

REPORT DOCUMENTATION PAGE			Form Approved OMB NO. 0704-0188		
<p>The public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA, 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.</p> <p>PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.</p>					
1. REPORT DATE (DD-MM-YYYY) 02-04-2013		2. REPORT TYPE Final Report		3. DATES COVERED (From - To) 1-May-2005 - 30-Apr-2013	
4. TITLE AND SUBTITLE Desert Tortoise Head-start Program at Twentynine Palms Marine Base			5a. CONTRACT NUMBER W911NF-05-1-0220		
			5b. GRANT NUMBER		
			5c. PROGRAM ELEMENT NUMBER 7MPFE5		
6. AUTHORS Kenneth An Nagy, Scott Hillard			5d. PROJECT NUMBER		
			5e. TASK NUMBER		
			5f. WORK UNIT NUMBER		
7. PERFORMING ORGANIZATION NAMES AND ADDRESSES University of California - Los Angeles Regents of the University of California, Los Angeles Office of Contract and Grant Administration Los Angeles, CA 90095 -1406			8. PERFORMING ORGANIZATION REPORT NUMBER		
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Research Office P.O. Box 12211 Research Triangle Park, NC 27709-2211			10. SPONSOR/MONITOR'S ACRONYM(S) ARO		
			11. SPONSOR/MONITOR'S REPORT NUMBER(S) 48444-EV.3		
12. DISTRIBUTION AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision, unless so designated by other documentation.					
14. ABSTRACT The TRACRS head-starting facility at MCAGCC Marine Base is functioning well as a hatchery-nursery. Hatching success (percentage of eggs laid that hatched and the hatchlings have emerged from the nest) continues to be about 70 to 80 %, and survivorship of hatchlings and juveniles has averaged over 80% per year. TRACRS now contains 402 juveniles aged 1-6 years, and 52 new hatchlings from 2012, for a total of 454. Some juveniles may be large enough to release (about 100-110 mm carapace length) by autumn of 2013, depending on rain and food availability.					
15. SUBJECT TERMS Threatened species, conservation, head-starting method, extinction, predation, mortality, survivorship					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT UU	15. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON Kenneth Nagy
a. REPORT UU	b. ABSTRACT UU	c. THIS PAGE UU			19b. TELEPHONE NUMBER 310-825-8771

## Report Title

Desert Tortoise Head-start Program at Twentynine Palms Marine Base

### ABSTRACT

The TRACRS head-starting facility at MCAGCC Marine Base is functioning well as a hatchery-nursery. Hatching success (percentage of eggs laid that hatched and the hatchlings have emerged from the nest) continues to be about 70 to 80 %, and survivorship of hatchlings and juveniles has averaged over 80% per year. TRACRS now contains 402 juveniles aged 1-6 years, and 52 new hatchlings from 2012, for a total of 454. Some juveniles may be large enough to release (about 100-110 mm carapace length) by autumn of 2013, depending on rain and food availability. However, as of January 2013, only 0.55 inches (19 mm) of rain has fallen at TRACRS since 1 July 2012, and there are no seedlings emerging yet, so releases may be delayed until 2014.

---

**Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:**

#### (a) Papers published in peer-reviewed journals (N/A for none)

<u>Received</u>	<u>Paper</u>
07/23/2012	1.00 Kenneth A. Nagy, Michael W. Tuma, L. Scott Hillard. Shell hardness measurement in juvenile desert tortoises, <i>Gopherus agassizii</i> , <i>Herpetological Review</i> , (09 2011): 0. doi:
07/23/2012	2.00 Christina M. Davy, Taylor Edwards, Amy Lathrop, Mark Bratton, Mark Hagan, Brian Henen, Kenneth A. Nagy, Jonathan Stone, L. Scott Hillard, Robert W. Murphy. Polyandry and multiple paternities in the Threatened Agassiz's desert tortoise, <i>Gopherus agassizii</i> , <i>Conservation and Genetics</i> , (09 2011): 0. doi:
<b>TOTAL:</b>	<b>2</b>

Number of Papers published in peer-reviewed journals:

---

#### (b) Papers published in non-peer-reviewed journals (N/A for none)

<u>Received</u>	<u>Paper</u>
-----------------	--------------

**TOTAL:**

Number of Papers published in non peer-reviewed journals:

---

#### (c) Presentations

Number of Presentations: 0.00

---

**Non Peer-Reviewed Conference Proceeding publications (other than abstracts):**

Received      Paper

**TOTAL:**

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

---

**Peer-Reviewed Conference Proceeding publications (other than abstracts):**

Received      Paper

**TOTAL:**

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts):

---

**(d) Manuscripts**

Received      Paper

**TOTAL:**

Number of Manuscripts:

---

**Books**

Received      Paper

**TOTAL:**

## Patents Submitted

---

## Patents Awarded

---

### Awards

Session in honor of Ken Nagy at the annual SICB (Society for Integrative and Comparative Biology) meeting, San Francisco, CA January 2013 (see <http://sicb.org/meetings/2013/>).

