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Heavy Metal Concentrations in Mojave Desert Tortoises (*Gopherus agassizii*) Related to a Mitigation Translocation Project, Ivanpah Valley, California, USA

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Abstract.—**The Mojave Desert Tortoise (*Gopherus agassizii*) is listed as threatened under the U.S. Endangered Species Act because of subsidized predation by other species, loss and degradation of its habitat owing to human activities, and disease. Potential exposure of toxic substances on *G. agassizii* that possibly impede recovery, however, have not been thoroughly investigated. To quantify concentrations of several heavy metals and examine possible adverse effects of heavy metal toxicity on *G. agassizii*, we analyzed blood samples using the Dried Blood Spot (DBS) method and soil samples from their locations in the Ivanpah Valley, California, USA. In most cases, heavy metal concentrations in blood never or rarely exceeded minimum detection levels (typically, 0%–7% of samples in a given season). In soils, several heavy metals (e.g., arsenic, lead, and thorium) exceed average crust composition, but none exceeded soil health guidelines. Furthermore, lead, selenium, iron, and arsenic concentrations were lower than, or within, published ranges for turtles, reptiles, and other vertebrates. We found a positive relationship between survival and selenium and iron concentrations but no relationship between metal concentrations and health indicators such as body condition and disease prevalence. Our results suggest that *G. agassizii* in our study area were not exposed to toxic levels or suffered adverse effects of heavy metals. The DBS method is minimally invasive and effective for the collection of blood samples from *G. agassizii*. Further analyses should explore how well samples collected by the DBS method reflect metal concentrations in other tissues.**

Key Words.—arsenic; cutaneous dyskeratosis; dried blood spot; endangered species; iron; lead; selenium; upper respiratory tract disease

Introduction

The Mojave Desert Tortoise (*Gopherus agassizii*) is listed as threatened under the U.S. Endangered Species Act. The primary threats identified include habitat loss and degradation due to anthropomorphic activities such as urbanization, military training, mining, and energy production (Lathrop 1983; Prose 1985; Lovich and Bainbridge 1999; Scott et al. 2020). These activities are thought to play a role in the increased spread and incidence of disease, subsidized and opportunistic predation, and increased mortality rates (Jacobson et al. 1991; Hunter et al. 2003; Berry et al. 2006; Cypher et al. 2018). The diversity of threats and an incomplete understanding of their contribution to population declines hampers recovery efforts (Averill-Murray et al. 2012). To mitigate the potentially harmful effects of some of these activities, particularly military training and renewable energy development, the current conservation strategy for *G. agassizii* is primarily translocation of individuals (Field et al. 2007; Esque et al. 2010; Lovich and Ennen 2011; Nussear et al. 2012; Lovich and Ennen 2017). Many studies following translocation efforts are now focusing on the effect translocation has on biological parameters and overall mortality rates of individuals (Drake et al. 2012; Farnsworth et al. 2015; Hinderle et al. 2015; Brand et al. 2016; Cypher et al. 2018).

The effects of several environmental (e.g., drought, temperature, and disease) and anthropogenic (e.g., subsidized predation and collisions with vehicles) factors on tortoise survival following translocation are well documented (Esque et al. 2010; Nussear et al. 2012; Lovich et al. 2014; Dickson et al. 2019). The adverse effects of exposure to toxic substances (e.g., heavy metals) on tortoises, however, have not been investigated in depth (Martínez-López et al. 2010).

To date, heavy metal toxicology studies on chelonians focus mostly on sea turtles (Pople et al. 1998; Saeki et al. 2000; Sakai et al. 2000; Kenyon et al. 2001; Roe et al. 2011). Because concentration levels of metals are necessary for successful embryonic development, the earliest studies focused on heavy metal monitoring in sea turtle eggs (Stoneburner et al. 1980; Sakai et al. 1995). The freshwater turtle and other reptile literature includes many studies that examine cause and effect relationships from point and non-point source pollution (Clark et al. 1998; Burger 2002; Henny et al. 2003; Bishop et al. 2010; Di Geronimo et al. 2018). The presence of chemical contaminants in sea turtles such as polycyclic aromatic hydrocarbons, polychlorinated biphenyls, and organochlorine pesticides are also receiving increased attention (Alam and Brim 2000; Innis et al. 2008; Harris et al. 2011). A much smaller body of literature exists that has evaluated toxicological biomarkers of exposure to heavy metals and chemical contaminants in terrapins or tortoises (Burger 2002; Allender et al. 2015).

