

**REPRODUCTION OF THE SMALLEST TORTOISE, THE
NAMAQUALAND SPECKLED PADLOPER, *HOMOPUS
SIGNATUS SIGNATUS***

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HABITAT SELECTION BY *SPHAERODACTYLUS NICHOLSI* (SQUAMATA: GEKKONIDAE) IN CABO ROJO, PUERTO RICO

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ABSTRACT: We studied habitat selection by the litter-dwelling gecko *Sphaerodactylus nicholsi* in the Cabo Rojo National Wildlife Refuge in southwestern Puerto Rico to determine how this small diurnal species exists in an apparently xeric habitat. We divided the 240 ha refuge into 956 quadrats each measuring 50 m × 50 m and selected 60 quadrats at random to search for geckos. The 60 quadrats yielded 33 discrete locations with sphaerodactylids that we called occupied sites. Occupied sites were located beneath the dense canopies of broad-leaved evergreen trees and were completely shaded. The average area of an occupied site was 103 m². We paired each occupied site with a nearby random site and compared relative sphaerodactylid density and habitat variables to determine features important in habitat selection. Occupied sites were in dense shade of evergreen trees and contained trees that produced large seeds or fruits; random sites commonly were exposed to sunlight, either directly or filtered through a thin deciduous canopy. Occupied sites had significantly lower daytime substrate temperatures, more leaf litter, less grass, less bare ground, and an order of magnitude more sphaerodactylids than the random sites. The four random sites with sphaerodactylids also had dense shade. Despite its small size, diurnal habit, and apparent vulnerability to dehydration, *S. nicholsi* thrives through selection of the scattered patches of relatively mesic environment within a xeric landscape.

Key words: Gekkonidae; habitat selection; metapopulation; Puerto Rico; *Sphaerodactylus nicholsi*

THE GEKKONID genus *Sphaerodactylus* includes the world's smallest amniotes (Hedges and Thomas, 2001; Thomas, 1965). Species of this genus often inhabit dry forests on Caribbean islands where their small body size, relatively large surface/volume ratio, and diurnal activity appear to make them vulnerable to thermal stress and dehydration. At least one of the smallest species, *S. parthenopion* (Virgin Islands Dwarf Sphaero, after Hedges, 2001), does not have the specific physiological adaptations for water conservation possessed by desert lizards and shows water loss rates similar to lizards from mesic habitats (Hedges and Thomas, 2001; MacClean, 1985). It has been argued that this species and other small sphaerodactylids survive through selection of relatively mesic microhabitats within larger xeric settings (Hedges and Thomas, 2001; Powell, 1999) along with adjustment of the reproductive cycle so that abundance of delicate hatchlings peaks during the wetter part of the year (López-Ortiz and Lewis, 2002).

Sphaerodactylus nicholsi (Nichol's Crescent Sphaero) is a small ground-dwelling gecko inhabiting dry coastal forests in southwestern

Puerto Rico. It ranges in snout-vent length (SVL) from 8.0 mm in hatchlings to 23.0 mm in large gravid females (López-Ortiz and Lewis, 2002). In the Guánica State Forest this species has been reported to be most abundant in deep leaf litter in humid ravines (Genet et al., 2001). The closest relative to *S. nicholsi* (Hass, 1991), *S. townsendi* (Townsend's Dwarf Sphaero), was reported to inhabit leaf litter, decomposing logs, and palm trash in eastern Puerto Rico (Gaa-Ojeda, 1983). Similar habitats are reported for sphaerodactylids from other islands. *Sphaerodactylus parthenopion* was captured hidden in moist microhabitats on the leeward side of Virgin Gorda (MacLean, 1985; Thomas, 1965), *S. becki* (Navassa Sphaero) was found during the day in areas of deep shade beneath leaf litter or protected by other objects (Powell, 1999), and *S. ariassae* (Jaragua Sphaero) of Isla Beata was found under leaf litter (Hedges and Thomas, 2001). These patterns are consistent with selection of mesic microenvironments by the geckos in order to limit exposure to physiologically challenging conditions of low humidity and high temperature.

The hot, dry climate of southwestern Puerto Rico was exemplified at the refuge weather station in the year of this study by monthly

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annual rainfall and temperature with 95% confidence intervals of 47.8 ± 23.5 mm and 26.9 ± 0.6 C (J. Schwagerl, personal communication). These conditions combined with the high surface to volume ratio of *S. nicholsi* meant choice of microhabitat by this litter-dwelling gecko should be critical to its survival. We looked for evidence of microhabitat selection to determine how this small diurnal species exists in an apparently xeric habitat. We compared microclimatic and other ecological variables at locations used by the geckos with values of the same variables at nearby randomly selected locations. The conditions at the random locations provided a null hypothesis against which to test our prediction that the geckos occupied mesic patches in a relatively xeric setting.

MATERIALS AND METHODS

The study was conducted at the Cabo Rojo National Wildlife Refuge located in the Subtropical Dry Forest Life Zone (Ewel and Whitmore, 1973) in southwestern Puerto Rico from June 1997, through May 1998. The study site was the 240 ha core area of the refuge that houses the administrative facilities and backs up to coastal mangroves and salt flats. The vegetation of the refuge was a mixture of secondary woodland with both deciduous and evergreen broad-leaved trees, grassland, and brush (Startek, 1997).

To assure that the area searched for geckos included a representative sample of habitat on the refuge, we divided the refuge into a grid of 956 quadrats (Krebs, 1989). Each quadrat measured 50 m \times 50 m (2500 m²). We used random numbers to select 60 quadrats to be searched for geckos.

The observer walked a zigzag pattern through each of the 60 quadrats searching the surface of the ground for *S. nicholsi*. When a gecko was found, the location was flagged for subsequent delimitation of the occupied site and estimation of relative population density. It quickly became apparent that geckos were associated with leaf litter beneath shady trees. Not all trees supported geckos, but no geckos were found outside patches of shaded litter during the searches of the quadrats. This observation was used to help delimit occupied sites once the presence of geckos was established.

An occupied site was a location within one of the quadrats where at least one individual of *S. nicholsi* was found. As geckos were not found beyond the densely shaded leaf litter zone beneath particular trees or clusters of trees, all occupied sites were patches of litter. We measured the length and width of each occupied site defining the length as the longest axis of the patch of shaded litter and the width as the longest axis perpendicular to the first. Area was calculated as the ellipse defined by these axes. All occupied sites were surrounded by a boundary area devoid of geckos that extended at least 5 m beyond the litter patch occupied by the geckos. The boundary area was not part of the occupied site. If two occupied sites were less than 5 m apart, they were collapsed into a single site.

An index of relative abundance (RA) was established for each occupied site (Brower and Zar, 1984). The RA was defined as the number of *S. nicholsi* seen per minute of searching with a constant level of effort. The observer would search with the same speed and intensity of search over each occupied site and divide the final count of geckos by the number of minutes to obtain the RA. As the observer moved systematically through the occupied sites the geckos could be counted as they emerged from the leaf litter, moved over the surface, and then disappeared back into the litter. The same person (RLO) carried out all RA measurements. Counts were conducted 0930–1100 h and 1430–1700 h.

As we wished to determine whether occupied sites were more mesic than average sites on the refuge, each occupied site was paired with a random site located 33 m away along a random bearing. The random sites were a representative sample of habitat on the refuge near occupied sites. All random sites were close enough to occupied sites that we believed geckos would not be absent simply because of distance from a source population. A random site did not have to fall within the quadrats used to search for occupied sites. The random sites were circles 5 m in diameter. If geckos were found in a random site, the area that they utilized was delimited and searched as in occupied sites for the estimate of RA. If no geckos were found in the initial circle, the random site was considered devoid of geckos.

