

## Crowding Affects Health, Growth, and Behavior in Headstart Pens for Agassiz's Desert Tortoise

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**ABSTRACT.** – Worldwide, scientists have headstarted threatened and endangered reptiles to augment depleted populations. Not all efforts have been successful. For the threatened Agassiz's desert tortoise (*Gopherus agassizii*), one challenge to recovery is poor recruitment of juveniles into adult populations, and this is being addressed through headstart programs. We evaluated 8 cohorts of juvenile desert tortoises from 1 to 8 yrs old in a headstart program at Edwards Air Force Base, California, for health, behavior, and growth. We also examined capacities of the headstart pens. Of 148 juveniles evaluated for health, 99.3% were below a prime condition index; 14.9% were lethargic and unresponsive; 59.5% had protruding spinal columns and associated concave scutes; 29.1% had evidence of ant bites; and 14.2% had moderate to severe injuries to limbs or shell. Lifetime growth rates for juveniles 1–8 yrs of age were approximately two times less than growth rates reported for wild populations. Tortoises in older cohorts had higher growth rates, and models indicated that high density in pens and burrow sharing negatively affected growth rates. Densities of tortoises in pens (205–2042/ha) were 350–3500 times higher than the average density recorded in the wild (< 1/ha) for tortoises of similar sizes. The predominant forage species available to juveniles were alien annual grasses, which are nutritionally inadequate for growth. We conclude that the headstart pens were of inadequate size, likely contained too few shelters, and lacked the necessary biomass of preferred forbs to sustain the existing population. Additional factors to consider for future reptilian headstart pens include vegetative cover, food sources, soil seed banks, and soil composition.

**KEY WORDS.** – density; *Gopherus agassizii*; growth; headstart; juvenile

Wild Agassiz's desert tortoises (*Gopherus agassizii*) have declined for decades due to numerous human activities (US Fish and Wildlife Service [US FWS] 1990, 1994, 2011; California Department of Fish and Wildlife 2016). Sources of habitat loss and degradation include urbanization, agriculture, livestock grazing, roads, mining, and energy development (US FWS 2010). Sources of negative effects to populations include heightened predation by subsidized predators (e.g., Boarman and Berry 1995; Esque et al. 2010) and disease (Homer et al. 1998; Jacobson et al. 2012, 2014). Limited recruitment of young tortoises into reproductive populations is a major barrier to recovery. As a result, augmentation of depleted populations by headstarting is now part of the desert tortoise recovery plan (US FWS 2011). However, headstarting has met with limited success as a conservation strategy for augmenting populations of threatened and endangered chelonians and other reptiles (e.g., Frazer 1992; Bowen et al. 1994; Heppell et al. 1996; Hayes et al. 2016). Head-starting programs for desert tortoises exemplify some of the major issues associated with this recovery strategy.

Four headstart programs were established to learn more about early life stages and to test the efficacy of rearing juvenile desert tortoises to augment wild populations (Morafka et al. 1997; Nagy and Hillard 2013; Nagy et al. 2015; Todd et al. 2016). These programs used field enclosures to house tortoises and employed similar methods for stocking headstart pens; females were brought to enclosures to lay eggs and returned to the collection site after egg deposition. The first project at the National Training Center, Fort Irwin, California, begun in 1990, initially experienced 90%–94% hatching success (Morafka et al. 1997). Annual survivorship declined after the first 3 yrs, however, and the decline was attributed to several factors including predation, drought, and overcrowding. The second project at Edwards Air Force Base (EAFB), California, initiated in 2002, included 3 phases. The first phase included developing and testing a technique to measure shell hardness in juvenile tortoises (Nagy et al. 2011), experimental releases of 1-yr-old tortoises, and testing the effect of artificial watering on growth and survival (Nagy et al. 2015). The results of the first phase of research at EAFB, conducted between 2003 and 2008, led

to recommendations for supplementing rain and for delayed release of smaller, yearling tortoises. Also during this phase, Davy et al. (2011) drew on 24 female adults and their clutches from the headstart pens to determine paternities of the neonates. The authors reported a high incidence of multiple paternities, one of the highest reported in tortoise species. The initial phase of EAFB research concluded in 2008, but headstarting operations continued in a second phase under the direction of EAFB and their contractors. During this phase, the objective was to maximize production of juvenile tortoises.

In 2011, EAFB requested that we evaluate their program, thus initiating the third phase. The evaluation focused on 5 topics about the juveniles in the headstart pens: 1) an assessment of health and behavior of juveniles as of October 2011; 2) an analysis of changes in tortoise densities from 2003 through 2012; 3) development of models to explore effects of tortoise density on tortoise growth and sharing of burrows; 4) a comparison of tortoise densities and tortoise growth with published data from other semiwild and wild populations; and 5) a discussion of potential actions for future headstart projects for the tortoise and other terrestrial, herbivorous reptiles.

## METHODS

*Study Site and Headstart Facilities.* — The headstart facility was located on EAFB, Los Angeles County, California (elev. 950 m) and was within the boundaries of critical habitat for the desert tortoise (US FWS 1994). The facility consisted of 5 enclosures covering 0.13 ha (1314 m<sup>2</sup>) and was arranged in 3 groups—2 enclosures that were separate and 3 that were clustered together (Fig. 1A; Appendix 1). During phases 1 and 2, the 5 enclosures were subdivided into 9 pens or pen subdivisions, ranging in size from 121 to 270 m<sup>2</sup>, using aluminum sheeting and plywood veneer (Appendix 1). Each enclosure was covered with mesh to exclude avian predators (e.g., Common Ravens [*Corvus corax*]) and reinforced with exclusionary fencing at the base and metal flashing along the guy wires to deter small mammalian predators from tunneling into the pens or entering the pens via guy wires (Fig. 1). The construction was sufficient to prevent tortoises from escaping the enclosures and from moving from one subdivision to another. Each independent pen had 1 entrance gate and the connected pens (Pens 3, 4, and 5) had a single gate. Gates were locked when pens were unattended. Thus, we considered the enclosures to be independent entities. Between 2003 and 2010 in phases 1 and 2, researchers brought ~ 24 females into the pens to lay eggs, and females produced 8 annual cohorts of juvenile tortoises. Davy et al. (2011) conducted a genetic study in 2003 and 2004 and described relationships between the female parents and the juvenile tortoises: “. . . a minimum of 50% of clutches . . . were sired by multiple males and a minimum of 50% of females were polyandrous.” The authors went on to say that evidence

from a single locus “. . . suggested multiple paternity and polyandry in all of the clutches and females.” Because no records were available on distribution of females and clutches among the pens in the first 2 phases of the study, we do not know the genetic relationships between the juveniles in the pens.