Studies that focus on *G. agassizii* have found higher concentrations of environmental contaminants in diseased tortoises than in healthy ones (Jacobson et al. 1991; Kristin Berry et al., unpubl. report), suggesting that these potentially toxic substances negatively affect tortoise health and survival (Chaffee and Berry 2006). Tissue samples collected from a mining-affected area (Kelly-Rand Mining District, Kern County, California, USA) and sites on or adjacent to military bases (Fort Irwin National Training Center and Edwards Air Force Base, USA) contain more bioaccumulated arsenic in scutes, and more exogenous particles in scute and lung tissue, compared with samples collected from areas of minimal land disturbance (Andrea Foster et al., unpubl. report). Several health-related factors with plausible links to contamination affect the morbidity and mortality of free-ranging *G. agassizii*. Cutaneous dyskeratosis (CD, or shell disease) is characterized by shell lesions (Jacobson et al. 1994, 2014; Homer et al. 1998; Christopher et al. 2003), and the major causes are nutritional deficiencies and environmental toxicosis affecting keratinized tissues (Berry 1997; Bruce Homer et al., unpubl. report). Elevated concentrations of various heavy metal toxicants, especially arsenic, are found in tissues of *G. agassizii* showing clinical signs of CD (Jacobson et al. 1994; Seltzer and Berry 2005; Bruce Homer et al., unpubl. report). Tortoises with and without CD have comparable copper, cadmium, and lead residue concentrations in the liver, but diseased tortoises have much higher concentrations of iron and mercury (Jacobson et al. 1991). Arsenic is understood to be a major cause of the high incidence of CD (Kristin Berry et al., unpubl. report). Other heavy metals associated with mining activities and vehicular traffic on nearby roads (e.g., gold, cadmium, mercury, lead, antimony, and tungsten) are locally abundant and occur in higher concentrations than arsenic in soils and/or plants in the region, especially in plants considered preferred dietary items for tortoises (Chaffee and Berry 2006). Desert Tortoises with CD show elevated concentrations of toxicants (e.g., barium, calcium, cadmium, chromium, magnesium, molybdenum, nickel, phthalates, and selenium) in the liver, kidneys, and plasma and/or nutritional deficiencies, such as low copper, zinc, selenium, and plasma vitamin A (Homer et al. 1998; Bruce Homer et al., unpubl. reports).

Another health-related factor that affects tortoise populations is Upper Respiratory Tract Disease (URTD), which is caused by two identified species of *Mycoplasma* bacteria and is one of the most extensively characterized infectious diseases of chelonians (Jacobson et al. 1991; Brown et al. 1994, 1995). The increasing prevalence of URTD, CD, and other signs of disease in *G. agassizii* and the potential role of adverse toxicological effects of metal contamination in their etiology require further investigation (Jacobson et al. 1991, 1994, 2014; Sandmeier et al. 2009). Information about emerging infectious diseases and environmental contaminants can be used to assess and manage the overall health of *G. agassizii* and their ecosystem (Bruce Homer, pers. comm.).

To establish baselines for high-quality habitats across the range of *G. agassizii*, it is essential to understand landscape-scale patterns and concentrations of potentially toxic substances, in addition to acute toxicity levels. Chaffee and Berry (2006) performed the only evaluation to date by comprehensively sampling soil and vegetation, but not tortoises, at multiple sites in the Mojave Desert. In addition, reference intervals for hematological (e.g., packed cell volume, hemoglobin concentration, and white blood cell count) and biochemical (e.g., glucose, triglycerides, and various enzymes) parameters have been developed for *G. agassizii* (Christopher et al. 1999), although no values exist for concentrations of heavy metals such as lead, arsenic, and copper. Published reference intervals provide guidelines for interpreting analyses on tissue samples from tortoises performed under a variety of environmental and physiological conditions.