---

### Graduate Students

<u>NAME</u>	<u>PERCENT SUPPORTED</u>
<b>FTE Equivalent:</b>	
<b>Total Number:</b>	

---

### Names of Post Doctorates

<u>NAME</u>	<u>PERCENT SUPPORTED</u>
<b>FTE Equivalent:</b>	
<b>Total Number:</b>	

---

### Names of Faculty Supported

<u>NAME</u>	<u>PERCENT SUPPORTED</u>	National Academy Member
Kenneth A Nagy	0.06	
<b>FTE Equivalent:</b>	<b>0.06</b>	
<b>Total Number:</b>	<b>1</b>	

---

### Names of Under Graduate students supported

<u>NAME</u>	<u>PERCENT SUPPORTED</u>
<b>FTE Equivalent:</b>	
<b>Total Number:</b>	

**Student Metrics**

This section only applies to graduating undergraduates supported by this agreement in this reporting period

- The number of undergraduates funded by this agreement who graduated during this period: ..... 0.00
- The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:..... 0.00
- The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:..... 0.00
- Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):..... 0.00
- Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:..... 0.00
- The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense ..... 0.00
- The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields: ..... 0.00

**Names of Personnel receiving masters degrees**

<u>NAME</u>
<b>Total Number:</b>

**Names of personnel receiving PHDs**

<u>NAME</u>
<b>Total Number:</b>

**Names of other research staff**

<u>NAME</u>	<u>PERCENT SUPPORTED</u>
L. Scott Hillard	0.50
<b>FTE Equivalent:</b>	<b>0.50</b>
<b>Total Number:</b>	<b>1</b>

**Sub Contractors (DD882)**

**Inventions (DD882)**

**Scientific Progress**

See Attachment

**Technology Transfer**

# FINAL REPORT

DESERT TORTOISE HEAD-START PROGRAM

AT TWENTYNINE PALMS USMC BASE

Award No: W911NF-05-1-0220

to

Marine Corps Air Ground Combat Center (MCAGCC),

Natural Resources and Environmental Affairs,

Twentynine Palms, California 92278

and

U.S. Army Research Office

Durham, North Carolina

Prepared by:

Kenneth A. Nagy, Ph.D., Research Professor of Biology

With contributions from

Scott Hillard, M.A., Field Research Coordinator.

Contact:

Dr. Ken Nagy, Research Professor of Biology

Department of Ecology and Evolutionary Biology

University of California, Los Angeles

621 Young Drive, South

Los Angeles, CA 90095-1606

(Office) 1 (310) 825-8771 (Fax) 1 (310) 825-9433

(E-mail) [kennagy@biology.ucla.edu](mailto:kennagy@biology.ucla.edu)

## SUMMARY

This report summarizes progress made from 2005 to 2012 at the head-starting facility at the Twentynine Palms Marine Base site (TRACRS) at Sand Hill Training Area. During the 2005-2006 winter, a predator-resistant (fenced and netted) facility enclosing natural habitat was successfully constructed. The facility consisted of three 100 ft. X 150 ft. enclosures, two of which were subdivided into 24 pens measuring 25 ft. X 25 ft. The resulting 48 pens were used as “private maternity rooms” to individually house pregnant, wild, female Agassiz’s desert tortoises (*Gopherus agassizii*) temporarily, while they laid their eggs in spring. The hatchlings that emerged from nests in autumn lived in those pens over winter, and were moved to the remaining “open” enclosure the next spring before egg-bearing females were again introduced to obtain a new cohort of hatchlings. Later, a new, fourth enclosure, 100 ft. X 300 ft., was built, and four 3000-gallon plastic water tanks, electric water pumps and generators, a storage building, a laboratory building, a perimeter security fence, and in 2012, external water and electrical power were added. All four enclosures have been irrigated as necessary to provide green annual plant food for juvenile tortoises throughout spring, especially during the drought years of 2007 and 2012, when no plants germinated outside the enclosures.

Seven annual cohorts of eggs, ranging in number from 110 to 187 in good rainfall years to 48 to 87 in drought years, have been obtained for TRACRS during the last seven years. Annual survivorship and growth rates of each cohort have been measured. Hatching success has hovered near 75%, and annual survivorship has increased as juveniles got older. Five years after hatching, 50% of offspring starting as eggs were still alive, which is much higher than presumed survivorship of wild, free-living juveniles. Growth rates at TRACRS have been about three times greater than average growth rates of juveniles raised without supplemental spring-time irrigation at another head-start facility. We anticipate that some juveniles will reach releasable size (100-110 mm carapace length) as early as eight years of age.

