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Head-started Agassiz's desert tortoises (*Gopherus agassizii*) achieved high survival, growth, and body condition in natural field enclosures

Running head: Tortoise growth and survival during head-starting

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ABSTRACT: We measured survival, growth and body condition of eight hatchling cohorts of desert tortoises in captivity over 11 years to evaluate head-starting methods. At 11 years of age, seven times as many of the first cohort had survived than if they were free-living tortoises. Improvements in predator control, food and water supplementation and pen structure increased survival from seven to 10 times that under wild conditions. Annual survival averaged 96%. Carapace length (CL) increased 6.95 mm per year, similar to that of free-living tortoises. Annual growth rates varied with calendar year (possibly reflecting food and water supply), age, cohort (year

hatched), mother, and in four dry years, with crowding. Most of the first cohort grew to a releasable size (CL >100 mm) by their ninth year. Body Condition Indices (CI) remained high, indicating little dehydration despite droughts in eight of the 11 years, because irrigation offered drinking opportunities. Head-started tortoises developed fully-hardened shells (≥98% of adult shell hardness) earlier (10.1 vs 11.6 years), but at a larger CL (117 vs 104 mm), than did free-living tortoises. Selective feeding in head-start pens decreased subsequent germination of favored wildflower species, apparently by reducing the natural seedbank. Consequently we reseeded and irrigated each autumn to promote subsequent spring food supply. We irrigated in early summer to enable drinking and ensuing consumption of dry, dead plants and Bermuda grass hay, a supplement. These procedures can greatly improve juvenile survivorship, and increase numbers of hard-shelled, midsized juveniles to help augment wild populations.

KEY WORDS: Survivorship . Predation . Emergence success . Density effects . Condition Index . Shell Hardness Index .

1. INTRODUCTION

Head-starting is one means to augment populations of Agassiz's desert tortoise (*Gopherus agassizii*), a Threatened Species (U.S. Fish and Wildlife Service 1994, 2011a). Here we quantify the effectiveness of head-start protocols evaluated on penned *G. agassizii* in the Mojave Desert of California. Head-starting should increase survival of tortoise eggs and young by

reducing or eliminating death by predation and the physical environment (e.g., dehydration and starvation during droughts) and by fostering juvenile growth and resistance to these threats (Morafka 1994, Morafka et al. 1997, Nagy et al. 1997, McGovern 2019). A primary goal is to add older, larger juveniles, with higher survival probabilities, to wild populations to enhance reproduction rates of local females and improve population rates of natural increase. This goal is consistent with specific recovery guidance under the Endangered Species Act (USFWS 1994, 2011a).

Desert tortoises have low egg and juvenile survivorship (Turner et al. 1986, 1987a, Karl 1999, Bjurlin and Bissonette 2004), but have high adult survivorship and long lives (Turner et al. 1984, 1986, 1987a, Curtin et al. 2009), as do many chelonians (e.g., Wilbur and Morin 1988, Congdon and Gibbons 1990, Kuchling 1999). But adult growth is slow, sexual maturity is reached late (ca. 12-20 years in female desert tortoises; Turner et al. 1987b, Germano 1994), and females have low fecundity (ca. 8 eggs per year; Turner et al. 1986, Mueller et al. 1998, Wallis et al. 1999, Lovich et al. 2015). These life-history traits predispose population vulnerability due to predation on eggs and young in areas experiencing unusually high predation. Recent studies indicate that juvenile and adult mortality have increased, and densities have declined in most Mojave Desert populations (Allison and McLuckie 2018). Consequently, increasing young tortoise survival, such as provided by head-starting efforts, should increase recruitment and help wild populations recover.

We built predator-resistant enclosures in an area of high tortoise density (Woodman et al. 2001) and good-quality tortoise habitat (Barrows et al. 2016) containing creosote bush, white bursage and galleta grass vegetation in the south-central Mojave Desert. The fenced enclosures, with overhead netting, excluded most terrestrial and avian predators. Initially, we kept living conditions as natural as possible for enclosed juvenile tortoises (i.e., native vegetation and substrate) to optimize their fitness after being released. However, subsequent droughts depleted native food supplies and threatened juvenile health and survivorship, so we supplemented food and water. The two main 'head-starting' factors we provided to juveniles were protection from predation and provision of adequate plant food and drinking water via a sprinkler irrigation system. We evaluated the effects of these relatively simple treatments on annual survivorship and growth while inside head-start enclosures, and compared them to the age-specific survivorships and growth rates of free-living *G*. *agassizii* (Turner et al. 1987a and 1987b, Medica et al., 2012, Nagy et al. 2015b). Additionally, we measured how shell hardness, which should convey resistance to predation by common ravens (*Corvus corax*) and other predators (Nagy et al. 2011), changed with body size and how it and growth were affected by irrigation.

2. MATERIALS AND METHODS

2.1. Study site and weather

In winter 2005-2006, we built three fenced enclosures, each a 30.8 x 46.2 m rectangle enclosing natural Mojave Desert vegetation, on a flat area in good desert tortoise habitat in the Sand Hill Range Training Area of the Marine Corps Air Ground Combat Center (MCAGCC), San Bernardino County, California. The head-start facility is known as TRACRS (Tortoise Research And Captive Rearing Site). Fencing consisted of 122-cm-wide, galvanized screen having 6-mm-square mesh, buried 61 cm deep, extending 61 cm above ground, and connected to the bottom of the 1.22- or 1.52-m-high section of 5.1-cm mesh cyclone fencing. The 6-mm-mesh prevented entry by digging rodents, large snakes and predatory lizards. A band of metal flashing 51-cm-high was fastened to the top of the 6-mm mesh and secured to the cyclone fence to exclude climbing rodents. Large birds were excluded by overhead netting (5.1-cm mesh). We constructed elevated-threshold doors for human entry and to block small animal entry. Each enclosure was surrounded with a short fence of 25-mm poultry mesh, 30-cm tall and 0.6 m outside of the enclosure fence, to prevent contact of diseased wild tortoises with the head-start enclosure mesh. A bigger, fourth enclosure, 30.8 x 92.4 m, also containing natural vegetation, was built in 2008 with the same 5.1-cm-mesh bird netting overhead, but with solid walls of corrugated metal sheeting that was 180-cm-tall and was buried 60-cm (Fig. 1).

We subdivided two of the small enclosures into 24 7.7 x 7.7 m pens each, by partially burying 61-cm-high metal sheeting. Similarly, we subdivided part of the larger, metal-walled enclosure into 24 pens (7.7 x 7.7

m). These 72 small pens served both as private enclosures for gravid females to nest and lay their eggs during springs of 2006 through 2013 and as pens for the females' hatchlings during their first winter. This allowed us to identify the mother of every hatchling (Nagy et al. 2016). Subsequently, we enlarged some 7.7 x 7.7 m pens by removing partition sections to form larger communal enclosures for entire juvenile year classes (or cohorts). We also moved some entire cohorts into larger, undivided enclosures. All enclosures used a sprinkler system to (1) supplement rainfall and (2) encourage plant germination and growth during drought years. Our rain gauges placed within sprinkler footprints measured an average of 12.7 mm (range 5.1 to 22.4 mm) of water per hour. When necessary, native fire ant colonies were controlled with ant-species-specific poison bait in tortoise-proof stations.

We used weather records from the National Weather Service (NWS) station nearby, approximately 10 km away, and having similar elevation and topography (Nagy et al. 2016). Using these records we calculated long-term average annual rainfall. Because desert rains can be localized, we installed three rain gauges within TRACRS to obtain more accurate precipitation data. Annual rainfall data are reported as total precipitation measured between 1 October and 30 September the following year.

2.2. Mycoplasmosis status

Prior to this study, URTD (Upper Respiratory Tract Disease), and specifically mycoplasmosis, was present in some tortoises at Sand Hill, so we used aseptic handling techniques (U.S. Fish and Wildlife Service, 2009). We examined female tortoises for clinical signs of URTD and took blood samples to quantify antibodies to *Mycoplasma agassizii* and *M. testudineum* (Christopher et al. 1999, US Fish and Wildlife Service 2011b). Adult plasma was analyzed for *M. agassizii* from 2006 to 2015, except 2014, and for *M. testudineum* in 2012 and 2013. Juvenile plasma was analyzed for *M. agassizii* in 2007, 2008 and 2009. Any juvenile that may have been exposed to *Mycoplasma* was quarantined until determined to be free of clinical sign and of antibodies to *Mycoplasma* spp. [via ELISA (enzyme-linked immunosorbent assay) tests--Brown et al. 1999, Christopher et al. 2003]. All juveniles hatched at TRACRS had negative ELISA results for *Mycoplasma agassizii* and were subsequently moved to experimental enclosures.

2.3. Food availability and food supplementation

We measured food availability as plant cover to avoid harming the food supply to our tortoises. Mid-April of 2006 to 2018 we measured annual plant cover (zero to 100%) of combined native and exotic species growing in one of our enclosures. We used a 1 m^2 Daubenmire square of PVC (polyvinyl chloride) pipe gridded (100 0.01m^2 squares) using fishing line cross-strung at 10-cm intervals. In each of the 24 pens, we placed the square within the irrigation spray zone and then outside the spray zone, with placement

judged to capture the representative cover in the spray zone and in the non-spray zone. For each year we calculated average annual plant cover as a) the sum of % cover for all annuals in each m^2 plot (n = 48), b) as the mean of dry (not irrigated) and wet (irrigated) % cover for each 7.7 x 7.7 pen (n = 24 each), and c) as the mean % cover for all 24 pens. We also measured species richness as the number of forb species in each m^2 plot and total numbers of herb species (i.e., forbs and grasses) in the enclosure (sum of all species in the 48 Daubenmire plots). Due to drought-induced shortages of herbs in spring of 2007, 2012 and 2013, we provided potted, nursery-grown plants (African daisies, *Osteospermum* spp. and Bermuda grass, *Cynodon dactylon*) to enclosures as needed. At the end of those seasons, we removed the potted remains. Beginning in 2012, we sowed seeds of native forbs (*Malacothrix glabrata*, *Chaenactis fremontii*, *Plantago insularis*, and *Salvia columbariae*) every autumn to replenish the soil seed bank with food species. Beginning in summer 2013, we added dry Bermuda grass hay to all occupied enclosures to supplement the dry herbs that tortoises were eating then. Head-start tortoises ate each of these species.

2.4. Egg procurement

Each spring from 2006 through 2016 we radio-tracked wild, adult female tortoises (transmitter model AI-2, Holohil Systems) that lived within 5 km of the head-start facility and used some ELISA-negative females as egg donors inside TRACRS. We examined females for oviducal eggs (via

palpation and primarily by x-ray radiography; MinXray Portable models HF8015 and X750G; Gibbons and Greene 1979; Wallis et al. 1999). When a female's radiograph showed moderately to heavily shelled (calcified) eggs, we transferred her to an individual TRACRS pen to oviposit. We provided each female at least two burrows to use as refugia or for nesting. Some females dug additional burrows. We avoided close monitoring of females to avoid influencing when and where they nested, and we did not search burrows for nests so as to avoid disrupting or altering egg placement and nest conditions. Both of these may influence incubation temperatures, potentially altering hatchling sex ratios (see Nagy et al. 2016 and references therein) and nest success. Females oviposited after 1 to 4 weeks at TRACRS, were offered water to drink, and were released to their home burrow. Radiography confirmed that many egg-donor females produced second clutches after being released.