We scored 20 habitat variables. Fourteen of the variables were measured in a 5 m diameter circle on all occupied sites and random sites. The circle was placed in the center of each occupied site. If a random site contained *S. nicholsi*, the 5 m circle was placed in the center of the occupied area. If the random site had no geckos, the circle was centered on the random point. Ground cover (seeds and fruits, grass, leaf litter, fallen branches, and bare soil) was estimated from five 0.25 m² random quadrats in each circle. The placement of each quadrat was determined by random polar coordinates with the center of the circle as the origin. Each quadrat was subdivided into 100 blocks measuring 25 cm², and variables were scored as present or absent in each of the 100 blocks to give an index of percent cover. Soil moisture (as percent of relative saturation) and acidity were measured with a Kelway HB-2 ® soil tester at each of the five random quadrats. Soil surface temperature was measured at the five random locations within each circle and averaged; air temperature 2 m above ground level at the center of the circle was measured at the beginning and end of the visit to each site and averaged. Elevation above sea level at the center of each circle was recorded. Soil textural types were described following Tarbuck and Lutgens (1993) from three random 0.108 m² locations in each 5 m diameter circle. Locations were selected by random polar coordinates. We removed leaf litter and visually assessed the soil surface. Sand, clay, alluvium, and humus were scored as present for the circle if they were found at any of the three locations.

Four variables based on functional categories of trees were measured on all occupied and random sites. Tree species and other vegetation except grass were identified for occupied sites, for inhabited random sites, and for the 5 m circle in uninhabited random sites. The tree categories, all scored as present/absent, were trees, trees leaving large seeds or fruit in the litter, evergreen trees, and deciduous trees.

Potential predators such as species of *Anolis*, *Hemidactylus*, and *Ameiva* were counted and identified using binoculars during a randomly selected hour on both occupied and random sites. The two final variables were tabulated as presence of anoles and hemidactylids on all occupied and random sites.

Statistical analysis was carried out with SPSS for Windows (SPSS, Inc., 1999) and Statistix 8 (Analytical Software, 2003). Measurements summarizing conditions in occupied and random sites were expressed as mean and standard deviation unless otherwise indicated.

RESULTS

Eighteen (30%) of the 60 surveyed quadrats contained *S. nicholsi*. In these 18 quadrats a total of 33 occupied sites was found, giving an average and 95% confidence interval of $\bar{x} = 0.55 \pm 0.27$ occupied sites per quadrat surveyed. The average area with confidence interval of an occupied site, measured as an ellipse, was $\bar{x} = 103.42 \pm 37.18$ m² (range: 15.7–487.7 m²). The occupied areas represented 2.2% of the 150,000 m² surveyed in the 60 quadrats and, therefore, of the entire Refuge.

The RA of the 33 occupied sites ranged from 1–19 ($\bar{x} = 4.1 \pm 4.19$ SD) geckos per minute. The Spearman rank correlation between ellipse area and RA of *S. Nicholsi* on the occupied sites was non significant ($r_s = -0.065$, $P = 0.718$). In the random plots RA ranged from zero to eight ($\bar{x} = 0.42 \pm 1.49$ SD) geckos per minute. Only four of the 33 random plots contained sphaerodactylids. The difference in average population density between occupied sites and random sites was statistically significant (Wilcoxon Signed Ranks Test: $Z = 4.566$; $P < 0.000$).

All but two of 33 occupied sites were completely shielded from direct insolation by broad-leaved evergreen tree canopies, sometimes the canopy of a single tree. The important species of evergreen tree were *Pithecellobium dulce*, *Tamarindus indicus*, *Bucida buceras*, *Hymenaea courbaril*, and *Guaiacum officinale*. The two occupied sites that did not have evergreen trees were shielded from the sun by overgrowth of the evergreen vine *Stigmaphyllon periplocifolium*. Evergreen trees shaded three of the four random sites occupied by *S. nicholsi* and the fourth contained a large dead shrub densely overgrown with leafy vines of *S. periplocifolium*. Deciduous trees, although they produced litter, did not alone allow occupation by *S. nicholsi*. Table 1 shows the high percent frequency of evergreen species on the occupied sites. The two most abundant

TABLE 1.—Plant species found on the 33 occupied sites and 33 paired random sites with percent frequency \pm standard error. E indicates evergreen; D indicates deciduous.

Species	Leaf retention	Percent of occupied sites	Percent of random sites
<i>Pithecellobium dulce</i> (Roxb.) Benth.	E	48.48 \pm 8.83	24.24 \pm 7.58
<i>Prosopis pallida</i> (H.&B. Ex Willd) Kunth	D	36.36 \pm 8.50	27.27 \pm 7.87
<i>Stigmaphyllon periplocifolium</i> (Desf.) A. Juss.	E	27.27 \pm 7.87	18.18 \pm 6.82
<i>Tamarindus indicus</i> L.	E	27.27 \pm 7.87	6.06 \pm 4.22
Shrubs		24.24 \pm 7.58	18.18 \pm 6.82
Cactaceae		18.18 \pm 6.82	12.12 \pm 5.77
<i>Bucida buceras</i> L.	E	18.18 \pm 6.82	6.06 \pm 4.22
<i>Hymenaea courbaril</i> L.	E	15.15 \pm 6.34	6.06 \pm 4.22
<i>Guaiacum officinale</i> L.	E	12.12 \pm 5.77	0
<i>Leucaena leucocephala</i> (lam.) Dewit	D	6.06 \pm 4.22	3.03 \pm 3.03
<i>Melicoccus bijugatus</i> Jacq.	E	6.06 \pm 4.22	0
<i>Ziziphus reticulata</i> (Vahl) DC.	E	6.06 \pm 4.22	0
<i>Bourreria succulenta</i> Jacq.	E	3.03 \pm 3.03	0
<i>Coccoloba diversifolia</i> Jacq.	E	3.03 \pm 3.03	3.03 \pm 3.03
<i>Crescentia linearifolia</i> Miers	D	3.03 \pm 3.03	0
<i>Randia aculeata</i> L.	E	3.03 \pm 3.03	0
<i>Pictetia aculeata</i> (Vahl) Urban	D	3.03 \pm 3.03	0
<i>Polygala cowellii</i> Blake	E	3.03 \pm 3.03	0
<i>Talinum triangulare</i> (Jacq.) Willd.	E	3.03 \pm 3.03	0
<i>Zanthoxylum monophyllum</i> (Lam.)	D	3.03 \pm 3.03	0
<i>Zanthoxylum flavum</i> Vahl	D	3.03 \pm 3.03	0

trees on the refuge, based on analysis of random sites, were the deciduous mesquite, *Prosopis pallida* and the evergreen *P. dulce*.

For several of the habitat variables given in Table 2, the occupied sites exhibited more mesic conditions than the average values for the refuge as given by the random sites. Significant differences were found for tree category, temperature, soil, and ground cover.

Trees were found on all 33 occupied sites and only 17 of the random sites. Evergreen species were found on 31 of the occupied sites and only 12 of the random sites. Trees that contributed large seeds or dry fruit to the substrate were found on 27 of the occupied sites and only 9 of the random sites. These species were the relatively abundant *P. dulce* and the relatively infrequent *T. indicus*, *Melicoccus bijugatus*, *Polygala cowellii*, *Randia aculeata*, and *Ziziphus reticulata*. Deciduous trees were equally frequent on occupied and random sites.