Specific research questions have included: Is Upper Respiratory Tract Disease (URTD) vertically transmitted from sick mothers to their offspring during the egg stage? How similar are the genomes of the two organisms that cause URTD (*Mycoplasma agassizii* and *M. testudineum*)? Are there “new” predators on juvenile desert tortoises at TRACRS? Are the sex ratios of cohorts hatching at TRACRS near 1:1? Can we alter cohort sex ratios by manipulating soil temperatures inside TRACRS? Do all the hatchlings from a single female have the same father? Do head-started tortoises survive well after release back into the field? So far, we have been unable to address experimentally the first two questions. Statistically insufficient numbers of URTD-positive pregnant females have been found at Sand Hill despite extensive searching



using volunteers for over three years. The prevalence of URTD symptoms has declined since earlier studies were done at Sand Hill. And colleagues in Florida have not been able to organize genome studies on *Mycoplasma* samples as of yet.

Regarding the question of predation inside TRACRS enclosures, five species of predators have been detected: weather-caused rips in the bird netting have allowed entry to a roadrunner, a burrowing owl and an antelope ground squirrel, and some tortoise mortality occurred before the netting problems were corrected. The remaining two predators were ants: native fire ants (*Solenopsis xyloni*—already known as hatchling predators from another study site) and common Harvester Ants, *Pogonomyrmex californicus*. Both have been controlled when and where necessary by cautious use of MaxForce® bait/poison. In 2009, we collaborated with Dr. G. Kuchling of Australia to determine endoscopically the gender of juvenile tortoises from the 2006, 2007 and 2008 cohorts. Surprisingly, most were female, and the proportion that was female increased over time, such that the 2008 cohort was nearly all female. In 2009, we conducted a controlled experiment to try to reduce nest temperatures via shading nest areas, and thereby increase the proportion of male hatchlings (these tortoises have temperature-dependent sex determination). Dr. Kuchling returned in 2011 to determine gender of 2009-cohort juveniles. There was no significant effect of shading on sex ratio. However, the 2009 cohort was mostly males, apparently due to the generally cooler weather in spring of that year. Genotyping results for mothers, their hatchlings, and local males that were potential fathers showed that most females were polyandrous and most clutches had more than one father (Davy et al., 2011). A severe drought in 2012 threatened the well-being of tortoises in the TRACRS facility—winter rain was insufficient to induce germination of any annual food plants anywhere at Sand Hill. But by irrigating inside enclosures, adequate plant germination and spring growth was achieved to support normal juvenile survivorship and growth through autumn. The last research question, about survivorship and growth after release, is the main focus of our efforts in the upcoming years.

The TRACRS facility is functioning well as a hatchery-nursery. Hatching success (percentage of eggs laid that hatched and the hatchlings have emerged from the nest) continues to be about 70 to 80 %, and survivorship of hatchlings and juveniles has averaged over 80% per year. TRACRS now contains 402 juveniles aged 1-6 years, and 52 new hatchlings from 2012, for a total of 454. Some juveniles may be large enough to release (about 100-110 mm carapace length) by autumn of 2013, depending on rain and food availability. However, as of January 2013, only 0.55 inches (19 mm) of rain has fallen at TRACRS since 1 July 2012, and there are no seedlings emerging yet, so releases may be delayed until 2014.

## INTRODUCTION

The long-term research project being done on desert tortoises (*Gopherus agassizii*) at the Marine Corps Base at Twentynine Palms is concerned with learning the biology behind ways to repopulate depleted areas of the Base and of other parts of the Mojave Desert with juvenile and sub-adult tortoises. In the wild, there is high mortality of tortoise eggs in the nests, and very high mortality of babies after hatching and upon emerging from the soil. Normally, perhaps only one or two babies reach sexual maturity for every 100 eggs produced by females. The opportunity for enhancing natural populations of tortoises by increasing egg and juvenile survivorship looks to be tremendous. Even a small decrease in the egg and juvenile mortality rates can double or quadruple the recruitment rate. This project is providing centrally-important guidelines for successful augmentation of existing tortoise populations via head-starting procedures.

We are increasing egg and neonate survivorship by using predator-excluding pens built in natural habitat to protect adult females that are vulnerable while they are digging their nests and laying their eggs, then to protect these eggs during incubation and hatching, and then to shield the hatchlings and growing juveniles from predators for varying lengths of time before their ultimate release to the wild. Aspects of disease monitoring are incorporated into these studies. Also, we are exploring the possibility of increasing growth rate, and thus decreasing the time required to reach predator-resistant size, by careful supplementation of natural rainfall to promote food plants to germinate in droughts, and to stay green longer into the summer dry season. In addition, in studies at Twentynine Palms Marine Base and at other sites, questions about early release, subsequent “half-way house” protection upon release, the season and distance of release, and the minimum age/size for successful survivorship upon release are being planned or investigated, along with genetic questions regarding multiple paternity and temperature-dependent sex determination.