2.5. Nest and egg success

We recorded the hatchling emergence events for each nest laid, and we uniquely marked emerging hatchlings on vertebral scutes with a permanent marking pen (Sharpie™) and a small printed label epoxied to one scute. Emergence success was calculated as number of hatchlings emerging out of the number of eggs laid (typically equaling clutch size from radiographs). We compared our emergence success measurements with the

life table value in Turner et al. (1987a) for free-living tortoises at Goffs, California.

2.6. Juvenile survivorship

We captured most juveniles twice each year, in Spring (late March, early April) and in Autumn (late August, early September), to measure survival and growth. Despite extensive search efforts, if we repeatedly ceased detecting individuals, we assumed they died shortly after they were last seen alive. Annual survivorship calculations were based only on those juveniles confirmed visually to be alive or dead (or repeatedly missing and assumed dead) a year later, and were compared to age-specific survivorship estimates for free-living juveniles (Turner et al. 1987a; Bjurlin and Bissonette 2004).

2.7 Growth measurements and analyses

We measured body mass to 0.1 g using portable digital scales and standardized orthogonal, straight-line shell dimensions to 0.1 mm with digital calipers (Nagy et al. 2002). These included carapace length (CL, the distal measure at nuchal and supracaudal scute notches), shell width (SW, the distal measure at notches between left and right marginal scutes 5 and 6), and shell height (SH, the distal, vertical measure of plastral and carapacial scutes measured perpendicular to the SW measure). We based growth measurements on shell lengths rather than body masses, which can vary widely due to differences in hydration, reproductive mass and gut fill rather

than somatic growth (Nagy and Medica 1986, Jacobson et al. 1993, Henen 1997, Nagy et al. 2002). We analyzed growth rates as annual changes in carapace length (CL), tested these for effects of age, year, cohort, mother and individuals, and we compared annual growth rates with those of juveniles in three wild populations (see 3.4 below for details).

2.8. Biomass density effects on growth rate

We used linear least-squares regression (using SPSS—Statistical Package for the Social Sciences) and analysis of covariance (ANCOVA, Zar 1999) to evaluate relationships of CL growth rates (i.e., mm y^{-1}, from autumn to autumn) to tortoise biomass density (= biodensity: g tortoise per m^2 ground surface) among pens. First, we estimated growth rates as the mean increase in CL per year for 19 to 37 pens per year, with a minimum of three individuals per pen. We estimated each pen's biodensity as the sum of autumn body masses, at the beginning of the 12 months, of all individuals in that pen, divided by ground surface area of that pen.

2.9. Body condition and shell hardness indices

We calculated body condition index (BCI) as the ratio of body mass (g) to shell volume (cm^3) estimated as the product of standardized carapace length, width and height (in cm, Nagy et al. 2002). For juveniles this index varies primarily with hydration state and gut fill. We analyzed autumn BCI (late August, early September) of all juveniles each year, as growth rates were greatest in spring, which is when body mass fluctuates considerably.

BCIs reflect dehydration or atrophy (BCI<<0.45), healthy or normal hydration and nutrition (0.45 to 0.60), and prime body condition (> 0.60; Nagy et al. 2002).

We measured shell compressibility using a tension-calibrated digital micrometer placed vertically at the middle of a juvenile's carapace and plastron, typically at the third vertebral scute (Nagy et al. 2011). Then we calculated shell hardness index (SHI) as 100*(CSH/USH), where CSH and USH are compressed and uncompressed shell height (0.01mm), respectively. Adult shells are incompressible (SHI = 100%) at the pre-set tension of the micrometer. We compared the age and size at which juveniles head-started with irrigation reached adult SHI with those of juveniles head-started without irrigation (Nagy et al. 2011).

2.10. Statistics

Results are indicated as mean $\pm$ standard deviation (SD) and sample size (n). Differences between means were evaluated using two-tailed Student's *t*-tests and one-way or two-way ANOVA (analysis of variance), considering probability values $p \leq 0.05$ statistically significant. We used least-squares linear regression to evaluate relationships between annual growth rates and plant cover as an index of annual food supply, precipitation (rain plus irrigation) as an index of annual water supply, and pen-specific tortoise mass (biodensity) as an index of annual food demand. We used General Linear Models ANOVA to evaluate main effects of year, age, cohort

and mother on growth rates, and nested (or hierarchical) ANOVA to evaluate age, cohort and mother effects, nested with effects of year, because the dataset was not crossed (Zar 1999). One-way Repeated Measure ANOVA provided the same main and post-hoc results as one-way ANOVA with mother as a random factor.

To compare regression slopes and elevations, we used analysis of covariance (ANCOVA; Zar 1999). We used non-linear, least-squares analyses (SigmaPlot 11) to analyze fit for curvilinear relationships (e.g., exponential rise to asymptotes), and Spearman Rank Order correlations (r_s) for other non-linear, heteroscedastic and non-normal data. We used Z tests to compare regression correlation coefficients (r^2; Zar 1999).

Juvenile shells harden asymptotically to adult values (Nagy et al. 2011), so the difference between juvenile and adult SHI (the same as compressibility) converges asymptotically on zero as juveniles grow. Logarithmic transform of this convergence results in linear relationships to tortoise age (years) and size (CL), enabling us to estimate the age and size at which juvenile shells reach 98% and 99% of adult SHI. We also used ANCOVA to compare SHI-to-CL regressions, and SHI-to-Age regressions, to evaluate their shell hardening trajectories to those of head-started tortoises experiencing only natural rainfall (Nagy et al. 2011). If slopes were similar among groups, we compared their elevations (at $p \leq 0.05$; Zar 1999). If two regression slopes differed significantly (t-test with $p \leq 0.05$), we used a

Zerbe test (Zerbe et al. 1982, Loehr et al. 2006) to determine at which covariate values (CL or Age) the two groups differed in elevation. We used the same procedures to test for growth rate differences among sites.

3. RESULTS

3.1. Rainfall, irrigation and plant cover

Annual rainfall during this 2006 to 2017 study averaged 5.76 cm, 56 % of the average (10.2 cm; Fig. 2) during the previous 30 years. However, rainfall varied considerably immediately before (2004-5 at 189 % and 2005-6 at 175 % of average) and during this study (2006 to 2017; 8 % to 117 % of average). There was a 4-year (2007 through 2010) and a 5-year (2012 through 2016) drought during our study (Fig. 2). Early in the study, we irrigated only when existing or anticipated drought conditions threatened the good germination and growth of herbs that tortoises eat. Later we also irrigated in late spring and late autumn of most years to provide drinking opportunities. More frequent irrigation was needed beginning in 2012, the start of the 5-year drought (Fig. 2).

Annual plant cover varied from less than one percent to over 36 %, and forb species richness varied from two to 19 species (Table 1). In 2012-2013, irrigation after January did not stimulate new germination.

3.2. Emergence success

Females laid 897 eggs in TRACRS during the first eight laying seasons, spring 2006-2013. Emergence success ranged from 68 % to 83 % per year and averaged 73.8 % (± 5.2 %, n = 8; Table 2). Annual emergence success was not correlated with rainfall, irrigation amounts, or average air temperature during mid-incubation (all $p > 0.39$). Our head-start process improved emergence success (73.8 ± 5.2 %, n = 8) compared to those in wild conditions at Goffs, California (55.2 %; $t_7 = 10.15$, $p = 10^{-5}$). With one exception (see Juvenile survival below), vertebrate predators apparently did not enter the enclosures. In regular inspections of nesting pens and burrows, we saw no nest disturbance or egg predation by vertebrates or invertebrates, including fire ants (*Solenopsis xyloni*) or other ant species, and no indirect evidence of predation (e.g., broken eggshells, dead embryos, digging, or footprints).

3.3 Juvenile survival

Juvenile survivorship inside TRACRS was high, averaging 96.0 % y^{-1} overall (Table 2). Average annual survivorship was not correlated to age ($r_s = 0.427$, $p = 0.198$, n = 11). Survivorship was low (66.7 %) in the 2009 cohort during the 2010 to 2011 period, during a predation event (see below). Annual survival was nearly 100 % in the latter years (2014-2017). For the three oldest cohorts, survival to nine years, the age when about half of the surviving individuals were large enough to release, was 48.6 juveniles per

100 eggs laid. As of autumn 2017, among all cohorts combined there were 50 live juveniles per 100 eggs laid.

We were able to determine causes of death for some juveniles. If carcasses were not found, absences from spring and autumn censuses indicated the tortoise died underground during winter. Inspection of the few smaller juvenile carcasses we found implicated death by ants and beetles attacking the exposed yolk sac and umbilicus, or soft, moist or incompletely closed umbilical scar. Additionally, older juveniles that were overturned or trapped in vegetation or fencing, likely overheated in the sun and died. We detected no dead juveniles that may have frozen after emerging to drink winter rain. The lowest annual survivorship (66.7 %, Table 2) occurred in the 2009 cohort during 2010-2011, probably from avian predation. In autumn 2010, overhead netting in their enclosure failed along seams stitched with non-UV-resistant twine, opening large holes. The gaps allowed several days access to a roadrunner (*Geococcyx californianus*), a burrowing owl (*Athene cunicularia*), and probably common ravens (*Corvus corax*), before we detected and closed the openings. Subsequently we found several predated carcasses of mostly 2009 juveniles and some 2010 cohort juveniles; several carcasses indicated signs of raven predation. We found the carcass of the roadrunner, which presumably could not escape, and we caught and released the owl unharmed. Additionally, horizontal support bars unique to the corrugated metal walls of enclosure 4, were near ground level and trapped small tortoises between the bar and wall or under the bar. Some of

these tortoises died of thermal exposure. We suspect these structures also contributed to the death of tortoises we found upside down near these bars. Following the removal of these bars, and continual netting inspection and repair efforts, mortality rates stabilized at very low levels in this enclosure.

3.4. Annual growth rates

The mean of all annual growth rates was 6.95 mm y^{-1} (SD $\pm$ 3.55, n = 3361). Annual growth rates of the eight cohorts averaged from 1.25 mm y^{-1} to 12.92 mm y^{-1} (Table A1). Individual growth rates were more variable, ranging from -2.4 mm y^{-1} (a shrinking shell) to more than +20 mm y^{-1}. Even growth rates among clutch mates varied considerably. For example, growth rates of five clutch siblings living together during the 2006-2007 year varied from 1.23 to 12.04 mm y^{-1} (mean 6.71 mm y^{-1} $\pm$ 4.21).

General Linear Model (GLM) ANOVA documented the main effects of year, age, annual cohort and mother (Table 3). The GLM ANOVA also matched univariate ANOVA test strengths and post-hoc tests that identified means significantly different from others (Student-Newman-Keuls test, all $p < 0.05$). There were many differences among years (e.g., low in 2013, high in 2016; Fig. 3), ages (mean growth rate correlated with age; Fig. 4) and cohorts (low in the 2011 cohort, high in the 2013 cohort; Fig. 5). Nested ANOVA confirmed the strength of year, age, cohort and mother on growth rates (Table 3). One-way repeated measures ANOVA (1WRMA) were balanced and complete for two separate analyses, year and age. 1WRMA

detected a very strong effect of year ($F_{10,2769} = 219.55$, $p < 10^{-30}$) and age ($F_{10,2769} = 123.59$, $p < 10^{-30}$), with post-hoc results nearly identical to those of the one-way ANOVA. The 1WRMA also quantified the strength of individuals ($F_{581, 2769} = 1.8841$, $p < 10^{-30}$). Simple linear regressions indicated that mean annual growth rates (Fig. 3) were correlated to annual plant cover (Table 1; $F_{1,9} = 10.7081$, $p = 0.0096$, $r^2 = 0.5433$) and precipitation (= rain plus irrigation, $F_{1,9} = 5.728$, $p = 0.04034$, $r^2 = 0.3889$), suggesting growth is more closely associated with food availability than to water availability. However, these two regression coefficients (r^2) were not different ($Z_{0.05,2} = 0.4264$, $p = 0.3349$). Similarly, nonlinear regressions, with growth rates increasing exponentially to an asymptote, were moderately strong for annual plant cover (Fig. 6), essentially nonexistent for precipitation ($F_{1,9} = 0.1372$, $p = 0.7197$, $r^2 = 0.0150$), and the two regression coefficients differed at $p = 0.0509$ ($Z_{0.05,2} = 1.6359$).

