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PROGRESS REPORT FOR 2009

The Health Status of Translocated Desert Tortoises (*Gopherus agassizii*) in the Fort Irwin Translocation Area and Surrounding Release Plots, San Bernardino County, California: Year 2

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The Health Status of Translocated Desert Tortoises
(*Gopherus agassizii*) in the Fort Irwin Translocation Area and
Surrounding Release Plots, San Bernardino County, California

Abstract. In spring of 2008, we translocated 158 adult and subadult tortoises (82 females and 76 males) from the Southern Expansion Area (SEA) to four plots located in the Superior-Cronese Desert Wildlife Management Area (DWMA) as part of the Desert Tortoise Health and Disease Research Project for the Ft. Irwin Expansion. Long-term objectives include modeling and predicting effects of translocation on survival of tortoises by health status, presence of infectious diseases and trauma, size and age class, and sex. Tortoises were placed in 4 health categories: 1) healthy or control tortoises, 2) tortoises with moderate to severe clinical signs of past trauma, 3) tortoises with moderate to severe clinical signs of shell disease, and 4) tortoises with moderate to severe clinical signs of upper respiratory tract disease but with no evidence of nasal discharge and negative laboratory tests.

As of December 2008, 43 of the initial 158 translocated tortoises had been found dead or had been salvaged for necropsy, and an additional 15 tortoises were missing. We started Year 2 in January 2009, with 100 live tortoises and 15 missing tortoises in the project. During 2009, we conducted health evaluations for clinical signs of health, disease, and trauma for 81 tortoises in the spring and 65 tortoises in the fall. In the spring 4 (4.9%) and 2 (2.5%) tortoises had positive or suspect ELISA tests for *Mycoplasma agassizii* and *M. testudineum,* respectively. In the fall 6 of 65 (9.2%) tortoises tested positive or suspect for *M. agassizii*; none had positive or suspect tests for *M. testudineum*. Overall during 2009, 9 of 81 individual tortoises (11.1%) had ELISA test results that were positive or suspect for *Mycoplasma* species. When weights of tortoises were compared for 2008 and 2009, spring weights were significantly higher than fall weights. In addition, weights in fall 2009 were significantly lower than weights in fall 2008.

Between January and December of 2009, 27 (23.5%) of the remaining 115 live and missing tortoises were found dead. Of the 27, 24 were probably killed by coyotes or other canids, one was killed by a vehicle, and 2 died of unknown causes. Overall, since the translocation began in March of 2008, 44.3% of tortoises have been found dead or were salvaged for necropsy. Combining data from 2008 and 2009, death rates were significantly higher on two plots, plots 3 and 5, than on plots 1.5 and 8. In contrast to 2008, in 2009 the size of a tortoise was not related to risk of death; the average carapace length did not differ from those still alive. Likewise, in contrast to 2008, in 2009 death rates did not differ between sexes. Death rates also did not differ significantly among the four health categories. At the end of 2009, an additional 20 tortoises (17.4%, 20/115) were missing.

We analyzed movement patterns for live tortoises between the time of initial release in spring 2008 and December 2009 (N = 68). Overall, the mean dispersal distance for males was twice that of females; likewise, males moved twice the total distances compared to females. Total distances moved were higher on plots 3 and 5 than

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on plots 1.5 and 8 but were not significantly different. However, the minimum total distances moved in 2009 were significantly less than in 2008. Females were more likely to use the same cover sites between 2008 and 2009 than males, a potential indication of settling.

INTRODUCTION

The desert tortoise (*Gopherus agassizii*) is a Federally- and State-listed threatened species. Critical habitat for the species occurs north and west of the Colorado River/Grand Canyon complex, including habitat on and adjacent to the National Training Center, Ft. Irwin, in the central Mojave Desert (U.S. Fish and Wildlife Service 1990, 1994). As part of the Ft. Irwin Translocation Project, an estimated 600 to 1000 tortoises have been or are planned to be translocated from the southern and western parts of the expanded Ft. Irwin base to areas outside the Ft. Irwin boundary (Esque et al. 2005).

The primary goal of this research project is to monitor the health and disease status of the translocated tortoises, with an emphasis on the spread of infectious disease. Because infectious diseases have been linked to declining desert tortoise populations (Jacobson et al. 1991; Brown et al. 1994, 1999; Homer et al. 1998; Christopher et al. 2003), the incidence of disease is a critical factor in determining success of translocation. We designed our project to measure the success of translocation, depending on health status of translocated tortoises. Specifically, the translocated tortoises were grouped into one of four health categories: 1) healthy or control tortoises, without moderate to severe clinical signs of infectious disease, trauma, or shell disease; 2) tortoises with moderate to severe clinical signs of past trauma; 3) tortoises with moderate to severe clinical signs of shell disease; and 4) tortoises with moderate to severe clinical signs of upper respiratory tract disease (URTD), but with no evidence of nasal discharge and negative laboratory tests.

Several long-term objectives are to be addressed during the life of the multi-year project. First, we are tracking and sampling tortoises for several years to model and predict the effects of translocation on survival by health status, size and age class, and sex. More specifically, we hope to determine whether or not translocatees in each of the four health categories develop new disease, more severe clinical signs of URTD, more severe cases of shell disease, or new trauma post-translocation. To better understand the epidemiology and distribution of mycoplasmosis or URTD in the Ft. Irwin Translocation Project area, the health status of tortoises and locations of tortoises that have previously tested positive or suspect for mycoplasmosis are being continuously monitored. As part of these analyses, we are also examining differences in survivorship and causes of death among health status categories; differences in survivorship among size and age classes, sexes, and translocation release sites; and differences in the pathogenesis of mycoplasmosis among size and age classes, sexes, and levels of anthropogenic impacts.

Second, the anthropogenic factors most likely to influence translocation success need to be identified and modeled. Anthropogenic factors include but are not limited to

roads, military maneuver areas, and rural or urban areas. Third, ecological factors, including landscape and topography, are other variables in the analysis. Both anthropogenic and ecological factors have the potential of affecting health status and degree of trauma of translocated tortoises. We will also explore differences in survivorship among size and age classes and sexes by comparing habitat conditions between initial capture sites and translocation release sites, including levels of anthropogenic disturbance at original home sites and release sites.

This report is a progress report summarizing the status of 158 tortoises that were translocated in the spring of 2008 and were subsequently monitored for health and disease (Berry et al. 2009). Briefly, in spring of 2008, a total of 82 females and 76 males were translocated from the Southern Expansion Area (SEA) to four plots located in the Superior-Cronese Desert Wildlife Management Area (DWMA). As of December 2008, 43 of the initial 158 translocated tortoises had been found dead (41) or salvaged for necropsy (2), and an additional 15 tortoises were unable to be located and were considered missing. We started the 2009 field season in January with 100 remaining tortoises. In addressing the previously stated objectives, we tracked the remaining translocated tortoises monthly, continued to search for missing tortoises, conducted health evaluations on the tortoises during spring and fall, analyzed movement patterns and use of cover sites, and determined causes of death for dead individuals. Our preliminary findings for 2009 are summarized below.

METHODS

Translocation

Between March 26 and April 8, 2008, 158 desert tortoises were translocated from the SEA to one of four designated plots (plots 1.5, 3, 5, and 8; see Fig. 1). These translocation plots, each about one square mile in size, are located outside the Ft. Irwin boundary and are within or bordering the Superior-Cronese DWMA. Prior to translocation, tortoises located in the SEA were fitted with radiotransmitters and were assigned to one of the following four health status categories based on previous health evaluations: 1) healthy tortoises, without moderate to severe clinical signs of infectious disease, trauma, or shell disease; 2) tortoises with moderate to severe clinical signs of past trauma; 3) tortoises with moderate to severe clinical signs of shell disease; and 4) tortoises with moderate to severe clinical signs of URTD, but with no evidence of nasal discharge and negative laboratory tests. Approximately 20 adult males and 20 adult females in each of the four health status categories were selected to be translocated (Berry et al. 2009). Tortoises that had previously tested positive for mycoplasmosis or had signs of nasal discharge were not considered for translocation.