During the day substrate temperatures were lower on occupied sites than on random sites. Occupied sites had significantly less grass and exposed soil expressed as percent substrate cover, and significantly more leaf litter than did random sites. Soil moisture did not differ between occupied and random sites, but occupied sites had significantly more humus.

Occupied and random sites also differed in presence of predators. Potential predators included other lizards, mongoose, and centipedes. *Anolis cristatellus* and *A. stratulus* were the only anoles found in the 60 survey quadrats. Both species were observed feeding on *S. nicholsi*. Presence of anoles was greater on occupied sites than on random sites; all occupied sites contained anoles. Adult anoles perched in trees or shrubs and went to the floor to feed or to move to another tree. Only juveniles were observed perched on leaf litter. *Hemidactylus mabouia* was equally frequent on occupied and random sites. Den holes of the teiid lizard *Ameiva exsul* were found on two occupied and six random sites, but *A. exsul* was observed foraging in occupied sites and never seen to do so in random sites. The Indian mongoose (*Herpestes auropunctatus*) was observed on four occupied sites and no random sites. Large (length >120 mm) centipedes (*Scolopendra* sp.) and tarantula-like spiders (*Cyrtopholis portoricensis*) appeared to be foraging on two occupied sites. Neither was seen eating *S. nicholsi*.

DISCUSSION

The small habitat patches occupied by *S. nicholsi* on the Cabo Rojo Refuge were

TABLE 2.—Comparison of habitat variables in 33 occupied sites and 33 paired random sites for *Sphaerodactylus nicholsi* at Cabo Rojo. Values given are mean \pm standard deviation. The substrate temperature comparison is based on 14 occupied and 14 random sites; the air temperature comparison is based on 12 occupied and 12 random sites. All 20 habitat variables are shown. Those variables with a significant difference ($P \leq 0.05$) between occupied and random sites according to Wilcoxon Signed Rank Test with Bonferroni correction for multiple comparisons are marked with an asterisk. The Bonferroni cutoff for 20 variables was $P \leq 0.0025$.

Habitat variables	Occupied sites	Random sites	significance
Trees			
Presence of trees	1.00 \pm 0.00	0.52 \pm 0.51	*
Presence of species with fruits	0.82 \pm 0.39	0.27 \pm 0.45	*
Presence of evergreen species	0.94 \pm 0.24	0.36 \pm 0.49	*
Presence of deciduous species	0.39 \pm 0.50	0.30 \pm 0.47	
Microhabitat			
Substrate temperature C	30.2 \pm 3.3	36.1 \pm 4.6	*
Air temperature C	31.3 \pm 2.3	33.5 \pm 2.4	
Percent soil moisture	37.54 \pm 30.5	35.92 \pm 33.05	
pH	6.51 \pm 0.33	6.47 \pm 0.40	
Elevation m	9.24 \pm 7.51	8.79 \pm 8.29	
Soil (frequency)			
Presence of clay	0.15 \pm 0.36	0.21 \pm 0.42	
Presence of sand	0.52 \pm 0.51	0.70 \pm 0.48	
Presence of alluvium	0.18 \pm 0.39	0.15 \pm 0.36	
Presence of humus	0.85 \pm 0.36	0.33 \pm 0.48	*
Substrates (% cover)			
Fruits or seeds %	7.86 \pm 20.08	0.12 \pm 0.37	
Leaf litter %	72.95 \pm 32.60	16.18 \pm 31.21	*
Grass %	10.02 \pm 14.20	51.67 \pm 33.78	*
Branches %	7.12 \pm 7.13	11.25 \pm 18.57	
Exposed soil %	4.41 \pm 11.20	20.93 \pm 23.34	*
Predators			
Presence of anoles	1.00 \pm 0.00	0.42 \pm 0.50	*
Presence of hemidactyls	0.58 \pm 0.50	0.21 \pm 0.42	

* Significant at $P \leq 0.05$ with Bonferroni correction for 20 comparisons.

characterized by protection from direct sunlight by evergreen canopies, thick leaf litter decomposing into humus, and trees that contributed large seeds or dry fruit to the litter. These interrelated features combined to create a microhabitat that offered relatively low, stable temperatures and a coarsely textured organic substrate within which the geckos could live. In comparison the random sites were characterized by exposure to sunlight, either directly or filtered through a thin seasonal canopy, primarily *P. pallida*. On these sites the leaf litter was less abundant, and the ground was commonly bare or supported grass. The random sites gave average conditions on the refuge. The differences between occupied and random sites demonstrated that the geckos selected mesic patches within a larger xeric setting.

The water loss rate for *S. parthenopion* described by MacLean (1985), and assumed

for other sphaerodactylids, should limit *S. nicholsi* to relatively mesic settings. While we did not find differences in soil moisture between occupied and random sites, the differences in substrate temperature will make the occupied sites a less desiccating environment. The average daytime substrate temperatures measured on the shady occupied sites (30.2 \pm 3.3 C) were below regular operating limits (32.5–39.5 C) for the heliothermic *Ameiva exsul* that inhabit the area (Rivera-Vélez and Lewis, 1994). A teiid foraging on an occupied site would need to shuttle out of the site and into the sun to maintain its preferred activity temperature. While we do not know preferred activity temperatures for *S. nicholsi*, we infer a range well below that of *A. exsul* because the gecko is active during the day in the perpetual shade of the occupied sites. The higher average daytime substrate

temperatures on random sites (36.1 ± 4.6 C), on the other hand, combined with radiative heating due to direct exposure to the sun could push body temperatures of sphaerodactylids to lethal levels.

The coarse leaf litter provided by the same trees that moderate temperature created a three-dimensional matrix in which the geckos could hide and forage. The remains of dry fruit in the litter, such as pods from *T. indicus* and *P. dulce*, that provided shelter and structure, may also have been a source of food for the invertebrates eaten by the geckos. The exposed soil on many random sites provided neither shelter nor food for geckos. The high percent cover of grass on random sites indicated exposure to full sunlight. Grass did not develop in the shade of occupied sites.

The open woodland of the refuge created discrete patches of acceptable habitat beneath the crowns of appropriate evergreen trees. These patches were scattered through a matrix of unacceptable habitat consisting of shrubs, grassy areas, and inappropriate deciduous trees, primarily mesquite (*P. pallida*). While mesquite was common on occupied sites, at those locations it always occurred in conjunction with the evergreen species that appeared to be essential for occupation by *S. nicholsi*. Mesquite was the most common tree on random sites and dropped fruit pods, but alone it never supported *S. nicholsi*. *Pithecellobium dulce* and *T. indicus*, on the other hand, cast both perpetual shade and fruit pods. They were two and four times more frequent on occupied sites than on random sites.

Anoles were more frequent on occupied sites than random sites, and *A. exsul* appeared to forage more on occupied sites. Both species were active during daylight. While both anoles and teiids were predators of sphaerodactylids, the greater abundance of the predators on occupied sites did not mean that they were there to eat *S. nicholsi*. They also may have selected the more mesic conditions, and the arthropod species consumed by them may have been more abundant on the relatively mesic sites. The nocturnal habit of *H. mabouia* may have accounted for its similar frequency on the shady occupied and relatively sunny random sites.