One-way ANOVA indicated a strong maternal effect ($F_{49,3311} = 2.510$, $p < 10^{-8}$) among the 50 mothers, but the p -value was larger than those for other univariate ANOVA ($< 10^{-30}$). Also, the offspring of only five mothers had significantly low (n = 1) or high (n = 4) means in SNK post-hoc tests. The one mother's group with low growth rates (mean = 5.01, SD = 0.350, n = 18) represented six years of data for 3 hatchings of 2011, which had the lowest rates of all cohorts (Fig. 5), occurred during the early, slow growth ages (Fig. 4), and hatched at the beginning of a five-year drought. The three mothers with high offspring growth rates (20 hatchlings, weighted mean =

8.87, SD =6.058, n = 83) had hatchlings in only 2012, 2013 or both, both occurring after the slowest-growing cohort (2011) and included a combination of years with the highest growth rates (2014-2017; Fig. 3). The four offspring of female 35 had high growth rates (mean = 8.59, SD =3.503, n = 32), hatched in 2008 (a moderate cohort year, Fig. 5), started growing before the 5-y drought, experienced improved irrigation and forage conditions (e.g., 2013-2016), and included an age effect (older tortoises, Fig. 4).

To compare growth rates (mm CL y^{-1}) of three free-living Mojave Desert populations and two head-start populations, including TRACRS' head-start population, we evaluated the slopes of linear regressions relating CL to age (up to 11 years, Fig. 7). The regression-based growth rates used annual mean CL measurements of estimated-age wild tortoises near Goffs, California (Turner et al. 1987a), known-age wild juveniles in Rock Valley, Nevada (Turner et al. 1987b, Medica et al. 2012), known-age wild and head-started tortoises at Fort Irwin, California (Nagy et al. 2015b, L.S. Hillard and M.W. Tuma, unpublished data), and known-age head-started juveniles at TRACRS. Growth rates were highest at Rock Valley and Goffs (estimated as the slopes of the regressions at 9.91 mm y^{-1} and 9.36 mm y^{-1}, respectively) and did not differ statistically from each other (ANCOVA $t_{19} = 1.131$, $p > 0.13$). Growth rates were lowest at Fort Irwin (enclosed: 4.19 mm y^{-1}; free-ranging: 4.38 mm y^{-1}) and did not differ from each other (ANCOVA $t_{10} = 0.363$, $p > 0.36$). The growth rate via regression slope of TRACRS juveniles

during their first 11 years (6.41 mm y^{-1}) was lower than those at Rock Valley and Goffs (both ANCOVA $t > 8.7$, $p < 0.000001$, df = 19 and 20, respectively), and higher than those at Fort Irwin (head-started ANCOVA $t_{15} = 6.41$, $p < 10^{-5}$; free-ranging $t_{15} = 6.12$, $p < 1 \times 10^{-6}$). Thus, the overall rate of growth of juveniles at TRACRS was intermediate between those of juveniles living in their natural habitats.

3.5. Density Effects on Growth Rate

Initial (previous autumn) biomass densities in separate pens ranged from a low of 0.27 g tortoise m^{-2} in 2006 to a high of 18.10 g m^{-2} in 2016. The only significant relationships between annual growth rate and initial juvenile biomass density were negative relationships that occurred during four years (2009-2010, 2010-2011, 2012-2013, and 2013-2014). We hypothesized that any year having a substantial food shortage should show a downward deflection in growth rate at high biodensities, beyond the point where food supply exceeds food demand. However, the results do not support this hypothesis; there was no clear deflection point or threshold (Fig. 8). Growth rates during 2012-2013 were unusually low (also see Table A1).

3.6. Body Condition and Shell Hardness Indices

The mean autumn BCIs ranged from 0.48 to 0.58 g body mass cm^{-3}. There were two instances where mean BCI values were slightly below 0.45, indicating very mild dehydration. One was the 2006 cohort in year 2007-8 (0.44), and the other being the 2008 cohort (0.44) in 2009-10, both during

years when precipitation and irrigation were low. However, during the drought year of 2009-2010, the average BCI of all juveniles was higher, at 0.50.

As juvenile CL (in mm) increased, SHI increased asymptotically toward adult SHI (i.e. 100 %; Fig. A1). The transform of SHI [ln (100 – SHI)] was linearly correlated to CL ($F_{1,626} = 2841$, $p < 10^{-5}$, $r^2 = 0.82$), with the regression equation ln (100 – SHI) = 4.489 – (0.0325 * CL). Similarly, the relationship between SHI and Age (in years) was significant ($F_{1,626} = 2605$, $p < 10^{-4}$, $r^2 = 0.81$), with an equation of ln (100 – SHI) = 3.107 – (0.238 *Age). The relationships of shell hardness ln (100 – SHI) to size (CL) for irrigated (this study) and unirrigated (from Nagy et al. 2011) head-started tortoises had similar slopes (ANCOVA $t_{665} = 1.01$, $p > 0.15$) but different elevations (ANCOVA $t_{665} = -5.55$, $p < 10^{-6}$). The unirrigated tortoises had the lower curve [ln (100 – SHI) = 4.039 – (0.0323 * CL)], indicating that their shell hardened at a smaller size than did shells of irrigated tortoises [ln (100 – SHI) = 4.479 – (0.0323 * CL)]; at the same CL, irrigated tortoises had softer shells than did unirrigated tortoises (Table 4). The slopes of shell hardness-to-age relationships (log-linear) differed between irrigated and unirrigated juveniles ($t_{665} = 4.436$, $p < 0.00001$). The slope for the irrigated tortoise equation [ln (100 – SHI) = 3.107 – 0.238 * Age; $F_{1,626} = 2605$, $p < 0.001$, $r^2 = 0.81$] was steeper than that for unirrigated tortoises [ln (100 – SHI) = 2.941 – 0.1946 * Age; $F_{1,39} = 271$, $p < 0.001$, $r^2 = 0.87$]. Above 7.3 years (Zerbe test, $p < 0.05$), irrigated tortoises had significantly harder shells than did unirrigated

tortoises. Additionally, we calculated the expected ages when unirrigated and irrigated juveniles reached 98% and 99% of adult SHI (Table 4). Shells of irrigated juveniles hardened 1.5 years faster, and at a larger size (12% larger), than did shells of unirrigated tortoises.

DISCUSSION

4.1. Survivorship

We demonstrated head-starting's ability to substantially enhance nest and egg success, and to increase juvenile survival when compared to the wild. Consequently, head-starting can potentially augment Agassiz's desert tortoise (*Gopherus agassizii*) populations and species recovery (USFWS 2011a) by providing releasable healthy juveniles that are past their highest mortality stages.

In natural habitats, emergence success (percent of emerging neonates per 100 eggs laid) was 55.2% (at Goffs, Turner et al. 1987a) and 68.9% (in the Sand Hill Training Area [Sand Hill] < 5 km from TRACRS, Bjurlin and Bissonette 2004). These two studies documented substantial nest predation by vertebrates (37% at Goffs and 26% at Sand Hill). At Goffs, egg and nest mortality were attributed to 1) infertile eggs (6.1 %), 2) broken eggs (6.6%) and 3) nest predation (32.1%; Turner et al. 1987a). At TRACRS, we observed no signs of vertebrate predation on nests, and emergence success was relatively high (73.8 % over 8 years; Table 2), but was not as high as expected from Goffs results in the absence of predation (87.3%). It is

possible that non-vertebrate organisms, such as subterranean invertebrates, fungus, or microbe infections reduced emergence success at TRACRS, or there may be spatio-temporal variation in nest and emergence success. In another head-start study (Nagy et al. 2015a), native fire ants swarmed, killed and consumed 30% of neonate tortoises underground while hatchlings emerged from nests; subsequent predator control measures were effective.

After nine years, the first cohort hatched at TRACRS (2006) had 42 live juveniles per 100 eggs laid. This is 6.6 times the expected number of nine-year-old juveniles (7.3 per 100 eggs) surviving in a wild population (from Goffs life table analysis, Turner et al. 1987a). After 11 years, the 2006 cohort had 37 live juveniles, which is 7.3 times the life table expectation of 5.06 alive had those eggs been laid in the field. Improvements in TRACRS structure, maintenance and husbandry over time have increased annual survival, such that the expected 11-year survival from 100 eggs, calculated using annual means of cohort survival (Table 2, bottom row), is 51 juveniles (Fig. 9). This is ten times the survival of 5.06/100 eggs expected in the wild (Goffs; Turner et al. 1987a).

The current survivorship of wild tortoises, especially neonates and small juveniles, may be much lower than in earlier studies of remote wild populations (i.e., Turner et al. 1987a). The recent density decline (50% from 2005 to 2014; USFWS 2015) in wild adult *G. agassizii* has pushed many populations near or below thresholds of viable population densities (USFWS

1994, USMC 2017, Berry and Murphy 2019). MCAGCC populations have experienced comparable declines over the past few decades (USMC 2017). The proportion of juveniles in wild populations also appears to have declined since 2007 (Allison and McLuckie 2018). This may be due largely to increased populations of, and prey switching by, subsidized predators [e.g., common ravens (*Corvus corax*) and coyotes (*Canis latrans*); Esque et al. 2010, Berry et al. 2013], but may also be affected by factors that can reduce fecundity (e.g., food availability; Turner et al. 1986, 1987a, Henen 1993, 1997, Lovich et al. 2015) and slow juvenile growth, among other causes. Consequently, head-starting may be central to bolstering the declining natural populations of Agassiz's desert tortoises. However, biologists express concern about post-release survival (e.g., Heppell et al. 1996, Siegel and Dodd 2000, Reed et al. 2009), with models suggesting that head-starting cannot, logistically and numerically, augment and sustain populations. We anticipate measuring survival rates of released head-started TRACRS juveniles in an effort to evaluate these uncertainties.

4.2. Mortality inside enclosures

Besides preventing deaths by large predators, dehydration and starvation, we reduced juvenile deaths several other ways. We eliminated detectable nest predation by ground squirrels by constructing fences with bands of slippery metal sheeting that rodents could not climb (Nagy et al. 2015a). Native Fire Ants (*Solenopsis xyloni*) were living inside enclosures in

low (non-predatory) densities. When ant nests increased in size or number (e.g., after considerable rain or irrigation in spring), we reduced ant densities by placing fire ant-specific toxic bait (with Hydramethylnon) inside tortoise-excluding stations, near ant nests. By removing the ground-level bars in enclosure four, we reduced trapping and overturning of tortoises, greatly reducing mortality due to exposure. Frequent monitoring and repair of the bird netting in all enclosures has apparently excluded predatory birds and eliminated associated juvenile deaths. Use of aseptic handling methods through 2017 contributed to the absence of clinical signs of mycoplasmosis in head-started tortoises.

