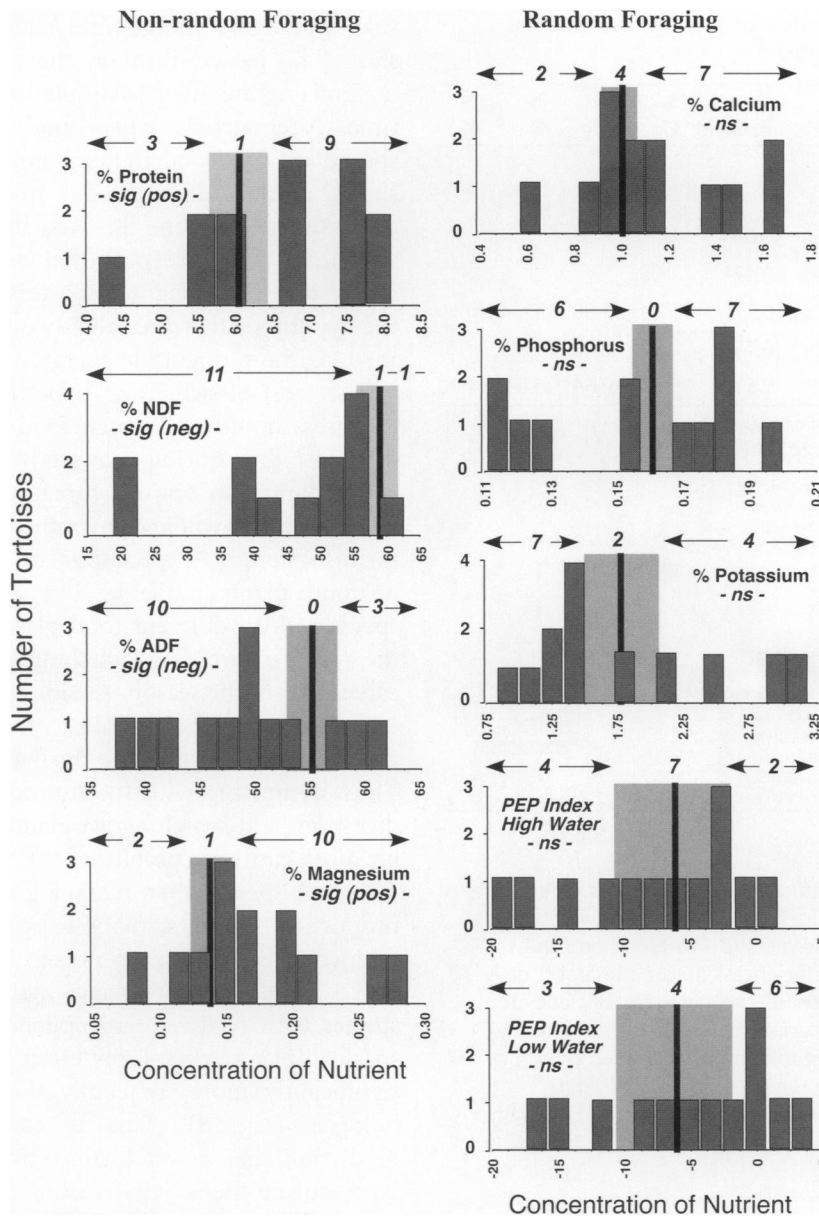


**Fig. 4** The mean cumulative percent of diets made up by plant species ranked in order of tortoise preference as determined by the relative number of bites of each species taken by 13 adult tortoises in nature. Thus, the mean percent of the entire diet which is composed of the highest ranked plant species is 44%, the mean percent of the entire diet made up of both the first-ranked and second-ranked species is 67%, etc. The 5 highest-ranked food species make up ~90% of the diets of the focal tortoises. Error bars are the 95% confidence limits.