Our ultimate objective is to make the head-starting procedure for desert tortoises into an effective conservation and management tool for use by government and conservation agencies on this Threatened Species.

## METHODOLOGY

We have previously built and operated head-starting enclosures at Fort Irwin National Training Center (FISS) and at Edwards Air Force Base (JHETSS), and now at the Marine Corps Air Ground Combat Center (TRACRS). In 2005 and early 2006, we built the first three predator-exclusion enclosures, and in spring 2006, we began “stocking” the 48 small pens with pregnant female tortoises from the surrounding Sand Hill area. Local female tortoises were “borrowed” from their home ranges for 2-3 weeks while they laid their first clutch of eggs inside a pen in one of the enclosures. Then, the females were returned to their home burrow unharmed, hopefully to produce and lay a second clutch within their individual home range. For the first three years, we attempted to obtain 24 disease-free females for the “control” (disease-free) enclosure, and 24 females having active Upper Respiratory Tract Disease (URTD) symptoms for the “disease” enclosure. We then protected the eggs and hatchlings inside TRACRS from predators, and did experiments to answer a variety of questions relevant to achieving successful repopulation efforts. During the first several years, small blood samples were collected from mothers producing large clutches, their hatchlings, and from local wild males that were potential fathers, and genomes (based on 17-20 microsatellite markers) were determined to evaluate paternity of clutches (see details in Davy et al. 2011).

In springs of 2006, 2007, 2008 and 2009, teams of searchers walked the Sand Hill area, in the south-western corner of the Marine Corps Air and Ground Combat Center, looking for adult female desert tortoises. GPS positions of females found were recorded, a field health profile was completed, including shell dimension and body mass measurements, and females were given a unique number if they did not already have one from previous studies. Some of these females were selected as potential egg donors, and were fitted with transmitters so they could be tracked and used over many years to provide eggs to TRACRS.

In 2006, 2008, 2009, 2010, and 2011, all five being moderate to excellent rainfall and wildflower years, about 25-30 telemetered females were recaptured from surrounding habitat. In 2007 and 2012, both severe drought years, fewer females were used, because some telemetered females did not produce eggs. In spring, females were located via radio-tracking, then x-rayed, palpated for eggs and blood sampled on the spot, and released where captured while awaiting test results to determine if 1) they had shelled eggs, and 2) if they were ELISA positive or negative (test for antibodies to *Mycoplasma agassizii*, a URTD-causing microbe). Procedures used for x-raying, palpating and blood-sampling these females at or near their capture site are described in more detail by Nagy et al.

(2007). If and when appropriate, females were moved into separate, private 25 ft. X 25 ft. (8 X 8 m) pens at TRACRS where two or three burrows per pen had been prepared for their selection. After weight loss and x-rays indicated that they laid their first clutch in the pens, they were telemetered (if not already) and released into a burrow at or near where they were captured. All of their locations have been determined via radio-tracking on a monthly schedule since then, or until transmitters were removed.

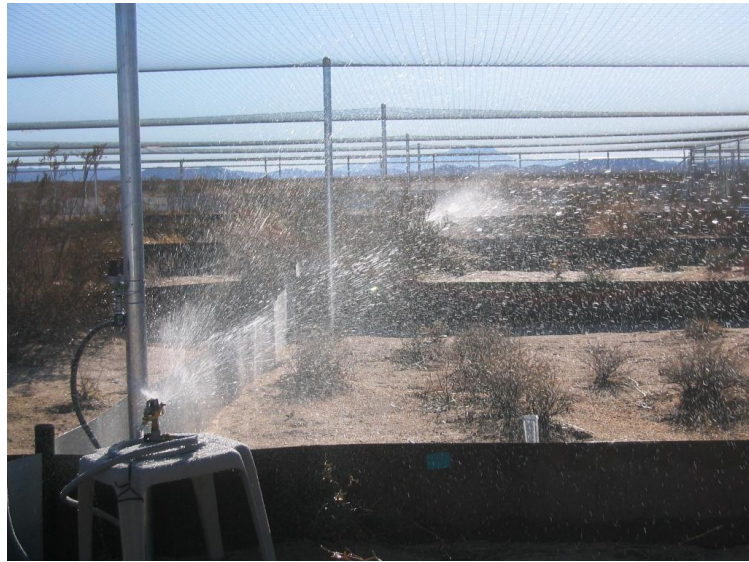
When hatchlings began appearing in autumn, they were assigned numbers, which were written on plastron and carapace with permanent marker and, later, by gluing paper squares with the printed number using clear epoxy in the center of a dorsal carapacial scute. Their unique number also identified their mother. Hatchlings were measured for shell dimensions and body mass, and body condition indices (CI, Nagy et al., 2002) were calculated as the ratio of body mass (grams) over the product of shell length, height and width (all in cm, to obtain an estimate of relative shell volume in  $\text{cm}^3$ ). They were then released back into the maternal enclosure. Growth rates were calculated as absolute and relative (percentage) increase in Midline Carapace Length (MCL). The next spring, following confirmation of disease-free health status (negative blood ELISA test and clinical examination), yearlings were moved to larger common enclosures while they grow large enough to release.