4.3. Body Condition and Shell Hardness Indices

BCI measurements on TRACRS juveniles indicated adequate-to-good hydration and gut fill (Nagy et al. 2002) during spring and during fall, especially after 2013 when we began irrigating enclosures more often through winter and spring. Those drinking opportunities supported subsequent food consumption and digestion, especially in late spring and autumn when only dry plants were available, and facilitated sustaining near-normal body mass. In contrast, free-living adult and juvenile desert tortoises typically transition from high spring BCI to reduced BCI in summer (normally a dry season, Nagy and Medica 1986, Nagy et al. 2002) and BCI remains low into autumn if there are no drinking opportunities. Summer rains support drinking, ensuing food consumption and increases in body and lipid mass

(Henen 1997, Henen et al. 1998). In another head-start study, juveniles without irrigation developed BCI lower than 0.40 and all died during a prolonged drought of 1.33 y (Nagy et al. 2015a). By irrigating every summer we insured TRACRS juveniles had opportunities to drink, eat and hopefully grow despite the frequent drought years. Mortality among free-living adults also increases during droughts (up to 40% dying per year; Turner et al. 1984, Peterson 1994, Longshore et al. 2003, Field et al. 2007, Lovich et al. 2014, Berry and Murphy 2019). Juvenile mortality should also increase in droughts and be higher than adult rates, especially given juveniles' small size, high surface-to-volume ratios, and high mass-specific metabolic rates (Nagy et al. 1997). Yet annual mortality rates for our head-start juveniles were < 17% during the 2007 to 2010 drought (excluding the 2010 avian mortality event in the 2009 cohort, Table 2), and < 8% in almost every cohort every year of the 2012 to 2016 drought (the 2013 cohort in 2014 was the exception). We irrigated more during the latter drought than in the prior drought. Thus, head-starting protected juvenile tortoises from death by dehydration, starvation and predation.

SHI of irrigated juveniles increased faster than in juveniles head-started without irrigation (Nagy et al 2011). Moreover, juveniles with irrigation also reached adult shell hardness at a larger size. Both of these benefits reduce head-starting duration and costs by allowing earlier releases and increased defenses to raven predation.

4.4 Growth rates

Despite irrigating to counter drought conditions (i.e., to hydrate tortoises and promote food plant growth), our juveniles' overall growth was not higher than average rates in wild juvenile tortoises but was comparable to rates in other irrigated, head-start facilities (4.2 mm y^{-1} to 11.9 mm y^{-1}; Nagy et al. 2015a, Nafus et al. 2017, Mack et al. 2018 and Tuberville et al. 2019).

4.41. Free-living versus head-start

Average annual growth rates of free-living juveniles in three natural populations were both higher and lower than the average growth rate at TRACRS (Fig. 7). The growth rate differences between the three field populations appear positively related to variation in annual rainfall amounts: Rock Valley, 160.2 mm rain y^{-1} (45-year average, Medica et al. 2012) and Goffs, 167.9 mm rain y^{-1} (during four years of the study, Turner et al. 1987a), and Fort Irwin, 48.0 mm rain y^{-1} (3-y average from 2005-2008; Nagy et al. 2015b). But these average annual growth rates of free-living juveniles include both high-rainfall years with much food available, and low (or no) rainfall years when herbs are absent. During "good" rainfall years, wild juveniles can grow up to twice as fast as the average rate (Medica et al. 2012, Hillard and Nagy unpublished obs.). At TRACRS, we tried to achieve a "good" herbaceous production year every year by irrigating. So why did TRACRS juveniles not achieve higher growth rates? To address this, we

examined the relationships between growth, total precipitation (rain plus irrigation), food availability, and tortoise biomass density.

4.42. Precipitation and food availability

Growth responses to precipitation (rainfall and irrigation) and food supply are essential to an understanding of the effectiveness of head-starting efforts and general tortoise biology. Although these responses are complicated or obscured by the large variation in growth rates (Table A1), the large individual variation is consistent with results from numerous studies of desert tortoise growth (e.g., Nagy and Medica 1986, Turner et al. 1987b, Medica et al. 2012), physiology (Nagy and Medica 1986, Peterson 1996, Henen 1997, Henen et al. 1998, Drake et al. 2012, Nafus et al. 2017, among others) and behavior (Woodbury and Hardy 1948, Medica et al. 1980, Nagy and Medica 1986; Nafus et al 2017, among others). Such large variation may appear extreme, but it is likely exaptive for desert reptiles (Bradshaw 1988 and 1997) and other ectotherms (Pough 1980), and central to their species' success in arid environments.

Nonetheless, the growth rates here correlated strongly to plant cover (an indicator of food supply) and correlated mildly to precipitation. Precipitation influences plant production in complex ways (e.g., Beatley 1974, Turner and Randall 1989) and young herbaceous plants are the primary food source as tortoises emerge from winter dormancy (Nagy and Medica 1986; Henen 1993, 2002, Lovich et al. 2015, and many others). We

expected tortoise growth rates to be related less to total annual precipitation, as the sum of rainfall and irrigation, than to plant cover, because the effects of precipitation timing and amounts on plant germination and growth vary considerably. Food plants also provide more than water to tortoises (Nagy and Medica 19868, Henen 1997), with egg production asymptotically related to the availability of annual plants (an indicator of plant production whether annual or perennial; Turner et al. 1987, Henen 1993, 1997, Lovich et al. 2015). At TRACRS, late fall and early winter precipitation > 15 mm could initiate germination of herbs but if little or no precipitation occurred by mid-February, germination was minimal despite subsequent irrigation (e.g., in 2012-2013; Fig. 10). Late spring precipitation sustained plant growth to May or June. Summer precipitation had little effect on winter herbs that survived through spring.

As our tortoises grew and their body mass increased, they ate more of their preferred plant foods early in the year, before the plants could flower, set seed and add these seeds to the soil seed bank. Consequently, plant species richness declined (especially in 2011-2012; Fig. 10), and the remaining plants were of low feeding preference or were non-native species. It was necessary to provide nursery-grown plants for food in springs of 2007, 2012 and 2013, and to sow native wildflower seeds every fall beginning in 2012. Winter rainfall and irrigation in 2012-2013 were too little and too late to stimulate germination, so spring 2013 also had low plant cover, necessitating provision of more nursery plants. Beginning in 2013, we

irrigated each fall immediately after seed sowing regardless of weather forecast and realized rainfall. This method increased plant cover and juvenile growth rates above those of the first seven years (Fig. 10). Plant species richness also increased in response to regular irrigation and seeding. We also irrigated briefly, 30-60 minutes, during summer so tortoises could drink, eat the available dry plants (Nagy and Medica 1986, Henen 1997, 2002), and eat the Bermuda grass hay we began providing each summer starting in 2012. With these modifications in irrigation, seeding and dry food supplementation, growth rates in TRACRS increased to levels seen in free-living juveniles during "good" years (Medica et al, 2012).

4.43. Biomass density and food supply

If growth rates were limited just by food supply and not influenced by food quality, we would suspect that annual growth rates would be lower in pens with greater densities of tortoise biomass. However, this did not occur in seven of the 11 years, and in three of the other four years (2009-10, 2010-11, 2013-14, Fig. 8), growth rates were near average (Table A1). Except for the very low growth rate in the year with the lowest plant cover, 2012-13, growth rates in the other 10 years varied little and seemed to plateau despite increasing food availability (i.e., plant cover; Fig. 6), suggesting food availability rarely limited growth. At Edwards Air Force Base (EAFB) in 2010 to 2012, head-start juveniles had very low growth rates (3.7 mm y^{-1}), but also had low condition indices, poor health, lethargy and high mortalities despite

some irrigation (Mack et al. 2018). The biomass densities at EAFB were (ca. 3 to 7 g m^{-2}, calculated from Mack et al. 2018) intermediate to those at TRACRS (Fig. 8). Herbaceous plants available at EAFB's facility, comprised primarily of three non-native annual grasses of low nutritional quality (Hazard et al. 2009, 2010), support Mack et al.'s (2018) suggestion that EAFB pens lacked sufficient mass of preferred herbs to sustain the animals. Hatchling desert tortoises grew at high rates (9.6 – 11.9 mm y^{-1}; Nafus et al. 2017) at an irrigation-equipped head-start facility that had a plant population with good native species richness and cover. There, first-year juveniles also showed maternal effects on growth rates, with larger mothers producing larger hatchlings that grew faster and had higher survivorship (Nafus et al. 2015).

Head-start overcrowding may limit juvenile growth inside head-start facilities via more than one means. Although maximum biodensities inside TRACRS (12-13 g m^{-2}; Fig. 8) were more than 100 times that of wild adults in the surrounding habitat (0.097 g m^{-2}, assuming 100 2.5-kg adults mi^{-2}, Woodman et al. 2001), growth rates inside TRACRS were low in only one of 11 years, and that was during the most severe food paucity (Fig. 8). Consequently, extreme food paucity and food composition (e.g., low availability of preferred forbs and grasses) provides one estimate of maximum biodensity in head-start enclosures.

Qualitatively, we detected early seasonal reductions of specific food plant species as juveniles emerged from winter brumation. We suspect they consumed their 'preferred' foods as seedlings, before those plants could grow, provide a larger source of food, and set seed that sustains the seed bank. Additionally, the remaining, 'less-preferred' plant species would be consumed less vigorously, subsequently propagating and competing with preferred species. Wild juveniles have much lower constraint on movements, so wild juveniles should have access to much greater areas to forage selectively on more nutritious foods.

In order to be of a manageable size and yet produce useful numbers of large juveniles for release, head-start facilities can have biomass density constraints that reduce food plant diversity, food plant productivity, and soil seed banks. We have countered these reductions by sowing seeds of preferred food plants in autumn and early winter and irrigating deeply and regularly after sowing through the ensuing May. Additionally, we controlled some seed-eaters (ants and rodents) but not small birds that easily ingressed through cyclone fencing and overhead nets.

5. RECOMMENDATIONS AND CONCLUSIONS

The conclusions and recommendations below are based primarily on results in this study, located in the south-central part of the Mojave Desert. Rainfall patterns and average annual precipitation amounts vary widely across the species range, from relatively high winter rainfall in the western

areas to low, mostly winter rainfall in the central Mojave area, and to relatively high summer rainfall in eastern parts of the range. Similarly, the species composition of tortoise food plants varies from mainly “winter annuals” in the west to a mixture of “winter” and “summer” annuals in the east (see Henen et al. 1997, Wallis et al. 1999, and references therein). We suggest that the recommendations below, which are based on results from a relatively low rainfall area with mainly winter rainfall, be applied considering regional differences in climate. In hindsight, some of these recommendations may now seem obvious, but our initial research strategy was to start with a protocol that was current, low-cost and simple to operate, but with the caveat to adaptively manage with more intensive procedures as our results indicated necessary for successful head-starting.

1). To help sustain soil seed banks in head-start enclosures, seeds of preferred plant species should be sown in autumn (October and November) of each year.

2). After sowing seeds, irrigation should commence in October or November, as desert rainfall is, and forecasts are, extremely variable and unpredictable (Louw & Seely 1982, this study).

3). Tortoises were more apt to remain above ground, eat and grow if they were hydrated (Nagy et al. 2015a). To provide drinking water, we recommend irrigating for at least 30 minutes as juveniles emerge from brumation in March, several times in spring (especially during droughts), in

early June before summer heat arrives, and in late August and September to enable drinking and eating before brumation. Dehydrated tortoises may emerge in winter rains to drink, and subsequently they may be prone to die from sudden cold exposure (B. Henen and Mark Bratton, independent observations).

