

# SEASONAL VARIATIONS IN THE FOOD HABITS OF THE SOUSS VALLEY TORTOISE, *TESTUDO GRAECA GRAECA*, IN AN ARID AREA OF WEST-CENTRAL MOROCCO

NAWAL HICHAMI<sup>1,2,3,4</sup> AND MOHAMMED ZNARI<sup>1,2</sup>

<sup>1</sup>Laboratory Water, Biodiversity and Climate Change, Research unit Ecology, Conservation Biology and Valorization of Animal Resources, Department of Biology, Faculty of Science, Semailia, Cadi Ayyad University, Bd Prince Moulay Abdellah, Post Office Box 2390, 40000 Marrakech, Morocco

<sup>2</sup>Natural History Museum of Marrakech, Cadi Ayyad University, Avenue Allal El Fassi, Marrakech, Morocco

<sup>3</sup>Laboratory of Biotechnology & Sustainable Development of Natural Resources, Faculty Polydisciplinary of Beni Mellal, University Sultan Mulay Slimane, Mghila Post Office Box 592, Beni Mellal 23000, Morocco

<sup>4</sup>Corresponding author; e-mail: nouna.hichami@gmail.com

**Abstract.**—Like other threatened species, detailed knowledge of the food habits of the Souss Valley Tortoises (*Testudo graeca graeca*) is important for its conservation and management. We determined the composition of the diet and its seasonal changes using fecal microhistological analysis in tortoises from an arid steppe-land of the central Jbilet Mountains, west-central Morocco, between spring 2011 and winter 2012. Tortoises were exclusively herbivorous with a specialist trend selecting rare, very rare, or some abundant plant species. Diets differed among seasons as indicated by Margalef's Richness, Shannon's Diversity, and Evenness indices. The spring diet is more diverse, with 16 plant species consumed versus just five and seven species in summer and autumn-winter respectively, with only five shared species among all seasons. Dietary overlaps are low between spring and other seasons and between summer and autumn. Conversely, there is moderate overlap between the summer diet and no difference between the autumn and winter diets. Trophic niche overlap is highest between spring, winter, and medium between other seasons, and there is a pronounced similarity between autumn and winter. The data we provide on seasonal variations in diet could be useful in developing management plans for tortoises and their natural habitat in arid lands with low productivity.

**Key Words.**—aridity; diet; fecal analysis; seasons; trophic niche overlap; management

## INTRODUCTION

The general dietary habits of the approximately 65 species of terrestrial chelonians in the world indicate that are predominantly 66% are exclusively herbivorous (about 50 species in Testudinidae and about 15 species in Geoemydidae and Emydidae; see Halliday and Adler 2002; Luiselli 2006a), while 33% are omnivorous, and just one species, the Common Box Turtle (*Terrapene carolina*) is predominantly carnivorous (Luiselli 2006a). In addition to general herbivory, previous tortoise diet studies also found that most tortoise species are generalist foragers feeding on a wide range of plant species (Loehr 2002; Rouag et al. 2008; Del Vecchio et al. 2011). They report dietary shifts between leaves and fruits and opportunistic inclusion of mushrooms, depending on the local and seasonal availability of food resources (Fowler de Neira and Johnson 1985; Loehr 2002; MacDonald and Mushinsky 1988; Moskovits and Bjorndal 1990). Dietary patterns vary among species; for example, some tropical species feed mostly on mushrooms, complementing their diets with a few plants and animals, both living

and carrion (Luiselli 2003, 2006a). There is some indication that a generalist foraging strategy may constitute an adaptation to allow for the consumption of toxic plants (Lagarde et al. 2002; Loehr 2002). Selective feeding strategies allow animals to take advantage of ephemeral and annual plant species with particularly high water, nitrogen, or protein content (Jennings 2002; MacDonald and Mushinsky 1988; Oftedal et al. 2002; Hichami and Znari 2023). In this regard, Jennings and Berry (2015) found that Desert Tortoises (*Gopherus agassizii*) in the Mojave Desert of California, USA, were selective foragers choosing food plants non-randomly throughout the foraging season, confirming their hypothesis that these tortoises depend on a few key plant species during different phenological periods of spring.

It is important to understand the habitat requirements of the Spur-thighed Tortoises (*Testudo graeca*) because they are declining in many parts of their range, including west-central Morocco (see Hichami and Znari 2021 for a review). They are globally classified as Vulnerable (VU) in the International Union for the Conservation of Nature (IUCN) Red List (IUCN 2016). While there have been different

studies on spring diet of the subspecies Souss Valley Tortoise (*T. g. graeca*) (Bailey and Highfield 1996; El Mouden et al. 2006; Hichami and Znari 2023), data on the seasonal change in their food habits are still lacking. In spring 2003, with exceptionally high primary productivity, El Mouden et al. (2006) found that at least 88 plant species (belonging to 46 families) were eaten by the Souss Valley Tortoises, mainly Asteraceae, Fabaceae, and to a lesser extent Poaceae, but just five species were preferred. Similarly, Hichami and Znari (2023) reported that in the springs of 2011 and 2012, 90% of the diet of each tortoise in the same area was composed of the first five to six ranked plant species. Thus, these tortoises tended to consume only about five to six species, out of more than 44 annual forbs and 27 species of grasses available at the study site. We used fecal microhistological analysis to investigate sexual and seasonal variations in the diet of free-ranging Souss Valley Tortoises in an arid steppe land of west central Morocco. We also estimated relative abundances of plant species in the spring and compared these with the frequencies of plant taxa consumed by tortoises.

#### MATERIALS AND METHODS

**Study area.**—The study site is a 33 ha, area located in the central Jbilet Mountains about 25 km north of Marrakech, in west-central Morocco (31°37' N, 8°02' W, and 580 m elevation). It is an arid region with a mean annual rainfall of about 240 mm, with most precipitation falling between September and February (El Mouden et al. 1999; Znari et al. 2002). The average air temperature in the hottest month (July) is 39.1° C and the minimal annual temperature averages 5° C and rarely reaches 0° C (Emberger 1933; Le Houérou 1989). Vegetation consists mainly of typical arid vegetation with dispersed shrubs comprised of Lotus Jujube (*Ziziphus lotus*), with sparse Morocco Gum (*Vachellia gummifera*) and Bridal Broom (*Retama monosperma*). Most of the habitat is open hard bare ground with stony soils in the flats, with low hillsides and shale outcrops surrounding small, sandy, pebbly, or stony wadis (riverbeds). Seasonal over-grazing by domestic livestock (sheep and goats) at the site strongly affects the vegetation structure. A simple visual inspection of the study area reveals two distinctive plant assemblages: a relatively rich and diversified plant community under *Ziziphus lotus* where there is protection against over-grazing due to the thorny structure of this shrub, and a less diversified plant community where these bushes do

not occur, with a low percentage of plant cover, apart from winter-spring of unpredictable exceptional rainy and more productive years.