The new enclosure at TRACRS (#4, measuring 100 ft. X 300 ft. and divided into thirds) was made available for use as a hatchery in 2009, and about half of the 24 individual pens available in the south 1 third (Encl. #4A) were used in 2009 and 2010. The large commons enclosure in the middle third (Enclosure #4B) received all of the 2009 cohort juveniles in late spring 2010, and the adjacent large commons enclosure at the North third (Enclosure #4C) received all of the 2010 cohort juveniles in spring 2011. The 2011 hatchlings were moved to a large commons area created in Enclosure #1 (#1B).

Fig. 1. The TRACRS facility. A: Aerial view, showing Encl #1 at lower right, Encls #2 and 3 lower left, and double-sized Encl #4 middle left, lower white object is storage shed, upper white is laboratory, 2 upper black objects are solar panels, with surrounding dark line being the perimeter security fence. B: Irrigating Encl #2, view from North. C: Inside Encl #4, view from south.



A



B



C

## RESULTS

Hatching success and survivorship at TRACRS have both continued to be relatively high. Hatching success (percentage of eggs that yielded a living hatchling visible on the surface of the soil) has been about 70% to 80% since the site began operation in 2006, and was 93% in 2011. Survivorship of hatchlings during their first year of life has been averaging from 86% to 100% in the different years (Table 1). And survivorship of juveniles during their second, third, fourth, and fifth years has averaged near 80% per year. Thus, 62% of the hatchlings appearing in 2006 were still alive six years later.

Table 1. Sequential survivorship of six cohorts of juvenile desert tortoises at the head-start facility at 29 Palms Marine Base.

	<b>2006 Cohort</b>	<b>2007 Cohort</b>	<b>2008 Cohort</b>	<b>2009 Cohort</b>	<b>2010 Cohort</b>	<b>2011 Cohort</b>
(as hatchlings)	1.00	1.00	1.00	1.00	1.00	1.00
As 1 year olds	0.89	0.95	1.00	0.87	0.86	0.94
As 2 year olds	0.77	0.93	0.83	0.58	0.74	
As 3 year olds	0.75	0.85	0.80	0.56		
As 4 year olds	0.65	0.83	0.78			
As 5 year olds	0.62	0.83				
As 6 year olds	0.62					

Growth rates under the frequent irrigation conditions at TRACRS were very high: about 3-5 times higher than juveniles at Edwards AFB (JHETSS) receiving natural rainfall only (Nagy et al., in press), and about 1.5 to 2 times higher than the growth rates in (minimally) rain-supplemented juveniles at JHETSS. The higher growth rates at TRACRS may be due primarily to the more frequent availability of drinking water from irrigation events at TRACRS.

A drought occurred during 2012, with only 0.94 inches (24 mm) of rain falling during the 12 months from 1 July 2011 to 30 June 2012. Natural areas adjacent to the TRACRS enclosures had no germination of annual plant seeds in 2011-2012. Our irrigation efforts inside TRACRS enclosures stimulated germination in areas around sprinklers in early spring 2012 (Fig. 2), and our continued periodic irrigation kept these plants green through the end of May, 2012. Some areas were clearly overgrazed by tortoises, and these will receive special attention in 2013.