4). Irrigation should continue about biweekly during spring (in the south-central region of the Mojave Desert) to sustain food plant growth, extend tortoise foraging on these plants, and foster production of food plant seeds.

5). Bermuda grass hay can be provided in dry months (ca. 1 June to 15 October) if dead forb and grass plant matter is sparse, as hydrated juveniles (like adults; Henen 1997) continue eating during summer.

6). Herbaceous plant growth should be monitored in enclosures to obviate or mitigate overgrazing, early senescence (e.g., in droughts), and loss of species diversity.

7). If new plants fail to germinate by February or March, add, irrigate, and resupply nursery-grown plants (e.g., *Gazania* spp. and grass sod) as a continuous fresh food supply through May.

8). Head-start enclosures should avoid use of horizontal bars or beams inside near ground level as they contribute to overturning, overheating and death.

9). The bird netting overhead must be inspected frequently to discover and repair degradation and damage caused by sunlight, strong winds, heat, and gnawing rodents, that enables bird depredation of juveniles.

10). Avoid overstocking tortoises (e.g., biomass density > ~ 5 g m^{-2}), which compromises tortoise growth, health, and survival, especially during droughts, and during conditions of low food diversity, quality and abundance.

11). This study shows that a head-start facility containing natural habitat and relatively high densities of young Agassizi's desert tortoises can be operated to promote good growth rates. These operations produce well-hydrated 11-year-old juveniles that number 7 to 10 times more than would survive from the same number of eggs laid in the wild.

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TABLES

Table 1. Annual plant cover (%: mean, SD) in 24 individual 59.3 m^2 TRACRS (Tortoise Research and Captive Rearing Site) pens each year. Forb species richness is indicated for the entire enclosure (number of species per 1,423 m^2).

Cover	YEAR 2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017
Mean	3.63	11.91	9.56	11.76	9.38	2.05	0.94	8.04	22.10	36.90	24.44
n		1	6	6	8	5	4	8	0	0	4
SD	2.55	4.26	3	8.55	7	7	4	2	8.16	7	8
Forbs	10	19	14	19	17	2	9	16	15	10	12

Table 2. Emergence success (Emergence: % of eggs laid with hatchlings emerging from nests) and annual survivorship (%) by age, of eight cohorts of juvenile desert tortoises raised in the TRACRS facility. Numbers of eggs obtained per year were 166, 48, 146, 187, 110, 112, 87, and 41 from 2006 to 2013 (with lower numbers during drought years).

		Age, years													
Cohort	Emergence	1	2	3	4	5	6	7	8	9	10	11	Mean	SD	n
2006	75.3	88.8	86.5	97.9	86.2	96.3	100.0	92.3	97.2	98.6	94.9	95.2	94.0	4.9	11
2007	83.3	95.0	97.4	89.2	100.0	100.0	100.0	97.0	100.0	100.0	100.0		97.9	3.5	10
2008	69.9	100.0	83.3	96.5	98.8	100.0	96.3	100.0	98.2	98.2			96.8	5.3	9
2009	67.9	87.4	66.7	95.9	97.2	98.6	100.0	98.5	100.0				93.0	11.4	8
2010	76.4	85.7	93.1	100.0	97.0	100.0	95.4	100.0					95.9	5.2	7
2011	74.1	95.2	100.0	93.7	100.0	98.6	100.0						97.9	2.8	6
2012	67.8	100.0	94.9	100.0	100.0	100.0							99.0	2.3	5
2013	75.6	96.8	86.7	100.0	96.2								94.9	5.7	4
Mean	73.8	93.6	88.6	96.7	96.9	99.1	98.6	97.6	98.9	98.9	97.5		96.0		
SD	5.2	5.6	10.6	3.8	4.6	1.4	2.2	3.2	1.4	0.9	3.6		5.9		
n	8	8	8	8	8	7	6	5	4	3	2		8		

Table 3. General Linear Model (GLM) indicates Main Effect of year, age, cohort (year hatched) and mother on 3361 annual growth rates (mm yr^{-1}) measured over 11 years on eight cohorts of juvenile tortoises in the head-start facility, TRACRS, at the Marine Corps Air Ground Combat Center, Twentynine Palms, California. Main Effect could not analyze interactions. Nested ANOVA (Nested) used variables age, cohort and mother nested within year (e.g., Mother-year indicated mother nested with year). Degrees of freedom indicated by df_1 and df_2. All $p < 10^{-30}$ except for the simple nesting of mother within year (Mother-year*, $p < 10^{-15}$). Other forms (e.g., age, cohort and mother simultaneously nested within year) were incomplete, unbalanced designs.

Main Effect	F	df_1	df_2
Year	148.09	10	3284
Age	75.01	10	3284
Cohort	16.16	7	3284
Mother	4.34	49	3284
Nested (indented)			
Year	7.44	40 6	2905
Mother-year*	3.65	49	2905
Year	140.48	10	2856

Cohort-year	17.56	49	285 6
Mother-year	1.75	44 5	285 6
Year	140. 48	10	285 6
Age-year	17.5 7	49	285 6
Mother-year	1.75	44 5	285 6

Table 4. Carapace length (mm) and age (years) at which irrigated (this study, n = 628) and unirrigated (Nagy et al. 2011, n = 41) juvenile desert tortoises attained shell hardness values of 98% and 99% of adult shell hardness. Juveniles were 1-12 years old (irrigated) and 2-16 years old (not irrigated).

Shell Hardness Index	98 % CL	99 % CL	98 % Age	99% Age
Not irrigated	10 4	12 5	11. 6	15.1
Irrigated	11 7	13 9	10. 1	13.1

FIGURES

Fig. 1. A head-start enclosure (left) subdivided into small pens (foreground) and larger pens (background) at the Tortoise Research And Captive Rearing Site (TRACRS) on the Twentynine Palms Marine Base. Springtime annual wildflowers (right), including preferred food species (desert dandelion (*Malacothrix glabrata*; yellow-flowers); and desert pincushion (*Chaenactis fremontii*; white-flowers).

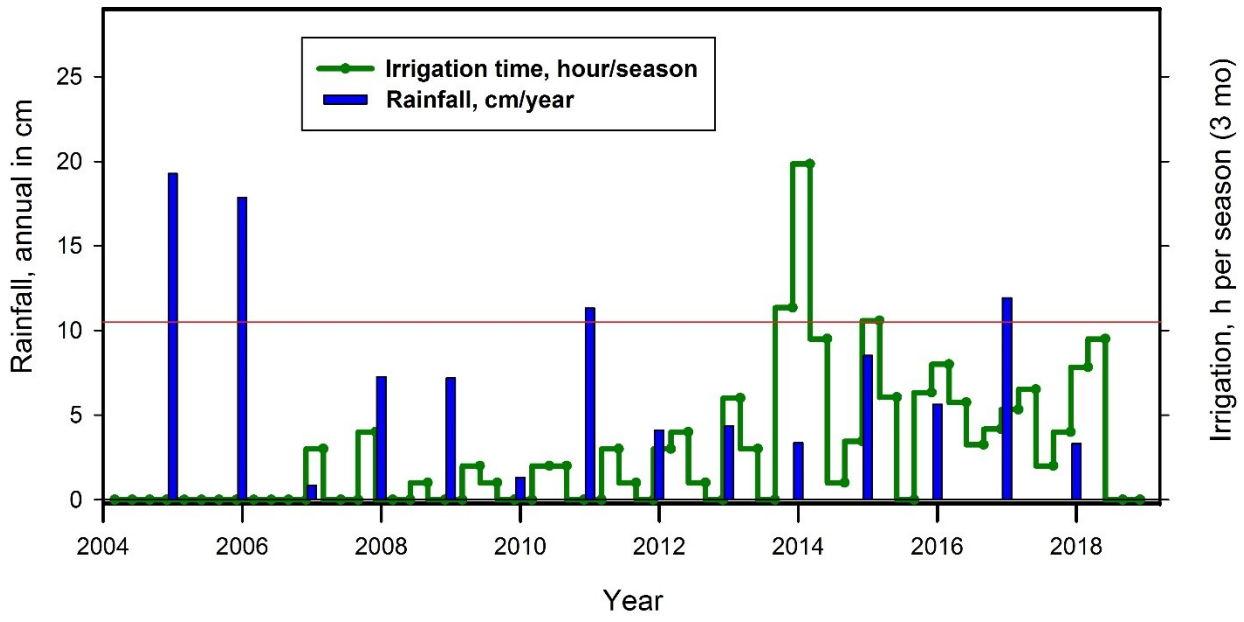

Fig. 2. Yearly rainfall (1 October to 30 September) measured at TRACRS (blue columns), 30-year average annual rainfall nearby (Expeditionary Air Field; red horizontal line), and hours of irrigation per 3-month season (green columns) from July 2004 to mid-2018. One hour of irrigation provided a 12.7 mm (ca. 0.5 inch) equivalent of ‘rainfall’.

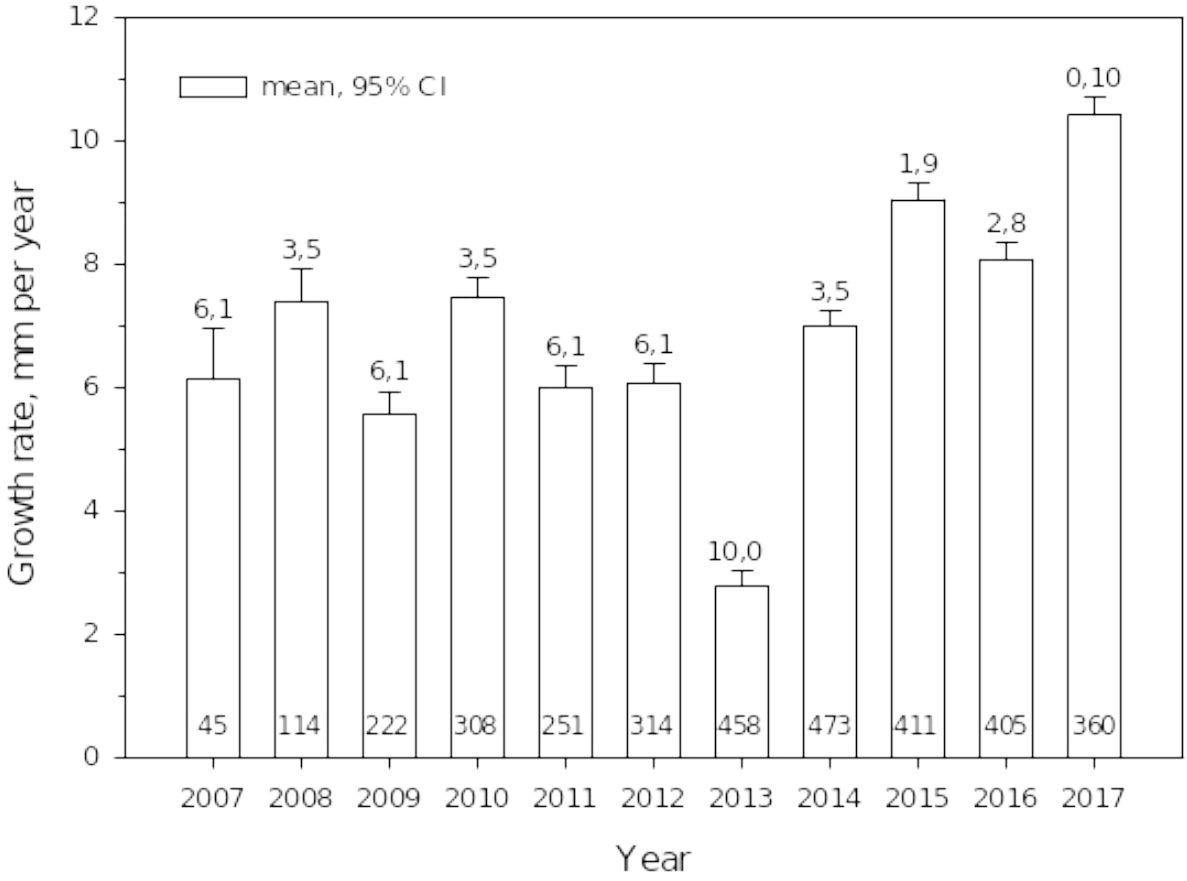

Fig. 3. Yearly variation in annual growth rates of juvenile head-start tortoises ($F_{10,3350} = 197.21$, $p < 10^{-30}$) at the Marine Corps Air Ground Combat Center's head-start facility, TRACRS. Annual growth was measured from October through September, matching the rainfall year and the first year of growth of hatchlings. Numbers at the upper 95% confidence limit indicate the number of means higher and lower than it (e.g., 2013 had 10 means higher and zero means lower) by Student-Newman-Keuls post-hoc comparisons ($p < 0.05$). Samples sizes are indicated within the bars. The effects of individual and year were very strong (two-way ANOVA $F_{581,2769} = 1.884$, $p < 10^{-15}$; $F_{10,2769} =$