**Diet analyses.**—We quantified the diets of 85 *T. g. graeca* by analyzing fecal pellets collected in spring (April-May 2011), summer (July-August 2011), autumn (October-Nov 2011) and winter (January-February 2012). Tortoises typically defecated during capture and handling, but when this did not happen; we placed tortoises individually in small indoor cages without food until the first defecation occurred (in some instances, for up to 72 h). We then returned tortoises and released them at their capture points in the field.

We oven-dried fecal pellet samples at 60° C for 48 h and stored them in plastic vials for subsequent analyses. We analyzed only the first fecal pellet excreted by each tortoise to avoid pseudo-replication. We ground individual fecal pellets in a mortar and then cleared them in a 0.05 ml solution of NaOH for 3 min. We washed the ground samples with distilled water over 400 and 800 µm sieves and collected the retained material afterward. We homogenized fecal pellets in a mortar, discolored them using a hypochlorite solution, and then sieved them under a series of 0.4–0.8 mm sieves (Chapuis 1980). Fragments longer than 0.4 mm were dried again, washed in a 50% (v/v) glycerin/water solution, repulverized the mass to make a fine, homogeneous sample, and then added this mass back to the sample to help produce a large number of fragments (Butet 1985).

We identified plant taxa by comparing the different features, dimensions, and arrangements of the epidermal cells, along with other taxonomic structures (e.g., cell forms and trichomes, stomata form) of the recovered fragments with those of reference materials prepared through the monthly collection of leaves, stems, flowers, and fruits of the plants found at the study site. This reference material contained 74 plant species and was available at the herbarium of the Natural History Museum of Marrakech - Cadi Ayyad University, Morocco. We used a practical flora of Morocco (Fennane et al. 2008) for the taxonomic identification of plants found at the study site. We also grouped all plant taxa identified in fecal pellets into their corresponding families. The fragments that we could not identify at the species level were referred to as unidentified and we did not include them in our analyses.

We used Rodde's technique (Butet 1985) to identify and count all fragments using light microscopy. We prepared three microscope slides for each sample and counted all fragments on three horizontal lines, separated by 2 mm, on each slide (El Mouden et al. 2006; Del Vecchio et al. 2011; Hichami and Znari 2023). We identified most plant fragments in the fecal material to species level using a photographic reference of epidermises identified from plant species that occur in the habitat of *T. g. graeca*; some fragments were identifiable only to higher taxonomic levels (i.e., Genus or Family).

We identified collected fresh plant materials using the same microhistological method of Baumgartner and Martin as above (see Chapuis 1980). The micro-morphological criteria for plant identification included the shape, size, and general features of cells, cellular inclusions, density and distribution of stomata, and the morphology of external epidermal structures. We quantified the availability of plant species in the study area by producing botanical relevés (lists of plant species and their corresponding relative abundance measurements in sampling plots) where the tortoises were encountered, and in randomly placed locations nearby. Details of this sampling are available in Hichami and Znari (2023). We performed plant sampling only during the spring (April-May 2011 and 2012) due to the difficulty of identifying many dried plant species. We assume that the number of plants that were too small to be counted in the spring but grew large enough to be important in autumn was insignificant. According to our field observations, we also assumed that plant mortality from spring to autumn did not significantly influence our results. We identified all plant species in each plot and estimated their abundance using the method of Braun-Blanquet (Barker 2001). The abundance, or the total number of individuals of a species in the area of the sample (quadrat), can be expressed as percentage cover classes that are usually: 1 = solitary and less than 1% cover (very rare); 2 = few with less than 1% cover (rare); 3 = 1–5% cover; 4 = 5–25% cover; 5 = 25–50% cover; 6 = 50–75% cover; 7 > 75% cover.

We quantified the biomass percentage of each plant species by estimating the mean mass of 20 randomly selected individuals, which we then multiplied by the total number of individuals of that species within a given quadrat. We then calculated the overall abundance of each species by summing the relative biomass obtained at each sampling quadrat. Seasonal values were used to calculate the frequency

of occurrence (%FO) as expressed as a percentage by the ratio of the number of fecal sample containing the food item *i* and the total number of fecal sample examined, and the relative frequency (% N) of each plant taxon (species or family) by season:

$$\% N = (n/N) * 100$$

where *n* is the number of identified fragments attributed to a given taxon in a given season, and *N* is the total number of identified fragments in that season. We also evaluated the diet diversity for each month and on a seasonal basis, using species richness (*D*; Margalef 1958), diversity (*H*; Shannon-Wiener 1949), and evenness (*E*; Pielou 1966) indices (Table 1). Finally, we used the Sørensen Similarity Index (*C<sub>s</sub>*), which takes the presence or absence of a species into account (Sørensen, 1948), and Pianka's Niche Overlap Index (*O<sub>jk</sub>*), which also takes species abundance into account (Pianka 1973), for comparing the dietary similarity or overlap between seasons (Table 1). Both indices range between 0 (no overlap) and 1 (complete overlap). Pianka's Index values were classified according to the scale proposed by Langton (1982), where  $0 < O_{jk} \leq 29$  is low overlap,  $30 \leq O_{jk} \leq 59$  is medium overlap, and  $O_{jk} \geq 60$  is high overlap.

**Statistical analysis.**—We performed all statistical analyses using STATISTICA software (version 10.0.1) for two-tailed tests and the statistical threshold alpha set at 0.05. In cases of multiple simultaneous comparisons, alpha was adjusted using Bonferroni correction (Rice, 1989). We used parametric tests for normally distributed data. We used the non-parametric Mann-Whitney U-test to compare the median values of plant availability in the habitat to the median values of availability for plants that tortoises consumed. Similarities in quantitative compositions of the diets between males and females and between pair of seasonal samples were assessed using Pianka's Index. We analyzed differences in richness, diversity, and evenness among seasons using the non-parametric Kruskal-Wallis (KW) test, followed by a multiple-comparisons post-hoc Duncan Multiple Range tests.