Tortoises were tracked daily, then weekly, and finally at least once per month after translocation using radio telemetry (Berry et al. 2009). Beginning in July 2008, all translocated tortoises were tracked on a monthly basis, unless behavioral or health reasons dictated more frequent checks. Upon locating tortoises during monthly tracking, critical data were recorded, including, but not limited to: date, weather conditions, time

observed, location in UTMs (NAD 83), behavioral observations, specific location of the tortoise (e.g., inside cover site, in open, under shrub), interactions with other tortoises, and general condition of the tortoise (e.g. appearing ill, stressed, lethargic, or healthy). When tortoises were located and found to be dead, the location, position, and condition of remains, along with evidence for cause of death were recorded and the remains were photographed.

Health Evaluations

Periodically, comprehensive health evaluations of each tortoise were conducted. In general, the health status of each tortoise was evaluated once in the spring (April 27 to May 4) and once in the fall (October 7 to October 27) in 2009, but these evaluations were more frequent for tortoises showing indications of illness or stress. The evaluations included recording data regarding posture, behavior, activity, recent trauma, and clinical signs of disease, such as URTD and cutaneous dyskeratosis, on the eyes, beak, nares, integument, and shell on a standardized seven-page form modified from an appendix in Berry and Christopher (2001). Length at the carapace midline (MCL) and weight of each tortoise were measured during evaluations, and digital photographs were taken of the eyes, beak, nares, plastron, carapace, and any unusual trauma or lesions. Blood and nasal lavage samples were also collected during health evaluations.

Samples of blood were drawn either by brachial venipuncture or from the subcarapacial site using standard protocols (Hernandez-Divers et al. 2002, Berry et al. 2006). Samples of blood that contained 15% or more of lymph were considered to be suboptimal because of the potential negative impact of dilution (e.g., Gottdenker and Jacobson 1995). Where possible, such samples were repeated with an objective of obtaining 90–100% blood with no lymph or only a trace of lymph (Berry et al. 2005). A nasal lavage was taken using standard protocols (Berry et al. 2006). Blood plasma and nasal samples were shipped to the Mycoplasma Laboratory at the University of Florida to determine the presence of antibodies to *Mycoplasma agassizii* or *M. testudineum* using enzyme-linked immunoassay (ELISA) tests (Schumacher et al. 1993; Brown et al. 1994, 2004; Wendland et al. 2007). Cultures and polymerase chain reaction tests (Brown et al. 2002) were also used.   The laboratory procedures are summarized in Berry (2006).

Three primary databases were constructed for each calendar year. One database is the monthly monitoring with dates and locations in UTMs. The second database summarizes tissue samples obtained and includes data on type of samples obtained (blood plasma, plasma/lymph, and nasal lavage), date of collection, volume of samples, results of ELISA tests for *M. agassizii* and *M. testudineum*, and results of polymerase chain reaction tests for positive or suspect cultures. The first two databases are being transmitted separately to Clarence Everly, permit holder, for the federal U.S. Fish and Wildlife Service permit. They contain all Ft. Irwin-related data sets. The third database contains the data collected from health evaluations, including clinical signs of disease and trauma. This database is still in the process of receiving quality assurance and control and will be provided at a later time.

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Movement Patterns

Two variables relating to movement patterns were calculated for the translocated tortoises. The first variable, dispersal distance, was calculated as the straight-line distance between the point of release and the location furthest from the release point at which the tortoise was located. The second variable, minimum total distance, was calculated as the summation of the straight-line distances between consecutive locations. Both of these measurements were calculated with straight-line distances and, as such, should be considered conservative estimates. Only live tortoises with known locations (i.e., those not dead or missing) as of December 2009 were used in these analyses (n = 68).

To determine the degree of settlement of translocated tortoises, the minimum total distance moved in 2008 was compared to that in 2009 for the 68 tortoises described above. Fidelity to cover sites was also examined (n = 68) by comparing summer (July and August) and winter (December and January) cover site locations for 2008 and 2009. The distance moved each month by these 68 tortoises was also plotted to examine seasonal and annual variation in movements patterns and differences between sexes. Finally, the number of tortoises still remaining on each plot (i.e. within the one square mile boundary of the initial release plots) was compared to the number of tortoises that have dispersed from the plot.

Data Analysis

We used repeated measures ANOVA to examine changes in weight within individual tortoises across seasons after translocation. A post hoc test was used to determine which seasons differed. Only tortoises with weight data for all four seasons (spring 2008, fall 2008, spring 2009, and fall 2009) were used in this analysis (n = 64).

One-way ANOVAs were used to compare movement variables (dispersal distance and minimum total distance) between sexes and among plots. A paired t-test was used to compare minimum total distances between 2008 and 2009. Because tortoises were released at translocation sites in March-early April 2008, we analyzed and compared movements from March-December of 2008 with movements from March-December 2009.

Fisher's exact tests were used to compare cover site fidelity between sexes, as well as death rates between translocation plots, between sexes, and between health categories (healthy, shell disease, URTD, or trauma). Fisher's exact tests were also used to compare the proportion of tortoises still remaining within plot boundaries among translocation sites and among sexes. One-way ANOVAs were used to compare the sizes (MCL) of tortoises that died to those still alive. All statistical tests were conducting using SYSTAT Software version 12.0 (SYSTAT Software Inc. 2007).

RESULTS

Summary of 2008

A total of 82 females and 76 males were translocated from the SEA to plots located in the DWMA. Of the 158 translocated tortoises, 21 females and 17 males were translocated to Plot 1.5, 21 females and 19 males were translocated to Plot 3, 19 females and 20 males were translocated to Plot 5, and 21 females and 20 males were translocated to Plot 8. As of December 2008, 43 of the initial 158 translocated tortoises were found dead (41) or salvaged for necropsy (2), and an additional 15 tortoises were unable to be located at the time and were considered missing. As of December 2008, the locations of 100 live tortoises were known. The sex ratio of these tortoises was 44 females and 56 males.

Health Evaluations

In January 2009, 44 females and 56 males were known to be alive; in December 2009, 32 females and 36 males were known to be alive. Comprehensive health evaluations were conducted on 81 translocated tortoises in the spring of 2009 (April 27 to May 4). Blood plasma and nasal lavage samples were also collected from each of these 81 tortoises. Three of these blood samples (3.7%) were a blood/lymph mixture, with at least 90% of the sample composed of blood; the remaining samples were composed of 100% blood. As of the end of spring of 2009, 55 tortoises had been found dead or salvaged for necropsy and 22 were unable to be located.

Comprehensive health evaluations were conducted on 65 translocated tortoises in the fall of 2009 (October 7 to October 27). Blood plasma and nasal lavage samples were also collected from each of these 65 tortoises. Eight of these blood samples were a blood/lymph mixture, with at least 95% of the sample composed of blood; one sample (from 4499F) was a blood/lymph mixture with 50% of the sample composed of blood; the remaining samples were composed of 100% blood. As of the fall of 2009, 69 tortoises had been found dead or salvaged for necropsy, 20 were unable to be located, and four were unable to be extracted from their cover sites for health evaluations.

Tests for Mycoplasmosis

In the spring of 2009, four (4.9%) of 81 tortoises had positive or suspect ELISA tests for *Mycoplasma agassizii* (Table 1). Three tortoises had suspect tests and one tortoise had a positive ELISA test for *M. agassizii*. Of the four tortoises with positive or suspect ELISA tests for *M. agassizii*, two were located on plot 8, one was on plot 1.5, and one was on plot 3 (Fig. 2). Additionally, two tortoises (2.5%) had positive or suspect ELISA tests for *M. testudineum*. One tortoise had a positive test and the other a suspect ELISA test for *M. testudineum*; both were located on plot 1.5 (Fig. 3). Of the 81 nasal lavage samples collected in the spring, all cultures were negative for both *M. agassizii* and *M. testudineum*.

