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Egg Size and Annual Egg Production by Female Desert Tortoises (*Gopherus agassizii*): The Importance of Food Abundance, Body Size, and Date of Egg Shelling

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ABSTRACT.—**We studied egg production in two Californian populations of desert tortoises, (*Gopherus agassizii*) in 1992 and 1993. One population inhabited the Desert Tortoise Research Natural Area (DTNA) in the western Mojave Desert, where most of the rain falls in the winter. The second population lived near Goffs, in the eastern Mojave, where annual precipitation is divided more evenly between winter and summer. Due to El Niño conditions, heavy winter rains fell at both sites in both years (1991–1992 and 1992–1993). Consequently, the biomass of spring annuals and annual egg production by tortoises were high in both years at both sites. There were no differences in reproductive output between years so we pooled data for both years to examine the relationship between egg-laying parameters (clutch size and frequency, annual egg production, egg size, etc.) and female size. Variation in annual egg production was due mainly to variation in clutch size, not clutch frequency. Annual egg production per female was lower at DTNA than at Goffs, because some adult females at DTNA did not produce eggs in some years. Females that did lay eggs produced the same number of eggs per year at both sites, even though females at Goffs were smaller (midline carapace length = 214 mm) than females at DTNA (MCL = 234 mm). Despite correction for these body size differences, the eggs produced at Goffs were smaller in all dimensions than eggs produced at DTNA. Smaller eggs and presumably smaller neonates may be related to the greater predictability of summer rain and consequent greater food supply for emergent hatchlings at Goffs. For adult females, food supply probably limits reproduction only during drought years. How can individual females vary their annual reproductive output? Our more extensive data for DTNA tortoises showed that larger females produced larger clutch sizes. In addition, by statistically removing the effects of body size we showed that larger clutches contained smaller eggs. Moreover, larger females produced eggs earlier in the year giving them a better opportunity to produce a second clutch that year. Thus, timing of first clutch was important. Still, much of the variation in reproductive output was not explained. Other characteristics of individuals (e.g., age, genetics, physiological maturity, home range quality, or forage selection) may explain some of the variation in reproductive output.**

Rainfall in the Mojave Desert is unpredictable and varies greatly with time and location. While the long-term average annual precipitation is about 150 mm, annual precipitation ranges from as little as three millimeters to as much as 400 mm. Rain often falls primarily in the winter, promoting a flush of annual plants in the spring (Beatley, 1974), but seasonal rainfall patterns usually vary considerably from year to year. Rainfall also differs between the eastern and western regions of the Mojave Desert (Nagy and Medica, 1986; Peterson, 1996a). Rainfall from July through September, when many desert tortoise eggs hatch, frequently exceeds 40 mm in the eastern Mojave, whereas the western Mojave averages 20 mm or less (National Oceanic and Atmospheric Administration, 1995). Thus, summer annuals are more likely to germinate and grow in the Eastern Mojave and, as a result, tortoise hatchlings in the Eastern Mojave are more likely than their western counterparts to find food and water before hibernating in autumn. We wondered whether variation in tortoise reproduction matched current resource availability for adults or food and water availability for hatchlings.

The nutrients for reproduction may come from nutrients consumed from the environment and from the animal's body stores. However, an animal confronted with unpredictable food and water supplies should not draw too heavily upon body reserves if doing so threatens its life. This is especially true for female desert tortoises because: (1) they require many years to mature (ca. 15 yr. Woodbury and Hardy, 1948) and, (2)

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a female's reproductive life may last 30 yr (Germano, 1994). In severe droughts 18 to 41% of adults may die (Turner et al., 1984; Peterson, 1994), so selection pressure is high. Also, if an individual's future survival and fecundity is affected by current reproductive output, there is a trade-off between the immediate fecundity benefits and the potential future losses that affects lifetime reproductive output (Williams, 1966; Charnov and Krebs, 1974; Dijkstra et al., 1990). As desert tortoises have no parental care (unlike most birds) and can be easily monitored for years at a time (unlike marine turtles), desert tortoises offer opportunities for new insights into reproductive effort. Reproductive output in a desert tortoise (*Gopherus agassizii*) depends on clutch size, possibly egg size, clutch frequency per breeding season, and the number of breeding seasons in the tortoise's lifetime.

In pioneering studies of reproductive output of free-living desert tortoises Turner et al. (1984, 1986) reported that female tortoises usually lay at least one clutch per year, that tortoises lay even when rainfall and food conditions are poor, that the upper ceiling on mean clutch frequency is about 2.0, and that mean clutch size is relatively low. The present radiographic study of reproduction in the desert tortoise builds on those of Turner et al. (1984, 1986). By measuring both egg number and egg size we attempted to answer a number of interesting questions concerning reproductive output in *Gopherus agassizii*. We were particularly interested in geographic variation, annual variation, body size effects, food availability effects, and the influences of reproductive event timing and *Mycoplasma* infections.

MATERIALS AND METHODS

Sites, Rainfall and Annual Plant Productivity.—In spring of 1992 and 1993 we used x-rays to measure egg production by free-living desert tortoises in the Eastern Mojave Desert (Fenner Valley near Goffs, California, elevation 680 m; see Turner et al., 1986 for details), and desert tortoises living on Section 8 (2.6 km^2) of the Desert Tortoise Research Natural Area (DTNA) in the Western Mojave Desert (elevation 750 m), about 8 km north of California City, California. The perennials creosote bush (*Larrea tridentata*) and burro bush (*Ambrosia dumosa*) dominated both sites, but there were notable differences between sites in the species and abundance of annual plants and the less common perennials.

In 1990, Section 8 was divided into four 64 hectare plots, using fencing to prevent tortoise movements among plots in order to measure the effects of water supplementation on tortoises. The original tortoises (residents) inhabited the two northern plots while the southern plots had the original tortoises (hosts) as well as those moved in from the adjacent Section 6 (guests). In this study we used tortoises from all plots and cohorts (residents, hosts, and guests). Irrigation on the western plots supplemented rainfall to produce a total winter precipitation between 150 and 200 mm—approximately double the 30 yr average annual rainfall for nearby Mojave (ca. 100 mm; NOAA, 1995). The third and final irrigation season, which started in October 1991, added 105 mm of supplementary water but was stopped in early February when 175 mm of rain fell. Thus, during the 1991–1992 winter the "dry" plots received 250 mm and the wet plots 355 mm of precipitation. Heavy rain fell again during the 1992–1993 winter, with nearby Randsburg receiving 336 mm. The heavy rainfall during both winters resulted from El Niño (ENSO) conditions.

Rainfall was measured on site with rain gauges containing mineral oil to trap and prevent the evaporation of the rainwater. In both years we estimated the biomass (g above-ground dry matter/m^2) of winter annuals at Goffs by clipping all annual plants growing in quadrants in late April or early May. We sampled fifty, 50 × 20 cm quadrants along two 100 × 2 m transects. The plants, separated into grasses and forbs, were placed in pre-weighed plastic vials and were later dried to a constant weight at 60 C. Similarly, we estimated plant biomass at DTNA in 1993 using thirteen, 50 × 20 cm quadrants in each of the four 64 ha study plots. A consulting firm supplied the DTNA data for 1990–1992.

Tortoise Handling.—Ten (1992) or 11 (1993) female tortoises at Goffs and 22 (1992) or 33 females (1993) at DTNA were captured and x-rayed. In 1993 we divided the DTNA tortoises into two groups that were sampled in alternate weeks. All of the tortoises carried radio-telemeters (SB-2; AVM Instrument Co., Livermore, California; 1–7% of body mass) glued to the first vertebral scute on the carapace. The general procedure was to locate a female and then, using a pair of sterile gloves, place her individually in a sterilized pan carried on a backpack. The sterile technique minimized the risk of transferring pathogens between tortoises. After capturing several females, we walked to a field laboratory (0–2.5 km away), x-rayed the tortoises and then returned them to their capture sites.