**Fig. 5** The number of plant species comprising the first, second, third, fourth, and fifth-ranked species taken by 13 adult tortoises. Thus, 9 plant species were the first ranked species by the 13 focal tortoises.

distribution of random diets (as was the case for NDF and ADF), then tortoises, on average, significantly exclude those components more frequently than would be expected by random foraging. Potassium, calcium and phosphorus appeared in diets in proportions approximately expected from random foraging (Fig. 6).



**Fig. 6** Frequency diagram of the percent of tortoises (out of 13 individuals) with diets differing in percent of dietary components. The histogram bars represent the observations concentration of nutrient for actual diets, and the mean and 95% confidence limit of the random diets are presented as a bold line and gray bar at the back of the graphs. The numbers above the histograms are the number of tortoises with diets less than, the same as, and greater than expected by random foraging. Graphs at the left of the figure are of dietary components consumed in abundances different from those expected as a result of random foraging, and the graphs at the right of the figure are for dietary components that were, on average, consumed randomly. In addition to particular nutrients, PEP indices (Oftedal 2002) were analyzed against random foraging. The “high” value of PEP indices assumes 85% water content in plants, and the low value assumes 50% water in plants.

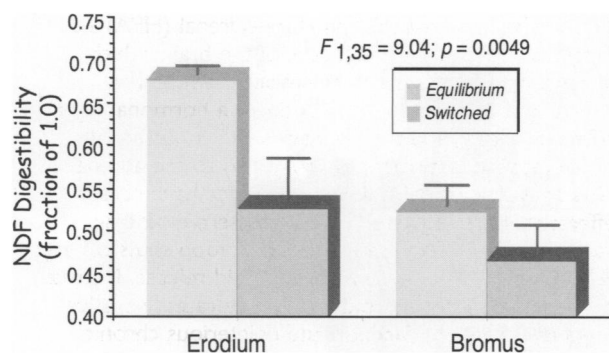
Interestingly, even when the sample of 13 observed tortoises consumed a particular nutrient in proportions expected from random foraging, individual tortoises, nevertheless, sometimes obtained much less, or much more, nutrient than expected by random foraging. Thus, there was always a great deal of individual variation among tortoises in terms of nutrients consumed.

There were many significant correlations among nutrients in actual diets. Thus, diets rich in protein were significantly high in all other nutrients but low in fiber (Table 1). Calcium is significantly correlated with potassium and magnesium (Table 1). Finally, the PEP indices of actual diets for tortoises were statistically indistinguishable from those of random diets (Fig. 6).

**Table 1** Correlation matrix of nutrients found in the actual diets of 13 tortoises

	% NDF	% ADF	% Protein	% Ca	% Mg	% P	% K
% NDF	1						
% ADF	0.43*	1.00					
% Protein	-0.61*	-0.51*	1.00				
% Ca	-0.31	-0.11	0.42*	1.00			
% Mg	-0.30	-0.24	0.52*	0.74*	1.00		
% P	-0.65*	-0.55*	0.75*	0.32	0.32	1.00	
% K	-0.36	0.04	0.53*	0.53*	0.64*	0.44*	1.00

Asterisks in each box indicate that the correlation is significant at the 95% probability level.



**Fig. 7** Mean digestive efficiencies of tortoises eating diets of a grass (*Bromus*) and a forb (*Erodium*) when tortoises were equilibrated to the diets for 1 month, and the comparative digestive efficiencies during the first 5 days after the diets are switched. The digestive efficiency for forbs (*Erodium*) and for grass (*Bromus*) was significantly lower (by ~50%) during the first 5 days after switching the diet. Error bars are the 95% confidence limits.

### Diet switching

The digestive efficiency for grass (*Bromus*) and forbs (*Erodium*) significantly reduced to approximately half during the first 5 days after tortoises were switched onto the new diets (Fig. 7).