## RESULTS

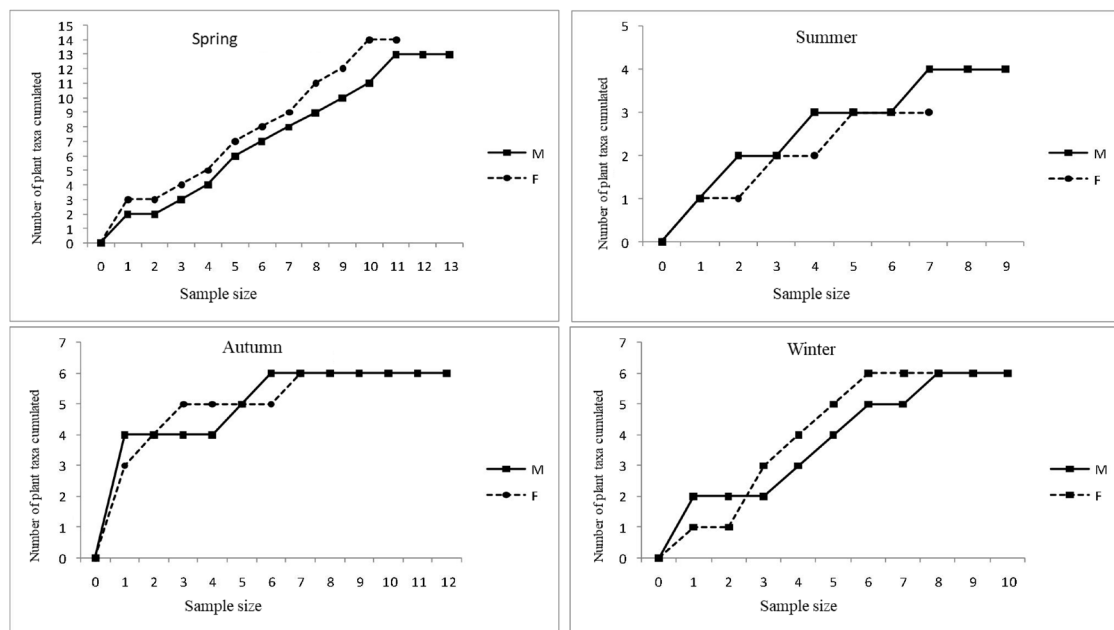
Overall, we obtained fecal pellets with identifiable remains from 85 individuals (38 males (M) and 47 females (F)) of 106 adult tortoises that we handled. We found 11 F and 15 M in spring, nine F and 10 M

**TABLE 1.** Niche metrics (richness, diversity, evenness, niche similarity, and niche overlap indexes) used in the present study.

Index name	Formula	Parameter definitions
Margalef's Richness Index D	$D = \frac{(S - 1)}{\ln(N)}$	S is the total number of species observed and N is the total number of counts.
Shannon-Wiener Index H'	$H' = -\sum_{i=1}^S p_i \cdot \log p_i$	$p_i$ is the proportion of the $i^{\text{th}}$ prey in the diet and logs to the base 2. S is the number of taxa in the diet (taxonomic richness).
Pielou's Evenness Index J'	$J' = \frac{H'}{\ln S}$	H is the corresponding Shannon-Wiener value. $\ln S = H_{\max}$ .  values of Pielou's index J' are constrained between 0 and 1, with values close to 1 indicating that all recorded taxa in the diet are close in numbers per site.
Sørensen Similarity Index (Cs)	$Ss = \frac{2a}{2a + b + c}$	a: number of species in sample A and sample B (joint occurrences), b: number of species in sample B but not in sample A, and c: number of species in sample A but not in sample B
Pianka's Niche Overlap Index	$O_{kl} = \frac{\sum_i^n p_{il} p_{ik}}{\sqrt{\sum_i^n p_{il}^2 \sum_i^n p_{ik}^2}}$	$O_{kl}$ is the resource overlap between species k and l, and since the index is symmetric $O_{kl} = O_{lk}$ . $p_{ib}$ represents the proportion of resource i that is used by species b (or in a different period by the same species). The index is a modification of one presented in MacArthur & Levins (1967).

in summer, 10 F and 12 M in autumn, and eight F and 10 M in winter. We pooled data for both sexes in the seasonal analysis of diet. Otherwise, sample sizes were not sufficiently large for adequate statistical comparisons, but samples were separated by sex

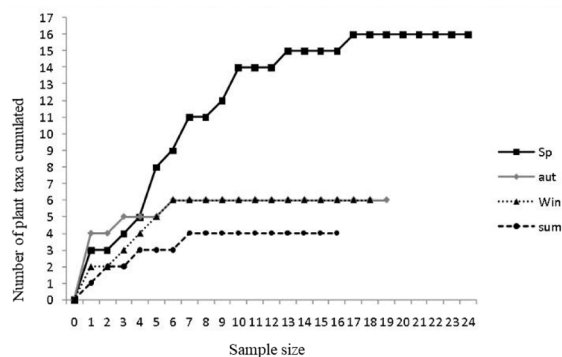
for an overall analysis (with pooled spring and autumn samples). Accumulation curves of the number of food species in relation to sample size in males and females (Fig. 1) and in the four seasons (Fig. 2) showed that plateaus were reached in all cases; hence,

**Figure 1.** Accumulation curves by season for food type categories ingested by male and female Souss Valley Tortoises (*Testudo graeca graeca*) in Morocco.

**TABLE 2.** Importance of food items in the diet of the Souss Valley Tortoise (*Testudo graeca graeca*) in Morocco over four seasons. Abbreviations are OF% = occurrence frequency (in %), N% = numerical frequency (in bold when > 5%), which is the percentage of fragment number per eaten plant species (mean  $\pm$  standard deviation, in %), and plant families are Ai = Aizoaceae, As = Asteraceae, Bo = Boraginaceae, Br = Brassicaceae, Fa = Fabaceae, La = Lamiaceae, Ma = Malvaceae, Po = Poaceae, Per = Primulaceae and Ra = Rhamnaceae. Plant species consumed are Purslane-leaved Aizoon (*Aizoon canariense*), Scarlet Pimpernel (*Anagallis arvensis*), Earring Vetch (*Astragal cruciatus*), Buckler Mustard (*Biscutella didyma*), Bermuda Grass (*Cynodon dactylon*), Blue Echium (*Echium plantagineum*), Devil's Thorn (*Emex spinosus*), Fern Leaf Lavender (*Lavandula multifida*), Lesser Kawkbit (*Leontodon saxatilis*), Sand Bird's Foot Trefoil (*Lotus arenarius*), Cheeseweed Mallow (*Malva parviflora*), Bur Clover (*Medicago polymorpha*), Tattered Medick (*Medicago laciniata*), Egyptian Sage (*Salvia aegyptiaca*), Geniculate Fescue (*Vulpia geniculata*), and Lotus Jujube (*Ziziphus lotus*).