Body Size Measurements.—We weighed a tortoise at capture (before it voided urine and feces) and again when we x-rayed her. Hibernation mass was measured between late October and early March when tortoises were not active. At least once during each spring we used calipers (±1 mm) to measure midline carapace length (MCL), midline plastron length (MPL), height, and width of each female. We recorded

MCL as the midline distance from the anterior edge of the nuchal scute to the posterior edge of the caudal scute; MPL was the midline distance from the notch on the gular scute to the notch of the anal scute. We measured height as the distance from the midline junction of pectoral and abdominal scutes (plastron) to the dorsal surface of the second vertebral scute (directly above the plastron location). Width was the distance between the junctions of marginal scute numbers four and five on the left and right sides. We calculated condition indices (CI) for each tortoise, by dividing body mass (g) by volume (cm^3) determined either by (1) the cube of MCL, or (2) by the product of MCL, width and height.

Radiographic Technique.—Our radiographic method followed those of Gibbons and Greene (1979) and Turner et al. (1986). At DTNA we used a portable x-ray machine (MinXray 300 Northbrook, Illinois). We placed the tortoise upright in its plastic pan, which in turn, was placed on the loaded film cartridge positioned to keep the "focus to film" distance constant at 651 mm. We used Polaroid 180 × 240 mm instant radiographic film (Polaroid, Cambridge, MA) with a Polaroid Instant Radiograph System (Model 85-06 cassette with rare earth intensifying screen and radiographic film processor) enabling us to develop the x-rays on site. Settings of 60 kVP (kilovolt peak) for 0.08 sec gave an acceptable exposure and reduced the tortoises' radiation exposures (2.4 milli-Ampere-sec) to 30% of that given by Turner et al. (1986) and about 2% of that used by Gibbons and Greene (1979). At Goffs we followed the technique of Turner et al. (1986) except the exposure was 10 mA at 75 kVP for 0.45 s with a "focus to film" distance of 559 mm. These exposures should be safe for the females and their embryos (Hinton et al., 1997).

It was easy to interpret clutch size, clutch frequency, and egg dimensions (termed egg-laying parameters) by viewing the radiograph on a light-box and using calipers to measure (±0.02 mm) the maximum length and width of each egg image. To derive the true egg size (length and width) we corrected for radiographic magnification using the technique of Graham and Petokas (1989) which requires knowing the "focus to film" distance and the "object (egg) to film" distance. We used mediolateral (lateral or side view) radiographs of nine gravid females (MCL range = 190–238 mm; 40 eggs) to measure the height of the eggs above the film ("object to film distance"). We found no relationship (no P-value < 0.05) between this height and female size (MCL: $F_{1,8} = 2.3$, $P = 0.18$, $r^2 = 0.25$; body mass: $F_{1,8} = 2.0$, $P = 0.20$, $r^2 = 0.22$) so we used the average height above the film (magnification corrected, mean ± SD: 29.7 ± 7.5 mm, $N = 9$) to correct for radiographic magnification in the standard (dorsoventral) radiographs. Because eggs vary in shape, we also calculated the volume and effective diameter (the diameter if the eggs were spherical) of each egg, using the equation of Coleman (1991):

$$\text{Volume} = \pi(W^2)(L)/6$$

where W and L are true width and length respectively.

Some clutches of eggs appeared in two or more serial radiographs of a tortoise, with the egg images being more visible in the latter radiographs as noted by Turner et al. (1986). This increased visibility may result from increased calcification of the shells, but egg lengths, widths, or volumes were not significantly different ($P = 0.3$) among serial radiographs.

Desert tortoise eggs are not spherical and their axes (short and long), as indicated by the mediolateral radiographs were often, but not always, parallel to the film plane. Thus, our measures of egg size, after correcting for radiographic magnification, tended to underestimate slightly the size of the eggs. In dorsoventral and mediolateral radiographs the egg image length and its film plane projection length were compared and we found that (1) the film plane lengths underestimated the image lengths by 4.6% (SD = 0.051; $N = 40$, nine females), and (2) the underestimate was not related to female MCL. Thus, the egg lengths we report are, on average, 4.6% shorter than the true egg lengths. Desert tortoise eggs are more round in cross section than in longitudinal section so our width measurements should be more accurate (i.e. <4.6% error) than the length measurements.

The first appearance of eggs each year was timed from an arbitrary date set at 12 April which was just before the first eggs appeared in radiographs in both years. We denoted the two-week x-ray intervals as 1.0 unit of time. Eggs appearing from 12–18 April were assigned time 0.5 and those from 19–25 April 19–25 time 1.0, and so on.

Statistical Analysis.—Of the 13 individual tortoises studied at Goffs, six were studied in both years. Similarly, 16 of 32 individuals at DTNA were studied in both years. Owing to this partial repeated measure and the risk of incorporating a bias (some tortoises with two years of data and others with just one), the egg production data were analyzed in several ways. For females studied in both years, we compared the body size measurements and egg-laying parameters between the two years using a paired t-test. We used regression analysis to examine the relationship between egg parameters and the

TABLE 1. Winter (October to March) and summer rainfall (June–September, in mm) and productivity (g dry matter / m^2; measured late-April or early-May) of winter annual plants from 1990 to 1993 at Goffs and at DTNA. The 1992 (Goffs) and 1993 (DTNA and Goffs) productivity data include one standard deviation (±SD).

	Year	1989–1990	1990–1991	1991–1992	1992–1993
Goffs	Winter	30	80	176	212
	Summer	44	39	2	1
	Total	86	119	226	224
	Productivity			39 ± 48	24 ± 22
DTNA-irrigated	Winter	155	251	275	210
	Summer	16	25	0	0
	Total	171	276	275	210
	Productivity	2.2	25	29	26 ± 14
DTNA-dry	Winter	22	150	245	210
	Summer	16	25	0	0
	Total	40	175	257	210
	Productivity	0.05	17	35	22 ± 17

different indices of body size. We first analyzed these relationships within each year and, afterwards, compiled an independent data set which included all of the tortoises studied in just one year plus a randomly selected year for those tortoises studied in both years. This data set had 13 and 19 tortoises at DTNA and eight and five tortoises at Goffs in 1992 and 1993, respectively. We also used this independent data set (IND), which is free of repeated measures, for comparisons that involved the main effect 'Site'. When analysis of variance showed significant differences between study sites, any further analyses (e.g., the regression of clutch size on body size) used the DTNA independent data set only because it has 32 tortoises compared to 13 for Goffs.

Because Goffs tortoises were smaller than those at DTNA, we used analysis of covariance (ANCOVA) for all between-site comparisons. To account for unequal sample sizes in data sets, we used a general linear model analysis of variance (ANOVA) to assess main effects and their interactions.

Unless stated otherwise the data and analyses are for egg-laying tortoises only. Those tortoises that did not lay eggs in either year are discussed separately. Statistical analyses were completed using Minitab 9.0 or Sigmastat for Windows 2.0 and were considered significant at $P < 0.05$.

RESULTS

Heavy rain fell uniformly over both the eastern and western Mojave deserts in the winters of 1991–1992 and 1992–1993 (Table 1). An abundance of green annual forage persisted at both sites during March, April, and much of May, but by June it had dried. At Goffs, forbs dominated the annual biomass in 1992 (forb and grass biomass = 28.2 and 10.7 g/m^2, respectively) and grasses dominated annual biomass in 1993 (forb and grass biomass = 6.3 and 17.8 g/m^2, respectively). We did not segregate the plant matter into species although we did observe that a forb (*Pectocarya* sp.) provided most biomass at Goffs in 1992, while in 1993 there was more variety of forb species. *Erodium cicutarium* was the most common forb at DTNA in both years while the introduced Mediterranean plant, *Schismus barbatus*, was the dominant annual grass at both sites in both years.