The conditions on the random sites defined the average habitat of the refuge near

occupied sites. The differences between random sites and occupied sites meant that *Sphaerodactylus nicholsi* was found on sites that were atypical of the refuge. As the 33 occupied sites represented only 2.2% of the surveyed area, we understand the geckos to have been distributed over the refuge as a metapopulation where subpopulations exploited appropriate habitat patches (Hanski, 1999). The successful reproduction that occurred at these sites (López-Ortiz and Lewis, 2002) may have made them sources of colonists for other sites.

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REPRODUCTION OF THE SMALLEST TORTOISE, THE NAMAQUALAND SPECKLED PADLOPER, *HOMOPUS SIGNATUS SIGNATUS*

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The smallest tortoise, *Homopus signatus*, is inadequately protected, and information on its reproductive ecology can facilitate effective conservation. We combined X-ray radiography and ultrasonography to assess the reproductive status of free-ranging female *Homopus signatus signatus* during August–September 2000 ($n = 30$) and September–October 2001 ($n = 29$). Females produced only single-egg clutches but can produce more than one clutch in a season. Most (ca. 75%) of the females were gravid each spring, so *H. s. signatus* probably has a seasonal pattern of egg production. This pattern may be related to the seasonal climate; summers are hot and dry, yet winter rainfall is moderately predictable. Females were gravid from August through October, but further analyses are necessary to characterize the entire reproductive season and quantify clutch frequency. Large females produced large eggs, compared to eggs of small females. The smallest gravid female had a straight-line carapace length of 84.1 mm while the largest female measured 110.0 mm. Compared to gravid females, nongravid females were in poorer body condition (body mass and mass relative to carapace length) only in 2000, when there was lower rainfall and plant availability than in 2001. The difference in body mass approximated the mass of one egg. The low clutch size and fecundity suggest that populations have low intrinsic rates of natural increase. This plus their limited range and specific habitat requirements make *H. s. signatus* vulnerable to anthropogenic impacts.

Key words: Clutch size; Eggs; Follicles; *Homopus*; Reproduction; Tortoise

A CONSERVATIVE life history pattern has enabled chelonians to persist for roughly 200 million years and inhabit terrestrial, fresh water, and marine habitats (Frazer, 2000). Part of the success of chelonians lies with their long lives, iteroparous reproduction, and low adult mortality rates. A trade-off is the long period required to reach maturity, with the

mortality risks of hatchlings and juveniles being potentially high (Frazer, 2000; Kuchling, 1999). Within this life-history pattern, females of various species show considerable variation in how they allocate their resources (e.g., energy) to egg production, with investments ranging from large clutches of small eggs to small clutches of large eggs (Ernst et al., 2000). Ironically, the threatened or endangered status of many chelonian species may be linked to their conservative life histories

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(Congdon et al., 1993, 1994). Therefore, studying chelonian life histories is central to efforts to conserve chelonians.

The speckled padloper (*Homopus signatus*) is inadequately protected (Branch et al., 1995) and listed as "Lower Risk/Near Threatened" by the IUCN (Hilton-Taylor, 2000). *Homopus signatus* is associated with rocky habitat in a small distribution along the west coast of southern Africa (Boycott and Bourquin, 2000; Branch, 1998). A variety of anthropogenic impacts, including mining, overgrazing livestock, poaching, and vehicular traffic, threaten these tortoises and their habitat (Boycott, 1989; Loehr, 2002a). In addition, climate-change models predict major changes to their habitat within the next 50 years (Midgley et al., 2001). For effective conservation action, we require information on the general and reproductive ecology of *H. signatus*. Data for captive *H. s. signatus* suggest that they produce only single-egg "clutches" and have a low fecundity, producing at most four eggs in late winter and spring (Loehr, 1999). However, there has been no study of *H. signatus* reproduction in the wild.

We studied the reproduction of free-ranging *H. s. signatus* in late winter and spring of 2000 and 2001. Their environment is very seasonal, with hot, dry summers and cool, moist winters (Le Roux and Schelpe, 1997; Loehr, 2003). Spring conditions are mild, with annual food plants growing in response to winter rains (Le Roux and Schelpe, 1997; Loehr, 2002b). Thus, we predict that *H. s. signatus* females have seasonal reproductive activity, with high levels of egg production and vitellogenesis in spring. Female size has a major influence on egg size and fecundity for many chelonians (Congdon and Gibbons, 1985; Iverson, 2002; Valenzuela, 2001). The small body size of *H. s. signatus* probably facilitates the use of many microhabitats in their rocky environment. However, a small body size may constrain egg size and fecundity. We hypothesize that large female *H. s. signatus* produce larger eggs, and more eggs, than small females produce. Annual variation in resources, such as rainfall and food availability, can influence body condition and reproductive output of females (Henen, 1997; Kuchling, 1999). Therefore, we completed radiographic analyses in both years to test for annual variation in reproductive

activity. To assess the importance of body condition to reproductive output, we compared indices of body condition between gravid and nongravid females.

MATERIALS AND METHODS

General Design and Measurements

We studied *H. s. signatus* near Springbok (2917DB), South Africa in 2000 (22 August to 22 September, 30 females) and 2001 (5 September to 3 October, 29 females). The field site was approximately 36,000 m² and included a rocky slope and adjacent flower fields (Loehr, 2002a). Rainfall and maximum air temperatures were obtained from the Springbok weather station, 2.5 km north of the study area. We captured females opportunistically to determine reproductive status via X-ray radiography and ultrasonography.

Captured individuals were promptly weighed (± 0.1 g, Soehnle Ultra digital balance), usually before they excreted urine or feces. For individuals caught more than once in a year, we report the highest body mass, which we used to calculate body condition. We used vernier calipers (± 0.1 mm) to measure straight-line carapace length (SCL, nuchal to supracaudal), straight plastron length (PL, midline, gular to anal), maximum shell height, and maximum shell width. Tortoise mass changes significantly with hydration state, food intake or excretion. Thus, we also estimated female size as shell volume, using a modified formula for an ellipsoid ($V_{\text{fem}} = \pi * \text{SCL} * \text{shell height} * \text{shell width} / 6000$, as cm³). We used two estimates of body condition: body mass divided by SCL (g mm⁻¹) and body mass divided by shell volume (g cm⁻³). Results for the latter index closely approximated the body densities (without air spaces) of desert tortoises and pond turtles (1.17 and 1.13 g cm⁻³, respectively; Henen, 1997, 2001).

For each tortoise, two field workers counted the scute laminae of the left, anterior-most costal scute (Germano, 1998; Germano and Bury, 1998; Loehr, 2002a). Specimens with eroded scutes or many compact laminae were classified as "old". We did not convert laminae counts to years because it is not known how many laminae form annually. Individuals were marked uniquely with small dots of black nail

polish on the carapace, and photographs were made for identification.

Radiography

Captive-bred *H. s. signatus* with SCL as small as 90 mm can be gravid (V. J. T. Loehr, unpublished data). To determine the minimum size for reproduction, we radiographed females as small as 75 mm at the Dr. Van Niekerk Hospital in Springbok. Females were radiographed dorso-ventrally (at 50 kV for 0.25 s at 50 mA), with 1 to 5 tortoises placed upright (i.e., on their plastrons) on each radiograph. Seven females were radiographed twice in one spring, 12–20 days after the first radiograph. Gravid females were easily identified from the eggshell images in the radiographs (Burbidge, 1967, as cited in Kuchling, 1999; Gibbons and Greene, 1979).