Taxa	Family	Spring		Summer		Autumn		Winter	
		OF%	N%	OF%	N%	OF%	N%	OF%	N%
<i>Aizoon canariense</i>	Ai	38.46	1.60 $\pm$ 0.89	--	--	22.22	11.06 $\pm$ 2.24	30.76	3.00 $\pm$ 0.74
<i>Anagallis arvensis</i>	Pr	7.69	0.13 $\pm$ 0.10	--	--	--	--	--	--
<i>Astragal cruciatus</i>	Po	11.53	0.46 $\pm$ 0.34	--	--	--	--	--	--
<i>Biscutella didyma</i>	Br	38.46	1.23 $\pm$ 0.68	--	--	--	--	--	--
<i>Cynodon dactylon</i>	Po	92.30	44.7 $\pm$ 12	83.33	28.3 $\pm$ 14.28	66.67	28.9 $\pm$ 2.80	92.30	43.5 $\pm$ 4.50
<i>Echium plantagineum</i>	Bo	11.53	0.43 $\pm$ 0.33	--	--	--	--	--	--
<i>Emex spinosus</i>	Po	3.84	0.03 $\pm$ 0.02	--	--	--	--	--	--
<i>Lavandula multifida</i>	La	3.84	0.13 $\pm$ 0.10	--	--	--	--	--	--
<i>Leontodon saxatilis</i>	As	65.38	5.25 $\pm$ 1.96	44.44	7.70 $\pm$ 6.79	44.44	24.7 $\pm$ 3.62	61.53	13.9 $\pm$ 2.26
<i>Lotus arenarius</i>	Fa	65.38	26.5 $\pm$ 10.17	42.56	5.89 $\pm$ 5.92	16.95	4.25 $\pm$ 2.90	18.59	3.43 $\pm$ 2.36
<i>Malva parviflora</i>	Ma	42.30	1.33 $\pm$ 0.66	--	--	--	--	--	--
<i>Medicago polymorpha</i>	Fa	53.84	3.98 $\pm$ 1.65	--	--	33.33	8.11 $\pm$ 1.40	23.07	5.29 $\pm$ 1.18
<i>Medicago laciniata</i>	Fa	3.84	0.16 $\pm$ 0.13	--	--	--	--	--	--
<i>Salvia aegyptiaca</i>	La	57.69	8.7 $\pm$ 4.36	88.89	56.3 $\pm$ 51.88	50.0	22.1 $\pm$ 2.88	61.53	29.6 $\pm$ 5.30
<i>Vulpia geniculata</i>	Po	3.84	1.03 $\pm$ 0.83	--	--	5.55	0.85 $\pm$ 0.20	15.38	1.28 $\pm$ 0.39
<i>Ziziphus lotus</i>	Rh	34.61	1.13 $\pm$ 0.66	22.22	1.83 $\pm$ 2.16	--	--	--	--
Others		54.16	3.18 $\pm$ 1.55	--	--	--	--	--	--
Diet Species richness			16+		5		7		7

we believe our sample sizes were adequate for analyzing dietary diversity. The most important families ranked by FO for both sexes with seasons combined



**FIGURE 2.** Accumulation curves for food type categories ingested by Souss Valley Tortoises (*Testudo graeca graeca*) of both sexes in spring (sp), autumn (aut), winter (wm), and summer (sum) in Morocco. Note that a plateau was reached in all cases, showing that tortoise dietary diversity was adequately described by sample sizes used.

were Poaceae, Fabaceae, and Asteraceae (Table 2). In spring, the most important families by FO were also Poaceae, Fabaceae and Asteraceae, respectively, and in the other seasons, Fabaceae was replaced by Lamiaceae and Asteraceae was absent in winter.

**Intersexual differences in diet.**—We identified at least 13 and 14 plant species in male and female fecal pellets in all seasons combined. The trophic niche breadths of both sexes were very similar (number of food taxa/number of fecal pellets examined: 0.34 in males versus 0.32 in females). Diet compositions of the two sexes were not significantly different, both qualitatively (both males and females ingested 13 out of 16 plant taxa that were eaten by tortoises and quantitatively (in terms of numerical frequency, %N; Table 2). Indeed, there was a significant positive relationship between the frequency of consumption of the various food items by males and females ( $r$



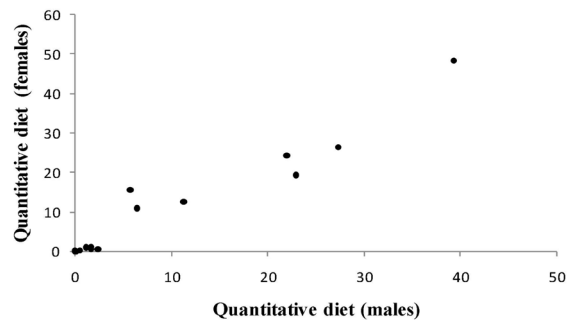


FIGURE 3. Comparison of diets of male and female Souss Valley Tortoises (*Testudo graeca graeca*) in Morocco. The percentage frequency of occurrence of each plant species in the diets of each sex is plotted in order of increasing frequency.

= 0.775,  $n = 25$ ,  $P < 0.001$ ; Fig. 3), and a medium dietary overlap value between sexes ( $O_{jk} = 0.43$ ).

**Seasonal differences in diets.**—In terms of numerical proportions (%N), six plant species accounted for more than 90% of the spring diet: in descending order these were Bermuda Grass (*Cynodon dactylon*), Sand Bird Foot Trefoil (*Lotus arenarius*), Egyptian Sage (*Salvia aegyptiaca*), Lesser Hawkbit (*Leontodus saxatilis*), and Cutleaf Medik (*Medicago laciniata*). In summer, only five plant species were consumed with Egyptian sage, Bermuda Grass, Lesser Hawkbit, and Sand Bird Foot Trefoil, representing 98% of the diet composition. Lotus Jujube was ingested at  $< 2\%$ . Finally, in autumn and winter, tortoises consumed seven plant species, the most important being Bermuda Grass, Egyptian Sage, and Lesser Hawkbit (Table 2).

The diet diversity based on Margalef's Index differed significantly among seasons ( $H = 23.51$ ,  $df = 3$ ,  $P < 0.001$ ) with the highest value recorded in spring (Table 3). In autumn,  $D$  was significantly lower as compared with the other seasons, except for summer. The Shannon Diversity Index also differed significantly among seasons ( $H = 16.17$ ,  $df = 3$ ,  $P < 0.001$ ). This index showed higher values during the spring season as compared with winter and autumn, while it was lower during the summer season (Table 3). Diet evenness ( $E$ ) also varied significantly among

seasons ( $H = 17.12$ ,  $df = 3$ ,  $P < 0.001$ ). The value of  $E$  reached a maximum in autumn and was minimal in spring (Table 3).

Sixteen plant species were included in the spring diet versus only five in the summer diet and seven in the autumn and winter diets. Tortoises consumed 80% of the plant taxa available over the four seasons (Table 2). Although diet composition differed markedly between spring and summer-autumn, in terms of taxonomic richness, the quantitative compositions of the diets (as revealed by the numerical frequencies of the various food items by tortoises) were not significantly different for all pairs of seasons ( $P > 0.05$  in all cases; Table 3).

The qualitative Sørensen Similarity Index ( $C_s$ ) was low, ranging from 0.40 to 0.54 between spring and each of the other seasons and between summer and autumn; it was medium ( $C_s = 0.66$ ) between summer and winter, while there was total similarity ( $C_s = 1$ ) between autumn and winter diets (Table 4). The degree of niche overlap was intermediate ( $O_{jk} = 0.53$ ) between spring and autumn. Niche overlap was high (0.67–0.92) between other comparisons of seasons (Table 4).