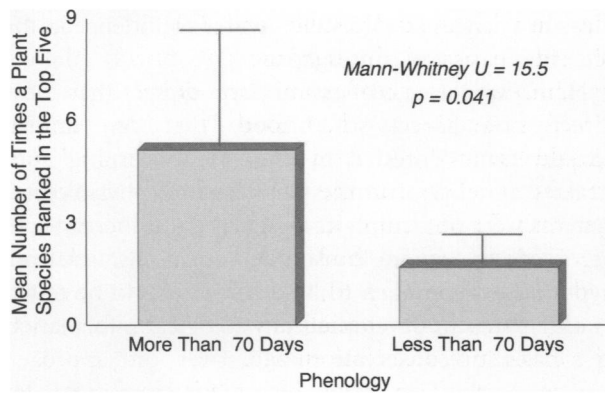
### Integrated resource acquisition hypothesis

Generally, food is available for desert tortoises in the Mojave Desert in spring, and sometimes in summer. Ordinarily, in years of adequate rain, food for tortoises will be available in excess until the plants dry and blow away. In years of inadequate rain, few or no plants germinate. Thus, generally, desert tortoises will either have an abundance of food, or they will have almost none (or sometimes none). Combining the predictions for desert tortoises from the optimal foraging and optimal digestion hypotheses, we get 2 salient predictions for environments, or years, of abundant food availability: (1) tortoises should avoid food items

that are highly indigestible and (2) food ingested should be passed through the gut quickly, thereby maximizing the rate of assimilation of energy per unit time. Alternatively, when food is scarce, tortoises should eat any food item encountered, and all food should be retained in the gut until all the energy can be assimilated from it. Actually, desert tortoises significantly avoided foods that were high in relatively indigestible fiber (Fig. 6), and this is concordant with the hypothesis that specialists would select foods with the maximum digestible energy.

The cost-of-switching hypothesis indicates that tortoises should not switch food types. The data on tortoises' diet during our study are simultaneously concordant with optimal foraging, optimal digestion and switching avoidance hypotheses. In particular, the tortoises definitely specialized on a small proportion of food plants available, but different individuals specialized on different food plants, and this may be the result of avoiding switching from the first foods eaten early in the season. Avoiding switching would be easiest, if tortoises specialize on plants that remain green for the longest time during the activity season. Thus, complying with the optimal foraging, optimal digestion, and switching avoidance hypotheses would be most easily accomplished, if tortoises specialized on plant species that remain green for the longest proportion of the activity season. We assessed the frequency with which top-ranked food species (species ranked in the top 5 for any individuals) were plant species with relatively long phenologies. Top-ranked species (plant species preferred by tortoises) were significantly more frequently those with long phenologies (Fig. 8). This is concordant with the prediction that desert tortoise avoid switching food types during the activity season.

In addition to specializing in foods with long phenologies, and specializing in foods high in protein and low in fiber, tortoises also significantly took foods that provided more magnesium, which are important elements for producing bones, shells and eggs. There was no evidence, however, that desert tortoises foraged in ways that maximized their PEP (Fig. 6). The fact that there is a great deal of correlation among the nutrients in the plants eaten by tortoises makes it difficult to specialize in particular nutrients. Taking foods rich in protein and low in fiber, however, will generally result in a diet with more digestible energy, calcium, magnesium, phosphorus and potassium as predicted by all optimal resource acquisition hypotheses except the PEP hypothesis. The PEP hypothesis is neither supported by the data on tortoises' diet, nor is it concordant with the other optimal resource acquisition hypotheses.