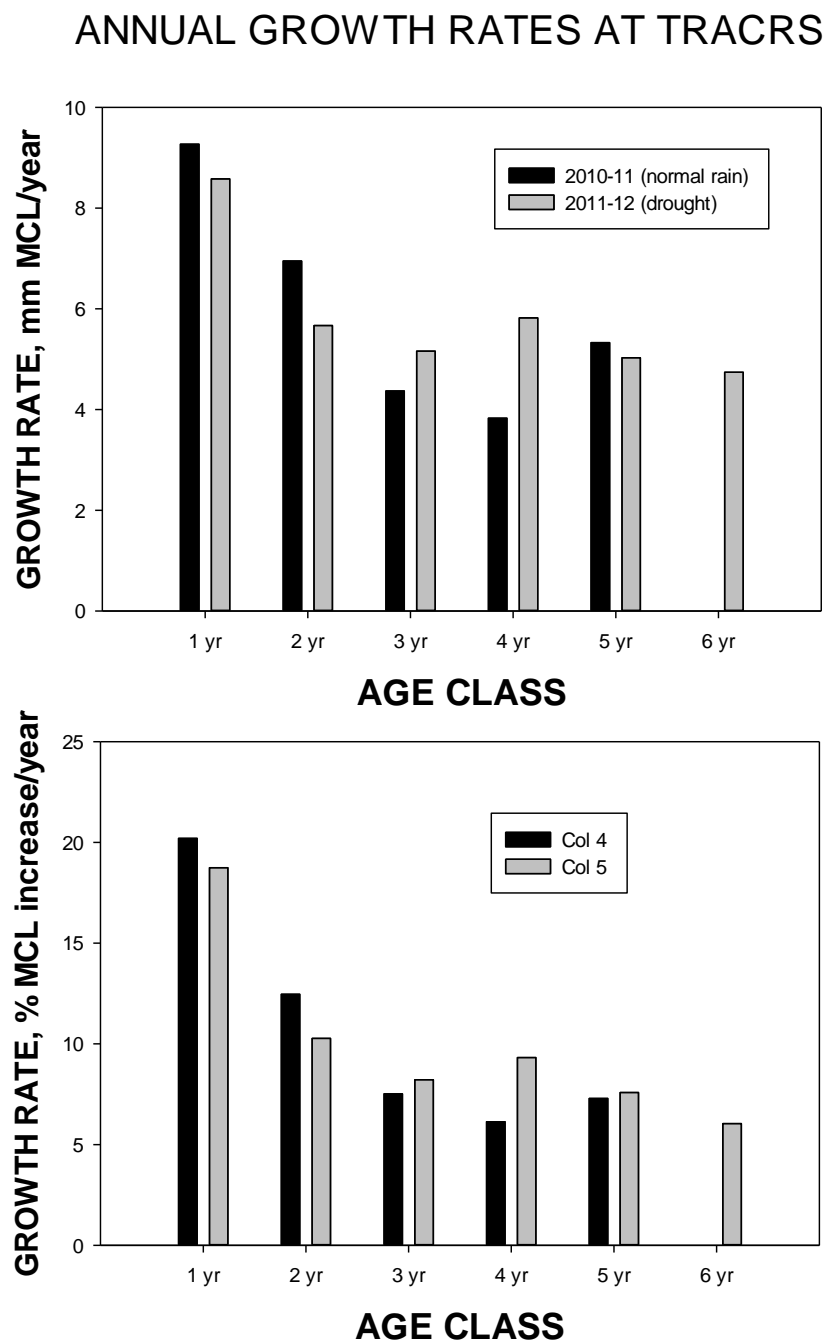
Fig. 2. Irrigation effects: annual plant germination and growth, and greening of shrubs, immediately around a sprinkler (on side of metal post) during a drought year at TRACRS.



Annual growth rates during the 2011-2012 drought were similar to those the previous, non-drought year (Fig. 1). Juvenile tortoises experiencing good conditions had relatively high growth rates their first year of life, somewhat lower rates their second year, and then growth rates stabilize for the next several years. This trend was apparent whether growth rates were expressed in absolute

units of mm increase in MCL per year or in relative units of percent MCL increase per year (Fig. 1). The increased irrigation in 2012 promoted rates of growth in all age cohorts that were superimposable upon rates measured during a year having average rainfall (Fig. 3).

Figure 3. Growth rates of six age cohorts of juvenile tortoises at TRACRS during a year having average rainfall (2011, black bars) and during a drought year (2012, grey bars) are shown, both as mean mm increase/year in midline carapace length (MCL) and as % MCL increase/year.





In the nearly six years since eggs and juvenile tortoises have occupied the TRACRS facility, only one “new” predator has been discovered. The earlier surprising discoveries at the Edwards AFB facility of predation on eggs and hatchlings by Antelope ground squirrels and then by native Fire Ants had taught us to be alert for any new and unexpected predation at TRACRS. The “new” predator is the common red and black Harvester ant *Pogonomyrmex californicus* (Fig. 4). So far these ants have apparently had only a minor effect on overall juvenile mortality at TRACRS—few carcasses have been found having ant-sized holes in their plastrons. The ants may only attack hatchlings that have still-wet and open or unhealed umbilical scars, or hatchlings that get stranded outside burrows due to cold weather and are vulnerable to attack. There has been some predation by birds inside TRACRS. In summer 2010, a single roadrunner and a single burrowing owl managed to get into the North enclosure for several days. Both species are known to kill and eat small tortoises. We found several juvenile tortoise carcasses in the North enclosure coincident with the presence of the two birds. Careful inspection of the enclosure netting and fencing revealed three holes that these birds possibly could have used, and these were closed. No predatory birds have since been seen inside. In 2012, we saw an antelope ground squirrel inside Enclosure #4, and there were some carcasses in Encl. #4C having tooth marks suggestive of rodent predation. Possible entry avenues were blocked immediately, and no squirrels or squirrel damage has been seen since.