The facility was located in a white bursage–creosote bush alliance (*Ambrosia dumosa*–*Larrea tridentata*) with other, less-common species of perennial shrubs (in descending order of occurrence: *Ephedra nevadensis*, *Lycium andersonii*, *Acamptopappus sphaerocephalus*, *Ambrosia salsola*), occasional clumps of one-sided blue grass (*Poa secunda* subsp. *secunda*), and alien annual grasses (California Department of Fish and Game 2010). We made qualitative observations and took photographs in October 2011, at the start of our research, to document status and production of annual plants (Fig. 1). The dominant annual vegetation was composed of alien grasses (*Bromus madritensis* subsp. *rubens*, *B. tectorum*, and *Schismus* spp.) and was dense under or within the dripline of shrubs and along edges of the subdividers and enclosures. Trails made by researchers and visitors were evidenced by relatively bare, compacted soils with few annual plants between shrubs. We did not gather quantitative data on vegetation inside the pens.

We acquired precipitation data from PRISM 800-m and 4-km data sets (PRISM Climate Group 2017). For 20 yrs preceding the study, the EAFB facility received an average of 160.3 mm of precipitation during the hydrologic year (i.e., 1 October–30 September); the majority (80%) occurred in fall and winter, between 1 October and 30 March. The fall–winter rains are important for annual plant production, a primary food source for juvenile tortoises (Ofstedal et al. 2002). In years of low rainfall, EAFB provided supplemental “rainfall” by artificially watering pens with a watering truck (see Nagy et al. 2015); however, no consistent records were available on dates or amounts of water delivered to the pens prior to our evaluation. In October 2011 and in April and October 2012, EAFB staff watered the pens immediately prior to health assessments of the tortoises. On 1 day in early March 2012, the pens also received water. The EAFB staff estimated the amount of water delivered to each pen at 1650–4400 l. Because of the timing of the watering in March, April, and October, the water did not stimulate germination of native annual forbs but did support growth and maintenance of alien annual grasses and perennial shrubs.

*Data Collection: Tortoise Health and Clinical Signs of Disease.* — The EAFB provided a database with metrics for each juvenile tortoise in the pens. Each juvenile was marked with an individual identification number prior to our study. In October 2011 and 2012, we removed and temporarily held tortoises to conduct health evaluations, matching the identification numbers with a list of tortoises held in each pen from the EAFB database. At capture, we noted pen identification, location within the pen, and activity. We first collected juveniles on the surface or those



**Figure 1.** Vegetation surrounding (A) and within (B) headstart pens at Edwards Air Force Base, California, in October 2011. The standing dried annual vegetation in B is composed primarily of alien annual grasses. Note the pathways devoid of annual vegetation; biologists created the pathways while looking for the juvenile tortoises during surveys of pens over multiple years.

easily removed from burrows. To facilitate remaining captures, we stimulated emergence by watering each pen with a hose attached to a water truck. To prevent emerging tortoises from drinking prior to the health evaluation, we patrolled the pen to collect them before they had an opportunity to drink. For a few juveniles removed postwatering, we recorded responses to watering, noting if they drank. In fall 2011, we noted that 1 juvenile drank and 2 others may have been able to drink; we did not remove these tortoises from the database used in the analysis because they represented a very small portion of the total sample.

To evaluate health, we used a protocol adapted from Berry and Christopher (2001). Using procedures to reduce spread of infectious diseases, we measured each juvenile for size (carapace length at the midline [MCL; mm], height, width, plastron length at midline), weight, and recorded activity levels as well as posture, body condition, clinical signs of disease, and trauma. We rated clinical signs of disease and trauma as none, mild, moderate, or severe, and separately for eyes, nares, beak, fore- and hind limbs, carapace, and plastron. We focused on clinical signs of diseases known to occur in wild desert tortoises such as upper respiratory tract disease (Jacobson et al. 2014), herpesvirus (Jacobson et al. 2012), cutaneous dyskeratosis (shell disease, characterized by discoloration and flaking of scutes) or necrosis (Jacobson et al. 1994, Homer et al. 1998), and starvation and dehydration (Berry et al. 2002). We took macro-scale digital photographs of each tortoise to document disease and trauma.

We used size and weight measurements to calculate growth rates and a condition index (CI) for each tortoise (Nagy et al. 2002). Growth rates (MCL or weight) for each tortoise in mm or g/yr were calculated as  $(X_2 - X_1)/(t_2 - t_1)$ , where  $X$  represents MCL or weight and  $t$  represents time. We calculated growth rates for the lifetime of each tortoise (i.e., growth from first measurement posthatching to October 2011) and for a 1-yr period (i.e., from October 2011 to October 2012). Condition indices in  $\text{g/cm}^3$  were calculated as  $(\text{Weight}/[\text{MCL} \times \text{Height} \times \text{Width}]) \times 1000$  (sensu Nagy et al. 2002). Then we identified juveniles in poor condition using one or more of the following criteria: 1) presence of moderate to severe clinical signs of disease or trauma; 2) lethargic and unresponsive; or 3) with CIs below a prime CI of  $0.64 \text{ g/cm}^3$  (a fully hydrated and well-fed wild desert tortoise) or below the CI of  $0.45 \text{ g/cm}^3$  (a dehydrated tortoise; Nagy et al. 2002).

*Data Collection: Tortoise Density and Burrow Sharing.* — We used historic records kept by EAFB to examine changes in counts of tortoises per pen over time (counts defined as density and converted to density/ha). In the historic records and prior to our involvement in the project, if a tortoise was not located for  $> 1$  yr, it was treated as dead because tortoises could not escape from the pens. For our analyses, we presumed death when a tortoise was not recaptured for metric measurements or health

evaluations for  $> 1$  yr. In 2011 and 2012, using the EAFB and our databases, we similarly counted numbers of juveniles in each pen using a temporary removal process. In 2011, we knew the total number and identification numbers of juveniles located in the pens in previous sampling periods. Thus, we sought to relocate all those individuals. We recognize that our density estimates are imperfect because a few marked tortoises expected to be in pens (based on histories from the second phase of research) were not located during our evaluations. These tortoises were probably deep in burrows, alive, dead, or dead and disintegrated. Because few “missing” individuals reappeared alive (15 between 2003 and 2011, none between 2011 and 2012), we assumed that missing tortoises died underground or disintegrated before they could be located. We calculated tortoise density as the number of tortoises counted in a given pen per unit area of each pen and then converted the figures to standard units of number of tortoises per hectare.