Plant productivity did not differ between the four treatment plots at DTNA in 1993 (all $t < 0.8$, $P > 0.4$ and df > 23) or between DTNA and Goffs in 1992 (95% confidence intervals for Goffs included the mean values for DTNA, Table 1) or 1993 (all comparisons: $t < 0.4$, $P > 0.71$ and df > 74). However, plant productivity was lower in 1993 than in 1992 for both sites (Goffs: $t = 2.0$, $P = 0.049$, df = 99; DTNA: 95% confidence intervals for 1993 excluded the respective means for 1992). There were also no significant treatment effects (i.e., among irrigated and dry plots or among resident, host, and immigrant groups) on any parameter of egg production. For example, ANOVA for total eggs (df = 35) showed no significant differences between cohorts ($P = 0.435$), plots ($P = 0.456$) or water ($P = 0.449$). Thus, mean data over all plots and cohorts were reported for DTNA (Table 2).

Egg-laying Patterns.—Females typically laid one clutch of eggs in late April or early May and most females (ca. 70%) laid a second clutch in late May or in June. Apparently, no female produced a third clutch. The proportion of mature females failing to form a clutch at all ranged from 0% at Goffs in 1992 to 25% at DTNA in the same year (Table 2). At DTNA in 1992, no clutches were visible in radiographs on the first trip (13–15 April) nor on the last trip (8–9 July); clutches were seen from late April to late June. At Goffs, the first images of eggs appeared in

TABLE 2. Mean (±SD) body size, body condition (CI), clutch parameters, egg parameters and coefficients of variation (CV, %) of egg-laying female tortoises at Goffs and at DTNA in 1992 and 1993. Average mass equaled the average of hibernation body mass and maximum spring body mass. Average CI equaled the average of hibernation CI and maximum spring CI, where CI = (body mass, g) × $(\text{MCL})^{-3}$. The size (number of eggs) and volume (cm^3) of second clutches were calculated only for DTNA females (1992: N = 12; 1993: N = 22) and Goffs females (1992: N = 7; 1993: N = 6) that laid second clutches. The mean egg lengths and widths were calculated from the number of eggs listed in parentheses (N). See Materials and Methods for details regarding egg sizes.

	Goffs 1992	Goffs 1993	DTNA 1992	DTNA 1993
Number	10	11	24	32
Egg layers	10	9	18	29
MCL (mm)	212 ± 15.0	212 ± 13.1	235 ± 20.3	234 ± 17.4
Average mass (g)	1907 ± 402	1758 ± 366	2598 ± 570	2596 ± 448
BM change (%) average	15.3 ± 6.4	37.0 ± 10.8	30.1 ± 5.3	12.7 ± 8.2
CI average (g/cm^3)	0.199 ± 0.011	0.174 ± 0.003	0.200 ± 0.016	0.203 ± 0.020
Clutch frequency	1.70 ± 0.48	1.67 ± 0.50	1.67 ± 0.49	1.76 ± 0.44
Clutch 1 size	4.2 ± 1.0	4.2 ± 1.2	4.4 ± 1.25	3.9 ± 1.05
Clutch 2 size	4.1 ± 2.5	4.7 ± 1.0	4.0 ± 1.8	4.0 ± 1.5
Eggs produced per tortoise	7.1 ± 2.8	7.3 ± 3.1	7.1 ± 2.7	7.0 ± 2.5
Clutch 1 volume (cm^3)	103.9 ± 24.9	108.4 ± 34.9	151.3 ± 52.2	129.9 ± 37.5
Clutch 2 volume (cm^3)	100.4 ± 66.7	111.1 ± 33.7	138.9 ± 63.1	130.5 ± 47.6
Time of first clutch	2.3 ± 0.8	2.0 ± 1.1	1.8 ± 0.8	1.6 ± 0.6
Time of second clutch	3.8 ± 0.8	3.5 ± 0.9	3.6 ± 0.5	3.8 ± 0.8
Total egg volume per tortoise (cm^3)	174.2 ± 80.3	182.5 ± 86.1	243.9 ± 105.9	228.9 ± 84.5
Egg length, clutch 1 (mm)	40.9 ± 2.4	40.9 ± 1.9	45.2 ± 3.3	45.5 ± 3.8
Egg width clutch 1 (mm)	34.0 ± 1.3	34.6 ± 1.7	37.7 ± 2.1	37.2 ± 2.3
(N)	(42)	(38)	(80)	(114)
Egg length clutch 2 (mm)	40.9 ± 2.42	39.7 ± 2.4	45.4 ± 4.8	44.6 ± 4.4
Egg width clutch 2 (mm)	34.1 ± 1.65	33.8 ± 1.5	38.2 ± 1.7	36.9 ± 2.0
(N)	(29)	(28)	(48)	(88)
CV clutch 1 size (%)	25	29	28	29
CV clutch 2 size (%)	55	22	44	39
CV clutch 1 volume (%)	24	32	35	28
CV clutch 2 volume (%)	66	30	45	36

radiographs from 29 April 1992 and the last clutch appeared on 24 June (the tortoise laid the latter between 7–27 July). There was a similar pattern in 1993 at Goffs: only one tortoise appeared gravid on 21 April and only one tortoise was still gravid on 29 June (her second clutch). Linear regression analyses indicated that the first clutches ($F_{1,43} = 15.48$, $P < 0.001$, $r^2 = 0.265$) and second clutches ($F_{1,31} = 4.29$, $P < 0.05$, $r^2 = 0.119$) appeared earlier on radiographs for larger tortoises than for smaller females. An analysis of covariance, with MCL as the covariate, showed no significant differences between sites or years in the time of appearance of the first clutch (Table 3). The timing of the second clutch was highly correlated to the timing of the first clutch ($F_{1,31} = 38.4$, $P < 0.001$, $r^2 = 0.553$; Fig. 1). After accounting for first clutch time ($F_{1,30} = 53.8$, $P < 0.001$) ANCOVA showed that Goffs tortoises produced their second clutches earlier than DTNA females produced their second clutches ($F_{1,30} = 7.55$, $P < 0.01$).

Body Size and Body Condition.—DTNA females were larger on average, than Goffs females for all body measurements (e.g., MCL: $F_{1,40} = 13.0$, $P < 0.001$, by ANOVA). Body mass varied considerably throughout each year (hibernation low to spring peak), but also varied widely between individuals (coefficient of variation, CV = 41%): some changed little (4%), while others increased mass by up to 56%. The average changes in mass, calculated as the mass increase from hibernation to peak mass in spring, ranged from 13% (DTNA, 1993) to 37% (Goffs, 1993). Mass changes showed a highly significant site x year interaction ($P < 0.001$, Table 3). In 1992, tortoises at DTNA were 30% heavier in spring than when they emerged from hibernation but in 1993 the gain was only 13%. Over the same time, Goffs tortoises changed by 15% and 37%, respectively.

Condition index (CI) estimates body density of tortoises and because adult tortoises grow slowly, changes in CI are determined primarily by fluctuations in body mass, which in turn are indicative of hydration status. We drew the same conclusions regardless of whether we calculated CI as body mass (g) divided by (1) the

TABLE 3. Statistical appraisal of body size, body condition index (CI), clutch parameters and egg parameters of egg-laying female tortoises at Goffs and at DTNA in 1992 and 1993. The results are from ANOVA and ANCOVA of the independent data set (free of repeated measures), which included all of the tortoises studied in just one year at each site plus a randomly selected year for those tortoises studied in both years. This gave 13 and 19 tortoises at DTNA and 8 and 5 tortoises at Goffs in 1992 and 1993, respectively.