Fig. 4. The common Harvester ant, *Pogonomyrmex californicus*, a new predator of small juvenile desert tortoises.

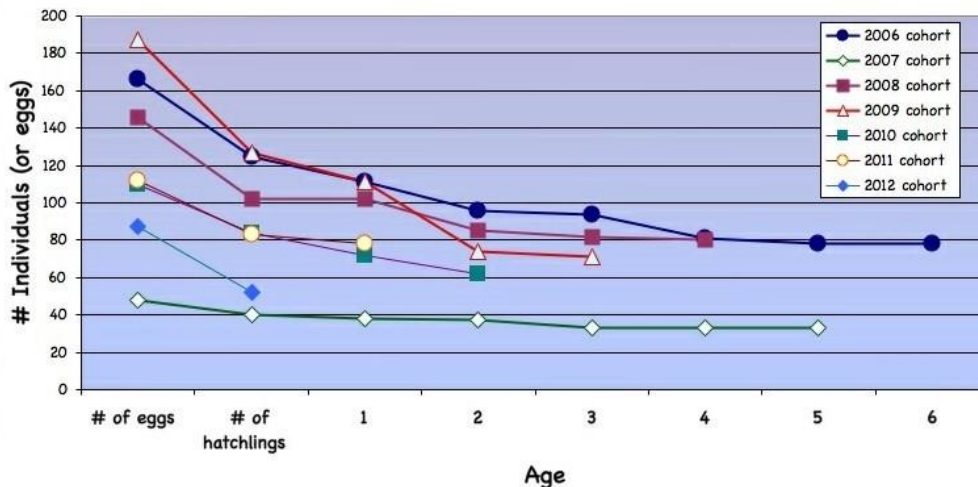


Results of genetic studies on hatchlings, and on their mothers and potential fathers in the Source Population of adult tortoises at Sand Hill have revealed that multiple paternity is common, and may be the rule, in the Sand Hill population. Most (58%) of egg clutches that had statistically sufficient numbers of typed hatchlings showed they had two or more fathers. These results are similar to results from the Edwards AFB hatchlings, and they support the conclusion of multiple paternity being present and common. These results have been published (Davy et al., 2011).

The 2009 endoscopy study of sex and sex ratio of three age cohorts of juveniles at TRACRS has shown that the sex ratios differ from a 50/50 ratio in favor of females. Also, the percentage that is females has apparently increased from 2006 to 2008, such that only one of the 37 juveniles studied from the 2008 cohort was male. The data are insufficient at present, however, to show a statistically significant time trend in sex ratio. In 2009, we conducted an experiment to shade and thus cool some burrows containing nests (experimental group) and not shade other nests (control group) to determine if the sex of hatchlings can be influenced this way. Dr. Gerald Kuchling visited TRACRS in spring 2011 to determine sexes via endoscopy, and he found that about 75% of the 2009 cohort was male. Interestingly, shading of burrows had no clear effect on sex ratios in 2009. Climate data for the 29 Palms Marine Base airfield show that ambient temperatures in the four springs could account for the variation in sex ratios among years. Temperatures in spring 2009 were lower than the other three years, and low incubation temperatures contribute to a shift to more males in a clutch of eggs. We will examine our temperature data measured at TRACRS itself, and analyze the sex ratio results in light of those data.

The TRACRS facility is functioning well as a hatchery-nursery. TRACRS now contains 402 juveniles aged 1-6 years, and 52 new hatchlings from 2012, for a total of 454. There has been a total of 856 eggs placed in TRACRS during the last seven years (Fig. 5). Thus, overall survivorship has been  $(100 \times 454/856 =) 53$  percent. But the best test of the effectiveness of a hatchery-nursery is whether or not tortoises raised in one are able to survive and breed successfully after being released in the field. We expect to begin that test soon. Some juveniles at TRACRS may be large enough (about 100-110 mm carapace length) to release by autumn of 2013, depending on rain and food availability. However, as of January 2013, only 0.55 inches (19 mm) of rain has fallen at TRACRS since 1 July 2012, and there are no seedlings emerging yet, so releases may be delayed until 2014.

Fig. 5. Sequential survivorship in seven annual cohorts of desert tortoises living inside a predator-resistant natural enclosure at TRACRS on MCAGCC. Mortality is highest (about 25%) from egg to hatchling, but mortality declines soon thereafter.