219.6, $p < 10^{-30}$, respectively), but the factorial analysis design was incomplete, preventing tests of interactions.

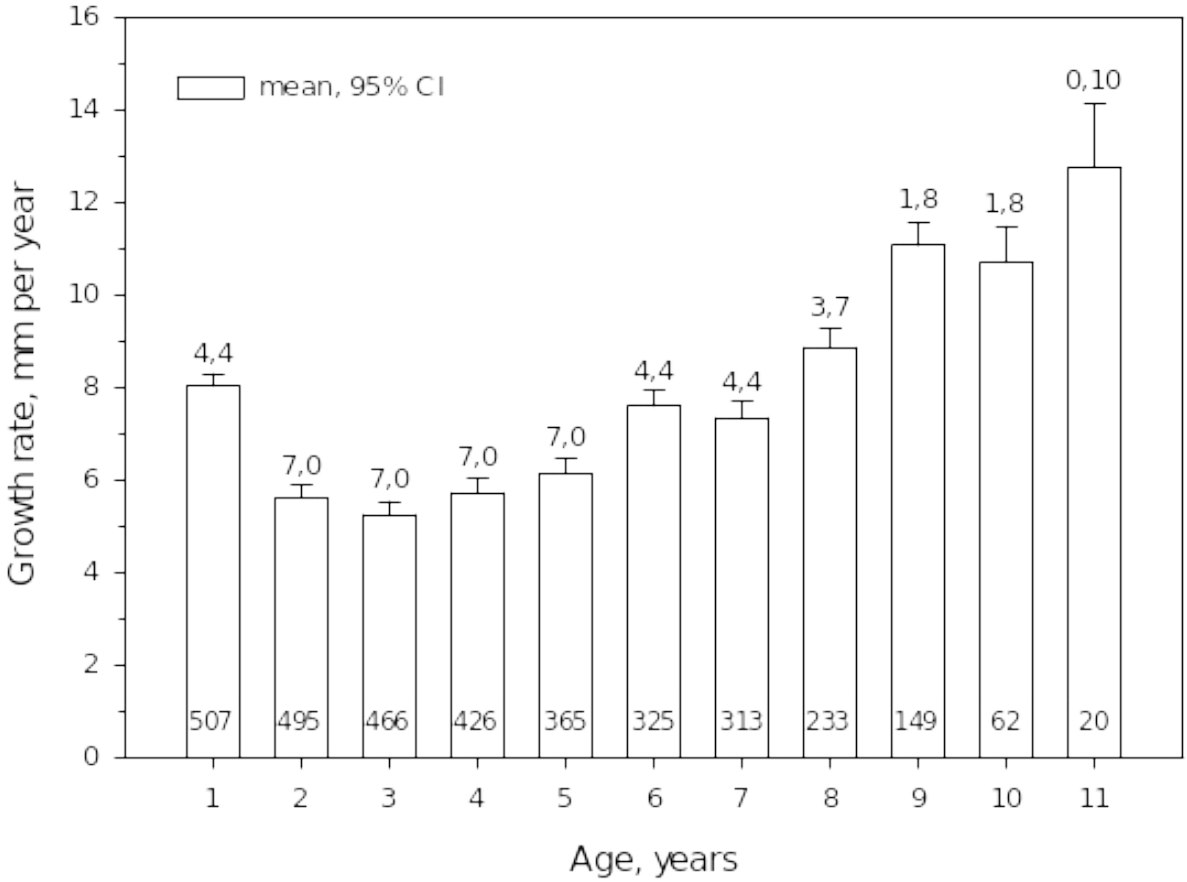

Fig. 4. Age variation in annual growth rate of juvenile desert tortoises (one-way ANOVA $F_{10,3350} = 88.14$, $p < 10^{-30}$) at the Marine Corps Air Ground Combat Center's head-start facility, TRACRS. Age is indicated relative to hatching, which was in September. Numbers at the upper 95% confidence limit indicate the number of means higher and lower than it (e.g., 11-years old had zero means higher and 10 means lower) by Student-Newman-Keuls post-hoc comparisons ($p < 0.05$). Samples sizes are indicated within the bars. Mean growth rates correlated with age (with first year: $F_{1,9} = 20.192$, $p = 0.001503$; $r^2 = 0.6917$; GR = 4.340 + 0.6263 * Year; and without first year: $F_{1,8} = 82.82$, $p = 0.000017$, $r^2 = 0.9119$; GR = 2.707 + 0.8303 * Year).

ANCOVA found no difference between the two regressions ($p > 0.3$ for slopes $t_{17} = 1.1616$ and $p > 0.5$ for elevations $t_{18} = 0.6552$).

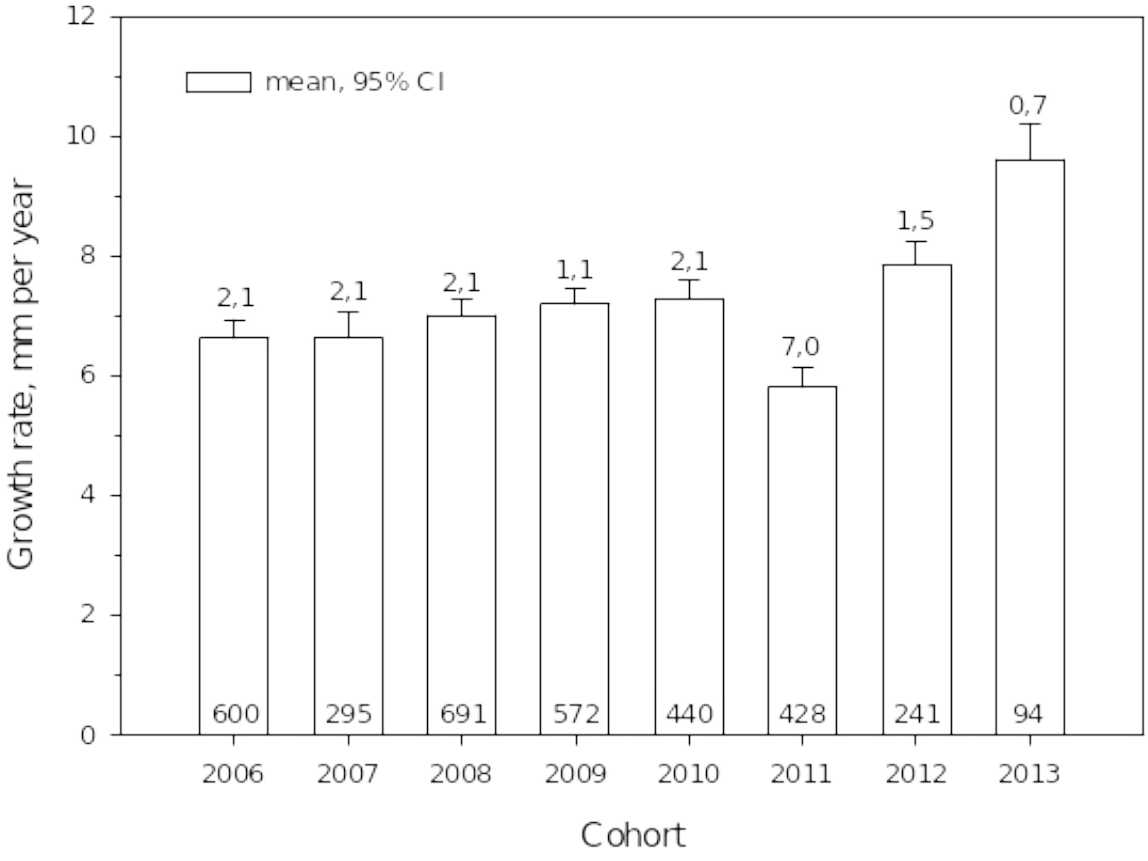

Fig. 5. Cohort variation in annual growth rates of juvenile desert tortoises (one-way ANOVA $F_{7,3353} = 18.98$, $p < 10^{-30}$) at the Marine Corps Air Ground Combat Center's head-start facility, TRACRS. Cohort refers to the year of hatching. Numbers at the upper 95 % confidence limit indicate the number of means higher and lower than it (e.g., cohort 2011 had 7 means higher and zero means lower) by Student-Newman-Keuls post-hoc comparisons ($p < 0.05$). Samples sizes are indicated within the bars.