Parameter	Site	Year	Site*year	Body size
MCL (mm)	$P < 0.001$ $F_{1,40} = 13.0$	$P = 0.81$	$P = 0.85$	—
Average mass (g)	$P < 0.001$ $F_{1,36} = 14.0$	$P = 0.72$	$P = 0.38$	—
BM change (%) average	$P = 0.31$	$P = 0.85$	$P < 0.001$ $F_{1,36} = 34.8$	—
CI average (g/cm^3)	$P < 0.05$ $F_{1,36} = 4.18$	$P = 0.169$	$P < 0.05$ $F_{1,36} = 4.61$	—
Clutch 1 size	$P = 0.245$	$P = 0.254$	$P = 0.251$	$P < 0.01$ $F_{1,40} = 9.45$
Clutch 2 size	$P < 0.05$ $F_{1,27} = 7.17$	$P = 0.58$	$P = 0.67$	$P < 0.01$ $F_{1,27} = 9.02$
Egg production per tortoise	$P < 0.05$ $F_{1,40} = 5.39$	$P = 0.79$	$P = 0.53$	$P < 0.001$ $F_{1,40} = 17.6$
Clutch 1 volume (cm^3)	$P = 0.67$	$P = 0.156$	$P = 0.151$	$P < 0.001$ $F_{1,40} = 34.8$
Clutch 2 volume (cm^3)	$P = 0.252$	$P = 0.753$	$P = 0.628$	$P < 0.001$ $F_{1,27} = 12.6$
Time of first clutch	$P = 0.88$	$P = 0.94$	$P = 0.43$	$P < 0.001$ $F_{1,40} = 8.54$
Time of second clutch	$P = 0.22$	$P = 0.89$	$P = 0.56$	$P < 0.05$ $F_{1,27} = 4.22$
Total egg volume per tortoise (cm^3)	$P = 0.52$	$P = 0.70$	$P = 0.63$	$P = 0.001$ $F_{1,40} = 28.3$
Egg length clutch 1	$P < 0.01$ $F_{1,40} = 8.36$	$P = 0.3$	$P = 0.40$	$P < 0.05$ $F_{1,40} = 4.17$
Egg width clutch 1	$P < 0.01$ $F_{1,40} = 7.74$	$P = 0.78$	$P = 0.54$	$P < 0.001$ $F_{1,40} = 18.9$
Egg length clutch 2	$P < 0.01$ $F_{1,27} = 8.55$	$P = 0.80$	$P = 0.22$	$P = 0.66$
Egg width clutch 2	$P < 0.001$ $F_{1,27} = 24.8$	$P = 0.19$	$P = 0.56$	$P < 0.05$ $F_{1,27} = 7.21$

cube of MCL, or (2) the product of MCL, width and height. Thus, in this paper we report CI's calculated by method 1 only. In 1993, Goffs tortoises had a lower average CI (Table 2) than DTNA tortoises, but there were no site differences in 1992, causing a significant site by year interaction (Table 3). The CI's at emergence from hibernation also showed a significant site by year interaction ($F_{1,34} = 17.3$, $P < 0.001$): in 1992, female tortoises at Goffs hibernated in better condition than those at DTNA while the opposite occurred in 1993. Maximum CIs were similar between sites in both years. There were no significant correlations between CI and any reproductive parameter.

Those reproductive parameters linked to tortoise size usually correlated with all four linear dimensions, namely MCL, MPL, width, and height. In these cases we reported the linear measurement that provided the highest coefficient of determination. As expected the linear measures of body size were highly correlated to each other. For instance, MPL = −0.810 + 0.945*MCL; $s = 0.542$; $r^2 = 0.916$; $P < 0.001$; $N = 45$.

Egg Number–Site and Body Size Effects (Tables 2, 3).—Because Goffs females were smaller than those at DTNA, we compared clutch size and egg measurements among sites using ANCOVA, with body size (e.g., MCL) as the covariate. The size of the first clutch did not differ among sites nor did it differ between early or late first clutches. The total number of eggs produced by a tortoise in any year (annual egg production, AEP) was positively related to its size, e.g., MCL (Fig. 2). Size corrected (via ANCOVA) AEP also depended on (1) location: Goffs tortoises had a greater AEP than DTNA tortoises ($P < 0.05$), and (2) the date that a female's first clutch appeared on a radiograph: the earlier the date, the greater the AEP ($P < 0.05$).

A general linear model analysis of variance

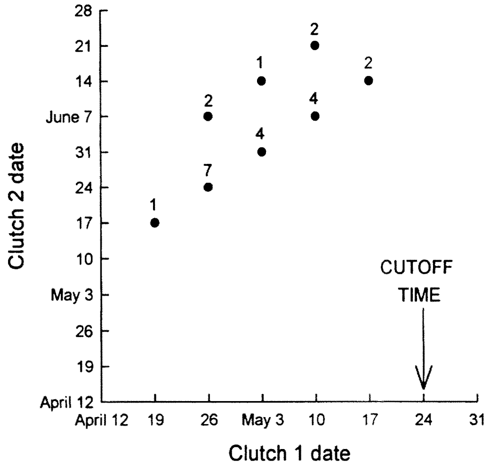

FIG. 1. Relationship between timing of first appearances of clutch 2 and clutch 1 in radiographs of the independent data set for DTNA desert tortoises. The timing of the second clutch depended largely on the timing of the first clutch. Also, a female producing her first clutch after May 24 (clutch 1 time = 3.0) did not produce a second clutch in that year. Numbers next to symbols indicate sample sizes.

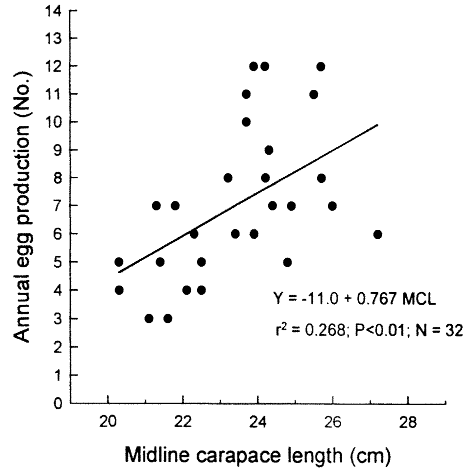

FIG. 2. Relationship between annual egg production and body size (midline carapace length, MCL) of female tortoises (*Gopherus agassizii*) at DTNA in 1992 and 1993 (DTNA independent dataset). Although the size of the first clutch was correlated to MCL (Y = $-3.34 + 0.323 \times$ MCL; $r^2 = 0.232$; $P < 0.01$, N = 32), the size of second clutches was not correlated to MCL.

on the IND data set (df = 42, see statistical analysis above) showed that females laying two clutches had longer plastrons ($P < 0.05$) and were wider ($P < 0.05$) than single-clutching females. However, tortoises at the extremes of body size provided exceptions to any rule about tortoise size and clutch frequency. For example, one large tortoise (MCL = 260 mm) produced only one clutch of seven eggs, while another large tortoise (MCL = 272 mm) laid a single clutch of six eggs. In contrast, the two smallest tortoises (190 and 203 mm MCL) each laid two clutches.

The number of eggs in the first clutch correlated with all linear measures of female body size but the predictive values are poor (Equation 1, Table 4). Similarly, total egg number was related to female size but with low r^2 values (Equation 2, Table 4). In contrast, the number of eggs in the second clutch did not correlate with any measure of tortoise body size.

Egg Size–Site and Body Size Effects.—After accounting for body size differences via ANCOVA (Table 3), analysis of the IND data set showed that Goffs tortoises produced first clutch eggs that were, on average, narrower ($P < 0.01$) and shorter ($P < 0.01$) than those of DTNA tortoises. The effect was more pronounced in second clutch eggs (width $P < 0.001$; length $P < 0.01$). Even though Goffs tortoises laid larger second clutches ($P < 0.05$) and more eggs in total ($P < 0.05$), than tortoises at DTNA, the smaller egg dimensions meant that tortoises at the two sites laid a similar volume of eggs overall ($P > 0.10$).

We used a linear regression analysis to further examine the relationships between egg size and tortoise size (Equations 5–18 in Table 4). However, owing to the differences in egg size between study sites, we confined our analysis to the random data set compiled for DTNA (N = 32). Of these tortoises 23 produced two clutches and nine tortoises produced just one clutch.