In 2011, we collected additional data on associations of tortoises at burrows (i.e., sharing burrows) because the EAFB team reported burrow sharing, and Morafka et al. (1997) had described the phenomenon as an indication of crowding in headstart pens at the National Training Center, Fort Irwin. Wild juvenile tortoises ( $< 140$  mm in carapace length) live a solitary existence in burrows (Berry and Turner 1986; K.H.B., unpubl. data, 2015) whereas adult individuals often share burrows or dens (e.g., Woodbury and Hardy 1948; Bulova 1994). For each pen, we categorized juveniles by location and association: 1) solitary, in the open and away from a specific burrow; 2) solitary, in a burrow; and 3) sharing a burrow with one or more tortoises, with identifications of the tortoises noted. We did not count numbers of burrows in each pen.

*Data Analyses.* — We were limited in some respects by the analyses we could perform because of the database available. We were aware that some juveniles in the pens were related, therefore raising issues of independence in the analyses. Unfortunately, the information available regarding distribution of females and resulting hatchlings was insufficient to address the topic. All statistical analyses were performed in R 3.2.2 (R Development Core Team 2016). Drawing on the EAFB database for 2003–2010 and our counts of tortoises in 2011 and 2012, we quantified the changes in tortoise densities over time using a linear regression model fitted to the densities in each pen or subdivision for each year. The model was fit using the *segmented* function in the *segmented* package (Muggeo 2017). We compared densities between years and pens using a 2-way analysis of variance (ANOVA) and conducted post hoc multiple comparisons with the *HSD.test* function in the *agricolae* package (Mendiburu 2016). Comparisons of size and weight were made between tortoises that shared a burrow and those that were solitary (for 2011 only) with a Mann-Whitney U-test.

We used a logistic general linear model (GLM) and linear mixed effects (LME) models to estimate effects of

tortoise densities in pens on burrow sharing and growth. We used GLM to estimate the likelihood that a tortoise shared a burrow and included pen, tortoise density, cohort (tortoises hatched annually between 2003 and 2010), and size (MCL) as variables. We presented the effect of each factor from the best fit GLM model as regression coefficients (i.e., beta coefficients) and as standardized beta coefficients, which were calculated using the *lm.beta* function in the *QuantPsyc* package (Fletcher 2015).

We used LME models to estimate effect of tortoise density and tortoise size (MCL) on lifetime growth (i.e., growth from first measurement posthatching to October 2011) while controlling for random variation between pens and tortoises. We also used an LME model to estimate the effect of tortoise density, tortoise size, and burrow sharing on short-term growth (i.e., growth from October 2011 to October 2012) while controlling for random variation between pens and tortoise. We used burrow sharing only as a factor in short-term models because we collected these data once, in 2011. For LME models of lifetime growth, we used average tortoise density of each pen each year from 2003 to 2011 and tortoise size in 2011. For single-year models, we used the average density and tortoise size from 2011 to 2012. Tortoise size was included to account for the allometric relationship between tortoise size and growth. Mixed effects models were fitted with the *lme4* package (Bates et al. 2016).

We selected among GLM and LME models containing different combinations of variables by using model performance, as measured by second-order corrected Akaike's information criterion ( $AIC_c$ ; Burnham and Anderson 2002). We compared models with the *AICcmodavg* package (Mazerolle 2015). We considered models with the lowest  $AIC_c$  as the best fit model (i.e., least loss of information) while considering additional models within 2  $AIC_c$  units (Burnham and Anderson 2002). We also compared models and estimated the relative importance of different parameters based on normalized Akaike weights ( $w_i$ ).

*Comparison of Densities and Growth of Tortoises in Pens with Those Variables in Wild Populations.* — We compared pen densities of juveniles at EAFB with estimated densities of juvenile and immature tortoises (densities calculated from mark-recapture data) known to occur in the wild on 4, long-term desert tortoise study plots in the 1970s and 1980s (from Morafka et al. 1997). In addition, we compared our data sets with known densities and growth rates from 2 studies involving enclosures: Fort Irwin headstart pens (Morafka et al. 1997) and pens with tortoises in Rock Valley, Nevada (Medica et al. 1975).

## RESULTS

*Precipitation and Vegetation.* — Precipitation for the hydrologic year varied during the overall study (2003–2012) and, on average (151.2 mm), was slightly lower than the preceding 20-yr mean (160.3 mm; PRISM Climate

Group 2017). In 2011 and 2012 (i.e., sampling years), precipitation for the hydrologic year was 245.4 and 90.0 mm, respectively, with 93.7% and 60.4% of precipitation occurring in fall and winter, respectively.

Based on our visual observations in 2011 and 2012, perennial shrubs did not exhibit new growth and few winter annuals germinated. The pens had high densities of standing dried alien annual grasses (*Schismus* spp., *Bromus* spp.) from previous seasons within and on coppice mounds of larger shrubs and along the edges of the headstart pens. Visual observations suggested that standing biomass of dried alien annual grasses was lower outside of the pens versus inside the pens (Fig. 1A). Few dried annuals occupied the intershrub spaces where soils appeared compacted from trails created by field biologists (Fig. 1B). Overall, we observed very few native annual forbs.

*Tortoise Growth and Health.* — In 2011, we temporarily removed and evaluated 148 juvenile tortoises that ranged in age from 1 to 8 yrs old. The average size and weight of juveniles was 59.0 mm (range, 45.5–114.1 mm) and 49.8 g (range, 18.2–282.5 g; Appendix 2). Lifetime MCL growth averaged 4.2 mm/yr (range, 0.0–8.9 mm/yr) and weight gain averaged 7.1 g/yr (range, –3.8–32.1 g/yr; Table 1). We resampled 109 tortoises in 2012 with an average size of 63.3 mm (range, 46.8–123.0 mm) and weight of 57.8 g (range, 17.6–314.1 g; Appendix 2). From 2011 to 2012, short-term MCL growth averaged 3.7 mm/yr (range, 0.6–7.8 mm/yr) and weight gain averaged 6.4 g/yr (range, –0.7–31.3 g/yr; Table 1). On average, size and growth decreased from older to younger cohorts (Appendix 2; Table 1), but 2 cohorts (2003 and 2006) appeared to grow better than the rest. For example, the average growth rate for the 2003 cohort was 7.2 mm MCL/yr, which was  $\geq 3$  mm/yr than other cohorts, except for the 2006 cohort (7.3 mm/yr). Weight gain followed a similar pattern (Table 1).