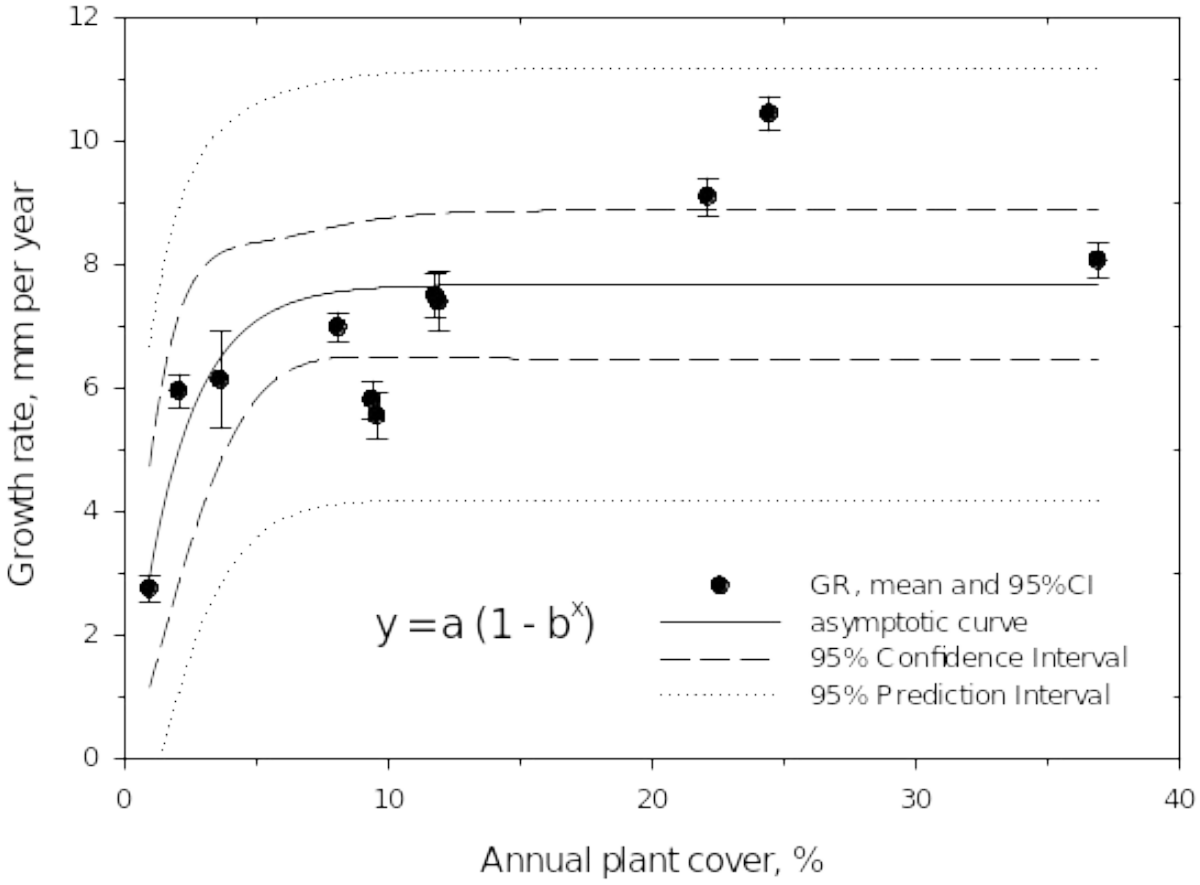

Fig. 6. Mean ± 95% Confidence Intervals (CI) of annual growth rates (GR, mm y^{-1}) of head-started tortoises each year in relationship to the annual plant cover (%) inside the enclosure that year, for 11 years. The nonlinear curve [$y = 7.674 * (1- 0.597^{x})$; $F_{1,9} = 10.62$, $p = 0.00986$, $R^2 = 0.5413$] was asymptotic (solid line) and bound by 95% Confidence Intervals (dashed line) and Prediction Intervals (dotted line).

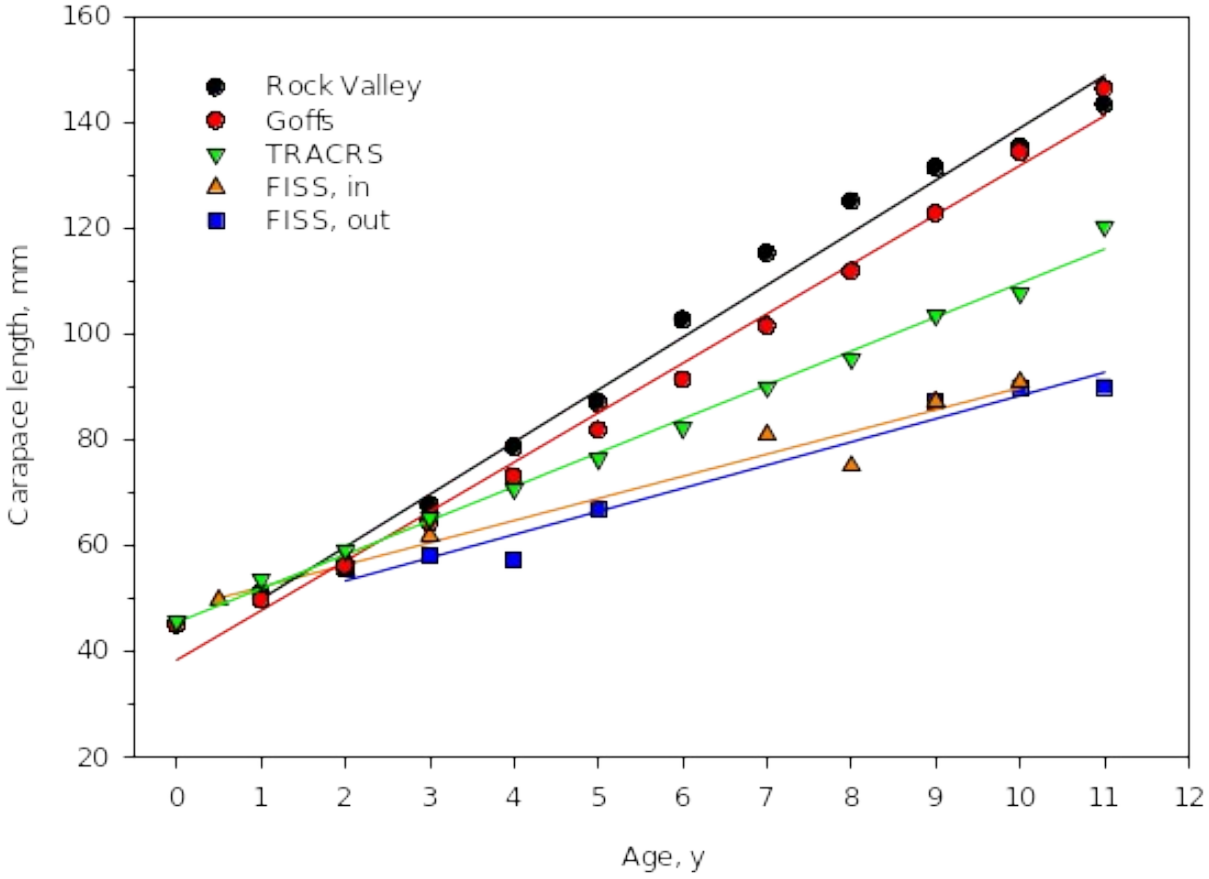

Fig. 7. Relationship of carapace length (CL) to age of known-age desert tortoises in four Mojave Desert populations. Least-squares regression equations were: CL = 40.0 + 9.91 * AGE for Rock Valley, CL = 38.4 + 9.36 * AGE for Goffs, CL = 45.5 + 6.41 * AGE for TRACRS, CL = 48.0 + 4.19 * AGE for FISS enclosed in pens, and CL = 44.6 + 4.38 * AGE for FISS free-ranging tortoises (out).

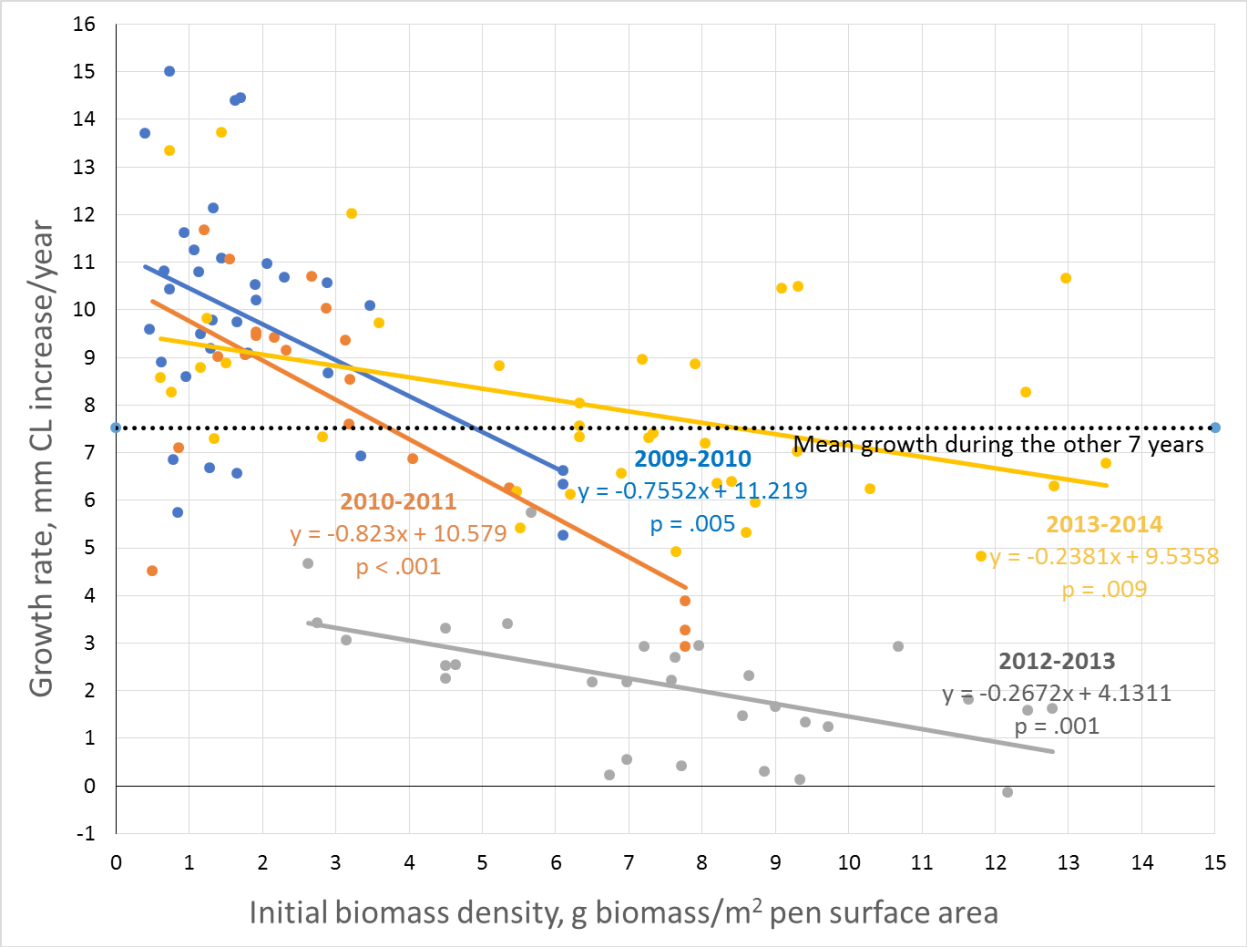

Fig. 8. Relationships of tortoise annual growth rates to tortoise biomass densities in four different years. Each point represents mean annual growth rate of all juveniles living in a given pen and the tortoise biomass density in that pen at the beginning of that year. Different colors represent different years. Data and associated linear regression lines are shown only for the four years having significant effects of biomass density on growth (all F >7.61, Adjusted r^2 > 0.152, p ≤0.009, n >18); all four years had below average rainfall. Growth rates during 2012-2013 were unusually low at all

biomass densities, and several groups of tortoises had growth rates near or below zero that year.