Female size also had an important bearing on egg dimensions, especially those of the first clutch. All of the following increased with increasing female size: egg length (Fig. 3a), egg width (Fig. 3b), egg volume (Table 4), and effective diameter (Table 4) of first clutch eggs, total volume of the first clutch (Table 4) and volume of both clutches combined (Table 4). For second clutches, however, only the mean width of eggs ($P < 0.05$) and total clutch volume (Table 4) were related to body size.

Annual Effects and Egg Size-Clutch Size Relationships.—In tortoises studied in both years we found no annual effects on the size of the first (N = 26) or second (N = 22) clutches or the total AEP (N = 26) ($P > 0.3$ for all comparisons; paired t-test). However, a test of this nature takes no notice of the change at the individual level—a tortoise laying four more eggs in 1992 than in 1993 is cancelled by another female doing the opposite. To use individual variation in the evaluation of annual variation, we correlated 1993 reproductive output (e.g., clutch size)

TABLE 4. Correlation statistics relating egg or clutch parameters to body size, timing of egg appearance and clutch parameters for random data sets from both sites (IND dataset; equations 1–4; N = 45) and the random data set for DTNA females (N = 32; equations 5–18). The latter data set was chosen for egg size measurements to remove effects of study sites on analyses. Equations 9, 11, and 13–15 are for multiple regression analyses. The column "$s_{y \cdot x}$" indicates the standard error of the estimate of y from x. Clutch size, body size (height, width and MPL), egg and clutch volumes, and linear egg measurements (width, length and effective diameter) were measured in units of number, cm, cm^3, and mm respectively.

Dependent variable	Independent variable	Intercept	Slope	$s_{y \cdot x}$	r^2	P	F	Equation
First clutch size	Height	−2.52	0.23	1.02	0.224	<0.001	12.4	1
Annual egg production	MPL	−6.55	0.66	2.45	0.206	<0.01	10.9	2
Clutch 2 size	Clutch 1 size	0.31	0.93	1.56	0.256	<0.005	10.3	3
Clutch 2 volume	Clutch 1 vol.	24.7	0.00077	47.1	0.302	<0.001	13.0	4
First clutch size	MPL	−1.61	0.27	1.08	0.161	<0.05	5.8	5
Mean egg width, clutch 1	MPL	19.1	0.86	1.70	0.434	<0.001	23.0	6
Mean egg volume, clutch 1	MPL	−6.99	1.89	4.164	0.381	<0.001	18.5	7
Mean effective diameter, clutch 1	MPL	23.2	0.78	1.70	0.384	<0.001	18.7	8
Mean egg length, clutch 1	MPL, Height	44.1	1.25 −2.551	2.41	0.311	<0.001 <0.01	6.6	9
Mean egg width, clutch 2	Height	26.2	1.14	1.41	0.172	<0.05	4.4	10
Total egg volume, clutch 1	Width Height	365.8	12.6 26.9	27.8	0.599	<0.01 <0.05	21.6	11
Total egg volume, clutch 2	MCL	−312.8	18.7	49.4	0.209	<0.05	5.6	12
Total egg volume, clutch 1 + 2	MCL Time 1	−138.7	20.5 −61.5	65.2	0.570	<0.05 <0.01	18.6	13
Mean egg width, clutch 1	MPL Clutch 1 size	18.2	1.00 −0.524	1.63	0.496	<0.001 0.069	14.3	14
Mean egg volume, clutch 1	MPL Clutch 1 size	−10,067	2408 −1907	3684	0.531	<0.001 0.005	16.4	15
Egg length, body size corrected	Clutch 1 size	50.7	−1.31	2.27	0.278	<0.01	11.5	16
Egg width, body size corrected	Clutch 1 size	43.0	−0.74	1.505	0.219	<0.01	8.4	17
Egg volume, body size corrected	Clutch 1 size	41.4	−1.91	3622	0.243	<0.001	9.7	18

against 1992 reproductive output and found a positive relationship for first clutch size ($F_{1,24} = 15.3$, $P < 0.001$, $r^2 = 0.389$) and total egg production ($F_{1,24} = 8.15$, $P < 0.01$, $r^2 = 0.254$) but no relationship for second clutch size ($P > 0.10$).

Clutch Frequency Effects On Egg Production.—Tortoises laying two clutches laid almost twice as many eggs as those laying one clutch (7.9 ± 2.3 eggs, N = 23 vs. 4.5 ± 1.4 eggs; N = 8; $F_{1,29} = 15.0$, $P < 0.001$). The difference was still significant ($F_{1,28} = 11.4$, $P < 0.01$) after the effect of body size (ANCOVA: MCL, $F_{1,28} = 7.5$, $P < 0.05$) was removed, although the adjusted means showed a narrowing of the gap between tortoises producing two clutches (7.6 ± 0.41 eggs) and those producing one clutch (5.0 ± 0.71 eggs).

Clutch Frequency Effect on First Clutch Size.—There was no difference between first clutch sizes of tortoises laying one or two clutches (two sample t-test: t = 0.84; $P = 0.42$; df = 9). However, again body size influenced the result (ANCOVA: MCL, $F_{1,28} = 14.2$, $P < 0.001$). The adjusted means after removing the body size effect (MCL) showed that one clutch layers laid a significantly larger clutch (4.8 ± 0.34 eggs; $P < 0.05$) than the first clutch laid by two clutch layers (3.9 ± 0.20 eggs).

Clutch Frequency Effect on Egg Size.—Tortoises that laid just one clutch laid smaller eggs (29.5 ± 1.67 cm^3) than those eggs in the first clutch (34.9 ± 0.99 cm^3) of tortoises laying two clutches ($F_{1,29} = 7.7$, $P < 0.010$). Again, body size had a significant effect (ANCOVA: MCL, $F_{1,28} = 7.8$, $P < 0.01$), and when this effect was removed the difference between the size of eggs laid by one clutch and two clutch layers (adjusted means: 30.5 ± 1.55 cm^3 vs 34.5 ± 0.90 cm^3) was smaller ($F_{1,28} = 4.8$, $P < 0.05$).

First Clutch Size Effect on Second Clutch Size.—Tortoises with first clutches ranging from 2 to 6 eggs also produced a second clutch, suggesting much variability. There was no relationship between clutch frequency and the size of the first

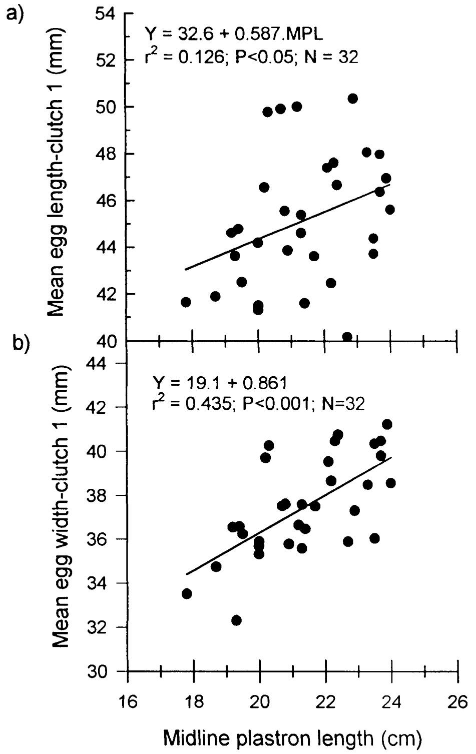

FIG. 3. Relationship of a) mean egg length and b) mean egg width of clutch 1 to midline plastron length of DTNA females (DTNA independent dataset).

clutch a tortoise produced (regression $P = 0.33$), but the multiple regression of clutch frequency against the number of eggs in the first clutch and MPL was significant for both clutch 1 size ($t = -2.11$, $P < 0.05$) and MPL ($t = 2.82$, $P < 0.01$): Total clutches = $-0.412 + 0.131$ (MPL) $-$ 0.155 (clutch 1 size); $r^2 = 24.6$, $F_{2,28} = 4.6$, $P < 0.05$. Thus, after removing the effects of body size, there was a negative relationship between the size of the first clutch and clutch frequency.