In the fall of 2009, six (9.2%) of 65 tortoises tested for *M. agassizii* had positive or suspect ELISA tests (Table 1). Three tortoises had positive tests and three tortoises

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had suspect ELISA tests for *M. agassizii*. All six tortoises were located on plots 1.5 or 8 (Fig. 4). Five of these tortoises had previous positive or suspect tests for mycoplasmosis (Table 1). All 65 tortoises tested for *M. testudineum* in the fall had negative ELISA tests (Fig. 5). Two tortoises (4024M and 4257F) which had previously tested positive and suspect, respectively, for *M. testudineum* in spring, were not available to be tested because they had been killed by predators. Results are not yet available for cultures from the 65 nasal lavage samples.

Weight

There was a significant effect of season on measured weight ($F_{3,189} = 132.0$, $p < 0.001$). The post-hoc test revealed weight was greatest in spring 2008 just after translocation, fell in fall 2008, increased back to initial levels in spring 2009, and fell again in fall 2009 (Fig. 6). Weight was not significantly different among the two spring seasons ($p = 0.964$), however it was significantly lower in fall 2009 compared to fall 2008 ($p = 0.001$).

Movements and Fidelity to Cover Sites

Summary statistics for dispersal distance and minimum total distance are reported in Table 2. The tortoise which has moved the most, 4143M translocated to plot 8, has been located on multiple dates just outside the Ft. Irwin boundary fence in the SEA, 12.6 km from its initial release location, and has moved a total distance of at least 18.8 km since its release. Overall, males have dispersed further from their release locations compared to females (means = 3256.4 m for males, 1517.9 m for females; $F_{1, 66} = 12.3$, $p = 0.001$). Males also had greater total distances moved compared to females (means = 6858.4 m for males, 3492.0 m for females; $F_{1, 66} = 23.9$, $p < 0.001$). Although the total distances that remaining live tortoises moved was greater on plots 5 (mean = 7403.3 m) and 3 (6020.8 m) compared to plots 1.5 (4899.8 m) and 8 (4778.4 m), these differences were not statistically significant ($F_{3, 64} = 1.5$, $p = 0.224$). Similarly, dispersal distance did not vary among translocations plots ($F_{3, 64} = 1.1$, $p = 0.351$).

The minimum total distance moved in 2009 (mean = 1854 m) was significantly less than that in 2008 (mean = 3222 m; $t_{67} = 4.837$, $p < 0.001$). Regarding use of cover sites, five of 68 (7.4%) tortoises have used the same cover site every season (summer and winter of 2008 and summer and winter of 2009), and an additional 36 (52.9%) tortoises have used the same cover site in at least two of these seasons. In contrast, 27 (39.7%) of 68 tortoises had minimal fidelity to sites and used a different cover site for each season examined. Females were more likely to use the same cover sites than males (Fisher's exact test, $p < 0.001$); 22 of 38 males used different cover sites for each season compared to just 5 of 30 females.

Eighteen tortoises still remain within the boundaries of their initial release plots. On plot 1.5, six tortoises still remain on the plot, compared to two on plot 3, one on plot 5, and nine on plot 8 (Table 3). However, when considering the total number of tortoises alive at each translocation site, the proportion of tortoises on plot to those off plot is not

significantly different among translocation plots (Fisher's exact test, $p = 0.801$). Additionally, the number of females remaining on the plots does not differ from the number of males (Fisher's exact test, $p = 1.00$).

There has been marked seasonal variation in movement. Tortoises moved the greatest distances in the spring months immediately following translocation (Fig. 7). Tortoises travelled large distances in the spring of 2009 and, to a lesser extent, in the fall seasons of 2008 and 2009. Tortoises were least active during summer and winter months. The distances moved in 2009 were noticeably less than those in 2008 for both the spring and fall seasons, respectively (Fig. 7). Corroborative with the previous analyses, in general males moved more than females in each month.

<div align="center">Mortality</div>

As of December 2009, 70 (44.3%) of the initial 158 tortoises had been found dead (68) or had been salvaged for necropsy (2). For 2009, the death rate of the 115 remaining tortoises (27 of 115), was similar (23.5%) but slightly lower than that of 2008, the year in which tortoises were first translocated (43 of 158, 27.2%). In 2009, 24 tortoises were probably killed by coyotes or other canids, and the causes of death were unable to be conclusively determined for three tortoises (Table 4). One of these tortoises, 4644F, had been missing for six months before its remains were located. When located, the carcass was crushed, the head and limbs were still remaining and intact, and there were no obvious signs of scavenging or predation (tooth marks, gnashes, tears). A relatively well-used, Bureau of Land Management-designated dirt road was approximately 300 m from where the carcass was located. The most likely cause of death, based on the condition of remains, was crushing by a vehicle. The tortoise was probably transported to the site by a person to conceal the death. The other two tortoises, 4548F and 4441M, were found dead in the open, with no evidence of predation; the head and limbs were still intact. Both tortoises moved large distances during the summer months prior to their deaths, and the expenditures of energy may have contributed to the causes of death.

Combining data for both sexes and both years, death rates varied significantly among translocation plots (Fisher's exact test, $p < 0.001$); 12 of the tortoises that died were located on plot 1.5, 24 were located on plot 3, 26 were located on plot 5, and eight were located on plot 8. More dead tortoises were females (42) than males (28), but the difference was not statistically significant (Fisher's exact test, $p = 0.126$). Death rates did not differ among health categories (i.e. groups to which tortoises were assigned prior to translocation; Fisher's exact test, $p = 0.7918$); 21 tortoises with clinical signs of shell disease died, followed by 17 tortoises with clinical signs of trauma, 16 healthy tortoises, and 16 tortoises with clinical signs of URTD. The size of a tortoise was not related to risk of death, as the average carapace length of tortoises that died did not differ from those still alive ($F_{1,137} = 1.719$, $p = 0.192$). However, tortoises that died in 2009 were larger than those that died in 2008 (mean MCL $\pm$ SE $= 246.5 \pm 4.7$ mm vs. 231.7 ± 3.7; $F_{1,68} = 6.05$, $p = 0.016$). Males were driving the statistical difference between years. Males dying in 2009 were significantly larger than those dying in 2008 (MCL $= 262.5 \pm 7.5$ mm vs. 226.3 ± 8.1 mm; $F_{1,26} = 10.67$, $p = 0.003$), whereas sizes of females were not

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significantly different between years (MCL = 226.5 ± 4.5 mm in 2009 vs. 234.0 ± 2.8 mm in 2008; $F_{1,40}$ = 1.98, p = 0.167).

For data from 2009 alone, there was a significant effect of translocation plot on death rates (Fisher's exact test, p = 0.005; see Table 4), with again the highest rates on plots 3 and 5. Seven of the remaining 32 tortoises on plot 1.5 died in 2009, compared to six of the remaining 16 on plot 3, ten of the remaining 15 on plot 5, and four of the remaining 31 on plot 8. In 2009 alone, there was no difference in death rates among the sexes (Fisher's exact test, p = 0.501); 11 of the remaining 45 females died compared to 16 of the 51 remaining males.

Three of the 43 tortoises found dead in 2008 (4014F, 4720F, 4011F) previously had suspect ELISA tests for mycoplasmosis. In 2009, eleven of the 27 tortoises found dead had previous positive or suspect tests for *M. testudineum* (2533M positive in spring 2009; 4024M suspect in spring 2009; 4136F, 2023M, 2557F, 4179F, 4644F, 4085F, 4106M, 4361M, and 4442M suspect in fall 2008). Several of these tortoises had suspect ELISA tests for *M. testudineum* from fall 2008, a season with an unexpectedly high number of suspect tests for this species (Berry et al. 2009).

Of the initial 158 translocated tortoises, 20 tortoises (17.4%, 20/115) were unable to be located in December 2009 and are considered missing. Of the 20 currently missing tortoises, six had their radiotransmitters detached by a predator or otherwise, and the radiotransmitter signals of the remaining 14 are inaudible at previously known locations. As of December 2009, the locations of 68 live tortoises were known. The sex ratio of these tortoises (32 females and 36 males) is not significantly different than the sex ratio in December 2008 (X^2 = 0.05, df = 1, p = 0.82).