**Spring food availability vs. relative importance in the diet.**—Plant species availability and relative abundances in the habitat in spring, were strongly unequal, with some taxa more or less abundant and others extremely rare (Table 5). Tortoise diets included mostly species that were rare (e.g., Bermuda Grass, Sand Bird's Foot Trefoil) to very rare (e.g., Lesser Hawkbit, Egyptian Sage), but species that were extremely abundant were much less consumed (e.g., Purslane-leaved Aizoon, *Aizoon canariense*), or were even avoided, such as Holly-leaved Eryngo (*Eryngium ilicifolium*), Arabic Mignonette (*Reseda arabica*), and Mount Atlas Daisy (*Anacyclus maroccanus*). Other species with medium relative abundances, such as Tattered Medick (*Medicago laciniata*) and Genuiculate Fescue (*Vulpia geniculata*) were eaten much less (Tables 2 and 5). Overall, the median abundances of plant species consumed by

TABLE 3. Mean  $\pm$  standard deviation indices of diet diversity for Souss Valley Tortoises (*Testudo graeca graeca*) in the central Jbilel Mountains, Morocco, during four seasons (sp = spring, su = summer, au = autumn, and wi = winter). The significant differences of comparisons of indexes  $D$ ,  $H$ , and  $E$  among pairs of seasons are based on Duncan Multiple Range tests.

Index	Spring (sp)	Summer (su)	Autumn (au)	Winter (wi)	Significant Comparisons
Species richness $S$	16+	5	7	7	
Margalef $D$	1.228 $\pm$ 0.32	0.839 $\pm$ 0.285	0.817 $\pm$ 0.409	0.929 $\pm$ 0.355	sp-su, sp-au, sp-wi, su-wi
Shannon $H$	1.092 $\pm$ 0.39	0.533 $\pm$ 0.210	0.673 $\pm$ 0.234	0.858 $\pm$ 0.436	sp-su, sp-au, sp-wi
Evenness ( $E$ )	0.697 $\pm$ 0.14	0.774 $\pm$ 0.172	0.825 $\pm$ 0.297	0.814 $\pm$ 0.123	sp-su, sp-au, sp-wi, su-au

**TABLE 4.** Univariate measures of seasonal dietary overlaps for Souss Valley Tortoises (*Testudo graeca graeca*) in Morocco using Sørensen Index (Cs) and Pianka Index (Ojk). The values in bold indicate high niche overlaps (> 0.60; see Materials and Methods).

Comparisons	Sørensen, Cs	Pianka, Ojk
Spring vs. autumn	0.40	0.53
Spring vs. winter	0.41	0.67
Spring vs. summer	0.51	0.79
Summer vs. autumn	0.54	0.77
Summer vs. winter	0.66	0.86
Autumn vs. winter	1.00	0.92

tortoises were higher than those that tortoises avoided ( $U = 633$ ,  $df = 38$ ,  $P = 0.012$ ).

### DISCUSSION

Our study showed that the Souss Valley Tortoise is strictly herbivorous in west-central Morocco, which is in accordance with previous investigations in the same study area (El Mouden et al. 2006, Hichami and Znari 2023). In addition, diet composition did not differ significantly between sexes. We also found that spring diet composition was more diverse than in the other seasons. The relative abundances of the few

plant species consumed by tortoises in spring were generally lower than those of some more abundant plant species that were not eaten. Some very abundant species were even avoided. This indicates that these tortoises were primarily specialists (selective feeders); however, they also fed, but much less often, on a few less abundant plant species.

The fact that Souss Valley Tortoises from the arid steppe lands of Jbilet Mountains are herbivorous is consistent with previously published data on the same subspecies in Morocco (Bayley and Highfield 1996; El Mouden et al. 2006; Hichami and Znari 2023) and other Mediterranean species such as Hermann's Tortoise (*Testudo hermanni*; Guyot and Lescure 1994; Huot Daubremont 1999; Del Vecchio et al. 2011). Andreu (1987) has also reported Spur-thighed Tortoises from southern Spain consuming arthropods (mainly insects: coleopterans, dipterans, hemipterans, and acari), molted skin (snakes), carapace bones and eggshells of tortoises, as well as carrion of various mammals and birds (i.e., Common Wood Pigeon, *Columba palumbus*; Lopez Jurado et al. 1979). We did not find carrion in any of our tortoise fecal pellets but may not have been detected because of the difficulty identifying unusual food items in tortoises.

**TABLE 5.** The availability (relative abundance) of all plant species in the central Jbilet, west-central Morocco, in spring 2011.

Species	Relative abundance	Species	Relative abundance
Purslane-leaved Aizoon ( <i>Aizoon canariense</i> )	530	Lesser Hawkbit ( <i>Leontodon saxatilis</i> )	76
Holly-leaved Eryngo ( <i>Eryngium ilicifolium</i> )	470	Psyllium Blond ( <i>Plantago ovata</i> )	74
Arabic Mignonette ( <i>Reseda arabica</i> )	400	Earring Vetch ( <i>Astragal cruciatus</i> )	73
Mount Atlas Daisy ( <i>Anacyclus maroccanus</i> )	346	Fern Leaf Lavender ( <i>Lavandula multifida</i> )	72
Golden Thistle ( <i>Scolymus hispanicus</i> )	276	Scarlet Pimpernel ( <i>Anagallis arvensis</i> )	66
Tattered Medick ( <i>Medicago laciniata</i> )	273	Egyptian Sage ( <i>Salvia aegyptiaca</i> )	52
Geniculate Fescue ( <i>Vulpia geniculata</i> )	261	Camel Grass ( <i>Andropogon schaeenanthus</i> )	41
Small-flower Stock ( <i>Mathiola parviflora</i> )	207	Moorish Wallrocket ( <i>Diploaxis assurgens</i> )	36
Cretan Weed ( <i>Hedynois cretica</i> )	182	Blue Echium ( <i>Echium plantagineum</i> )	35
Bermuda Grass ( <i>Cynodon dactylon</i> )	157	False Sowthistle ( <i>Reichardia tingitana</i> )	28
Devil's Thorn ( <i>Emex spinosus</i> )	146	Hairy Rupturewort ( <i>Herniaria cinerea</i> )	24
Mexican Fleabane ( <i>Erigeron karvinskia</i> )	145	Lotus Jujube ( <i>Ziziphus lotus</i> )	20
Sicilian Star-thistle ( <i>Centaurea sulfurea</i> )	145	Clovenlip Toadflax ( <i>Linaria bipartita</i> )	16
Redstem Stork's Bill ( <i>Erodium praecox</i> )	142	Onion Weed ( <i>Asphodelus tenuifolius</i> )	15
Fern Leaf Lavender ( <i>Lavandula multifida</i> )	126	Buckler Mustard ( <i>Biscutella didyma</i> )	10
Cheeseweed Mallow ( <i>Malva parviflora</i> )	117	Blue Fescue Grass ( <i>Festuca gluca</i> )	7
African Carline Thistle ( <i>Carlina involucrata</i> )	113	European Umbrella Milkwort ( <i>Tolpis burbata</i> )	6
Sand Bird's Foot Trefoil ( <i>Lotus arenarius</i> )	111	Bristlefruit Hedgeparsley ( <i>Torilis leptophylla</i> )	1
Virgin's Mantle ( <i>Fagonia cretica</i> )	110	Birdcage Thistle ( <i>Atractylis cancellata</i> )	1
Cardo-pallottola ( <i>Echinops spinosus</i> )	86		