The average CI in 2011 was 0.52 g/cm<sup>3</sup> (range, 0.22–0.66 g/cm<sup>3</sup>), with little difference between cohorts (Appendix 2). The majority of tortoises ( $n = 147$ , 99.3%) were below the prime CI value of 0.64 g/cm<sup>3</sup>, but most ( $n = 138$ , 93.2%) were above the value of CI expected for a dehydrated tortoise (0.45 g/cm<sup>3</sup>; Nagy et al. 2002). In 2012, a below-average rainfall year, the average CI dropped to 0.49 g/cm<sup>3</sup> (range, 0.37–0.72 g/cm<sup>3</sup>). Similar to 2011, the majority ( $n = 107$ , 99.1%) of tortoises were below the prime CI but more individuals ( $n = 28$ , 25.9%) were below the dehydrated CI.

Most tortoises had one or more mild to severe clinical signs typical of poor condition. Twenty-two tortoises (14.9%) showed one or more behavioral characteristics or postures typical of clinically ill tortoises: unresponsive to stimuli, limbs and head hanging loose, eyes not open or only partially so, and lethargy. The most common clinical sign was a prominent and protruding spinal column, present in 88 (59.5%) of the tortoises. These tortoises had mildly concave, semitransparent vertebral and costal

**Table 1.** Lifetime ( $n = 148$ ) and short-term ( $n = 109$ ) growth and weight gain (with standard error [SE]) for 8 cohorts of juvenile desert tortoises in headstart pens at Edwards Air Force Base, California. Growth was measured by carapace length at the midline (MCL). Lifetime values were from first measurement posthatching to October 2011. Short-term values were from measurements taken in 2011 and 2012.

Cohort	$n$	MCL (mm/yr)			Weight (g/yr)		
		Mean (SE)	Min	Max	Mean (SE)	Min	Max
<b>Lifetime</b>							
2003	3	7.2 (0.4)	6.7	7.9	27.5 (2.5)	23.5	32.1
2004	3	4.1 (1.2)	1.7	5.8	9.6 (3.4)	2.9	13.2
2005	13	4.4 (0.4)	2.3	7.7	9.3 (1.0)	3.9	17.1
2006	3	7.3 (0.4)	6.6	8.0	19.1 (1.8)	16.7	22.6
2007	20	4.8 (0.3)	2.8	7.3	9.0 (0.8)	4.1	16.9
2008	30	3.3 (0.2)	1.4	5.9	5.1 (0.5)	0.0	11.0
2009	40	4.1 (0.3)	-0.2	8.4	6.9 (0.5)	2.7	19.2
2010	36	4.0 (0.3)	0.4	8.9	4.2 (0.6)	0.0	13.8
All	148	4.2 (0.1)	0.0	8.9	7.1 (0.4)	0.0	32.1
<b>Short-term</b>							
2003	3	7.0 (0.4)	6.5	7.8	27.0 (2.2)	24.6	31.3
2004	2	5.3 (0.1)	5.2	5.4	11.7 (1.7)	10.0	13.3
2005	11	4.2 (0.4)	2.2	7.1	8.2 (1.3)	3.1	17.5
2006	3	6.8 (0.6)	5.8	7.7	19.7 (3.3)	13.3	24.5
2007	18	4.4 (0.2)	2.6	6.1	7.9 (0.6)	4.3	14.2
2008	21	2.9 (0.3)	1.0	4.8	4.0 (0.5)	-0.1	8.2
2009	27	3.5 (0.3)	0.6	6.9	5.6 (0.7)	-0.1	15.6
2010	24	2.9 (0.2)	1.0	4.2	2.9 (0.5)	-0.7	9.5
All	109	3.7 (0.1)	0.6	7.8	6.4 (0.5)	-0.7	31.3

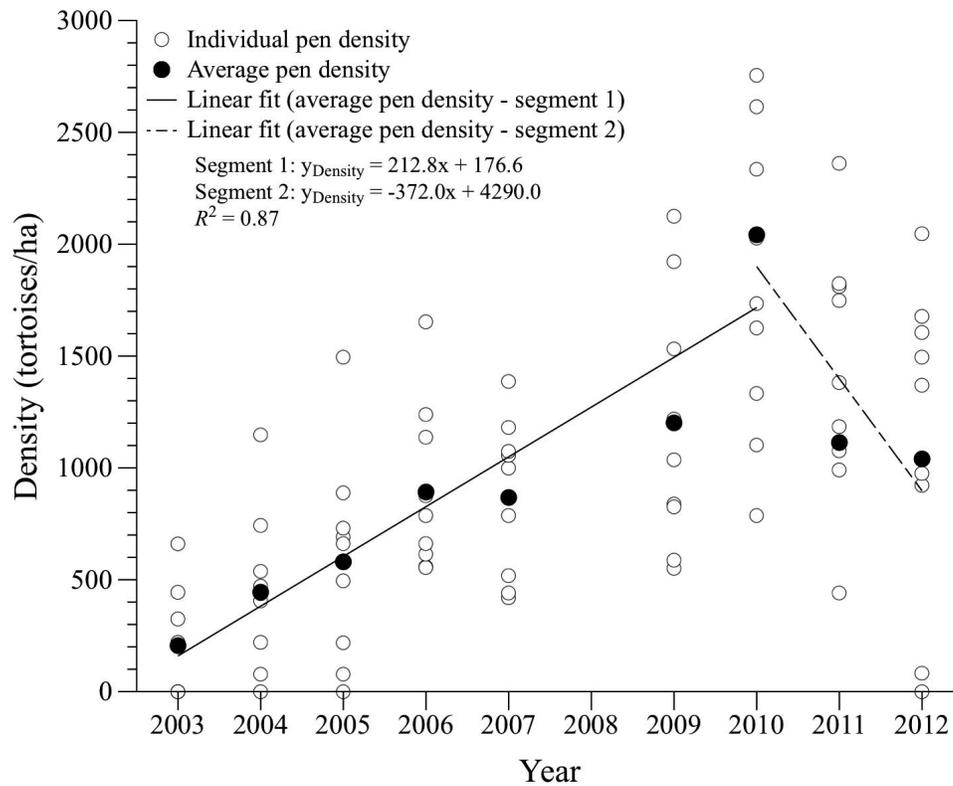
scutes of the carapace outlining the ribs. Moderate to severe clinical signs of cutaneous dyskeratosis, a disease characterized by discoloration and flaking of the integument, was evident on 8.8% of limbs, 18.9% of heads, and 16.9% of shells. No tortoise had a nasal discharge, a typical clinical sign of upper respiratory tract disease; however, 40 (27.2%) juveniles had mild to severely occluded nares. Moderate to severe ocular signs—edema of the palpebrae or periocular areas and ocular discharge—occurred in 21.6% and 35.4% of tortoises, respectively.