DISCUSSION

The results for the second year of the SEA translocation project reveal that the death rate of translocated tortoises is still high. In January 2009, 115 tortoises were known to be alive or missing. By the end of 2009, 23.5% of the tortoises had died and an additional 17.4% either remained missing or were newly missing. Overall, in December 2009, 40.9% had either been found dead or were still missing. Combining the data from 2008 and 2009, from the time of initial translocation of 158 tortoises in March-April of 2008, 70 (44.3%) tortoises have died and an additional 20 (12.7%) are missing.

As in the first year, predation by coyote continues to be the primary cause of deaths (Table 4). Overall, death rates were highest in the months immediately following translocation in 2008 and in the spring and fall of 2009 (Fig. 8). These time frames correspond to when tortoises were active and spending more time above-ground (i.e., just after translocation to a novel location, foraging in spring, and seeking mating opportunities in late summer/fall; see Fig. 7). Correspondingly, death rates were lowest in the winter of 2008 and summer of 2009 when tortoises spent more time in well-developed cover sites. While death rates were higher among females and smaller tortoises in 2008 (Berry et al. 2009), this was not the case in 2009. There is an apparent

trend that predation was initially highest among small females, but now larger males are also targets of predation (Fig. 9). This pattern may be an artifact of fewer females on the study plots after the initial wave of predation, or it may signify that coyotes have increased their abilities to successfully prey upon the larger male tortoises.

Disease may be an important factor in predation. A substantial portion of the tortoises that died in 2009 (40.7%) had previously tested positive or suspect for mycoplasmosis after being translocated. This figure includes all tortoises in the project, regardless of health group. We need to conduct further research and analysis on effects of health and disease on survival.

Between 2008 and 2009, the proportion of tortoises with suspect or positive ELISA tests increased for *M. agassizii* but decreased for *M. testudineum*. In the spring and fall of 2009, 4.9% and 9.2% of tortoises had positive or suspect ELISA tests for *M. agassizii*. These proportions of ELISA suspect and positive tortoises for *M agassizii* are higher than in 2008 (Berry et al. 2009) and higher than reported for 669 tortoises sampled in and around the SEA in 2007 (Berry and Mack 2008). Similar to findings in 2008 (Berry et al. 2009), tortoises with positive or suspect tests for *M. agassizii* are concentrated on or near plots 1.5 and 8 (Figs. 2 and 4). Three individuals had multiple positive or suspect tests for *M agassizii* during 2008 and 2009 (Table 1).

In the spring of 2009, two tortoises (2.5%) had positive or suspect ELISA tests for *M. testudineum*. These two tortoises were killed by predators during summer and thus could not be sampled in fall. All remaining tortoises had negative tests for *M. testudineum* in the fall. While the proportion of tortoises with positive or suspect tests in spring of 2008 and 2009 are similar, there is a notable discrepancy when comparing rates from the fall seasons of the same years, 31.5% in 2008 vs. 0% in 2009 (Berry et al. 2009). Shifts from positive or suspect ELISA tests for *M. testudineum* to negative status may be due to the quality of blood samples and dilution with lymph, the virulence of *Mycoplasma* spp., timing of sampling in fall, variations in the tests, or other factors.

Weight can be an important indicator of overall health (Henen et al. 1998; Christopher et al. 1999, 2003; Berry et al. 2002). Weight may reflect hydration status, expenditures of energy, availability of food and water, ability of a tortoise to find food and water, and health status. The seasonal differences in weight between spring, summer, and fall observed in the SEA tortoises are comparable to previous studies of desert tortoise populations; weight is generally higher in the spring than in fall (Christopher et al. 1999). However, the decrease in weight between the 2008 and 2009 fall seasons is of concern, and weight should continue to be monitored in conjunction with health assessments or more frequently.

The data on movement patterns of translocated tortoises will be useful for determining the appropriate size for future translocation release sites, the effects of translocation on behavior, and potentially, the effects of habitat type and quality on behavior. Our preliminary results show that translocated tortoises may disperse up to 13 km from their release location within the first two years. Therefore future managers and

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scientists responsible for designing and managing translocations should consider translocation sites with a buffer zone of suitable habitat at least this large in each direction. Additionally, only 18 of the initial 158 tortoises have not dispersed from the one square mile release plots, indicating the need for translocation sites with much larger areas of quality habitat. In some regards, the translocated tortoises in this study have exhibited movement patterns similar to those reported in previous studies. Differences exist between sexes, with males moving more than females (Berry 1986, O'Connor et al. 1994), and differences exist between seasons, with higher activity levels in the spring and fall compared to the summer and winter when temperature extremes and/or lack of water limit above ground activity (see Fig. 7; Henen 1997, Henen et al. 1998, Nagy and Medica 1986). Tortoises moved less in 2009 compared to 2008, the year in which tortoises were first translocated, and some tortoises have repeatedly used the same cover sites. These results suggest that some translocated tortoises have begun to "settle" into the new sites and may be establishing home ranges, a first step in assimilating with the resident population. Also of note is that movements were greater (statistically in 2008 [Berry et al. 2009], but not for both years combined) on plots 3 and 5 compared to plots 1.5 and 8. Plots 3 and 5 also had higher death rates, and the possible relationship between increased movement and risk of mortality deserves further attention.

Continued work on this project will be directed at addressing the previously stated objectives. Health, including prevalence of mycoplasmosis and other diseases, weight, and general condition, of translocated tortoises will continue to be monitored at regular intervals by incorporating clinical signs of disease recorded during health evaluations with ELSIA test results. Signs of trauma and shell disease, along with signs of URTD, will be analyzed to determine the effects of translocation and anthropogenic impacts on these variables and whether or not incidences of disease and trauma have increased since translocation. The survival and movement patterns of translocated tortoises will continue to be monitored to assess the success of translocation. Finally, habitat characteristics, including topography, foraging and cover site availability, and levels of anthropogenic impacts, will be compared between initial capture sites and translocation release sites as well as among the four translocation plots.

RECOMMENDATIONS

1. This report does not contain a complete analysis of all health data for the translocated tortoises, between the time of translocation and December 2009, e.g., the analysis of changes in clinical signs between seasons and years. This analysis will be conducted as time permits.

2. The abnormally high death rates that began shortly after the initial translocation in March and April of 2008 have continued, and have again risen to high levels in the fall of 2009. The high death rates are primarily the result of canid (coyote) predation. The result has been loss of a significant portion of the sample population. Scientists have reported high death rates of tortoises from predators in other Ft. Irwin studies and in other research projects in California and Nevada during the last few years, and have summarized findings in a draft manuscript for the open literature (Esque et al.,

unpublished paper).   Little or no action has been taken (depending on the site) by managing agencies to mitigate the impact to tortoise populations. In our study, which is in critical habitat, we designed the health and disease project to provide valuable information for recovery efforts and to mitigate some impacts of the translocation. Unfortunately, the high death rates have compromised the quality and quantity of data, as well as our ability to achieve many of the initial research objectives. Many elements of the research project will need to be repeated in future translocation efforts using a more robust sample if we are to achieve our initial goals.

3. Based on the unpublished manuscript by Esque et al. on predation, the high death rate from translocatees appears to be influenced by proximity to urban/rural areas and topographical features.   There may be other local factors that contribute to elevated populations of coyotes and other predators of tortoises, including proximity to old agricultural fields, roads, trails, and recreation. The younger and smaller subadult and adult tortoises are probably more vulnerable than larger, older tortoises. We need to explore and analyze any and all factors that may affect predation of tortoises and the success of the future translocation of tortoises from the Western Expansion Area to the Western Expansion Translocation Area prior to moving tortoises.

4. Based on unexplained deaths of two tortoises during 2009 (4548F in September 2009, 4441M in August 2009), we may need to increase the health sampling of tortoises from twice per year to three or four times per year or once per season. Additional sampling may be limited to weighing the tortoises and conducting an abbreviated health evaluation (no drawing of blood or taking a nasal lavage).

5. The ELISA test for *M. testudineum* needs to be validated for *G. agassizii*. (This recommendation is repeated from Berry et al. [2009]). This research project is a very high priority, is essential to resolving questions about translocation, and should be undertaken with appropriate financial support as soon as possible. Until the test is validated, we will have continuing questions about the test and cut-off points for suspect and positive titers. We will be able to make better decisions about translocatees if the validation research has been completed.