Nevertheless, when present in tortoise fecal pellets, carrion tends to be over-represented (Moskovits and Bjorndal 1990). None of the studies mentioned above on Souss Valley Tortoises from west-central Morocco reported tortoises feeding on carrion, although Bailey and Highfield (1996) found tortoises in the Souss Valley fed on cattle droppings.

Several other tortoises in the family Testudinidae are entirely herbivorous, such as the Namaqualand Speckled Tortoise (*Homopus signatus signatus*; Loehr 2002; Luiselli et al. 2003), the Radiated Tortoise (*Astrochelys radiata*) from Africa (Leuteritz 2003), the Russian Tortoise (*Testudo horsfieldi*) from central Asia (Lagarde et al. 2002), and the Gopher Tortoise (*Gopherus polyphemus*) from North America (MacDonald and Mushinsky 1988). A considerable portion of Souss Valley Tortoise diet consisted of plant species from the families Poaceae, Fabaceae, Asteraceae, and Lamiaceae, with plants from the Poaceae, Fabaceae, and Asteraceae consumed mainly in spring. Thus, our data are consistent with those presented for the same population in the spring of 2003 by El Mouden et al. (2006), and similar to the Souss Valley population in the Admine Forest of southwest-central Morocco, where tortoises also foraged on cultivated plants (Bailey and Highfield 1996).

There is a clear preference for vegetative parts of plants by tortoises in the genus *Testudo*, particularly during spring (Rouag et al. 2008; Del Vecchio et al. 2011; Hichami and Znari 2023). The lack of fruits in the spring diet may be due to the unavailability of fruits in this season (at least during the sampling period), actual preference for vegetative parts, or both. Problems of microhistological identification of fruits could also be involved. Vegetative parts may be chosen because of the availability of certain nutrients (Phosphorus, Sodium, and Calcium), particularly in dicots (Carey et al. 1985). Leaves of plants in the family Fabaceae may be selected preferentially because of their relatively high protein content (Lewis et al. 2005; Hichami and Znari 2023). Leaves from a variety of plant species (primarily Bermuda Grass and Lesser Hawkbit) dominated the diet in all seasons, whereas Egyptian Sage became important in the summer and secondarily in autumn and winter.

The dietary similarity between male and female Souss Valley Tortoises is consistent with data from other studied populations of Spur-thighed Tortoises, and other primarily herbivorous tortoises. For example, the dietary composition of the two sexes does not differ significantly in Spur-thighed Tortoises

from Algeria (Rouag et al. 2008; Aissa and Adel 2021) or west-central Morocco (Hichami and Znari 2023; El Mouden et al. 2006), in Hermann's Tortoises from central Italy (Del Vecchio 2011), Bell's Hinge-back Tortoises (*Kinixys belliana*) from Nigeria (Luiselli 2003, 2006a), or Gopher Tortoises from the USA (MacDonald and Mushinsky 1988). In tortoises reintroduced to the Seychelles, male and female Aldabra Giant Tortoises (*Aldabrachelys arnoldi*) had similar diet diversity and uniformity (Gibson and Hamilton, 1984; Pemberton and Gilchrist 2009).

Seasonal dietary differences revealed in our study are the first to demonstrate a significant seasonal change in the diet of Souss Valley Tortoises. Both the Margalef's and Shannon's indices varied significantly among seasons (with the highest and lowest values observed in spring and summer, respectively), and seem to be good diversity indicators of the temporal data series analysis, from changing environments such as arid steppe-lands. In contrast, the evenness index was maximal in spring, indicating a high equitability and commonality of plant species in the diet while in summer and other seasons, the lower values were indicative of a predominance of rarely eaten plant species. Moreover, there was a low qualitative trophic niche overlap between spring and each of the other seasons and between summer and autumn; it was medium between summer and winter, and a total similarity between autumn and winter. The degree of quantitative niche overlap was intermediate between spring and autumn to high between spring and winter and between autumn and winter. Such seasonal variation in the diet composition in the Souss Valley Tortoises reflects the large change in plant availability and productivity in the arid steppe lands of west-central Morocco.

Our data show that plants in the families Poaceae (primarily Bermuda Grass) and Fabaceae (primarily Sand Bird Foot Trefoil) were consumed heavily during spring, which also was found in spring 2003 for these tortoises (El Mouden et al. 2006; Hichami and Znari 2023). We suggest that the seasonal shift away from plants in the family Poaceae was due to low water content in grasses at other times of the year. Consuming dry grasses can cause negative water and nitrogen balances in tortoises from arid environments (e.g., Desert Tortoise, Henen 1997; Nagy et al. 1998; Russian Tortoise, Lagarde et al. 2002), and we presume that similar changes occur in the arid Mediterranean climate of west-central Morocco, which has almost no rainfall during the summer. Indeed, the dominant plant life forms (*sensu stricto*; Raunkiaer 1934) in



our study area were annuals, which accounted for 85% of the total plant species, typical of arid areas in the Mediterranean bioclimate.

Overall, it is most likely that these seasonal dietary shifts are due to changes in resource availability as observed in other terrestrial herbivorous chelonians that appear to be dietary generalists (Mason et al. 1999; Kabigumila 2001; Lagarde et al. 2002; Loehr 2002; Del Vecchio et al. 2011). For instance, in late March to mid-April, Russian Tortoises fed almost exclusively on the most abundant and accessible plant species available at that time, Sickie-fruited Hornbeam (*Ceratocephalus falcatus*), and from mid-April to mid-May shifted to plants in the family Papaveraceae, Brassicaceae, and the aster, Linear Koelpinia (*Koelpinia linearis*), plants that tended to dominate the annual plant community at that time (Lagarde et al. 2002).