First and Second Clutch Sizes and Volumes.—The size and volume of the second clutch (Equations 3 and 4; Table 4) were positively related to the size and volume of the first clutch, respectively. The positive relationship between the residuals produced from the regression of clutch size on MCL ($r^2 = 0.191$, $F_{1,21} = 4.97$, $P < 0.05$) showed that the sizes of the clutches were related in a way that was independent of body size. In other words, independent of body size, tortoises that produced a small first clutch were more likely to produce a small second clutch. A similar study of total clutch volume showed that, after removing the effects of body size, there was no consistent relationship between the volumes of the first and second clutches ($r^2 = 0.105$, $F_{1,21} = 2.45$, $P = 0.14$).

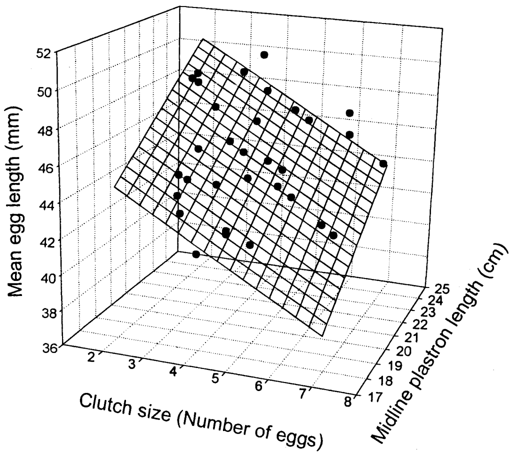

FIG. 4. Relationship of mean egg length of clutch 1 to midline plastron length (MPL; $P < 0.01$) and clutch size (clutch 1; $P < 0.01$) of DTNA females (DTNA independent dataset) analyzed by multiple linear regression [Length = 30.5 + 0.943(MPL) $-$ 1.31(Clutch size); $F_{2,31} = 8.47$, $r^2 = 0.369$, $P < 0.01$]. The negative slope for clutch size indicates a tradeoff between egg length and clutch size, which was also statistically significant for mean egg volume (Equation 15, Table 4) but not for mean egg width ($P = 0.069$; Equation 14, Table 4).

Egg Size and Number Tradeoff.—The total volume of clutch 1, clutch 2, and their combined volume increased significantly with clutch size ($r^2 = 0.73, 0.88, 0.87$; $F_{1,31} = 81, 155, 191$; $P < 0.001$ for all relationships, respectively). In absolute terms, there was no relationship between first clutch size and its mean egg width, or volume (regression: $P = 0.84$, and 0.56, respectively) although eggs of large clutches tended to be shorter ($P = 0.09$). For first clutches however, when the significant effect of body size (MPL, $t_{29} = 3.6$, $P < 0.001$) was removed by multiple regression, mean egg length (Fig. 4; $t_{29} = -3.34$, $P < 0.01$) and mean egg volume (Table 4; $t_{29} = -3.1$, $P < 0.01$) decreased with increasing clutch size (negative slope for clutch size in Fig. 4). Mean egg width also tended to decrease with increasing clutch size (equation 14, Table 4; $t_{29} = -1.89$, $P = 0.07$). Equations 5–7 (Table 4) and the equation from Fig. 3a allow the calculation of body size corrected estimates of first clutch size, mean egg width, mean egg volume and mean egg length, respectively. The correlations between body size corrected mean egg size (length, width and volume) and body size corrected first clutch size were significant (Equations 16–18 in Table 4), with egg size decreasing with increasing clutch size. There were no such trade-offs for the second clutch either on an absolute (simple linear correlation analysis) or a mass-corrected basis (using multiple regression, ANCOVA or correlation analysis of body size corrected values).

Respiratory Disease Effects.—Sixteen of the 29 egg laying tortoises at DTNA in 1992–1993 had positive ELISA titres for antibodies against *Mycoplasma agassizii* (the pathogen responsible for upper respiratory tract disease in *Gopherus agassizii*). An analysis of covariance, with MPL as the covariate, showed that antibody status had no effect on any reproductive parameter including egg number, egg size, or the time of appearance of shelled eggs in the oviduct ($P > 0.3$; df = 27).

Discussion

Nutrients.—Reproductive output for most animals is probably influenced by both environmental resource availability, such as food availability in spring, and maternal nutrient reserves (e.g., Doughty and Shine 1998). The patterns of nutrient acquisition for, and nutrient allocations to, reproduction for desert tortoises are complicated by their ability to: (1) enlarge some follicles to full size prior to hibernation (Rostal et al., 1994), (2) produce more than one clutch of eggs per year (Turner et al., 1986; Henen, 1997), and (3) reabsorb non-ovulated follicles (i.e., follicular atresia; Rostal et al., 1994) at the end of spring. As for many vertebrates (Scott et al., 1976; Congdon et al., 1983; Noble, 1991; White, 1991), desert tortoise follicles probably contain large amounts of lipid and protein, so female tortoises probably allocate lipids and protein to vitellogenesis before they hibernate. Female desert tortoises ovulate in April, May, and June, then add albumen and eggshells (which are probably mostly protein and calcium carbonate, respectively; Palmer and Guillette, 1991), and then lay. Because the nutrients for both pre- and post-hibernatory development of oocytes probably come from a combination of female diet and body reserves, the abundance and timing of dietary nutrient sources should influence reproductive nutrient allocation. Results of this study permit evaluation of (1) the importance of environmental resource availability in spring, (2) body size, (3) effects of timing of reproduction, (4) between-population differences in reproductive output that are consistent with historical rainfall patterns, (5) a trade-off between egg size and clutch size, and (6) *Mycoplasma agassizii* infection.

Rain and Food.—The availability or biomass of annual plants, the primary food source for desert tortoises in the Mojave Desert (Nagy and Medica, 1986; Henen, 1994, 1997), depends on rainfall. Winter annuals germinate when rainfall for late-September to January exceeds 26 mm. The biomass of winter annual plants produced in a given year increases as total winter rainfall increases, but asymptotes at ca. 60–100 g/m^2 when total winter rainfall (September–March) exceeds 100 mm (Turner and Randall, 1989). Mean clutch frequency (Turner et al., 1986) and annual egg production (Henen, 1994) were correlated to the logarithm of winter annual biomass. This semi-logarithmic relationship illustrates the sensitivity of clutch frequency and annual egg production to changes in winter annual biomass when biomass is below 1–2 g/m^2, but mean clutch frequency and annual egg production approach asymptotic levels (ca. 2 clutches and 7–9 eggs per female per year; Table 2) at higher biomass levels. Although annual biomass at both sites was higher in 1992 than 1993, annual biomass greatly exceeded 2 g/m^2 in both years, explaining why reproductive output was near asymptotic levels at both sites in both years. This apparent ceiling on reproductive output suggests that factors besides spring food availability (e.g., body size constraints, length of egg laying season, pre-hibernatory food availability, and maternal nutrient reserves) may also limit clutch frequency and annual egg production in desert tortoises.

Although winter rainfall, winter annual biomass, and reproductive output were at the high end of the range measured previously at Goffs (Turner et al., 1986), this study followed two relatively dry years (1990 and 1991) in the Mojave Desert. In 1990, only 20% and 30% of the normal winter rainfall fell at DTNA and Goffs, respectively, and few annual plants grew at DTNA in 1990 (Table 1). Early spring rain at both sites in 1991 compensated for an unusually dry winter and produced a flush of annual plants in April and May at both sites (Table 1; B. T. Henen et al., 1998). Such events typically allow desert tortoises to rehydrate (Nagy and Medica, 1986; Peterson, 1996b) and to improve their body condition (Henen, 1997), so females may have entered hibernation in good body condition in 1991 and 1992. Our data set was insufficient to evaluate whether the pre-hibernatory food availability and quality in 1991 and 1992 affected reproductive output in spring 1992 and 1993 respectively. With two consecutive years of high food availability, the lack of greater reproductive output in the second year (1993) is surprising, but is consistent with the findings of Turner et al. (1986), that three consecutive "good" years yielded no increase in egg production. It is not clear why some females did not produce a third clutch in 1993.