6. Quality of Habitat (a recommendation repeated from Berry et al. [2009]). The quality of habitat where translocated tortoises were placed is a topic that needs to be addressed as soon as possible. Were the locations appropriate and if not, why not? As we can see from our data, death rates were highest on plots 3 and 5 and movements of tortoises from their original release points were highest on plot 3 and lower on plots 1.5. The soils, surficial geology, vegetative cover and composition of shrubs, elevation, and potential food sources should be evaluated retrospectively for each release site and for the original home sites as soon as possible to reveal critical factors essential to improving the chances for successful translocations. We plan to initiate such a study in 2010.

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Table 1. Previous ELISA test results for desert tortoises with positive or suspect tests in 2009. Green cells represent negative status, orange cells represent suspect, and red cells represents positive.

ID	Sex	Plot	*M. agassizii*				*M. testudineum*			
			Sp08	Fa08	Sp09	Fa09	Sp08	Fa08	Sp09	Fa09
4410	M	8	red	red	red	red	green	orange	green	green
2040	M	8	green	red	orange	red	green	green	green	green
4166	F	1.5	red	orange	orange	orange	green	green	green	green
4423	F	3	green	green	orange	green	green	green	green	green
2533	M	1.5	green	green	green	N/A	green	green	red	N/A
4024	M	1.5	green	green	green	N/A	green	green	orange	N/A
4257	F	1.5	green	green	green	red	green	green	green	green
4300	M	1.5	green	green	green	orange	green	red	green	green
4611	F	8	green	green	green	orange	green	orange	green	green

Table 2. Summary statistics for movement variables of translocated desert tortoises from March 2008 through December 2009.

	Maximum (m)	Minimum (m)	Mean (m)	SD	N
Dispersal distance	12,567.3	275.2	2,438.3	2,203.6	68
Minimum total distance	18,814.4	1,070.7	5,274.2	3,280.7	68

Table 3. Counts of translocated desert tortoises that are still remaining (On Plot) or that have dispersed (Off Plot) from the boundaries of their initial release plots.

	On Plot		Off Plot		
Plot	M	F	M	F	Total
1.5	5	1	8	10	24
3	1	1	5	3	10
5	0	1	5	1	7
8	4	5	8	10	27

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Table 4. Summary of translocated desert tortoises found dead in 2009.

ID	Sex	Plot	MCL	Date Located	Notes
2038	F	1.5	214	22-Sep-09	Likely predation by coyote
4136	F	1.5	201	20-Oct-09	Likely predation by coyote
4162	F	1.5	227	22-Sep-09	Likely predation by coyote
4554	F	1.5	211	4-May-09	Likely predation by canid
2533	M	1.5	260	13-Aug-09	Likely predation by coyote
4024	M	1.5	255	22-Sep-09	Likely predation by coyote
4060	M	1.5	275	22-Oct-09	Likely predation by coyote
2557	F	3	206	4-May-09	Likely predation by coyote
4179	F	3	240	24-Feb-09	Likely predation by coyote
2023	M	3	267	22-Apr-09	Likely predation by coyote
4158	M	3	266	22-Apr-09	Likely predation by coyote
4239	M	3	274	22-Apr-09	Likely predation by coyote
4640	M	3	263	4-May-09	Likely predation by coyote
2550	F	5	211	23-Sep-09	Likely predation by coyote
4288	F	5	229	18-Mar-09	Likely predation by coyote
4548	F	5	227	23-Sep-09	Cause of death unknown; no signs of predation
4556	F	5	280	21-Oct-09	Likely predation by coyote
4644	F	5	232	23-Apr-09	Crushed shell, probable vehicle kill
4073	M	5	262	14-Aug-09	Likely predation by coyote
4108	M	5	266	14-Apr-09	Likely predation by coyote
4129	M	5	284	23-Sep-09	Likely predation by coyote
4291	M	5	262	21-Oct-09	Likely predation by coyote
4442	M	5	273	08-Dec-09	Likely predation by coyote
4085	F	8	223	15-Apr-09	Likely predation by coyote
4106	M	8	265	16-Apr-09	Likely predation by coyote
4361	M	8	211	15-Apr-09	Likely predation by coyote
4441	M	8	246	18-Aug-09	Cause of death unknown; no signs of predation

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Fig 1. Overview map of the Ft. Irwin Southern Expansion Area and translocation plots.