Almost 30% of the tortoises had signs of mild to severe trauma. Evidence of ant bites (pinchers and heads still attached) from 2 unidentified species of ants was common on the integument of limbs, soft tissues of the neck, axillary and inguinal areas, cloaca, and tail. The mean number of ant heads/tortoise was 1.7 (range, 1–10). Multiple signs of trauma, probably caused by rodents, were evident on different parts of the body of 21 (14.2%) tortoises. Lesions ranged from raw areas of integument on limbs and missing scales to loss of entire scutes with exposed bone. Some injuries were in the process of healing whereas others appeared to have blackened, necrotic tissue. Two tortoises were missing left hind feet, the result of either injury or congenital defect. Another tortoise had damage to the beak, shell, and limbs and was subsequently found dead.

*Tortoise Density and Burrow Sharing.* — Tortoise densities in pens increased linearly over time until 2010, at which time a break point occurred and densities began to decrease (Fig. 2). Average density ranged from 205 tortoises/ha in 2003 (count of 30 individual tortoises) to 2042 tortoises/ha in 2010 (count of 261 individual tortoises), after which densities decreased to 1040

tortoises/ha by 2012 (count of 142 individual tortoises). Density of juveniles varied significantly between years and pens (2-way ANOVA; “Year”,  $F_{8,64} = 14.17$ ,  $p < 0.001$ ; “Pen”,  $F_{8,64} = 4.70$ ,  $p < 0.01$ ). The decrease in tortoise density after Fall 2010 was a result of a die-off (Fig. 2). Many tortoises were located dead or died underground and not seen again. During our study period (October 2011–2012), 14 tortoises were found dead; 4 of these tortoises had been evaluated in 2011. More tortoises likely died underground, contributing to a lower number of resamples in 2012. The 4 dead tortoises evaluated in 2011 ranged in age from 1.1 to 7.0 yrs. Their average CI ( $0.55 \text{ g/cm}^3$ ; range,  $0.52\text{--}0.58 \text{ g/cm}^3$ ) was below the prime CI and their average growth was minimal (MCL =  $2.4 \text{ mm/yr}$ , range =  $1.7\text{--}3.1 \text{ mm/yr}$ ; weight =  $5.7 \text{ g/yr}$ , range =  $2.9\text{--}11.8 \text{ g/yr}$ ). These tortoises also had multiple clinical signs of disease including abnormal peeling of the skin and integument, a protruding and prominent spine, concave scutes, and edema of the eyelids.

Of the 148 tortoises removed from the pens for evaluation in 2011, 35 (23.6%) were located in the open and 113 (76.4%) were in burrows. For those in burrows, 56 (49.6%) were solitary and 57 (50.4%) shared a burrow with  $\geq 1$  to 6 tortoises. Tortoises in shared burrows were significantly smaller than tortoises found alone: 7 mm smaller MCL and 19 g lower in weight (Mann-Whitney U-test, Wilcoxon rank sum test;  $W = 1309$ ,  $p = 0.013$  and  $W = 1313$ ,  $p = 0.001$ ). We did not count the potentially available burrows in each pen or pen subdivision nor identify potential places where burrows might be constructed. Available, unused burrows or potential sites for burrows, coupled with densities, may have affected sharing of burrows.



**Figure 2.** Linear relationship between tortoise density and time (years) in headstart pens and pen subdivisions at Edwards Air Force Base, California. Data on densities were not available for 2008.

*Models.* — The likelihood for a juvenile to share a burrow was best predicted by tortoise density and size of the juvenile (Table 2). The beta coefficients for the best fit model indicated that tortoises were more likely to share a burrow when tortoise density increased and that they were less likely to share burrows as they grew larger. Standardized beta coefficients showed that with a 1 standard deviation (SD) increase in the predictor variable, the relationship between burrow sharing and MCL was approximately 2.5 times stronger than the relationship with tortoise density.

Tortoise size and density were important variables for short-term and lifetime growth (Table 3). Both growth metrics were explained best by a model containing tortoise size as a single variable; however, a second model containing tortoise density was within two AIC<sub>c</sub> units, suggesting density was also an important factor affecting growth. The beta coefficients for the model including both size and density showed that short-term

and lifetime growth (per MCL) were positively associated with tortoise size (10.5% and 18.5% increase per millimeter increase in MCL, respectively) and negatively associated with tortoise density (−0.05% and −0.09% decrease per increase in density of 1 tortoise/ha) (Table 4). Similarly, short-term and lifetime weight gain were positively associated with tortoise size (4.1% and 6.2% increase per millimeter increase in MCL, respectively) and negatively associated with tortoise density (−0.02% and −0.03% decrease per increase in density of 1 tortoise/ha).

*Comparisons of Density and Growth in Pens with Data from Wild Populations.* — The average density of tortoises in the nine pens ranged from 205 to 2042 tortoises/ha over the duration of the study. These values far exceed those estimated by mark–recapture methods for wild juvenile and small immature tortoises on nearby long-term study plots, where densities were estimated at < 1 tortoise/ha (Morafka et al. 1997). Likewise, densities

**Table 2.** Results of the best fit logistic generalized linear model predicting the likelihood that a juvenile desert tortoise was sharing a burrow in headstart pens at Edwards Air Force Base, California. Variables included in the model were density in pens (tortoises/ha) and tortoise size (carapace length at the midline [MCL]; millimeters).