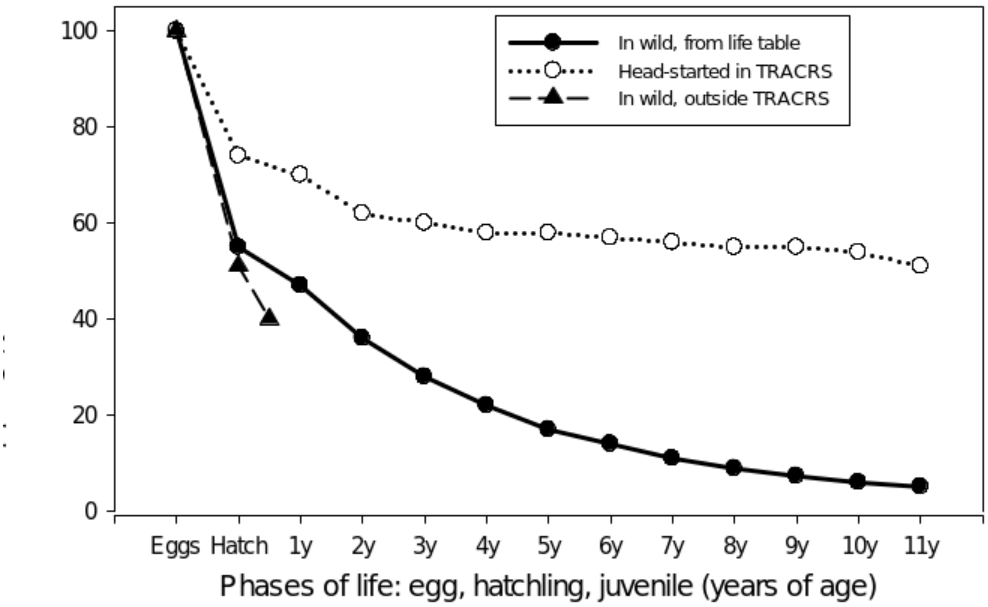

Fig. 9. Survivorship curves for Agassiz's desert tortoises, *Gopherus agassizii*, while free-living (filled symbols) and while being head-started in field enclosures (open circles, as calculated using consecutive, mean annual survivorship values in Table 2). At eleven years, 51 % of head-start (this study) and 5.06 % of wild (filled circles, Goffs study site; Turner et al. 1987a) offspring would survive. Nest predation in the wild near TRACRS (Bjurlin and Bissonette 2004; triangles) was similar to that at Goffs (Turner et al. 1987a), and ensuing pre-brumation predation was high.

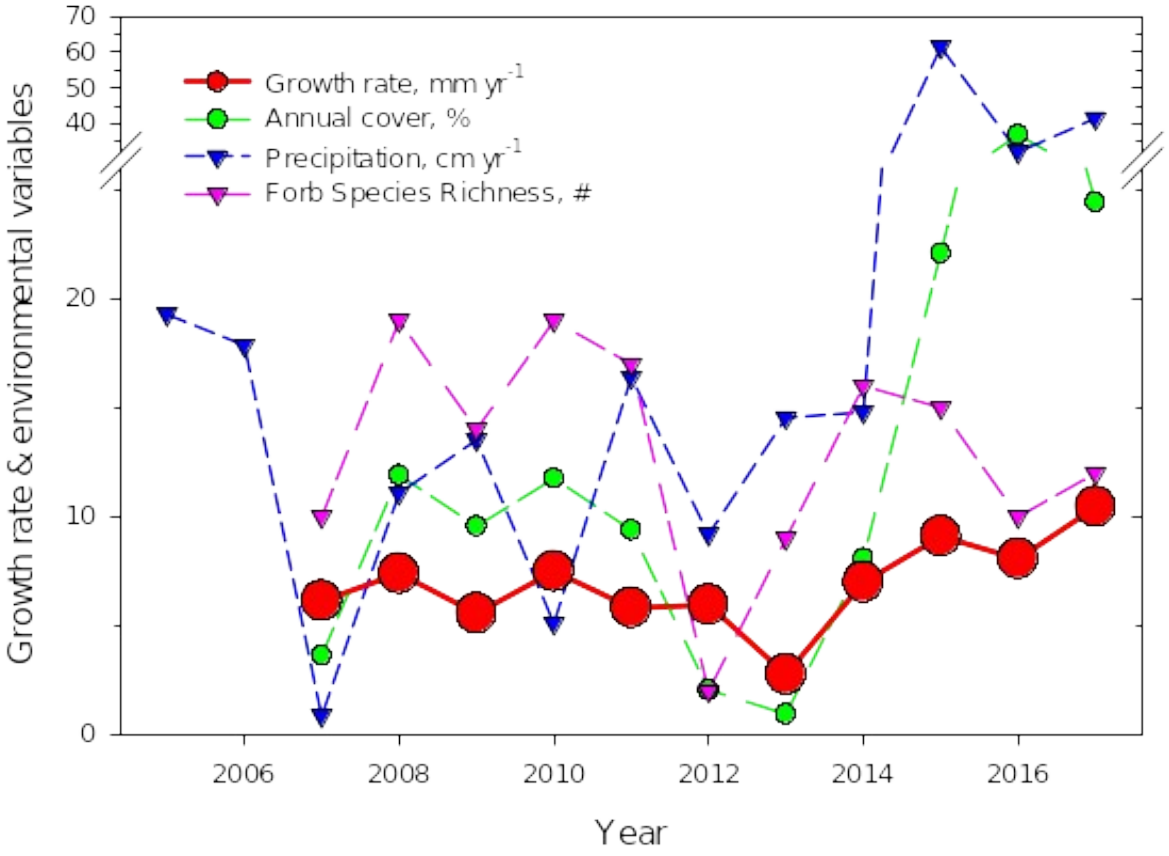

Fig. 10. Annual growth rates of juvenile tortoises (red: mm y^{-1} increase in CL), indices of natural plant food supply (green: % of ground covered by resident annual plants, excluding imported food supplements added in 2007 and 2012-17) and water supply (blue: cm y^{-1} of rainfall + irrigation), and species richness of resident forbs (pink: # species 1423 m^{-2}) inside TRACRS head-start enclosures.

APPENDIX

Fig. A1

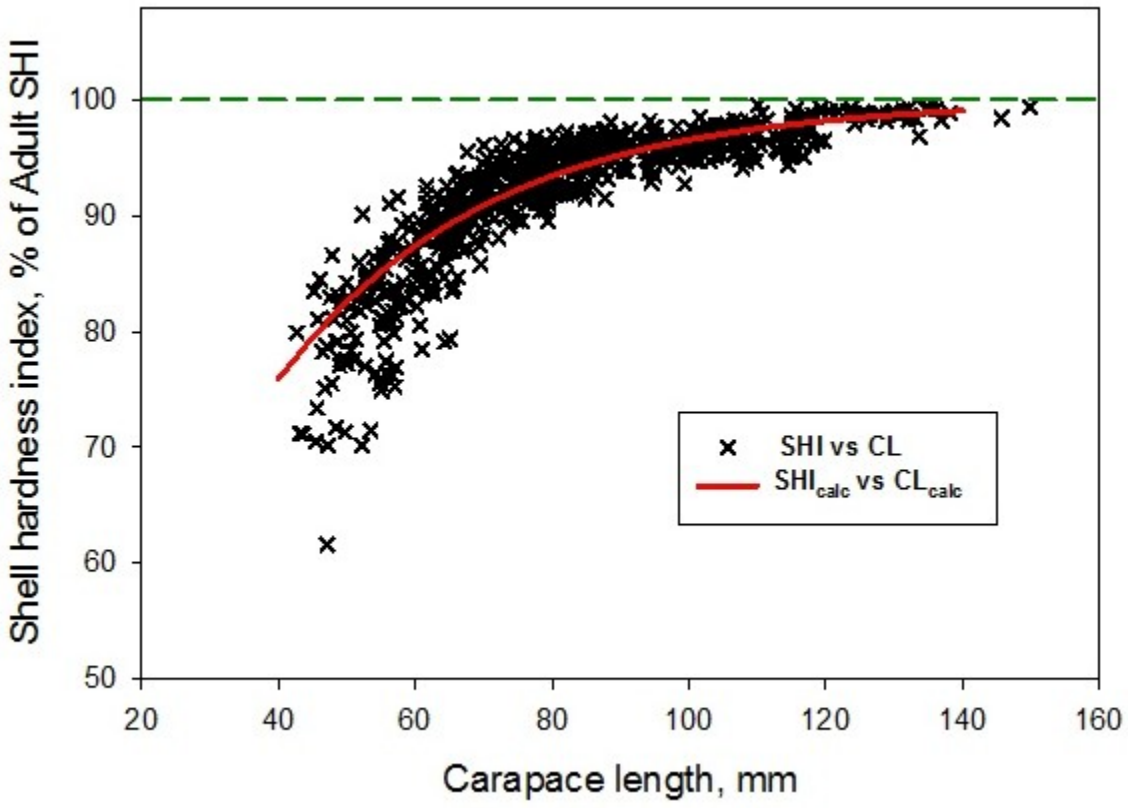

Figure A1. Relationship between shell hardness (SHI) and shell size (carapace length, CL) of one to 11 year old desert tortoises living in natural-habitat enclosures, and experiencing natural rainfall plus irrigation. The red curve represents the transformed linear regression equation calculated for a semilog analysis. The dashed blue line represents a fully-rigid adult shell defined as being incompressible by our digital micrometer.

APPENDIX

Table A1. Annual growth rates (mm y^{-1} in carapace length) of eight cohorts of desert tortoises in MCAGCC's head-start facility over 11 years (total n = 3361). Values are means (SD, n), with overall means (SD, n) shown for cohorts (right column) and year (bottom row). Superscripts at overall means indicate the number of means significantly higher and lower (e.g., 2 higher and 1 lower for Cohort 2006) from Student-Newman-Keul's post-hoc comparisons that followed One Way ANOVA.

Cohort	Year	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	Cohort total
2006	Mean	6.13	7.39	4.74	6.51	2.93	4.97	1.69	7.21	11.36	9.69	12.75	6.87 ^{2,1}
	SD	2.52	2.64	2.85	2.50	2.59	1.32	1.50	2.66	3.07	2.54	4.19	2.85
	n	45	80	90	82	15	15	77	71	68	37	20	600
2007			7.40	4.13	5.25	3.26	5.12	2.01	7.52	9.86	9.38	12.18	6.59 ^{2,1}
			2.51	2.37	1.97	1.74	2.19	1.88	1.78	2.79	3.33	3.27	2.85
			34	35	33	20	20	33	32	32	31	25	295
2008				6.84	6.32	4.12	5.32	3.29	8.04	10.20	9.05	11.71	7.19 ^{2,1}

			2.58	2.67	2.03	2.47	1.71	2.61	2.11	2.83	2.34	3.05
			97	87	72	72	82	81	77	73	50	691
2009				9.78	6.26	4.97	2.54	6.39	9.02	7.53	10.13	7.06[1,1]
				3.00	2.05	2.07	1.71	2.47	2.17	2.29	2.23	3.07
				106	73	66	69	67	68	66	57	572
2010					9.07	5.67	3.42	6.27	8.57	7.65	10.58	7.24[2,1]
					2.42	2.52	1.99	2.17	2.51	2.31	1.99	3.04
					71	64	66	62	57	59	61	440
2011						8.51	1.25	5.42	4.88	6.03	8.86	5.60[7,0]
						2.94	0.95	2.13	1.70	2.24	2.67	3.08
						77	78	77	56	71	69	428
2012							5.75	7.32	9.62	8.30	9.07	7.45[1,5]
							3.46	2.95	2.01	2.41	2.40	2.97
							53	59	34	42	53	241
2013								9.93	7.47	9.04	11.55	7.90[0,7]
								3.12	2.96	2.32	1.96	2.87
								24	19	26	25	94
Ann	6.24	7.60	5.58	7.44	5.89	6.33	2.92[1]	7.08	9.12	8.17	10.5[0]	6.95

	ual total	6,1	3,5	6,1	3,5	6,1	6,1	0,0	3,5	1,9	2,8	,10	
		2.89	2.96	2.98	2.98	2.92	2.91	2.89	2.82	2.82	2.80	2.80	3.55
		45	114	222	308	251	314	458	473	411	405	360	336 1

1
2