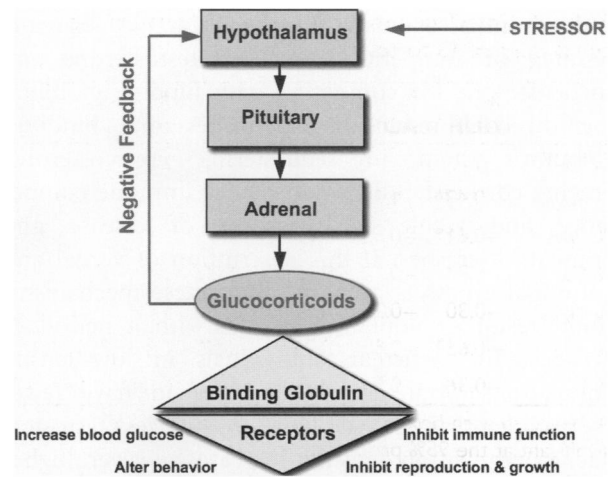


**Fig. 8** The mean number of times that a plant species eaten by monitored tortoises ranked within the top 5 most favorite food types in relation to the phenology of the plant species. Phenologies were divided into those species that remained green for more than 70 days during the growing season, and those species that remained green for less than 70 days during the growing season. Error bars are the 95% confidence limits.

## Stress, disease and population dynamics

The desert tortoise was originally federally listed as an endangered species because a respiratory disease seemed to have become a dangerous epidemic causing population declines in some parts of the range, and that hypothesis remains important as conservation activities are considered today (Tracy and others 2004). Elucidating and clarifying the known, and partially known, relationships among resource acquisition, stress and disease may provide insights that will be helpful to conservation programs. There is solid evidence that stress reveals itself in the production of “stress hormones” such as glucocorticoids, and that this production of glucocorticoids (Fig. 9) can be adaptive to organisms because it can increase blood glucose that might be needed to respond to the stress (Romero 2004). Free glucocorticoids, however, also can alter behavior, suppress reproduction and growth, and inhibit immune function (Dunlap and Schall 1995; Guillette and others 1995; Hopkins and others 1997; Fowler 1999; Sapolsky and others 2000; Romero and Wikelski 2001, 2002), and high titers of glucocorticoid correlates with stress and can predict increased mortality (Romero and Wikelski 2001).

The physiological system that deals with stress has 2 regulatory pathways: the HPA (hypothalamus, pituitary, adrenal glands) pathway responsible for sensing stress and producing glucocorticoids, and the protein-binding process that binds plasma glucocorticoids with a binding globulin. Protein binding may



**Fig. 9** The hypothalamic–pituitary–adrenal (HPA) axis. Stress is detected by the cortex of the brain, which sends a neuronal signal to the hypothalamus. The hypothalamus subsequently produces a hormonal signal to the pituitary, and the pituitary sends a hormonal signal (adrenocorticotropin or ACTH) to the adrenal to release glucocorticoids. Glucocorticoids have several effects that are regulated by corticosteroid-binding globulins (CBG). A negative feedback loop shuts off the HPA pathway leading to glucocorticoid release. If stress persists and glucocorticoids remain elevated, negative feedback ceases to function and deleterious chronic effects of glucocorticoids begin. After Romero (2004).

be a way for the system to respond quickly to excesses of glucocorticoids, which can have several detrimental effects in terms of growth, reproduction and immune competence. Thus, while stress can result in the HPA producing high levels of glucocorticoids, binding globulins can attach to the glucocorticoids and reduce the steroid’s activity for both positive and/or negative effects (Romero 2004). Ordinarily, up to 90% of the corticosterone in the blood can be attached to binding globulins, which further suggests that binding globulins represent a control mechanism for this response to stress.

The important glucocorticoid that responds to stress in desert tortoise is corticosterone, and the chemical structure of corticosterone is very similar to the sex hormone testosterone (Hammond 1995; Breuner and Orchinik 2002). Not only are these 2 hormones similar in structure, but the binding globulin for controlling plasma titers of corticosterone also bind with testosterone (Hammond 1995). Furthermore, the seasonal dynamics of corticosterone and testosterone in desert tortoise are virtually identical (Lance and others 2001). This similarity in form and function between corticosterone and testosterone creates a potentially dangerous outcome for populations of desert tortoises. In particular, if increased production of testosterone during vernal

and autumnal agonistic and reproductive behaviors resulted in competition between testosterone and corticosterone for combining with binding globulin, then this could reduce the effectiveness of the binding-globulin system in sequestering corticosterone. Freeing corticosterone could reduce immune competence and result in outbreaks of disease and population crashes. If this description of vernal and autumnal events is accurate, then these mechanisms could result in population cycles with a period of decades. In particular, individuals in low-density populations would be more likely to have fewer agonistic encounters, and thus produce less testosterone. As population densities increase, however, higher titers of testosterone could increase the vulnerabilities of individuals to disease. If behavioral, physiological and ecological mechanisms occur according to this model, then population crashes and cycles could be normal processes in the biology and ecology of desert tortoises. Recognizing this is critically important as management and conservation plans are developed.