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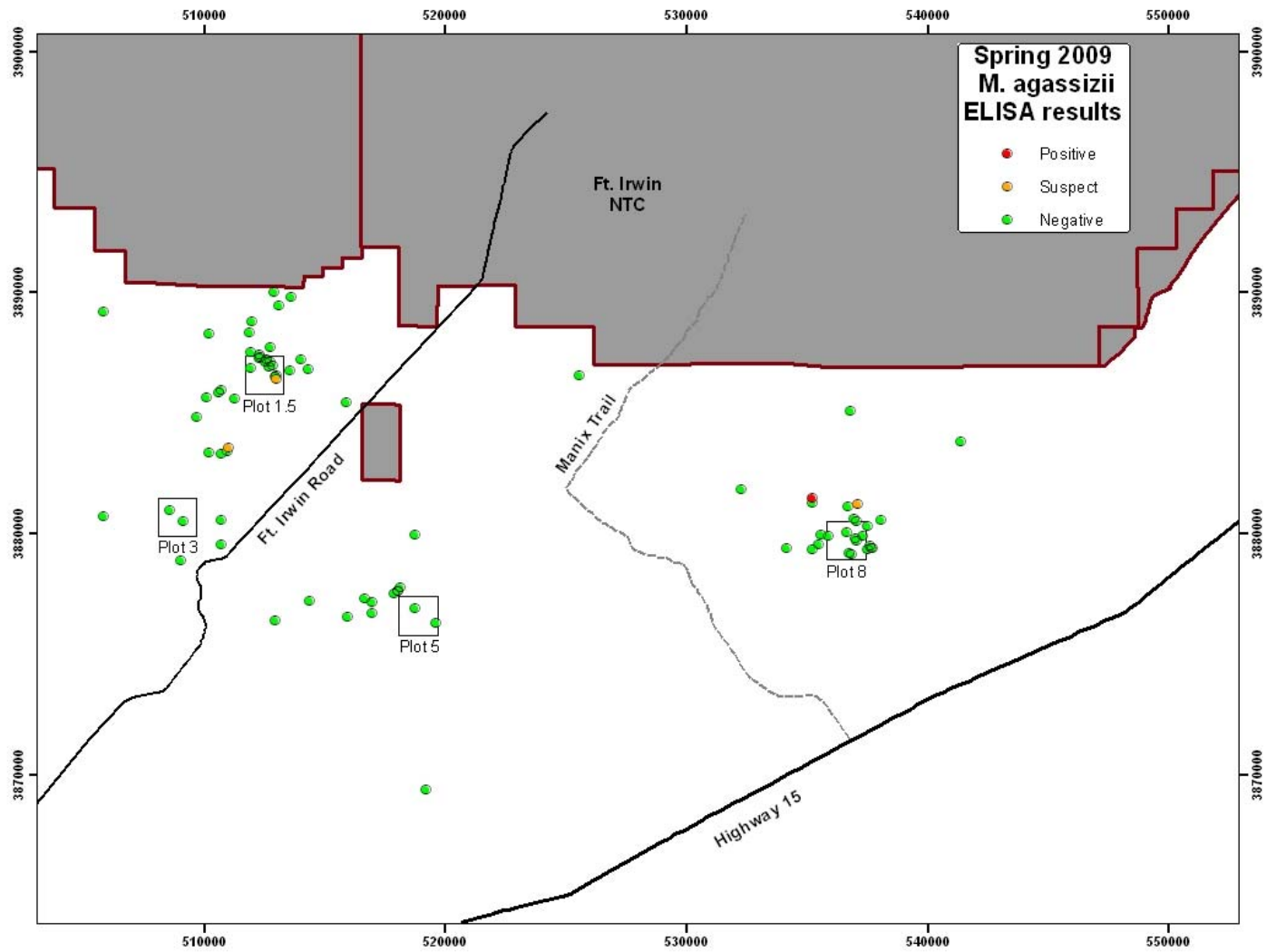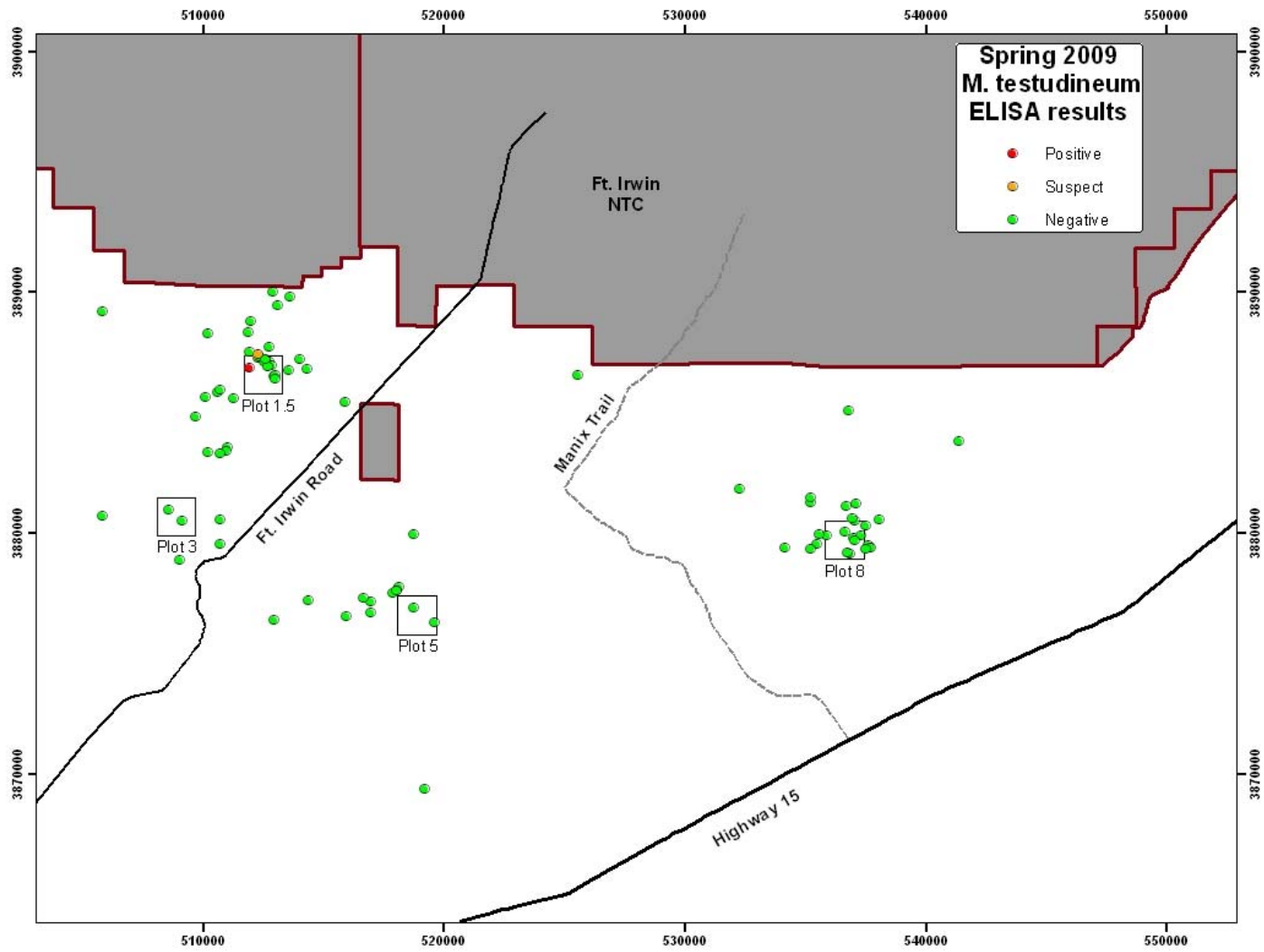
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Fig 2. Results of ELISA tests for *Mycoplasma agassizii* from desert tortoises sampled in spring of 2009.

Fig 3. Results of ELISA tests for *Mycoplasma testudineum* from desert tortoises sampled in spring of 2009.
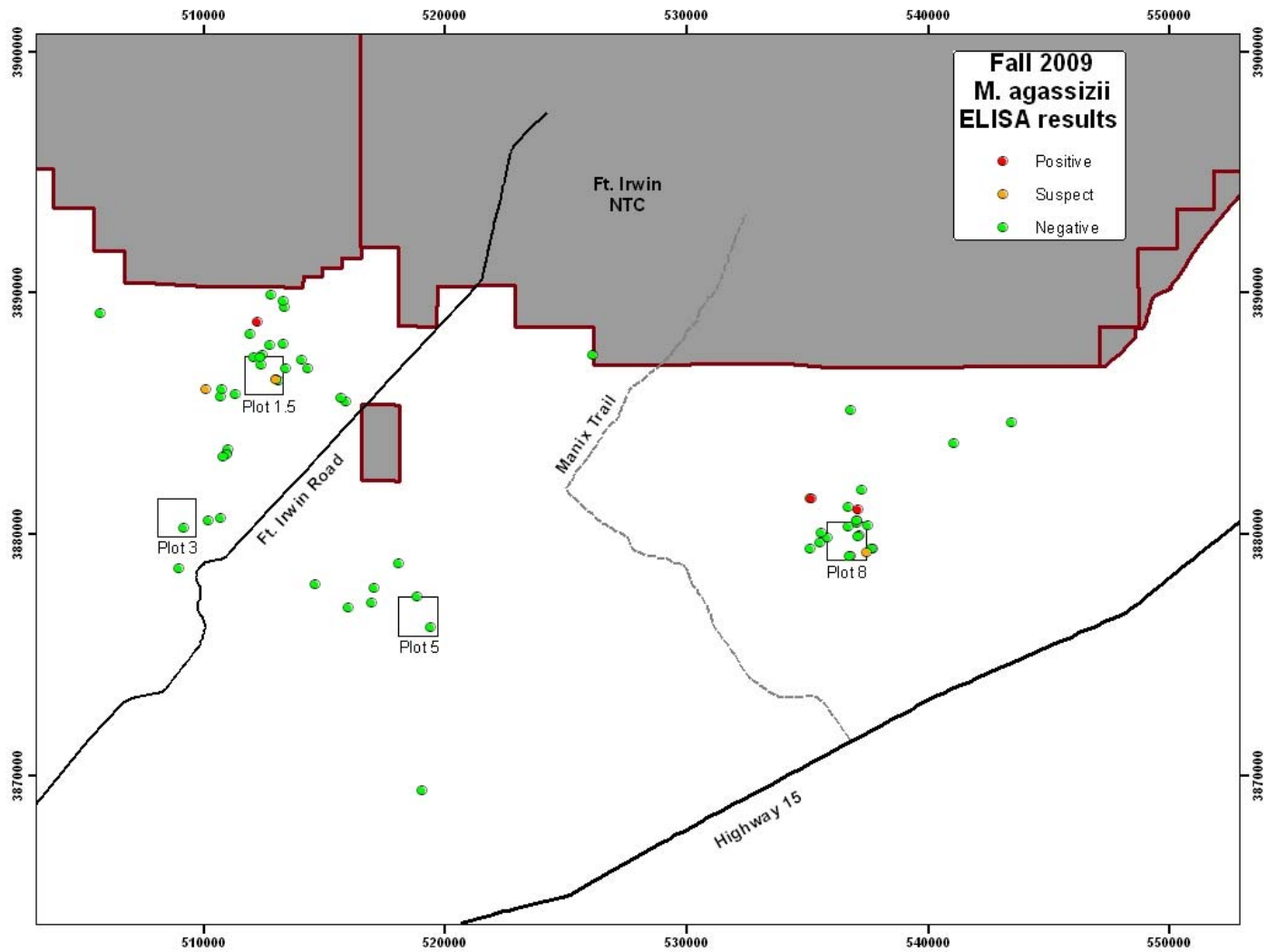
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Fig 4. Results of ELISA tests for *Mycoplasma agassizii* from desert tortoises sampled in fall of 2009.