The length and width of egg images (± 0.05 cm) were measured from the radiographs and converted to actual egg dimensions using the ratio of true plastron lengths to plastron lengths in the radiographs. For six females, we could not measure the plastron lengths reliably in the radiographs. Therefore, ratios were averaged for all possible tortoises on each radiograph to obtain robust values, the average being used to calculate true egg lengths and widths on that radiograph (e.g., true egg length = radiograph egg length * ratio). The plastron and egg were different distances to the film, probably causing us to overestimate egg size slightly. The average correction for the 18 radiographs (mean ± 1 SD = 0.993 ± 0.007) was only 1% different than that estimated using the Graham and Petokas (1989) method (using a focus to film distance of 1000 mm, an egg radius of 12.46 mm [Table 1], and an estimated distance between film and egg of 5 mm). Due to the large eggs and small shell heights, we assumed that the long axis of the eggs were parallel to the film plane. The calculated egg length and width were used to estimate egg volume for nonspherical eggs (Coleman, 1991): Volume = π (L) (W²) / 6, where L and W represent egg length and width, respectively.

Ultrasonography

Although radiography helps us determine the size and number of eggs in the oviduct, oviducal eggs represent only a fraction of

TABLE 1.—Mean (± 1 SD and range) size, scute laminae, body condition, and egg sizes of female *Homopus signatus signatus* (SCL ≥ 84.1 mm) in 2000 and 2001. There were no significant annual effects (Student's *t*-test or Mann-Whitney rank sum test, $P > 0.25$) but some means differed between gravid and nongravid females in 2000^a.

	Mean	SD	Range	<i>n</i>
Straight carapace length (SCL, mm)	92.6	6.09	84.1–110.0	55
Shell height (mm)	40.2	2.94	33.1–48.0	55
gravid 2000*	40.7	2.77	36.7–48.0	19
nongravid 2000	37.8	2.94	33.1–40.9	7
gravid 2001	40.8	2.99	37.3–47.2	18
nongravid 2001	39.5	3.09	35.2–44.1	6
Shell width (mm)	68.8	5.45	58.85–83.07	54
Shell volume (cm ³)	135.8	28.7	90.7–225.0	54
Body mass (g)	151.4	30.0	94.4–240.9	55
gravid 2000*	157.6	30.8	113.1–240.9	19
nongravid 2000	128.8	20.7	103.8–161.1	7
gravid 2001	160.1	31.1	110.5–230.9	18
nongravid 2001	141.4	16.4	122.3–163.9	6
Mass/SCL	1.62	0.219	1.12–2.19	55
gravid 2000**	1.68	0.218	1.34–2.19	19
nongravid 2000	1.43	0.152	1.22–1.62	7
gravid 2001	1.69	0.208	1.28–2.11	18
nongravid 2001	1.56	0.135	1.39–1.71	6
Mass/Volume (g/cm ³)	1.12	0.0784	0.910–1.280	54
Scute laminae (number)	–	–	8–13 ^b	32
Egg length (cm) ^c	3.46	0.192	3.07–3.89	35
Egg width (cm) ^c	2.49	0.156	2.22–2.86	35
Egg volume (cm ³) ^c	11.3	1.74	8.4–15.4	35

^a For gravid-nongravid comparisons in 2000, $P < 0.05$ (*) or $P < 0.01$ (**); two-way ANOVA, Student's *t*-test, or Mann-Whitney rank sum tests.

^b Scute laminae counts for nongravid females ranged from 9 to 13, both groups had females scored as worn or "old", possibly > 13 laminae.

^c Egg length and width possibly overestimated by 1%, volume by 2%.

a female's reproductive mass and investment. Radiography can be complemented with ultrasonography, which allows views of ovarian follicles (vitellogenic and atretic), corpora lutea, and eggs, with their components (e.g., yolks), in early stages of shell calcification. Yolk size provides a good estimate of the size of preovulatory follicles, follicles that are large enough for ovulation. The presence of preovulatory follicles suggests that more clutches may be produced in that season. Thus, ultrasonography can quantify potential for future clutches, and help quantify total reproductive investment.

On 21 and 22 September 2000, we used a PIE 100 Veterinary Ultrasound scanner (Philips, Maastricht, Netherlands) with a curvilinear, intravaginal probe to scan the reproductive tissues of 14 female *H. s. signatus* (SCL = 84.1 to 110.0 mm). We used a water bath for ultrasound transmission (Kuchling, 1999) and scanned females from the left inguinal, right inguinal, and cranial acoustic windows (Henen and Hofmeyr, 2003). We counted follicles and measured follicle, yolk and egg sizes with the scanner's electronic calipers (± 0.01 cm). The smallest yolk had a diameter of 2.12 cm or a spherical volume of 4.99 cm^3 , which we used as the minimum size for preovulatory follicles. To evaluate the stages of follicle development, we expressed follicle volume as a fraction of preovulatory size ($\text{POS} = 4.99 \text{ cm}^3$).

Several yolks and follicles had hollow appearing cores due to low echogenicity. However, all follicles and hollow centers had regular outlines. Thus we regarded follicles with "hollow centers" as vitellogenic follicles. We probably did not detect all follicles, as some may have been in acoustic shadows of other structures (Henen and Hofmeyr, 2003; Kuchling, 1989, 1999; Robeck et al., 1990).

Statistics

We summarized results, as means and standard deviations, only for adult females. Our two samples, 2000 and 2001, were independent but thirteen females were studied in both years. We had insufficient repeated measures within and across years to support two-way repeated measures analyses of variance and thus could not simultaneously test these results for effects of year and reproductive state on female size and condition. Consequently, we used paired *t*-tests to assess annual effects on these females' body mass, condition and egg size. We also used these data in independent sampling analyses, including standard two-way ANOVA, Student's *t*-tests and analyses of covariance, for several reasons. First, recapture results did not bias statistical results; means for these females did not differ from means of other females in 2000, 2001 or both years combined ($P > 0.05$ for all Student's *t* or Mann-Whitney rank sum tests). Additionally, (a) females were not telemetered so recapture success followed an independent

sampling design, (b) the number of females caught in both years was low (13 females including 5 gravid females) so the potential for bias was small, (c) paired *t*-tests showed no effects of year, except for a weak effect on egg width and female shell height ($P = 0.045$ and 0.049 , respectively, see Results) and (d) neither egg width nor shell height differed between years for females caught in only one year (egg width: $t_{17} = 0.47$, $P = 0.65$; shell height: $t_{29} = 0.29$, $P = 0.78$).

We first used two-way ANOVA to evaluate simultaneously the main effects of year and reproductive state (gravid or nongravid) on measures of body size (e.g., SCL) and body condition. If a two-way ANOVA failed tests of normality or homoscedasticity, we evaluated separately the main effects on group means using Student's *t*-tests or Mann-Whitney tests as appropriate (i.e., whether group means met parametric assumptions). Correlations were evaluated using linear regression analysis. We used analysis of covariance (ANCOVA) to determine if regression slopes and intercepts were affected by year and reproductive state. The ordinate was female body mass and the covariate was SCL or V_{fem} . If regression slopes differed (*t*-test), the ANCOVA stopped, otherwise we proceeded to test for differences in regression elevations (*t*-test; Zar, 1999). We verified ANCOVA results with statistical software (Statistica 99), which also allowed simultaneous testing for effects of year, reproductive state and the interaction of year and reproductive state (*F*-test). Similarly, egg size data were evaluated using Student's *t* or Mann-Whitney tests, linear regression, and ANCOVA. We performed Student's *t*-tests and Mann-Whitney tests using StatsDirect (StatsDirect Statistical Software, UK), and the ANOVA using Statistica '99 (StatSoft, Tulsa, Oklahoma, USA). *P* values < 0.05 were considered statistically significant.