### Science, physiology, ecology and conservation

In this paper, we have shown that the field of physiological ecology has become pivotally important in understanding threats to the persistence of sensitive species in conservation. Physiological data (without analyses or syntheses), however, are frequently used as part of “informed opinion” that is used, in turn, to guide management decisions. For example, conservation agreements, recovery plans and habitat conservation plans are commonly based upon “informed opinion” rather than upon published scientific analyses. Sometimes informed opinion is the only available basis for the urgent needs in conservation, but there are limits to the power of using untested hypotheses to guide conservation. Here, we have illustrated a particular approach to using science to inform conservation. Specifically, we have used theory and experiments to test hypotheses that form new syntheses of physiological and ecological knowledge and new theory that can engender confidence in a scientific basis for decisions concerning conservation. It is important to be cautious about the fact that untested hypotheses can lead conservation managers to propose initiatives that are dangerously opposite of that which would be proposed with sounder scientific advice. Thus, we propose recognizing the difference between untested ideas and knowledge devolving from tested hypotheses—especially knowledge that integrates well into a fabric of scientific theory (revealing connections to several areas of science and several

ideas in science) should yield greater confidence in the scientific basis for conservation.

Here, we selected several hypotheses that bear directly on aspects of biology that can inform conservation. Untested hypotheses concerning constraints and opportunities imposed by physiological systems were not simply accepted as truth thereafter to be used to guide conservation actions. Instead, hypotheses were taken to be propositions to be tested as a first step in developing physiological information as a basis for conservation. Moreover, our approach was explicitly focused on scientific assessments of how anthropogenic modifications of the environment (for example, domestic grazers or weedy plant species, which could potentially modify available nutrients to herbivorous wildlife) impact physiological processes that could be important for populations.

Guided by the principles stated here, we assembled existing hypotheses pertinent to resource acquisition by desert tortoises because there have been many assertions that anthropogenic change (for example, introducing domestic grazers to the Mojave, facilitating invasions of nutrient-poor weedy plants to the Mojave) has negatively affected populations of desert tortoises. We explicitly assembled hypotheses about the many levels of resource acquisition including behavior, physiology and ecology. Our tests of those hypotheses have led to a new synthesis we call an integrated theory of resource acquisition for desert tortoises (or any reptilian herbivore for that matter). The synthesis asserts that when food is available to desert tortoises, individuals should specialize in plant species that tend to be relatively low in fiber and remain green for long periods during the activity season.

When dietary needs are not easily met, tortoises could become stressed, and stress can lead to a compromised immune system and susceptibility to disease. Knowing how this system works also points to a means of assessing when environmental change can lead to stress and disease. Measurement of unbound corticosterone can indicate stress and the potential for contracting disease. Measurement of plasma corticosterone could be incorporated into population-monitoring programs to assess the efficacy of conservation actions.

Finally, our syntheses evoke a new hypothesis. The fact that both testosterone and corticosterone are sequestered from being free in blood plasma by the same binding globulins, suggests a hypothesis that could lead to natural population crashes and population cycles with very long periodicities. If such cycles occur, they need to be factored in as conservation actions are considered insofar as some population

fluctuations in desert tortoises may be natural phenomena as opposed to those of anthropogenic origin. Regardless of the cause of population fluctuation, knowing that some fluctuation may be natural should help understand anthropogenically caused fluctuations and assist in guiding management decisions.