Fig 5. Results of ELISA tests for *Mycoplasma testudineum* from desert tortoises sampled in fall of 2009.
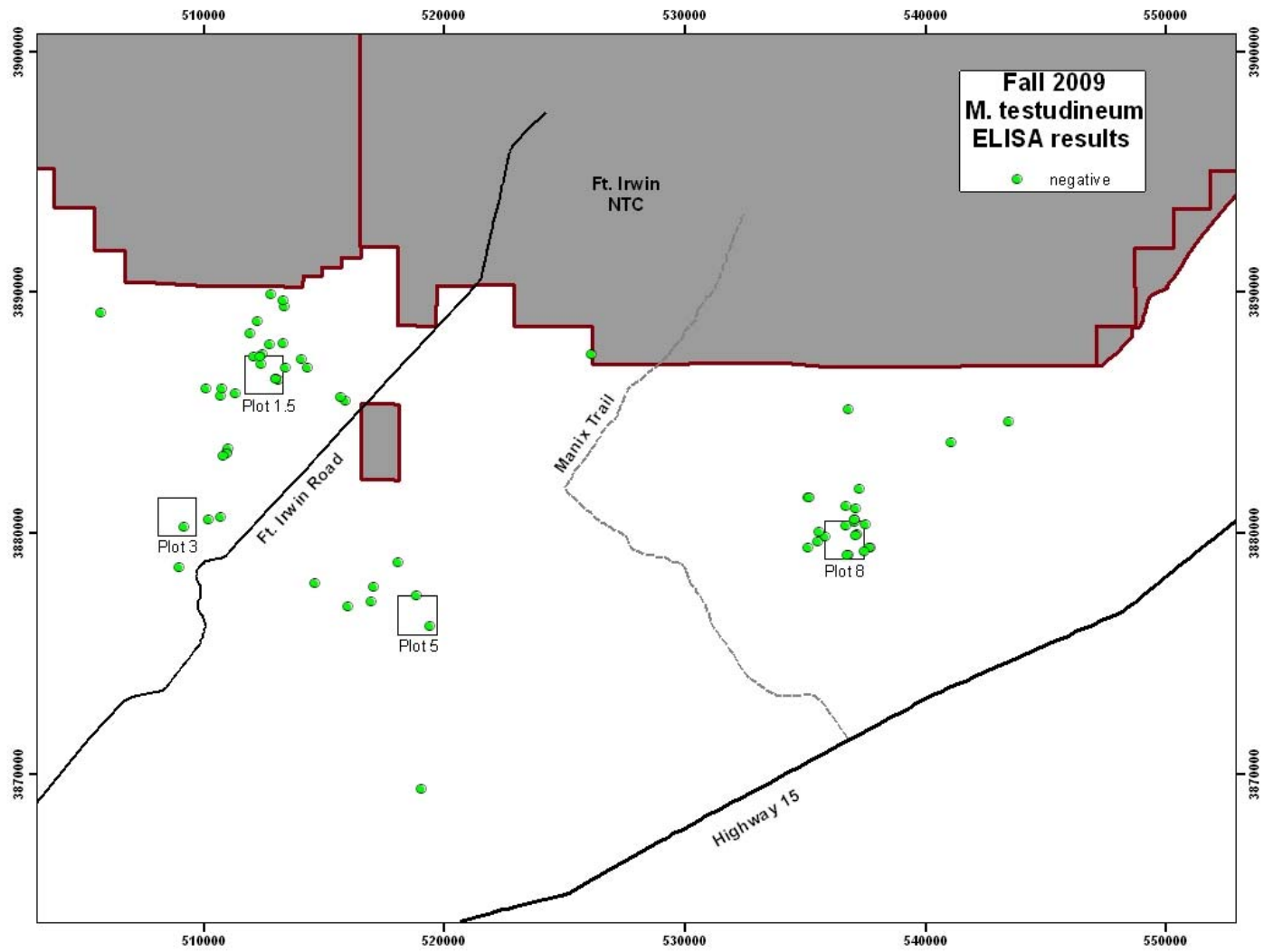
Gowan and Berry. 2009. Progress Report on the Health Status of Translocated Tortoises in the Southern Expansion Area.

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Fig 6. Mean (± SE) weight of desert tortoises (n=64) in four seasons post-translocation.

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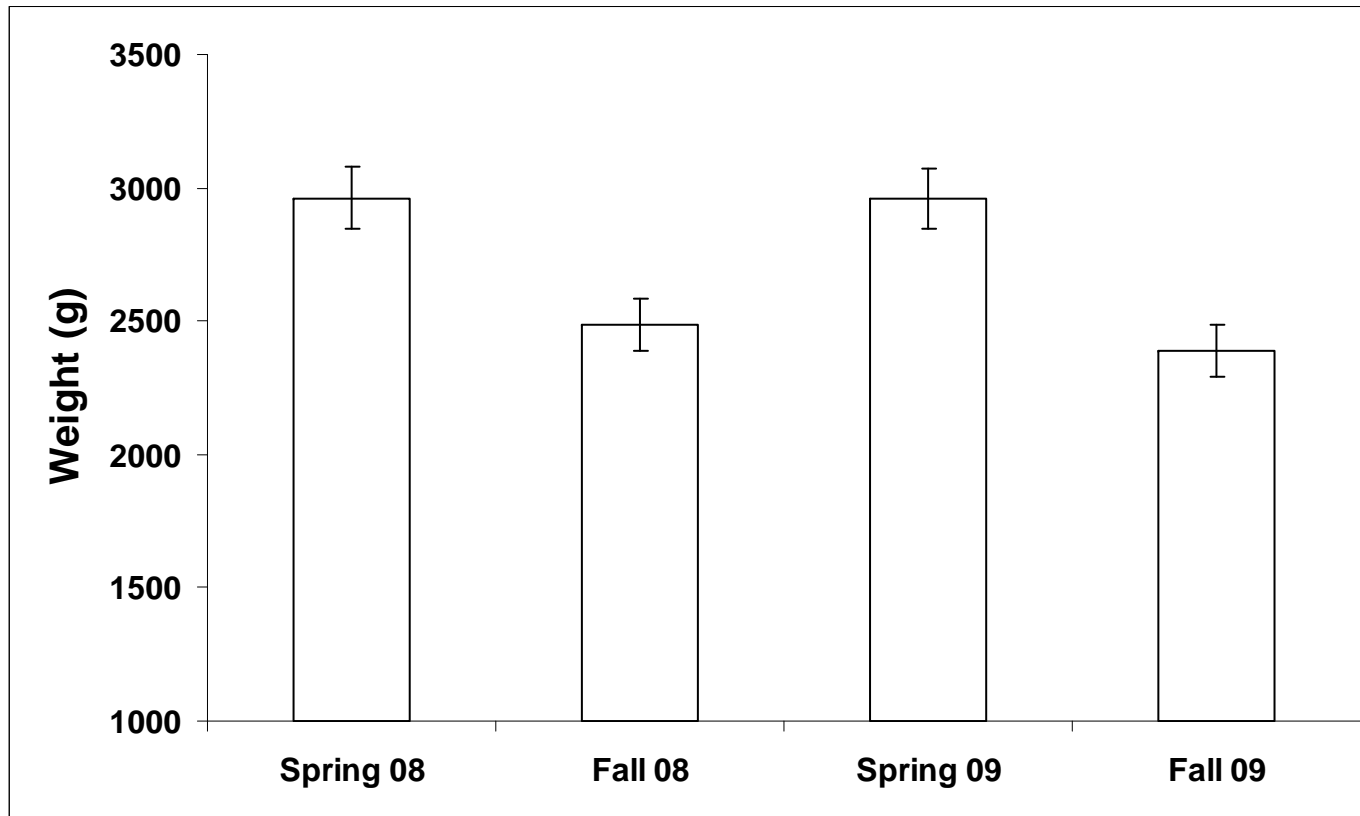
Fig 7. Mean (± SE) distances moved by desert tortoises for each month after translocation.