RESULTS

Rainfall and Air Temperature

Annual rainfall in 1999 (201 mm), 2000 (163 mm), and 2001 (226 mm) were lower than the long-term average for Springbok (1990–1998: 247 ± 69.1 mm, range 168 to 376 mm). The rainfall for 2000 was the lowest of this 12-year period, primarily due to low rainfall in autumn

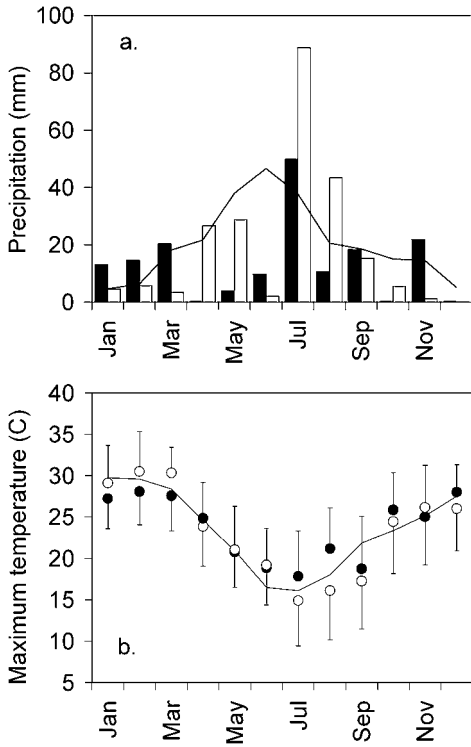


FIG. 1.—Monthly rainfall (a) and maximum air temperature (b) in 2000 (solid bars and circles) and 2001 (open bars and circles) for the Springbok weather station (2.5 km north of the field site). Temperatures are indicated as means \pm 1 SD. The solid lines represent long-term averages (1990–1998).

and winter (Fig. 1a). Maximum temperatures were similar in the two study periods (Student's *t*-test, $P > 0.05$) and monthly maxima were similar among 2000, 2001, and the long-term average (Fig. 1b).

Reproductive Activity

The smallest gravid female had a SCL of 84.1 mm and 9 scute laminae (Table 1). Two smaller females, one captured in 2000 and one in 2001, were not gravid (SCL = 77.8 and 80.0 mm, mass = 89.4 and 94.4 g, and 8 and 9 scute rings, respectively). All subsequent results are for individuals with SCL \geq 84.1 mm, the minimum SCL for a gravid female.

Most females were gravid in spring 2000 (73%; 19 of 26 females) and 2001 (75%; 18 of 24 females). Of the 12 females radiographed in both years, five had eggs in radiographs in 2000 and 2001. All 14 females scanned ultra-

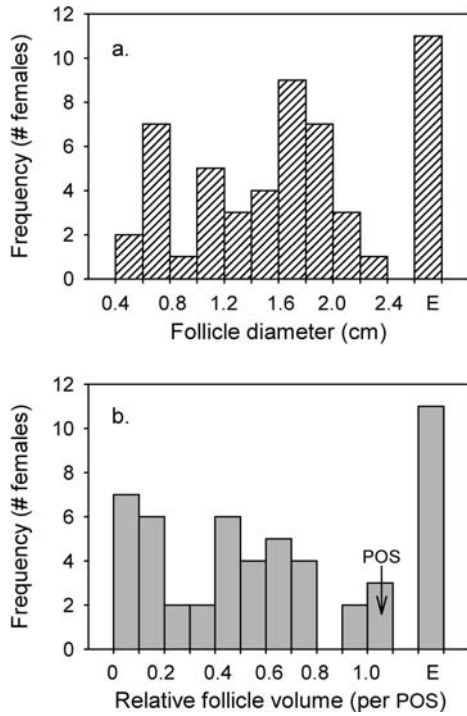


FIG. 2.—Size frequency distribution of vitellogenic follicles in 14 female *Homopus signatus signatus*. Follicle sizes are presented as (a) diameter and (b) volume relative to preovulatory size (POS). The E represents oviducal eggs.

sonographically in September 2000 contained vitellogenic follicles; 11 of those females were gravid with egg yolk diameters of 2.12 to 2.39 cm (2.23 ± 0.09 cm; $n = 8$). Follicle diameters ranged from 0.47 to 2.26 cm (1.46 ± 0.52 cm; $n = 53$; Fig. 2a) and follicle volumes ranged from 0.05 to 6.04 cm^3 (2.21 ± 1.69 cm^3 ; $n = 53$). The frequency of follicle volumes showed three size groups (Fig. 2b). Several females (64%) contained small vitellogenic follicles (<0.2 POS) while 86% had medium to large follicles (0.4 to 0.8 POS) and 29% had follicles larger than 0.9 POS. Follicle volumes did not differ between gravid and nongravid females (Student's *t*-test, $P = 0.16$).

The evidence for multiple clutches per year included: (a) one female with different eggs on two consecutive radiographs (based on the level of eggshell calcification), and (b) three gravid females also containing follicles of preovulatory size. Additionally, several females contained more than one large follicle and one

TABLE 2. Relationship of female body mass to female size (SCL and V_{fem}) for gravid female (G) and nongravid female (NG) *Homopus signatus signatus* during 2000 and 2001. Regression statistics include slope, intercept, coefficient of determination (r^2), F values and degrees of freedom (df)*. Also indicated are regression results for female mass minus egg mass and for female mass minus egg and follicle mass, assuming densities of 1 g cm^{-3} for eggs and follicles.

	Slope	Intercept	r^2	F_{df}
SCL				
G 2000	4.60	-271	0.872	$F_{1,17} = 115.9^{***}$
NG 2000	3.73	-205	0.885	$F_{1,5} = 38.42^*$
G 2001	4.28	-243	0.896	$F_{1,16} = 138.2^{***}$
NG 2001	5.13	-324	0.845	$F_{1,4} = 21.79^*$
Volume				
G 2000	1.03	12.8	0.918	$F_{1,17} = 189.5^{***}$
NG 2000	0.853	26.9	0.976	$F_{1,5} = 206.8^{***}$
G 2001	0.903	32.3	0.912	$F_{1,16} = 165.5^{***}$
NG 2001	0.630	61.2	0.777	$F_{1,4} = 13.91^*$
Body mass - egg mass				
SCL	4.18	-243	0.880	$F_{1,48} = 355.2^{***}$
V_{fem}	0.885	23.6	0.910	$F_{1,48} = 466.5^{***}$
Body mass - egg and follicle mass				
SCL	4.20	-253	0.936	$F_{1,10} = 145.6^{***}$
V_{fem}	0.886	13.8	0.952	$F_{1,10} = 199.2^{***}$

* Regressions were significant at $P < 0.05$ (*) and 0.0005 (***)

gravid female had four follicles larger than 0.9 POS. The three nongravid females had only small to medium follicles (<0.7 POS), although one of these females laid an egg before being scanned ultrasonographically.

Individuals had one to six follicles (3.8 ± 1.3 ; $n = 14$), but large females had more medium and large follicles than did small females. The number of follicles exceeding 2.0 cm^3 (0.4 POS) was correlated with both body mass ($r^2 = 0.376$; $F_{1,12} = 7.230$; $P = 0.020$) and SCL ($r^2 = 0.296$; $F_{1,12} = 5.048$; $P = 0.044$).