Follicular atresia (Rostal et al., 1994) may increase the flexibility of female reproductive responses to the extreme variations in environmental conditions, especially food availability. Females that develop many follicles, compared to those developing few follicles, are more likely able to quickly respond to favorable spring conditions (e.g., high biomass of winter annuals) and produce many eggs. In unfavorable spring conditions, females may reabsorb extra follicles because they cannot obtain the nutrients, and may lack the nutrient reserves, that are necessary to convert those follicles to eggs. Thus follicular atresia may underlie some of the variability in reproductive output that is correlated with the biomass of winter annuals. Females may incur some cost of developing and subsequently reabsorbing extra follicles, so there may be an optimal number of follicles to develop prior to hibernation. This, in turn, is probably affected by female body size or body condition.

Body Size.—Maternal body size strongly influences reproductive output. Turner et al. (1986) reported that female body size affected clutch frequency and clutch size, whereas Mueller et al. (1998) found that female size affected clutch size and annual egg production, but not clutch frequency. Our results indicate that body size affects reproductive output in many ways. Maternal body size affected annual egg production, primarily through effects on clutch frequency and the size of the first clutch, egg size, and the volume of egg clutches. For many chelonians (Landers et al., 1980; Gibbons, 1982; Rowe, 1994; and others cited in Bjorndal and Carr, 1989), clutch size is related to maternal body size. In none of these chelonians, however, did body size explain more than half of the variation in clutch size. Nonetheless, reproductive output is determined by more than just clutch size.

The effects of body size on clutch size, egg size, and clutch volume in desert tortoises were statistically significant for the first clutch although egg width and clutch volume were also related to female body size for second clutches. Turner et al. (1986) found that clutch size for first and second clutches was correlated to female size (MCL) in 1983 and 1984, but not in 1985, when only the size of the first clutch was correlated to MCL. The 1985 results were consistent with our results and are consistent with the use of both body reserves (water and protein; Henen, 1994, 1997) and springtime food intake to support reproductive allocations. Large females may be able to develop more follicles and store more nutrients (e.g., water, protein, and calcium) than smaller females.

Smaller females tended to produce fewer clutches and lay clutches later in the spring. Small females probably had smaller body nutrient reserves (e.g., water and protein) than did large females (see Henen, 1994, 1997), and a larger portion of the nutrients for their eggs, compared to large females' eggs, may come from winter annuals consumed after emergence from hibernation. Relative to large females, the reproductive output of small females may thus be more dependent upon the availability of winter annuals in the spring. Winter annuals senesce quickly and desiccate by June (the peak laying period) and females emerge from hibernation in March or April, so there may be little time for females to acquire nutrients prior to ovulation which peaks in May (Rostal et al., 1994; Henen and Oftedal unpubl. data). There appears to be great reproductive advantage for females to grow large.

In general, it appears that most female desert tortoises produce eggs every year, albeit fewer in drought than in wet years (Henen, 1997). This is consistent with a "bet-hedging" approach to reproduction: females should try to produce at least some eggs each year because females cannot predict whether their eggs will hatch during favorable or unfavorable conditions (Henen, 1997). However, some females do not produce eggs every year, even under the extremely favorable spring conditions of 1992 and 1993. All of the females we studied exceeded the minimum MCL threshold (178 mm) required for reproduction (F. B. Turner, K. H. Berry, D. C. Randall and G. C. White, unpubl. results) but two Goffs females and three DTNA females produced eggs in only one of the two years, and two DTNA females did not produce eggs in either year. The DTNA and Goffs females that produced eggs in only one year had lower body masses in the year that they failed to produce eggs. In an extremely dry year at Goffs, however, the females that did not produce eggs had the smallest MCL, body mass, total body water content, and nonlipid body dry matter content (Henen, 1994, 1997). Thus, the non-layers in the present study may have had insufficient body reserves to develop follicles or produce eggs for one or both years, despite their size. Additionally, other factors contributing to individual variation (e.g., home range quality, genetics, immaturity or sterility, senescence, or mating opportunities) may explain the lack of egg production by these females. After removing the effect of body size, Turner et al. (1986) found that individual variation in clutch size was still significant, indicating a strong individual influence on reproduction.

Body size may affect reproductive output in ways other than nutrient reserves or nutrient acquisition. Female body size in desert tortoises explained more of the variation in egg width than egg length (Fig. 3), suggesting that physical constraints (e.g., size of the oviduct or pelvic girdle) may limit egg size, limit egg width more than egg length, and affect the formation of optimally sized eggs (see below). This was supported by second clutch data where egg width, but not egg length, was correlated to body size.

Pelvic girdle size may affect the size and shape of eggs of other chelonians, with greater plasticity in egg length potentially allowing females to allocate different amounts of resources to their eggs (Congdon and Tinkle, 1982; Bjorndal and Carr, 1989; Long and Rose, 1989; Congdon and Gibbons, 1990a; Rowe, 1994; Nieuwolt-Dacanay, 1997).

As suggested for three species of *Testudo* (*T. graeca, T. marginata,* and *T. hermanni*; Hailey and Loumbourdis, 1988), the size of the female's shell in *Gopherus agassizii* may limit the number of eggs that she can carry. Meienberger et al. (1993) found that gravid females ate less than nongravid females and males (after accounting for body size), suggesting that the gut and oviduct compete for body space in gravid females. Alternatively, it is conceivable that the endocrinological state of gravid females may influence female appetite or foraging levels, which in turn may decrease females' risks of predation.

Timing.—The Mojave Desert is defined by low rainfall and is characterized by extreme seasonal and annual variability in water and food resources for animals (Turner et al., 1986; Henen, 1997). Due to El Niño (ENSO) conditions in winter 1991–1992 and 1992–1993, heavy winter rains fell at both sites, and winter annual plant production was at the highest levels recorded at Goffs (Turner et al., 1986; Henen, 1997), and at similarly high levels at DTNA. This fostered the high reproductive output of females at both sites and probably enhanced female body condition compared to the drought years prior to this study (see Peterson, 1996b; Henen, 1997). Nonetheless, there appeared to be a time constraint affecting the reproductive output of females at both sites (see also Mueller et al., 1998). Females that did not produce a clutch of eggs by the last week of May in either year were unable to produce a second clutch of eggs in that year, limiting their overall reproductive output. Body size influenced how early females could lay their first and second clutches of eggs. Large females produced more eggs (Fig. 2) partly by laying two clutches per year, which, in turn was due to them laying earlier first clutches (Fig. 1). The size of female nutrient reserves, the number of follicles developed prior to hibernation, and the rate of springtime nutrient acquisition (especially between clutches) may all underlie this time constraint upon reproductive output. The heavy winter rains, abundance of winter annuals and the persistence of green annual plants through spring of both years probably explains why the late May "cutoff" was consistent in both years. The phenology of food plants usually varies much more between years, with winter annuals either growing less, flowering, setting seed, senescing, and desiccating earlier, or not germinating at all, in years following dry winters. Thus, we might expect the cutoff to vary amongst years.

Inter-population Effects.—In many species of chelonians, there is variation between populations or subspecies in several life history traits, including components of reproductive output (e.g., clutch size, clutch frequency, egg composition; Gibbons et al., 1982; Congdon and Gibbons, 1983; Hailey and Loumbourdis, 1988; Lindeman, 1996 and 1997; Nieuwolt-Dacanay, 1997; Mueller et al. 1998 and others; see reviews by Congdon and Gibbons, 1990b; Gibbons and Greene, 1990; Iverson, 1992). In general, this variation is related to body size or local environmental conditions (e.g., latitude, temperature, rainfall, diet). In fact, body size differences are also probably related to local environmental conditions (Case, 1976).