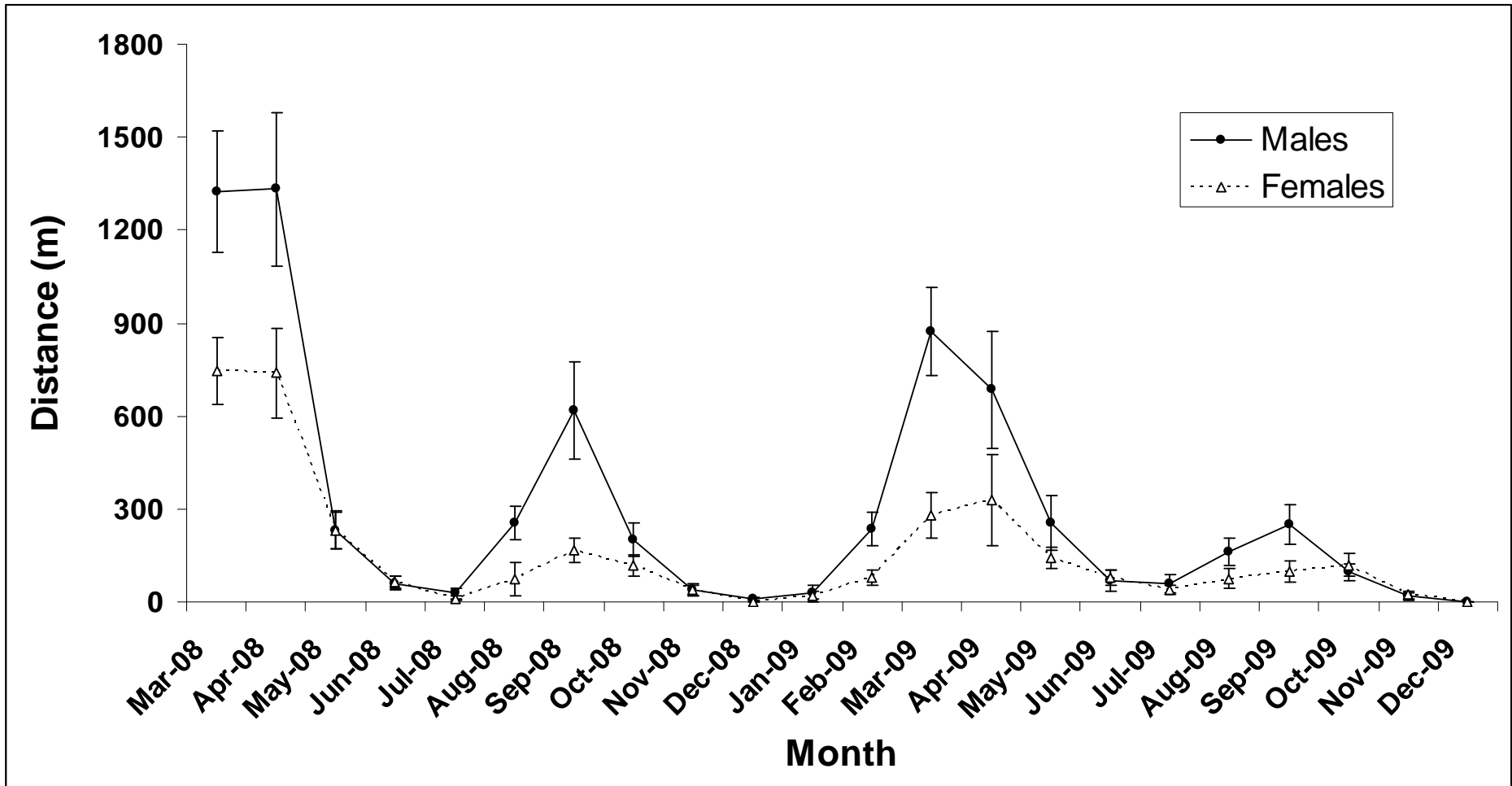Gowan and Berry. 2009. Progress Report on the Health Status of Translocated Tortoises in the Southern Expansion Area.

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Fig 8. Percent of desert tortoises found dead (# dead/# remaining) by season for the first 20 months after translocation.
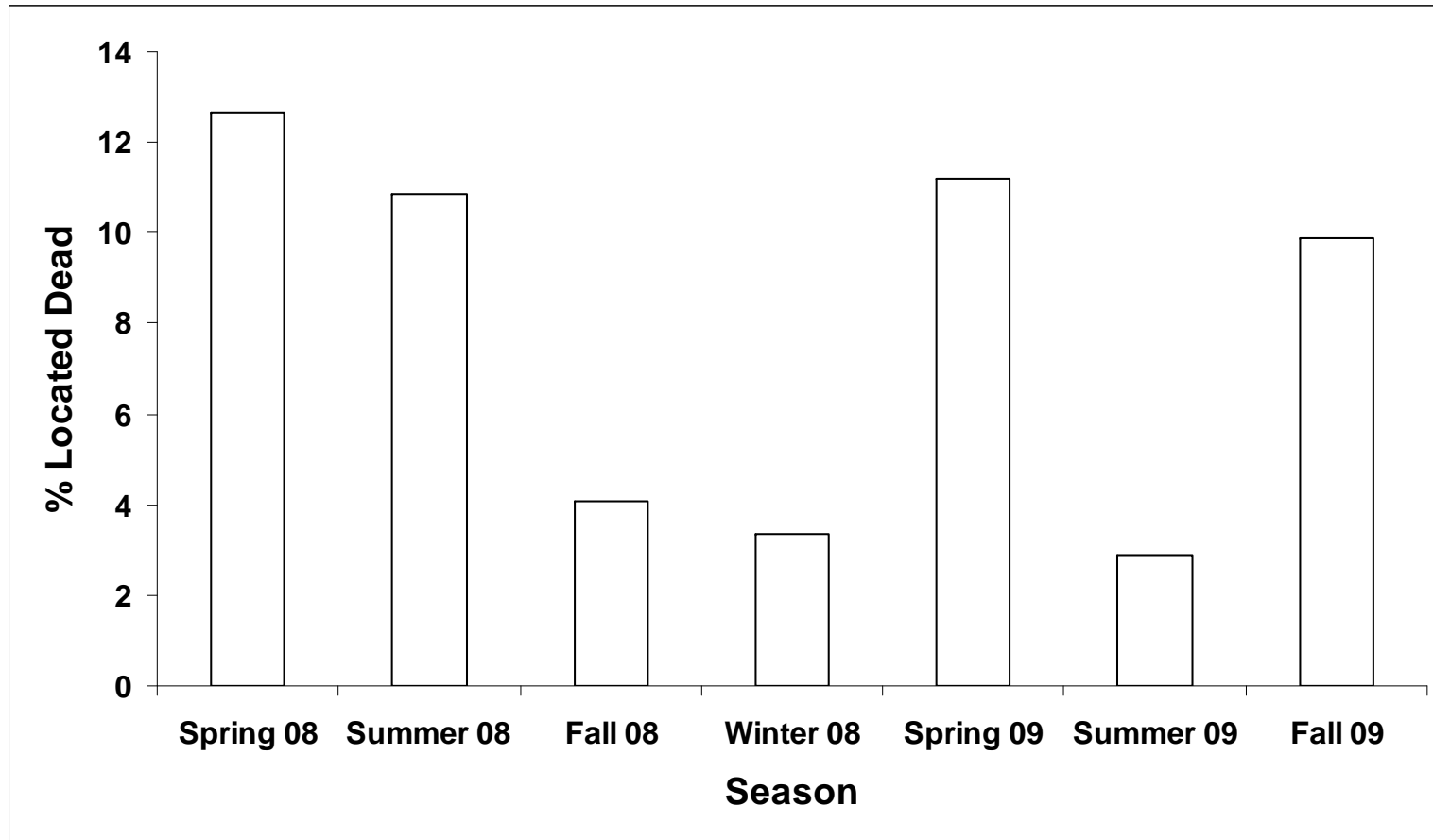
Fig 9. Mean MCL (carapace length at midline, mm) of desert tortoises located dead for each month after translocation. Note the increase in size of males found dead over time and the decrease in size of females.