Body Condition and Annual Effects

Body size and condition may vary among years and influence reproduction. Nongravid females had lower body masses, shell heights and body mass to SCL ratios than did gravid females (all $P < 0.05$). These differences were primarily due to significant differences occurring in the drier year (2000, i.e., low values for nongravid females, Table 1); significant differences were not detected in 2001. No measure

of body size or condition differed between females caught in both years and females caught in only one year (2000, 2001 or both years combined; all $P > 0.08$, for Student's t and Mann-Whitney tests). Additionally, body size and condition did not differ between years for females caught in both years (all paired $t < 2.14$, $df = 12$ and $P > 0.05$), except shell width was marginally larger in 2001 than in 2000 ($t_{12} = 2.19$, $P = 0.049$; 2000: $68.55 \pm 5.97 \text{ mm}$ and 2001: $69.21 \pm 6.15 \text{ mm}$).

Female mass was correlated with SCL and shell volume (Table 2), requiring ANCOVA to evaluate whether relationships differed between years, and between gravid and nongravid females. For regressions of female mass to SCL or shell volume, there were no annual differences in slopes or elevations (all $t < 1.35$, $df > 49$ and $P > 0.09$), suggesting that body condition did not differ between years. However, regression elevations, and thus female mass or condition, tended to be higher for gravid females, than for nongravid females, in 2000 (SCL: $t_{23} = 2.69$, $P = 0.007$; V_{fem} : $t_{23} = 2.12$, $P = 0.023$) and both years combined (SCL: $t_{47} = 2.60$, $P = 0.009$; V_{fem} : $t_{47} = 2.55$, $P = 0.008$), but not in 2001 (SCL: $t_{21} = 0.814$, $P = 0.22$; V_{fem} : $t_{21} = 1.36$, $P = 0.096$). No slopes differed in these ANCOVA (all $t < 1.462$, $df > 20$ and $P > 0.079$) and there was no significant interaction between year and reproductive state (all $F_{1,45} < 2.3$ and $P > 0.14$).

At the mean SCL in 2001 (93.3 mm), the predicted body mass was 155.7 g and 154.5 g for the gravid and nongravid female regressions, respectively. This difference in body mass (1.2 g; 8.0 g when using V_{fem} as the covariate) was less than the mass of an egg if we assume an egg density of 1 g cm^{-3} (Table 1). For 2000, however, the predicted mass of gravid and nongravid females differed by 14.7 g (12.4 g for the V_{fem} based prediction), essentially the mass of one egg.

The body mass of nongravid females did not differ from the mass of gravid females after subtracting egg mass, for each year and both years combined, whether using SCL or V_{fem} as the covariate (ANCOVA, all slope and elevation comparisons, $t < 1.54$, $df > 19$ and $P > 0.075$). However, when SCL was the covariate, we found a small but statistically significant interaction between year and reproductive state (ANCOVA for year: $F_{1,45} = 0.932$, $P =$

0.339, reproductive state: $F_{1,45} = 0.119$, $P = 0.732$ and interaction $F_{1,45} = 4.21$, $P = 0.046$), primarily due to body mass increases by nongravid females from 2000 to 2001 (Table 1). For ultrasound females, we also estimated somatic mass as body mass minus egg and follicle mass, assuming follicle densities of 1 g cm^{-3} . The variation (r^2 value) in somatic mass that was explained by variation in SCL or shell volume was slightly higher than the r^2 values for whole body mass regressions on SCL or shell volume (Table 2). Sample sizes were too small for ANCOVA comparisons of gravid and nongravid somatic masses.

Egg Size

Clutch size did not vary (clutch size = 1) and egg length, width and volume did not differ between years (Table 1; all $P > 0.25$). Egg width for the five females found gravid in both years increased slightly from 2000 to 2001 (paired $t_4 = 0.045$; 2000: mean \pm 1 SD = 25.36 ± 1.62 mm, 2001: 26.38 ± 1.68 mm) but for other females, egg width did not differ between years (Student's $t_{17} = 0.47$, $P = 0.65$; 2000: 24.40 ± 1.33 mm, $n = 9$; 2001: 24.71 ± 1.53 mm, $n = 10$). The coefficient of variation (i.e., SD/mean, %, Table 1) was roughly the same for egg length (5.5%) and egg width (6.3%), but higher for egg volume (15.4%).

Egg width was correlated with SCL, body mass, the ratio of mass to SCL, shell height and shell width ($r^2 = 0.44$ to 0.67 ; all $F > 25.7$, $P < 0.001$ and $n = 34$ or 35). However, egg width was not influenced by the ratio of mass to female volume (V_{fem} ; $F_{1,33} = 0.21$, $P = 0.65$). Egg volume was also correlated with female SCL, body mass, body mass to SCL ratio, body mass to V_{fem} ratio, shell height and shell width ($r^2 = 0.44$ to 0.67 ; all $F > 26$, $P < 0.001$, and $n = 35$). The relationships of egg width (and egg volume) to SCL, body mass and V_{fem} did not differ between years (ANCOVA, $P > 0.1$ for all slope and elevation tests).

In contrast, egg length was not strongly correlated with female size. Egg length was weakly correlated with body mass ($r^2 = 0.14$, $F_{1,33} = 5.46$, $P = 0.026$) but not correlated with female shell dimensions or condition indices (all $F_{1,33} \leq 6.52$, $P \geq 0.06$). The relationship of egg length to body mass did not differ between years (ANCOVA slope and intercept tests, $P > 0.05$). For gravid females

caught in both years, sample sizes were too small for ANCOVA comparisons of egg size between years.

DISCUSSION

Reproductive Activity and Cycle

Previously, there was nothing known about the reproduction of free-ranging *H. s. signatus*. A large proportion of the female *H. s. signatus* in our study was gravid in both years (73 to 75%). However, this proportion may underrepresent values for both years because (a) gravid status was probably not measured for the entire reproductive season, (b) early stage oviducal eggs are not detectable radiographically, and (c) some females may have been gravid before or after evaluation with radiography or ultrasonography. Captive *H. s. signatus* have a seasonal reproductive cycle (Loehr, 1999). A high reproductive activity (percent gravid) would be consistent with chelonians having seasonal reproductive cycles (Licht, 1984). Seasonally reproducing species can have similar or higher levels of reproductive activity than we found for *H. s. signatus* (e.g., *Testudo graeca* 93%, Díaz-Paniagua et al., 1996; *Gopherus agassizii* 75–100%, Wallis et al., 1999).

Reproductive cycles of chelonians are quite diverse. Many temperate-zone and subtropical chelonians initiate vitellogenesis in late summer and ovulate their first clutch in spring (Licht, 1984; Moll, 1979). A few temperate species nest in autumn–winter and some subtropical and tropical species initiate vitellogenesis in spring and oviposit in autumn (Kuchling, 1999). The high incidence of small vitellogenic follicles in September 2000 suggests a new vitellogenic cycle in early spring for *H. s. signatus*. The three size classes of follicles imply that vitellogenesis is a discontinuous process, with medium and large follicle classes representing the new cycles of a previous season (e.g., autumn) or a previous year.

We do not know the onset of the reproductive season for free-ranging *H. s. signatus*. Captive females can lay eggs in late winter and spring (Loehr, 1999) and wild females were gravid throughout August to October. Free-ranging *H. areolatus*, a closely related species, can be gravid from May through November (B. T. Henen, M. D.