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## References

- Belovsky GE. 1984. Moose and snowshoe hare competition and a mechanistic explanation from foraging theory. *Oecologia* 61:150–9.
- Bjorndal KA. 1997. Fermentation in reptiles and amphibians. In: Mackie RI, White BA, editors. *Gastrointestinal microbiology*. New York: Chapman and Hall. p 199–230.
- Blaustein AR, Romansic JM, Scheessele EA, Han BA, Pessier AP, Longcore JE. 2005. Interspecific variation in susceptibility of frog tadpoles to the pathogenic fungus *Batrachochytrium dendrobatidis*. *Conserv Biol* 19:1460–8.
- Breuner CW, Orchinik M. 2002. Plasma binding proteins as mediators of corticosteroid action in vertebrates. *J Endocrinol* 175:99–112.
- Brown JH, Maurer BA. 1989. Macroecology: the division of space among species on continents. *Science* 243:1145–50.
- Carey C. 2005. How physiological methods and concepts can be useful in conservation biology. *Integr Comp Biol* 45:4–11.
- Chown SL, Gaston KJ, Robinson D. 2004. Macrophysiology: large-scale patterns in physiological traits and their ecological implications. *Funct Ecol* 18:159–67.
- Cowles RB, Bogert CM. 1944. A preliminary study of the thermal requirements of desert reptiles. *Bull Am Mus Nat Hist* 83:265–96.
- Dawson WR. 2005. George A. Bartholomew's contributions to integrative and comparative biology. *Integr Comp Biol* 45:219–30.
- Dean-Bradley K. 1995. Digestive flexibility in response to diet quality in hatchling desert tortoises, *Gopherus agassizii*. Masters Thesis. Ft Collins, CO: Colorado State University.
- Despopoulos A, Silbernagl S. 1991. *Color atlas of physiology*. 4th edition. Stuttgart: Georg Thieme Verlag.
- Donoghue S. 2006. Nutrition. In: Mader DR, editor. *Reptile medicine and surgery*. 2nd edition. St Louis, MO: Elsevier. p 251–98.
- Dunlap KD, Schall JJ. 1995. Hormonal alterations and reproductive inhibition in male fence lizards (*Sceloporus occidentalis*) infected with the malarial parasite *Plasmodium mexicanum*. *Physiol Zool* 68:608–21.
- Emlen JM. 1966. The role of time and energy in food preference. *Am Nat* 100:611–7.
- Esque TC. 1994. Diet and diet selection of the desert tortoise (*Gopherus agassizii*) in the northeast Mojave Desert. MS Thesis. CO: Colorado State University. p 243.
- Esque TC, Peters EL. 1994. Ingestion of bones, stones and soil by desert tortoises. In: Bury RB, Germano DJ, editors. *Biology of North American tortoises*. Reston, VA: US Department of Interior National Biological Survey, Fish and Wildlife Research 13. p 105–12.
- Feder ME, Bennett AF, Burggren WW, Huey RB. 1987. *New directions in ecological physiology*. Cambridge: Cambridge University Press.
- Fowler GS. 1999. Behavioral and hormonal responses of Magellanic penguins (*Spheniscus magellanicus*) to tourism and nest site visitation. *Biol Conserv* 90:143–9.
- Glander KE. 1981. Feeding patterns in mantled howling monkeys. In: Foraging behavior, ecological, ethological, and psychological approaches. New York: Garland STPM Press. p 231–58.
- Groom MJ, Meffe GK, Carroll CR. 2005. *Principles of conservation biology*. 3rd edition. Sunderland, MA: Sinauer and Associates.
- Guillette LJ, Cree A, Rooney AA. 1995. Biology of stress: interactions with reproduction, immunology and