In previous studies on the spring diet in the arid central Jbilet Mountains, the Souss Valley Tortoises were found to be rather specialist herbivores, feeding on few (5–6) plant species (El Mouden et al. 2006; Hichami and Znari 2023). Hichami and Znari (2023) showed that the plants consumed were selected, based on a high index value of Potential Excretion of Potassium (PEP), which indicates plants that are rich in proteins and water, but poor in potassium, which is toxic to tortoises. On the other hand, Spur-thighed Tortoises from northern Algeria were found to be generalists and opportunists. They frequently fed upon several of the most available plant species and rarely upon the least available plant species; however, they avoided other plant species that were common or abundant (Rouag et al. 2008). Similarly, some other plant species common in the field were actively avoided, probably because of the presence of toxic substances (El Mouden et al. 2006) and/or their very low PEP index (Hichami and Znari 2023). In contrast, many species of rare food plants in the habitat, including some frequently consumed species, were well represented in the diet of the tortoise. This was also the case with the Desert Tortoise (Jennings and Berry 2015).

We found that tortoises focused mostly on leguminous species (Poaceae), which could be considered nutritious foods because of their high nitrogen content. Tortoises in arid steppe lands of west central Morocco, however, only consumed plants in a hydrated state until the late spring-early summer. As a result, most of the plants (annuals and herbaceous perennials) were dry at that time, and most tortoises stopped their foraging activity. This

is in accordance with the observations previously reported for the Desert Tortoise in the Mojave Desert of California, USA (Jennings and Berry 2015).

The knowledge of seasonal changes in diet composition concerning the availability of plant taxa could be useful in developing management plans, not only for tortoise populations, but also for the landscape itself. Moreover, this kind of landscape often features a mosaic of low-intensity agriculture and natural and structural elements (e.g., field margins, hedgerows, patches of Lotus Jujube, *Ziziphus lotus*, and Bridal Broom, *Retama monosperma*, and rock outcrops), which should be protected as they play important ecological roles in protecting plant and animal species of conservation concern. Herbaceous perennials, which were rare in our study site but important in the tortoise diet, may be of great interest in sustaining tortoise populations during drought periods when annuals are absent (mostly from early summer to mid-autumn).

*Acknowledgments.*—This work was partly funded by a grant from the British Ecological Society to MZ. In addition, we are very grateful to the Ph.D. students (Tarik Louzizi and Salwa Namous) of our lab who helped collect tortoises in the field.

## LITERATURE CITED

- Aissa, B.H. and M. Adel. 2021. Feeding behaviour of a *Testudo graeca whitei* population in mergueb nature reserve, Algeria. *Journal of Biodiversity, Conservation, Bioresources, and Management* 7:55–60.
- Andreu, A.C. 1987. Ecología dinamica poblacional de la tortuga mora, *Testudo graeca*, en Doñana. Ph.D. Dissertation, University of Seville, Spain. 86 p.
- Bailey, J.R., and A.C. Highfield. 1996. Observations on ecological changes threatening a population of *Testudo graeca graeca* in the Souss Valley, southern Morocco. *Chelonian Conservation Biology* 2:36–42.
- Barker, P. 2001. A Technical Manual for Vegetation Monitoring. Resource Management and Conservation, Department of Primary Industries, Water and Environment, Hobart, Tasmania.
- Butet, A. 1985. Méthode d'étude du régime alimentaire d'un rongeur polyphage (*Apodemus sylvaticus* L., 1758) par l'analyse microscopique des fèces. *Mammalia* 49:455–483.
- Carey, E.E., B.C. Tripathy, and C.A. Rebeiz. 1985. Chloroplast biogenesis: modulation of monovinyl and divinyl protochlorophyllide biosynthesis

- by light and darkness in vitro. *Plant Physiology* 79:1059–1063.
- Chapuis, J.L. 1980. Méthodes d'étude du régime alimentaire de Lapin de garenne *Orytolagus cuniculus* (L.) par l'analyse microscopique des fèces. *Revue d'Ecologie Terre et Vie* 34:159–198.
- Del Vecchio, S., Russell, L. Burke, R. Lorenzo, M. Capula, and L. Luiselli. 2011. Seasonal changes in the diet of *Testudo hermanni hermanni* in central Italy. *Herpetologica* 67:236–249.
- El Mouden, E.H., T. Slimani, K. Ben Kaddour, F. Lagarde, A. Ouhammou, and X. Bonnet. 2006. *Testudo graeca graeca* feeding ecology in an arid and overgrazed zone in Morocco. *Journal of Arid Environments* 64:422–435.
- El Mouden, E.H., M. Znari, and R. Brown. 1999. Skeltochronology and mark-recapture assessments of growth in the North African agamid lizard (*Agama imparealis*). *Journal of Zoology of London* 249: 455–461.
- Emberger, L. 1933. Nouvelle contribution à l'étude de la classification des groupements végétaux. *Revue Générale de Botanique* 45:473–486.
- Fennane, M. 2008. Les recherches floristiques au Maroc: passé riche, présent difficile et avenir incertain. *Prospectives Universitaires*, Université Mohammed V-Agdal, Rabat 1:109–116.
- Fowler de Neira, L.E., and M.K. Johnson. 1985. Diets of Giant Tortoises and Feral Burros on Volcan Alcedo, Galapagos. *Journal of Wildlife Management* 49:165–169.
- Gibson, C.W.D., and J. Hamilton. 1984. Population processes in a large herbivorous reptile: the Giant Tortoise of Aldabra atoll. *Oecologia* 61:230–240.
- Guyot, G., and J. Lescure. 1994. Etude préliminaire du comportement alimentaire en enclos semi-naturel chez la tortue d'Hermann. *Bulletin de la Société Herpétologique de France* 69–70:19–32.
- Halliday, T., and K. Adler. 2002. *The New Encyclopedia of Reptiles and Amphibians*. 2nd Edition. <https://doi.org/10.1093/acref/9780198525073.001.0001>.
- Henen, B.T. 1997. Seasonal and annual energy budgets of female Desert Tortoises (*Gopherus agassizii*). *Ecology* 78:283–296.
- Hichami, N., and M. Znari. 2023. Interannual variations in diet and nutritional value of plants consumed by the Souss Valley Tortoises, *Testudo graeca graeca*, in west-central Morocco: are they really selective foragers? *Amphibia-Reptilia* 44:167–181.
- Huot-daubremont, C. 1999. Observations du régime alimentaire de la Tortue d'Hermann en semi-liberté dans le Massif des Maures (Var). *Bulletin de la Société Herpétologique de France*, vol. 92:45–52.
- International Union for the Conservation of Nature (IUCN). 2016. IUCN Red List of Threatened Species, 2016-2. <https://www.iucnredlist.org>.
- Jennings, W.B. 2002. Diet selection by the Desert Tortoise in relation to the flowering phenology of ephemeral plants. *Chelonian Conservation and Biology* 4:353–358.
- Jennings, W.B., and K.H. Berry. 2015. Desert Tortoises (*Gopherus agassizii*) are selective herbivores that track the flowering phenology of their preferred food plants. *PLoS ONE* <https://doi.org/10.1371/journal.pone.0116716>.
- Kabigumila, J. 2001. Sighting frequency and food habits of the Leopard Tortoise, *Geochelone pardalis*, in northern Tanzania. *African Journal of Ecology* 39:276–285.
- Lagarde, F., X. Bonnet, J. Corbin, B. Henen, K. Nagy, B. Mardonov, and G. Naulleau. 2002. Foraging behaviour and diet of an ectothermic herbivore: *Testudo horsfieldi*. *Ecography* 26:236–242.
- Langton, R.W. 1982. Diet overlap between the Atlantic Cod, *Gadus morhua*, Silver Hake, *Merluccius bilinearis* and fifteen other northwest Atlantic finfish. *Fisheries Bulletin* 80: 745–759.
- Le Houérou, H. 1989. Classification éoclimatique des zones arides (s.l.) de l'Afrique du Nord. *Ecologia Mediterranea* 15:95–144.
- Leuteritz, T.E.J. 2003. Observations on the diet and drinking behaviour of radiated tortoises (*Geochelone radiata*) in southwest Madagascar. *African Journal of Herpetology* 52:127–130.
- Loehr, V.J.T. 2002. Diet of the Namaqualand Speckled Padloper, *Homopus signatus signatus*, in early spring. *African Journal of Herpetology* 51:47–55.
- Lopez Jurado, L.F., J.M. Ttalavera Torralba, I. Gonzalez, J.A. Macivor, and A.G. Alcazar. 1979. Las tortugas terrestres *Testudo graeca* y *Testudo hermanni* en España. *Naturalia Hispanica* 17:1–63.
- Luiselli, L. 2003. Seasonal activity patterns and diet divergence of three sympatric Afrotropical tortoise species (genus *Kinixys*). *Contributions to Zoology* 72:211–220.
- Luiselli, L. 2006. Resource partitioning in the communities of terrestrial turtles: a review of the evidence. *Revue d'Ecologie (Terre et Vie)* 61:353–365.
- MacDonald, L.A., and H.R. Mushinsky. 1988. Foraging ecology of the GopherTortoise, *Gopherus polyphemus*, in a sand hill habitat. *Herpetologica* 44:345–353.
- Margalef, R. 1958. Information theory in ecology. *General Systems* 3:36–71.