Hofmeyr, and G. Kuchling, unpublished data). Similarly, *H. s. signatus* may also be ready for ovulation in autumn or early winter. The follicle classes may represent clutches to be laid in subsequent years (Agassiz, 1857 as cited in Moll, 1979) or different clutches within a reproductive season (Moll, 1979), depending upon rates of vitellogenesis. The class of large follicles that we detected in *H. s. signatus* probably represented clutches of the current reproductive season. The medium-sized follicles could represent later clutches of the current season or clutches of the next reproductive season.

Captive *H. s. signatus* can produce up to four clutches per season (Loehr, 1999). Our results indicated multiple clutches for wild females, including potentially five clutches for the gravid female with four large follicles in 2000. This female could have been gravid beyond December 2000, based upon estimated clutch retention times for captives (25 to 43 d, Loehr, 1999 and unpublished results). However, further study over an annual cycle is needed to clarify the onset and duration of reproductive activity in *H. s. signatus*.

Body Size Effects

The minimum SCL for reproduction was smaller than previously recorded for captives (90 mm, V. J. T. Loehr, unpublished data). Yet this SCL (84.1 mm) was still a large percentage of the largest female's SCL (110.0 mm; 76%), suggesting a considerable delay in reaching maturity. Although the smallest gravid female had nine scute laminae, little is known about longevity in free-ranging *H. s. signatus*. A slightly larger congener, *Homopus areolatus*, may live 28 years in captivity (Hughes, 1986). Understanding the relationship between fecundity and age would be useful information for demographic analyses, but erosion limits the utility of scute laminae as an age marker. Body size can often serve as an adequate surrogate in demographic analyses, and was important to the reproductive potential of free-ranging *H. s. signatus*.

The number of follicles, and potential for number of eggs produced, was correlated with female size (SCL and body mass). Additionally, female size influenced egg size, which may result in larger hatchlings. By producing larger

and probably more offspring, large females may have a disproportionately large effect upon each cohort of the population. Thus, there may be considerable selection for a large body size or related traits (e.g., growth rates) in female *H. s. signatus*. This is supported by the large sexual dimorphism in body size (male SCL \leq 87.5, Boycott and Bourquin, 2000). However, it would be useful to know whether large eggs result in large hatchlings (e.g., Spotila et al., 1994) and whether large hatchlings have higher survivorship than do small hatchlings (see Congdon et al., 1999; Iverson and Smith, 1993; Janzen, 1993).

Body Condition and Environment

Namaqualand is arid and has hot dry summers; ambient temperatures can exceed 45 C (Lovegrove, 1993). However, rain falls with a moderate predictability, primarily during winter months (ca. 60–70%, Lovegrove, 1993; Schulze, 1997). Thus *Homopus s. signatus* females may have evolved to prepare regularly for winter rains and the concomitant plant production. This may help explain, despite the harsh dry summers, the high reproductive activity in 2000 and 2001. It may also explain the lack of annual differences in female mass, female condition, and egg size. Other terrestrial chelonians reduce egg production in response to low rainfall (e.g., *Terrapene carolina bauri* Dodd, 1997; *T. ornata luteola* Nieuwolt-Dacanay, 1997), but rainfall must be extremely low before desert tortoises (*Gopherus agassizii*) reduce egg production (Henen, 1997; Turner et al., 1986).

Annual differences in rainfall and vegetation (2000 < 2001) did not cause annual differences in egg size. Yet the body mass, and body mass to SCL ratio, of nongravid females in 2000 was low compared to that of gravid females in 2000 and nongravid females in 2001. The additional rain and vegetation of 2001 probably helped nongravid females increase their condition towards that of gravid females. The difference between gravid and nongravid body mass in 2000 appeared to be the mass of one egg (ANCOVA). Comparisons of somatic mass estimates also suggested that gravid and nongravid body masses differed by the amount of reproductive tissue. However, follicle volumes did not differ between gravid and nongravid

females scanned in 2000. A more extensive analysis of the reproductive season, female mass and female condition is needed.

Reproductive Strategy

The delayed maturity and iteroparous reproduction of *H. s. signatus* is consistent with many chelonian life histories. *Homopus s. signatus* produced single-egg clutches, as do other small, rock-dwelling tortoises (*H. boulengeri*, Boycott and Bourquin, 2000; the Nama padloper "*H. bergeri*", Boycott and Bourquin, 2000; Schleicher and Loehr, 2001; *Malacochersus tornieri*, Darlington and Davis, 1990; V. J. T. Loehr, personal observation). The small body and flat shape of *H. s. signatus* probably facilitates the use of many microhabitats, including crevices, enhancing survivorship in their rocky environment. However, this size and shape limits body volume and probably constrains egg size and fecundity. Females of *Homopus s. signatus* produce large eggs, so the trade-off with female survivorship occurs through clutch size and fecundity. A few other tortoise species typically produce single-egg clutches (*Chersina angulata*, Branch, 1998; *Pyxis arachnoides*, Zwartepoorte, 2000, V. J. T. Loehr, personal observation; *Pyxis planicauda*, K. Buley and R. Gibson, unpublished data). Those species are larger than *H. s. signatus* and do not inhabit rocky areas, so other factors contribute to those females producing single egg clutches.

Moderately predictable rainfall can support high reproductive activity and reproductive strategies involving single-egg clutches. Placing an investment in a large egg could be risky in an unpredictable environment, but large eggs may result in large hatchlings that are able to survive the hot and dry summers. Females that produce single-egg clutches can still alter clutch frequency in response to the current availability of resources. If current availability is limited, then females need not commit to producing another egg, thereby conserving resources. Whether follicles can be used as "nutrient stores" is unknown. Atresia was not prevalent in our analysis, but we scanned females only during a very brief period. Additionally, spreading eggs over different clutches could also reduce predation risks (on females or nests) for each clutch.

Egg size is another variable in reproductive strategy. *Homopus s. signatus* eggs are 31% larger than eggs of *H. areolatus* (B. T. Henen, M. D. Hoffmeyer, and G. Kuchling, unpublished data). However, *H. areolatus* females are 10 to 33% larger than *H. s. signatus* females (based on SCL and body mass, respectively; B. T. Henen, M. D. Hoffmeyer, and G. Kuchling, unpublished data). The smaller egg of *H. areolatus* may be linked to its more mesic habitat (M. D. Hofmeyr, B. T. Henen and V. J. T. Loehr, unpublished data).

Conservation

Clutch size equaled one, and females had follicles for additional eggs, but our data are limited in explaining annual fecundity. Based on clutch size, numbers of preovulatory follicles and egg retention times, fecundity appears low in *H. s. signatus*. These factors, plus the several years required to reach maturity, imply that *H. s. signatus* has a low intrinsic rate of increase (r). The ability of populations to maintain themselves can easily be surpassed by anthropogenic impacts. *Homopus s. signatus* are associated with rocky outcrops, so populations are isolated over the species' range. Thorough population surveys have not been completed for decades (see Greig and Burdett, 1976). Our data on the fundamental reproductive abilities suggest that this species is vulnerable, warranting current assessments of its population status and conservation potential (see also Boycott and Bourquin, 2000; Branch et al., 1995). Given recently raised concerns of global warming and consequent potential for climate and habitat changes in southern Africa (Midgley et al., 2001), this reassessment is urgent.

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