- intermediary metabolism. In: Warwick C, Frye FL, Murphy JB, editors. Health and welfare of captive reptiles. London: Chapman and Hall. p 32–81.
- Hammond KA. 1989. The role of diet quality and energy need in the nutritional ecology of a small herbivore. PhD dissertation. Fort Collins, CO: Colorado State University.
- Hammond GL. 1995. Potential functions of plasma steroid-binding proteins. *Trends Endocrinol Metab* 6:298–305.
- Henen BT, Peterson CC, Wallis IR, Berry KH, Nagy KA. 1998. Desert tortoise field metabolic rates and water fluxes track local and global climatic conditions. *Oecologia* 117:365–73.
- Hopkins WA, Mendonca MT, Congdon JD. 1997. Increased circulating levels of testosterone and corticosterone in southern toads, *Bufo terrestris*, exposed to coal combustion waste. *Gen Comp Endocrinol* 108:237–46.
- Jarchow JL. 1984. Veterinary management of the desert tortoise, *Gopherus agassizii*, at the Arizona-Sonora Desert Museum: a rational approach to diet. In: Proceedings of the Desert Tortoise Council. p 83–94.
- Lance VA, Grumbles JS, Rostal DC. 2001. Sex differences in plasma corticosterone in desert tortoises, *Gopherus agassizii*, during the reproductive cycle. *J Exp Zool* 289:285–9.
- MacArthur RH, Pianka ER. 1966. On optimal use of a patchy environment. *Am Nat* 100:603–9.
- McArthur ED, Sanderson SC, Webb BL. 1994. Nutritive quality and mineral content of potential desert tortoise food plants. United States Department of Agriculture, Forest Service Intermountain Research Station, Research Project INT-473. p 26.
- Meffe GK, Carroll CR. 1994. Principles of conservation biology. Sunderland, MA: Sinauer and Associates.
- Meffe GK, Carroll CR. 1997. Principles of conservation biology. 2nd edition. Sunderland, MA: Sinauer and Associates.
- Minnich JE. 1977. Adaptive responses in the water and electrolyte budgets of native and captive desert tortoises, *Gopherus agassizii*, to chronic drought. In: Proceedings of the symposium of the Desert Tortoise Council. p 102–29.
- Minnich JE. 1979. Comparison of maintenance electrolyte budgets of free-living desert and gopher tortoises. In: Proceedings of the symposium of the Desert Tortoise Council. p 166–74.
- Nagy KA, Medica PA. 1977. Seasonal water and energy relations of free-living desert tortoises in Nevada: a preliminary report. In: Proceedings of the Desert Tortoise Council. p 152–7.
- Nagy KA, Medica PA. 1986. Physiological ecology of desert tortoises in southern Nevada. *Herpetologica* 42:73–92.
- Oftedal OT. 2002. The nutritional ecology of the desert tortoise in the Mohave and Sonoran deserts. In: Van Devender TR, editor. The Sonoran desert tortoise. Natural history, biology and conservation. Tucson, AZ: University of Arizona Press.
- Oftedal OT, Allen ME. 1996. Nutrition as a major facet of reptile conservation. *Zoo Biology* 15:491–7.
- Oftedal OT, Hillard S, Morafka DJ. 2002. Selective spring foraging by juvenile desert tortoises (*Gopherus agassizii*) in the Mojave desert: evidence of an adaptive nutritional strategy. *Chelonian Conserv Biol* 4:341–52.
- Owen-Smith N, Mills MGL. 2005. Manifold Interactive influences on the population dynamics of a multispecies ungulate assemblage. *Ecol Monogr* 76:73–92.
- Penry DL, Jumars PA. 1986. Chemical reactor analysis and optimal digestion. *Bioscience* 36:310–15.
- Penry DL, Jumars PA. 1987. Modelling animal guts as chemical reactors. *Am Nat* 129:69–96.
- Peterson CC. 1996. Ecological energetics of the desert tortoise (*Gopherus agassizii*): effects of rainfall and drought. *Ecology* 77:1831–44.
- Rand AS. 1978. Reptilian arboreal folivores. In: Montgomery GG, editor. The ecology of arboreal folivores. Washington, DC: Smithsonian Institution Press. p 115–22.
- Rhoades DF, Gates RG. 1976. Toward a general theory of plant antiherbivore chemistry. In: Wallace J, Mansell RL, editors. Biochemical interactions between plants and insects. *Recent Adv Phytochemistry* 10:168–213. New York: Plenum Publishing.
- Richter CP. 1943. Total self-regulatory functions in animals and human beings. *Harvey Lect* 38:63–103.
- Romero ML. 2004. Physiological stress in ecology: lessons from biomedical research. *Trends Ecol Evol* 19:249–55.
- Romero LM, Wikelski M. 2001. Corticosterone levels predict survival probabilities of Galapagos marine iguanas during El Niño events. *Proc Natl Acad Sci USA* 98:7366–70.
- Romero LM, Wikelski M. 2002. Exposure to tourism reduces stress-induced corticosterone levels in Galapagos marine iguanas. *Biol Conserv* 108:371–4.
- Rozin P. 1977. The significance of learning mechanisms in food selection: some biology, psychology, and sociology of science. In: Barker LM, Domjan M, Best MR, editors. Learning mechanisms in food selection. Waco, TX: Baylor University Press. p 557–92.
- Sapolsky RM, Romero LM, Munck AU. 2000. How do glucocorticoids influence stress-responses? Integrating permissive, suppressive, stimulatory, and adaptive actions. *Endocr Rev* 21:55–89.
- Schoener TW. 1971. Theory of feeding strategies. *Annu Rev Ecol Syst* 11:369–404.
- Scholander PF. 1990. Enjoying a life in science. Fairbanks, AL: University of Alaska Press.
- Sibley RM. 1981. Strategies of digestion and defecation. In: Townsend CR, Callow P, editors. Physiological ecology: an evolutionary approach to resource use. Oxford, UK: Blackwell Scientific Publications. p 109–39.
- Stephens DW, Krebs JR (1986). Foraging theory. Princeton, NJ: Princeton University Press.
- Tracy CR, Averill-Murray R, Boarman WI, Delehanty D, Heaton J, McCoy E, Morafka DJ, Nussear K, Hagerty B, Medica P. 2004. Desert tortoise recovery plan assessment. Available at <http://www.brrg.unr.edu/dtrpac/reports/DTRPACreport.pdf>.