Predictor variable	$\beta$ (std. $\beta$ )	SE	$z$	$p$	% Change in odds of sharing a burrow		
					% Change	90% interval	Predictor change
Density	0.001 (0.78)	0.0004	2.01	< 0.05	0.1	0.02 to 0.15	1 tortoise/ha increase
MCL	−0.08 (−2.04)	0.02	−3.36	< 0.001	−7.7	−12.2 to −3.9	1 mm increase

**Table 3.** Models ranked according to corrected Akaike's Information Criterion ( $AIC_c$ ) using a linear mixed effects model for short-term and lifetime growth measures (carapace length at the midline [MCL] and weight) of juvenile desert tortoises in headstart pens at Edwards Air Force Base, California. Models are ranked by second-order corrected  $AIC_c$  and listed from best to worst with the number of model parameters (K), log likelihood (LL), difference in corrected  $AIC_c$  relative to the best model ( $\Delta AIC_c$ ), Akaike weight ( $\omega AIC_c$ ), and cumulative weight (Cum wt). Lifetime growth models included fixed effects of MCL and pen density (Density). Short-term models contained an additional fixed effect of burrow sharing (Share). Both groups of models contained random effects for cohort and pen.

Response	Model no.	K	LL	$AIC_c$	$\Delta AIC_c$	$\omega AIC_c$	Cum wt
MCL (short-term)	3	5	-197.86	406.31	0.00	0.57	0.67
	2	6	-197.45	407.73	1.42	0.28	0.85
	1	7	-196.90	408.92	2.61	0.15	1.00
	4	4	-217.57	443.53	37.23	0.00	1.00
Weight (short-term)	3	5	-141.81	294.20	0.00	0.61	0.61
	2	6	-141.55	295.92	1.72	0.26	0.86
Square root-transformed	1	7	-141.04	297.19	2.99	0.14	1.00
	3	4	-153.78	315.94	21.74	0.00	1.00
	3	5	-245.66	501.73	0.00	0.54	0.56
MCL (lifetime)	2	6	-244.75	502.09	0.36	0.46	1.00
	4	4	-273.55	555.39	53.65	0.00	1.00
	3	5	-63.20	136.82	0.00	0.63	0.63
Weight (lifetime)	2	6	-62.66	137.92	1.11	0.37	1.00
	4	4	-112.63	233.54	96.72	0.00	1.00
	Sqrt-transformed	2	6	-62.66	137.92	1.11	0.37
	4	4	-112.63	233.54	96.72	0.00	1.00

Model 1: Response  $\sim$  MCL + Density + Share + (1|Cohort) + (1|Pen).

Model 2: Response  $\sim$  MCL + Density + (1|Cohort) + (1|Pen).

Model 3: Response  $\sim$  MCL + (1|Cohort) + (1|Pen).

Model 4: Response  $\sim$  1 + (1|Cohort) + (1|Pen).

exceeded figures reported for semiwild pens at Fort Irwin and Rock Valley, Nevada (Medica et al. 1975). On average, growth rates in the EAFB pens (4.2 mm/yr) were lower than the mean recorded at Rock Valley (9.1 mm/yr) and in a wild population at the Desert Tortoise Natural Research Area (Kern County, California) sampled several times over a 34-yr period (9.6 mm/yr; K.H.B., unpubl. data, 2016).

## DISCUSSION

*Tortoise Health.* — In 2011, most juvenile tortoises in the EAFB pens had one or more moderate to severe clinical signs commonly seen in individuals with malnutrition, starvation, or dehydration. The clinical signs were similar to those reported for 4 juvenile tortoises salvaged and necropsied from headstart pens at Fort Irwin; necropsies confirmed the causes of illness and death as malnutrition, starvation, and dehydration (Berry et al. 2002). Some juveniles also experienced trauma from ants and rodents. Values for CIs were mostly below the prime CI, but remained above those of dehydrated tortoises. However, in 2012, additional tortoises fell below the CI

for dehydration, likely a result of below-average precipitation and in spite of efforts to artificially supplement water (previously shown to promote growth and CI values during periods of drought; Nagy et al. 2015).

Diet and availability of forage may provide another explanation for poor body condition. Nutritional deficiencies or toxicosis are potential sources of shell disease (cutaneous dyskeratosis) observed on limbs and shells of some tortoises (Jacobson et al. 1994; Homer et al. 1998). The predominant food plants accessible to juveniles were alien annual grasses (*Schismus* spp., *Bromus* spp.) and a few plants of the alien forb, *Erodium cicutarium*. Alien grasses may have contributed to the nutritional deficiencies. Hazard et al. (2009, 2010) conducted experimental studies on digestibility and nutritional values of native and alien forbs and grasses in juvenile tortoises using 2 native species (*Stipa hymenoides* [perennial grass], *Malacothrix glabrata* [forb]) and 2 alien species (*Schismus barbatus* [grass], *E. cicutarium* [forb]). Substantial nutritional differences existed between forbs and grasses, regardless of whether native or alien. When eating forbs, juveniles gained more minerals and increased rapidly in weight, whereas on the grass diets, juveniles lost body mass,

**Table 4.** Model results (beta [ $\beta$ ] coefficients, standardized  $\beta$  coefficients, and percent change) for the effect of density and desert tortoise size on short-term and lifetime growth measures (carapace length at the midline [MCL], millimeters; weight, grams) in headstart pens at Edwards Air Force Base, California. std. = standardized.

Response	Density in pens			Tortoise size		
	$\beta$ (std. $\beta$ )	SE	% Change	$\beta$ (std. $\beta$ )	SE	% Change
MCL (short-term)	-0.0005 (-0.10)	0.0006	-0.05	0.10 (0.68)	0.01	10.5
Weight (short-term)	-0.0002 (-0.06)	0.0002	-0.02	0.04 (0.56)	0.01	4.1
MCL (lifetime)	-0.0009 (-0.17)	0.0006	-0.09	0.17 (1.17)	0.02	18.5
Weight (lifetime)	-0.0003 (-0.12)	0.0002	-0.03	0.06 (0.96)	0.004	6.2

volume, and body nitrogen and phosphorus. Drake et al. (2016) reported that captive juvenile tortoises eating forbs grew more and had higher survival rates and immune functions than did tortoises fed grasses (the alien *Bromus rubens* or the native *Festuca octoflora*).