DTNA and Goffs have roughly the same latitute and altitude (DTNA: N 35°14′, W 117°51′, 750 m; Goffs: N 34°51′ W 115°09′, 680 m) but differ climatically with respect to summer rainfall, which apparently occurs more regularly in the eastern Mojave (Goffs), than in the Western Mojave (DTNA; Nagy and Medica, 1986; Peterson, 1996a). To confirm this we evaluated 30 yr summer rainfall data (July-September for 1965–1994) for three NOAA stations around Goffs (Mitchell Caverns, Needles Airport, and Parker Reservoir), four stations around DTNA (Inyokern, Mojave, Randsburg, and Tehachapi) and two stations (Daggett Airport and Victorville Power Plant) that were roughly half way between DTNA and Goffs, thus representing the central region of the Mojave Desert (NOAA, 1995). Multiple linear regression analysis ($F_{3,8}$ = 10.8, $P = 0.013$, $r^2 = 0.87$) indicated that summer rainfall across the Mojave was highly correlated to elevation ($t = 3.80$, $P = 0.013$) and longitude ($t = 5.03$, $P = 0.004$) but not latitude ($t = 0.47$, $P = 0.66$), with regression estimates of 7 and 54 mm of July-September rain being typical for DTNA and Goffs, respectively. We do not know the rainfall threshold for stimulating summer annual plants to germinate, but winter annuals require about 25 mm (Turner and Randall, 1989). If the thresholds are similar, we would predict that enough rain would fall at Goffs, but not at DTNA, in summer to stimulate the germination and growth of annual forbs. Do these differences in local conditions affect reproductive output of the tortoise populations? After accounting for body size differences, we found that Goffs females produced (1) smaller eggs (length and width) in both clutches (although the relationship was pronounced in second clutches), (2) more eggs in the second clutch, (3) more eggs each year, and (4) their second clutches earlier than DTNA females produced their second clutches (after accounting for time of the first clutch). These results are consistent with the Mojave summer rainfall paradigm. Goffs females may be able to produce smaller eggs than do DTNA females, without reducing their reproductive success, because their eggs are more likely to hatch during favorable conditions (summer rain and summer annual forage) and their hatchlings are more likely to have an opportunity to feed on succulent summer annuals before winter. Thus, they would need less nutrient reserves than DTNA hatchlings to survive the first fall and winter. By producing smaller but more eggs at Goffs, females may be increasing their fitness through increased numbers of offspring produced. The earlier production of the second clutch could enable Goffs hatchlings to emerge from nests to capitalize upon summer rains and summer annuals before the onset of winter. By laying (and presumably hatching) later at DTNA, hatchlings may be able to conserve some of their large nutrient reserves for surviving through winter. Similarly, the low July–September rainfall (31 mm; Rundle and Gibson 1996) in Nevada may explain why Mueller et al. (1998) found that Goffs females tend to produce more eggs than similarly sized females in Nevada. Determining whether these site differences in reproductive output are adaptive, and are adaptive for these reasons, will require additional study. Considerable genetic variation exists among tortoise populations in the Mojave Desert (Rainboth et al., 1989; Britten et al., 1997).

In a Sonoran population of *Gopherus agassizii* in 1993 (Murray et al., 1996), clutch frequency was low (ca. 0.78) compared to DTNA and Goffs for the same year (and earlier Goffs data; Turner et al., 1986). Clutch size ranged from 3 to 9 eggs per gravid female (mean = 5.7 ± 0.07 (SE), N = 7), clutch size was not correlated to MCL, and mean egg width (36.5 ± 0.30, N = 36) may have been slightly larger than that recorded for Goffs females in 1983-5 (35 mm, uncorrected for magnification; Turner et al., 1986) and 1992–1993 (Table 2). Also, Sonoran hatchlings might be able to take advantage of the reliable summer rains (ca. 85 mm; Murray et al., 1996).

Optimal Egg Size.—Theories about optimal egg size (e.g., OES and developmental plasticity; Congdon and Gibbons, 1990a; Nieuwold-Dacanay, 1997) vary in their success in explaining the size of eggs produced, clutch size, and the possible tradeoffs between the two measures. The accuracy of predictions of the OES theory falls when factors besides nutrient availability (e.g., morphological constraints, which may occur for female desert tortoises and many other chelonians: Congdon and Tinkle, 1982; Bjorndal and Carr, 1989; Rowe, 1994; and Nieuwolt-Dacanay, 1997) serve as additional constraints to egg size (see Congdon and Gibbons, 1990a). OES predicts that "... within a population the amount of variation in reproductive output should result primarily from variation in the number of offspring produced and secondarily from variation in egg size." (Congdon and Gibbons, 1990a). In this study, the variation in clutch size was three to four times that for egg volume, supporting the major prediction of OES.

On an absolute scale, egg length (clutch 1 eggs only) and egg width (first and second clutches) were correlated to female body size but egg length and width (clutch 1) were not correlated to clutch size. These results are consistent with the physical constraint hypothesis (see also Nieuwolt-Dacanay, 1997). However, after accounting for body size effects (via multiple regression analysis, ANCOVA, and correlations of body size corrected egg and clutch sizes), egg size and clutch size were inversely related (strongly for length and volume but weakly for width), indicating a strong trade-off between egg number and egg size. This agrees with OES, assuming that females have limited resources available for a particular reproductive bout (Congdon and Gibbons 1990a).

According to developmental plasticity theory for egg size (see review by Congdon and Gibbons, 1990a), if environmental variability is unpredictable (e.g., rainfall and food in deserts), natural selection should favor flexibility in reproductive responses. Accordingly, egg size might vary among reproductive bouts (e.g., clutch one versus clutch two) within a single year. In this study, evidence for egg size variation was weak. Many reproductive output patterns were true for the first clutch but not for the second clutch. There did not appear to be an obvious explanation for whether this was due to developmental plasticity or simply due to greater variations in second clutch parameters.

A pertinent life history component that is not well documented for most chelonians is the importance of egg size to hatchling size and hatchling survivorship or viability (Congdon and Gibbons, 1990b; Morafka, 1994). It is known for some chelonians (Congdon and Gibbons, 1990a, b; desert tortoises, Spotila et al., 1994) that hatchling size is correlated to egg size. The early growth rate of desert tortoise hatchlings does not seem to be affected by hatchling size, so that large hatchlings tend to always be larger than small hatchlings in the same early growth period (Spotila et al., 1994). Generally, chelonian survivorship is low for hatchlings and juveniles and high for adults (Congdon and Gibbons, 1990b) and limited data for desert tortoises (Germano, 1994) are consistent with this trend. Thus a large egg size may convey some advantage to the hatchling, but the effect is not clear-cut yet.

Mycoplasma agassizii.—The lack of significant influence of *M. agassizii* antibodies on the reproductive output of free-ranging females in this study agrees with the findings on desert tortoises living in seminatural captive conditions in Nevada (D. C. Rostal, V. A. Lance, J. S. Grumbles and I. M. Schumacher pers. comm.). Compared to captive, sero-negative females, however, captive, sero-positive females tended to have lower plasma estradiol levels and retarded follicular growth during the second year of study. Also, non-laying sero-positive females had lower estradiol levels than did seropositive females that laid eggs.

Our data should be treated with caution because seropositive animals do not always show clinical signs (Lederle et al., 1997; Schumacher et al., 1997). Seropositive females may be most severely affected when physiologically stressed by environmental conditions (e.g., droughts; Peterson, 1994, Lederle et al., 1997). Long term measurements of egg production by seropositive females under stressful and nonstressful conditions should clarify how *M. agassizii* affects reproductive output.

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