- Tracy CR, Dean Bradley K, Zimmerman LC, Castle K, Tracy CR. 2006. Rates of food passage in the digestive tract of young desert tortoises: effects of body size and diet quality. *Chelonian Conserv Biol*. Forthcoming.
- U.S. Fish and Wildlife Service. 1994. Desert tortoise (*Mojave population*) recovery plan. Prepared by: Brussard PF, Berry KH, Gilpin ME, Jackson ER, Morafka DJ, Schwalbe CR, Tracy CR, Vasek FC. Desert Tortoise Recovery Team for Regions 1, 2, and 6, USFWS, Region 1-Lead Region, Portland, Oregon. p 73 + 9 appendices.
- van Marken Lichtenbelt WD, Wessenlingh RA, Vogel JT, Albers KBM. 1993. Energy budgets in free-living green iguanas in a seasonal environment. *Ecology* 74:1157–72.
- Van Soest PJ. 1982. Nutritional ecology of the ruminant. Van Soest PJ, editor. Ithaca, NY: Cornell University Press. 1994.
- Van Soest PJ. 1994. Nutritional ecology of the ruminant. Ithaca, NY: Cornell University Press.
- Wilson EO. 1988. Biodiversity. Washington, DC: National Academy Press.
- Zahorik DM. 1977. Associative and non-associative factors in learned food preferences. TX: Baylor University Press. p 181–200.
- Zahorik DM, Haupt KA. 1977. The concept of nutritional wisdom: applicability of laboratory learning models to large herbivores. TX: Baylor University Press. p 45–70.
- Zahorik DM, Haupt KA. 1981. Species differences in feeding strategies, food hazards and the ability to learn food aversions. New York: Garland STPM Press. p 289–331.