*Short-term and Lifetime Growth.* — When comparing EAFB tortoises to juveniles kept in pens in Nevada and estimates from wild populations (Medica et al. 1975; K.H.B., unpubl. data, 2016), on average both the Nevada and wild tortoises increased in size by more than double compared with the lifetime average for the EAFB tortoises. The average annual growth in Nevada juveniles differed by year and was greatest following winters of high precipitation (Medica et al. 1975, 2012). During our phase of the EAFB study, precipitation was slightly lower than the long-term average and precipitation from 2011 to 2012 was 40% below average. Consequently, short-term growth (3.7 mm/yr) over this period decreased compared with the lifetime average (4.2 mm/yr).

Our models indicated 2 additional variables that influenced juvenile growth: tortoise size and pen density. Juvenile desert tortoises and other chelonian species generally exhibit rapid growth that slows at sexual maturity (Mushinsky 2014). In the EAFB pens, the larger juveniles with higher growth rates tended to belong to early cohorts, whereas juveniles in later cohorts tended to have slower growth rates. The lack of rapid growth by later cohorts of juveniles highlighted the effect of pen density, which increased with the addition of each cohort. Increased densities have led to declines in growth in other headstarted species including Mona Island iguanas (*Cyclura cornuta stejnegeri*; Pérez-Buitrago et al. 2008) and tuataras (*Sphenodon punctatus*; Goetz and Thomas 1994).

Maternal body size is a factor not included in our models and has potential for influencing size and growth of juveniles. In a recent study of desert tortoises, Nafus et al. (2015) reported positive correlations with maternal body size and hatchling size, and hatchling size and growth. Larger mothers tended to produce larger hatchlings and larger hatchlings tended to have higher growth rates compared with smaller ones. Pedrono et al. (2001) observed a similar pattern in the ploughshare tortoise, *Geochelone yniphora*. In the EAFB study, multiple wild females living nearby were brought to the headstart pens to deposit eggs over several years (Nagy et al. 2015), and cohorts were composed of juveniles from several mothers. Unfortunately, due to insufficient maternal data, we were unable to determine if similar correlations existed between individual mothers, their clutches, and surviving hatchlings.

*Sharing Burrows.* — Although burrow sharing is a common behavior in adults (as noted earlier), it is an aberrant behavior in juvenile tortoises and likely evidence of crowding. In a study of 1403 wild juvenile and small immature tortoises from 18 study sites, Berry and Turner (unpubl. data, 1986) observed that each tortoise found

associated with a burrow was solitary. In the first headstart program at Fort Irwin, 35% of juveniles shared burrows with 2 or more tortoises (Morafka et al. 1997). At the EAFB headstart facility, 50% of juveniles using a burrow were in shared burrows in sampling undertaken in a single year. Our models indicated that smaller tortoises were more likely to share a burrow, and the behavior of burrow sharing may suppress growth. Juveniles sharing burrows in 2011 were significantly smaller and weighed less than those not living in multiple-occupancy burrows.

Sharing burrows may affect juveniles differently, depending on location inside the burrow. A tortoise at or near the burrow entrance has more access to rainfall and forage, whereas those individuals deep inside are limited by the activity of those in front of them. Thus, they are likely to have limited opportunities to eat and drink, may be prevented from exiting by ill and dying individuals, and may grow more slowly. Tortoises at or near the burrow entry, however, would be exposed to greater temperature extremes and larger fluctuations in daily temperatures than those located deep in the burrow (Mack et al. 2015). Exposure to such extremes of temperature and moisture may have physiological consequences leading to imbalances in water, energy, and electrolyte budgets (Nagy and Medica 1986; Henen et al. 1998). Other plausible alternatives for explaining shared burrows exist, e.g., lack of unused burrows, lack of suitable places to dig new burrows, and size and dominance or alpha behavioral traits of the neonate or juvenile. Some tortoises in cohorts may be more likely to share burrows because they lack alpha behavioral traits. These topics would benefit from additional research.

*Capacity of the Pens.* — The capacity of headstart pens to support healthy, growing juveniles is dependent on sufficient resources (e.g., shelter, food, water, space) for the density and sizes of individuals. The evidence that densities in the EAFB pens surpassed available resources includes 1) poor condition of the juveniles, as measured by clinical signs (e.g., protruding spinal columns, concave scutes on carapace) and CIs; 2) behavior (e.g., burrow sharing); 3) high death rates; and 4) limited growth. In addition, informal observations suggest that the pens likely lack sufficient cover and variety of forage species. A similar situation occurred at Fort Irwin pens in the 1990s, when counts of juveniles increased annually to 344 with the addition of newly hatched cohorts (Morafka et al. 1997). Sharing of burrows occurred, mortality increased, and some tortoises died of starvation and dehydration (Morafka et al. 1997, Berry et al. 2002). Densities of juveniles in the wild in the 1970s and 1980s in California studies and densities of juveniles at the Rock Valley, Nevada, pens provide a guide: in both cases, densities were < 1 juvenile/ha (Table 1; Medica et al. 1975; Morafka et al. 1997; K.H.B., unpubl. data, 2012).

*Conservation Implications.* — Our models indicated that high densities of tortoises in pens could negatively affect the success of headstarting efforts. Future head-

starting programs may benefit from collecting and analyzing data on factors affecting growth and health. Examples include size and health condition of maternal females; numbers of clutches and clutch sizes produced; hatching times and disposition of each hatchling in a clutch; available forage (natural or provided); health, growth, and survival of each juvenile; available burrows in headstart facilities; and behavioral traits of juveniles. Data collected at higher resolution would increase the predictability and accuracy of models. For example, models could address how short-term growth changes over time with changes in predictive variables (e.g., density and food resources) in contrast to our models, which provide a snapshot in time.