- Mason, M.C., G.I.H. Kerley, C.A. Wheatherby, and W.R. Branch. 1999. Leopard Tortoises (*Geochelone pardalis*) in Valley Bushveld, Eastern Cape, South Africa: specialist or generalist herbivores? *Chelonian Biology and Conservation* 3:435–440.
- Moskovits, D.K., and K.A. Bjorndal. 1990. Diet and food preferences of the tortoises *Geochelone carbonaria* and *G. denticulata* in northwestern Brazil. *Herpetologica* 46:207–218.
- Nagy, K.A., B.T. Henen, and D.B. Vyas. 1998. Nutritional quality of native and introduced food plants of wild Desert Tortoises. *Journal of Herpetology* 32:260–267.
- Oftedal, O.T., L.S. Hillard, and D.J. Morafka. 2002. Selective spring foraging by juvenile Desert Tortoises (*Gopherus agassizii*) in the Mojave Desert: evidence of an adaptive nutritional strategy. *Chelonian Conservation and Biology* 4:341–352.
- Pemberton, J.W., and J.S. Gilchrist. 2009. Foraging behavior and diet preferences of a released population of Giant Tortoises in the Seychelles. *Chelonian Conservation and Biology* 8:57–65.
- Pianka, E.R. 1973. The structure of lizard communities. *Annual Review of Ecology and Systematics* 4:53–74.
- Pielou. 1966. Shannon's formula as a measure of specific diversity: its use and misuse. *American Naturalist* 100:463–465.
- Raunkiaer, C. 1934. *The Life Forms of Plants and Statistical Geography*. Clarendon Press, Oxford, UK.
- Rice, W.R. 1989. Analyzing tables of statistical tests. *Evolution* 43:223–225.
- Rouag, R., C. Ferrah, L. Luiselli, G. Tiar, S. Benyacoub, N. Ziane, and E.H. El Mouden. 2008. Food choice of an Algerian population of the Spur-thighed Tortoise, *Testudo graeca*. *African Journal of Herpetology* 57:103–113.
- Shannon, C.E., and W. Weaver. 1949. *The Mathematical Theory of Communication*. University of Illinois Press, Urbana, Illinois, USA.
- Sorensen, T. 1948. A method of establishing groups of equal amplitude in plant sociology based on similarity of species content and its application to analyses of the vegetation on Danish commons. *Biologiske Skrifter/Kongelige Danske Videnskabernes Selskab* 5:1–34.
- Znari, M., E.H. El Mouden, and H. Francillon-Vieillot. 2002. Long-term variation in reproductive traits of Bibron's Agama, *Agama impalearis*, in Western Morocco. *African Journal of Herpetology* 51:57–68.



**NAWAL HICHAMI** holds a Ph.D. in Ecophysiology and Conservation Biology from Cadi Ayyad University, Marrakech, Morocco. She is an Assistant Professor of Ecology and Conservation Biology at the Faculté Polydisciplinaire, Béni Mellal. Nawal has over 10 y of research experience in animal ecology, as well as in animal biology, zoology, anatomy, biodiversity, general ecology and conservation biology. Her research interests include diet, ecophysiology and reproduction, as well as various aspects of conservation ecology in vertebrate taxa such as fish, turtles and mammals. Nawal has supervised several undergraduate dissertations and published dozens of articles in journals. She has given more than 20 presentations at professional conferences. (Photograph courtesy of the Photo Center).



**MOHAMMED ZNARI** has earned Ph.D.s in Ecology from the Pierre & Marie Curie University, Paris, France, and the Cadi Ayyad University (CAU), Marrakech, Morocco. He is Professor of Ecology and Conservation Biology in the Faculty of Science-Semlalia, Marrakech, CAU. He is also the curator of Vertebrate Zoology at the Natural History Museum of Marrakech, CAU. Mohammed has over a 30-y of experience in ecological research and more recently in conservation biology (15+ y), but also in morphometrics, molecular phylogeography, and ecological modeling. His research interests are in feeding ecology, ecophysiology, and developmental and breeding biology along with different aspects of conservation ecology in various vertebrate taxa. Mohammed has been involved in several international research-conservation projects, and received different international fellowships (Fulbright, DAAD, and Averroes). He was a member of the International Committee of the World Congress of Herpetology and is currently Vice-president of the Moroccan Society of Herpetology, and Vice-president of the French-Moroccan Association ZAATOT: for the monkeys and humans in the High Atlas mountains. He has produced dozens of papers in peer-review journals, and he has contributed to international scientific meetings. (Photograph courtesy of the Photo Center).