## DISCUSSION

Predation on TRACRS nests or eggs, hatchlings and juveniles has been very low—only some apparent mortality of hatchlings by fire ants, harvester ants, and by two invading birds (a roadrunner and a burrowing owl) has been seen. Early in 2012, a single Antelope ground squirrel found a way into Enclosure #4, and apparently caused the death of one or more juveniles in the 2009 cohort and perhaps also in the 2010 cohort. We sealed all potential entrance sites, and placed “rat guards” on the guy wires anchoring Enclosure #4, and the ground squirrel did not enter the enclosure thereafter. The fence screening and flashing, the overhead bird netting, and the precautionary Fire Ant poison baiting stations apparently have been very effective so far. We continue to monitor nests and tortoises carefully for any signs of predation.

The continued high survivorship of the 2006 and 2007 cohorts of juvenile tortoises, and now of the 2011 and 2012 cohorts through 2012, indicates that the expected negative effects of a serious drought on a head-starting facility can be avoided. Increased irrigation, both in amount of water added per irrigation event in early spring of 2007, and in irrigation frequency alone in early spring of 2012, to stimulate germination, and then increased frequency of watering events later in spring after germination occurred, completely counteracted the increase in juvenile deaths that was expected from the substantial mortality observed in older juveniles in natural rainfall enclosures at JHETSS over the

same time period (Nagy et al., in press). However, the drought still affected our head-starting success by reducing the egg productivity of donor females, which did not have access to supplemental irrigation while living in the field.

The effects of a severe drought on the six age cohorts of juvenile tortoises at TRACRS was successfully nullified mainly by increasing the frequency of irrigation events, and by watering earlier in the year to initiate germination. Juveniles that received only natural rainfall at JHETSS during a similar drought in 2007 had growth rates that were zero or negative (Nagy et al. in press; see also Loehr et al. 2007). The lack of an obvious drought effect on growth rates of tortoises during the 2012 drought year adds more detailed evidence than does survivorship data that irrigation can counter some expected negative influences of a drought. These juveniles were not simply “toughing it out”, they were actually doing well during this stressful period.

Overall, the relatively high rates of growth by juveniles at TRACRS since 2006, as compared with juveniles at the JHETSS facility, both irrigated and natural rainfall only, have continued through 2012, despite the drought. Each year, the juveniles at TRACRS have received additional applications of irrigation water, both during summer and (especially) in autumn just before entering hibernation. The wildflowers do not respond strongly to these shortened watering events in the hot season. However, these events have allowed juvenile tortoises to drink the water, and most juveniles take advantage of these opportunities. Hydrated tortoises can continue to eat vegetation that has died and dried out, whereas dehydrated tortoises stop eating dry foods in mid-summer (Nagy and Medica, 1986). We suspect that the additional drinking opportunities are mainly responsible for the higher growth rates in TRACRS juveniles as compared to JHETSS juveniles which got irrigation during springtime only.

Protection from predation is promoting quite high hatching success and survivorship percentages at TRACRS, as compared to those that occur in nature. Our measurements indicate so far that, starting with 100 eggs, TRACRS is producing about 50 five-year-olds. We anticipate that survivorship of juveniles older than three years will get progressively higher as they age, so that perhaps as many as 35-40 releasable-sized juveniles will be available per 100 eggs laid at TRACRS.

We will continue to stock TRACRS with eggs and hatchlings from healthy females in 2013, drought permitting, so that tests of long-term questions can be done in future. These questions include: What is the best release method? Do relocated then released tortoises settle in? How soon do head-started tortoises show courtship and breed successfully? Are their offspring healthy?

## PUBLICATIONS

- Davy, C. M., T. Edwards, A. Lathrop, M. Bratton, M. Hagan, B. Henen, K. A. Nagy, J. Stone, L. S. Hillard, and R. W. Murphy. 2011. Polyandry and multiple paternities in the Threatened Agassizi's desert tortoise, *Gopherus agassizii*. *Conservation Genetics* 12:1313-1322.
- Loehr, V.J.T., M.D. Hofmeyr, and B.T. Henen. 2007. Growing and shrinking in the smallest tortoise, *Homopus signatus signatus*: the importance of rain. *Oecologia* 153:479-488.
- Nagy, K. A., and P. A. Medica. 1986. Physiological ecology of desert tortoises in southern Nevada. *Herpetologica* 42: 73-92.
- Nagy, K. A., B. T. Henen, D. B. Vyas, and I. R. Wallis. 2002. A condition index for the desert tortoise (*Gopherus agassizii*). *Chelonian Conservation and Biology* 4:425-429.
- Nagy, K. A., S. Hillard, and S. Dickson. 2007. Desert tortoise head-start studies: Annual Report for 2006, to U.S. Fish and Wildlife Service Permit TE-085050-1.
- Nagy, K.A., L.S. Hillard, S. Dickson, and D.J. Morafka. In press. Effects of added "rain" on survivorship, body condition and growth of head-started hatchlings and on survivorship of desert tortoise (*Gopherus agassizii*) yearlings released to the open desert. *Herpetological Conservation and Biology*.