To avoid overstocking and to maximize growth and health, evaluation of habitat characteristics of potential headstart sites would be productive. Data about the numbers, species composition, and canopy cover of perennial shrubs are essential because wild juveniles place 79% of their burrows beneath large shrubs (Berry and Turner 1986). Shrub cover is also critical for other threatened and endangered species (e.g., Lagarde et al. 2012; Attum et al. 2013). Evaluations of soil fertility, trace element analysis of soils (Seltzer and Berry 2005; Chaffee and Berry 2006), and estimates of atmospheric nitrogen deposition and its effects (Brooks 2003; Allen et al. 2009; Schneider and Allen 2012) provide additional information essential to understanding the environment in which juveniles may be reared, forage quality, grow rates, and whether the site is appropriate. Available forage is a critical factor for tortoises and for management of other threatened and endangered species. For example, captive-reared juvenile *Geochelone yniphora* kept in prerelease enclosures lost body mass, potentially because of diet (Pedrono and Sarovy 2000). Loss of a preferred food item, a cactus, was suspected to reduce carrying capacity for the endangered San Salvador rock iguanas, *Cyclura rileyi rileyi* (Hayes et al. 2016). Desert tortoises rely on preferred annual forbs as the principal source of energy and nutrition, and have food preferences similar to adults, but are more limited in accessibility of foods because of their small sizes and delicate beaks (Morafka and Berry 2002; Oftedal 2002; Oftedal et al. 2002; Jennings and Berry 2015). Alien annual species compose about 66% of the biomass of the annual flora in tortoise critical habitat in the western, central, and southern Mojave Desert regions during wet years and 91% in dry years (Brooks and Berry 2006). Alien annual species effectively compete with native annuals for resources (Brooks 2000) and have altered the foods available to tortoises over the last several decades (Oftedal 2002). Alien annuals have overwhelmed the soil seed bank in parts of California's deserts (Schneider and Allen 2012) and may have done so inside the EAFB pens. Seed banks are an important consideration when selecting pen locations unless juveniles are to be fed a commercial or other diet.

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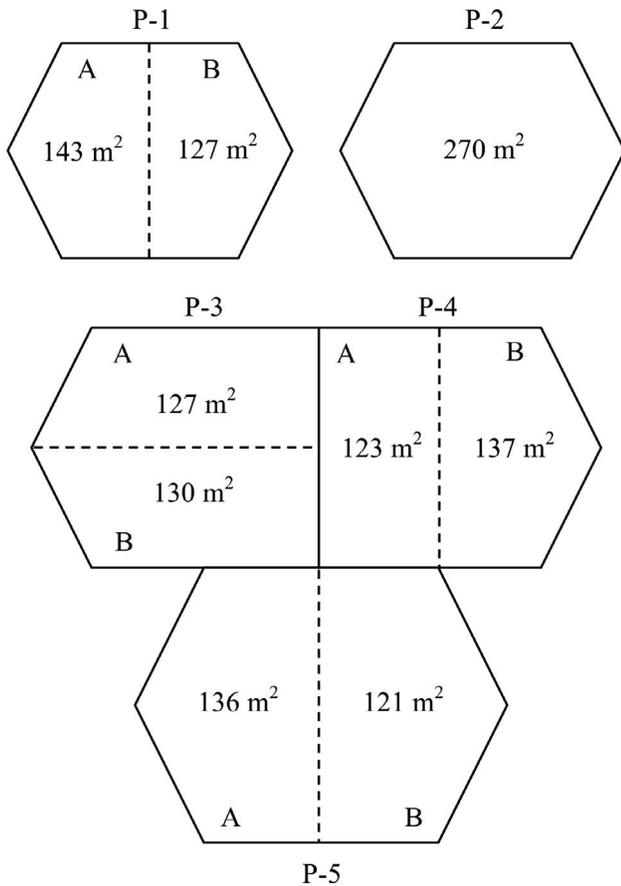
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**Appendix 1.** Diagram of the headstart facilities for desert tortoises at Edwards Air Force Base, California. Five enclosures were subdivided (dashed line) into 9 pens using plywood and metal flashing.

**Appendix 2.** Measurements of carapace length at the mid-line (MCL), weight, and condition indices (CI) for 148 juvenile desert tortoises in 2011 and 109 resampled tortoises in 2012 in headstart pens at Edwards Air Force Base, California.

Year	Cohort	n	MCL (mm)			Weight (g)			CI (g/mm <sup>3</sup> )		
			Mean (SE)	Min	Max	Mean (SE)	Min	Max	Mean (SE)	Min	Max
2011	2003	3	109.4 (2.4)	106.8	114.1	248.7 (18.7)	218.0	282.5	0.54 (0.02)	0.50	0.56
	2004	3	75.0 (8.5)	57.9	83.5	91.8 (23.1)	45.7	116.6	0.52 (0.01)	0.50	0.55
	2005	13	72.2 (2.3)	57.7	90.0	81.1 (6.0)	47.8	127.0	0.50 (0.03)	0.22	0.57
	2006	3	84.3 (1.9)	80.7	86.9	121.9 (8.7)	109.7	138.8	0.52 (0.01)	0.50	0.54
	2007	20	66.5 (1.1)	58.4	77.6	61.5 (3.3)	38.2	94.0	0.51 (0.01)	0.46	0.60
	2008	30	56.3 (0.9)	48.4	67.6	40.8 (1.8)	24.1	63.2	0.51 (0.01)	0.41	0.61
	2009	40	54.3 (0.6)	47.5	64.8	36.1 (1.0)	24.8	61.5	0.52 (0.01)	0.40	0.61
	2010	36	50.2 (0.4)	45.5	56.1	28.8 (0.8)	18.2	43.4	0.52 (0.01)	0.43	0.66
	All	148	59.0 (1.0)	45.5	114.1	49.8 (3.0)	18.2	282.5	0.52 (0.01)	0.22	0.66
2012	2003	3	115.9 (3.6)	112.0	123.0	275.8 (19.2)	255.5	314.1	0.52 (0.01)	0.50	0.54
	2004	2	89.2 (1.8)	87.3	91.0	119.1 (16.1)	103.0	135.1	0.45 (0.03)	0.42	0.48
	2005	11	75.8 (3.0)	59.4	95.2	83.7 (9.3)	46.6	150.1	0.47 (0.01)	0.44	0.50
	2006	3	89.5 (3.2)	83.7	94.8	147.4 (20.3)	108.9	177.9	0.54 (0.04)	0.47	0.59
	2007	18	69.5 (1.2)	59.4	77.7	65.7 (3.4)	47.4	96.9	0.48 (0.02)	0.42	0.72
	2008	21	58.1 (1.2)	49.1	67.1	41.7 (2.4)	24.1	58.9	0.49 (0.01)	0.41	0.60
	2009	27	57.3 (0.8)	50.7	69.1	39.7 (2.1)	23.1	71.5	0.49 (0.01)	0.39	0.61
	2010	24	52.3 (0.5)	46.8	57.2	30.6 (1.3)	17.6	49.0	0.49 (0.01)	0.37	0.63
	All	109	63.3 (1.3)	46.8	123.0	57.8 (4.5)	17.6	314.1	0.49 (0.01)	0.37	0.72