As part of efforts to mitigate the development and operation of the Ivanpah Solar Electric Generating System (ISEGS), an approximately 400-megawatt-capacity facility in the Ivanpah Valley, California, USA, *G. agassizii* were translocated from within the ISEGS boundaries to adjacent areas, where they were monitored for 5 y (2012–2017) to determine the effects of short-term, short-distance translocation on survival and other demographic parameters (Farnsworth et al. 2015; Dickson et al. 2019). In accordance with the monitoring requirements outlined in the 2011 Biological Opinion

(U.S. Fish and Wildlife Service [USFWS] 2011a) on the effects of translocating *G. agassizii* from the ISEGS, researchers developed a comprehensive Effectiveness Monitoring Program (EMP) to characterize conditions that influence the survival of translocated tortoises (Dickson, B.G., B.P. Wallace, R.D. Scherer, M.E. Gray, and A. Kissel. 2017. Process- and scale-based determinants of survival for translocated Mojave Desert Tortoises in the Ivanpah Valley, California. https://www.cspinc.org/public/CSP_ISEGS_Tortoise_Report_5yr_FINAL.pdf.), including the potential adverse effects of exposure to toxic heavy metals (Cohn, B.R., and K. Herbinson. 2017. Environmental toxicant and contaminant monitoring. https://www.cspinc.org/public/CSP_ISEGS_Tortoise_Report_5yr_FINAL.pdf.; Brian Cohn et al., unpubl. report). The EMP also examined local and landscape-scale environmental variables, characteristics of individual tortoises (e.g., body size, sex, and biannual health exams), and other factors, in addition to translocation status.

The Ivanpah Valley has been considered excellent habitat for *G. agassizii*, with some of the highest population densities being found in the East Mojave Desert (Turner et al. 1984); however, several anthropogenic activities could be sources of contamination, such as legacy elements from mining operations (e.g., arsenic and uranium; Chaffee and Berry 2006) and lead from automobile exhaust from traffic on Interstate 15 (I-15), which passes through the Ivanpah Valley. From 1988 to 1993, Colosseum, Inc. conducted mining and cyanide leaching of gold and silver ore in the Clark Mountain Range west of the ISEGS, within the old Clark Mountain Mining District, which, over the past 120 y, has produced silver, gold, copper, lead, tungsten, and fluorite (Environmental Protection Agency 1992). In addition, the Mountain Pass rare earth mine on the southern flank of the Clark Mountain Range, adjacent to the ISEGS, was one of the largest producers of rare earth elements in the world and was mined on a large scale between 1965 and 1995 (Haxel et al. 2002; Castor and Hendrick 2006; Castor 2008). In 1998, after a series of wastewater spills, chemical processing at this mine was discontinued. This wastewater was piped to evaporation ponds near the dry bed of Ivanpah Lake and contained a significant concentration of thorium and its decay products (Clark et al. 1998; Henny et al. 2003; Ault et al. 2015). Subsequently, the Desert Tortoise Research Facility was constructed as part of a settlement to satisfy parkland mitigation obligations for effects of the mine on *G. agassizii*. The mine has changed hands from corporation to corporation and has opened and closed several times since 2002. Increased anthropogenic pressure on *G. agassizii* in the Ivanpah Valley is the result of the construction of three active renewable energy facilities, including the ISEGS. The footprints of these facilities degrade and fragment habitat, and interrupt linkages between *G. agassizii* conservation areas in California and Nevada states (Bureau of Land Management 2002; Lovich and Bainbridge 1999; USFWS 2011a,b; Dutcher et al. 2020).

In this study, to examine the possible adverse effects of heavy metal toxicity on *G. agassizii*, we collected and analyzed tortoise blood samples and quantified concentrations of several heavy metals within a broader, biannual health assessment program. We also comprehensively analyzed soil samples from *G. agassizii* habitats throughout the study area to accompany concurrent tissue samples. Our data inform establishment of baseline landscape-level patterns in heavy metal concentrations detected in tissues of *G. agassizii* and their habitat.

Materials and Methods

Study area.—The Ivanpah Valley mostly contains Bureau of Land Management-administered land and is about 75 km southwest of Las Vegas, Nevada, USA (Fig. 1). In addition to a concentrated solar thermal power plant, the ISEGS facility includes fences surrounding the project footprint that prohibit the passage of *G. agassizii*. The area is also intersected by I-15 and includes two other solar power plants, elevated power transmission lines and towers, paved roads accessing the ISEGS, numerous unpaved roads, and a golf course. Elevation across the Ivanpah Valley ranges from 790 to 1,830 m, with vegetation consisting of Mojave Desert Scrub dominated by Creosote Bush (*Larrea tridentata*) and White Bursage (*Ambrosia dumosa*). The annual rainfall is low (about 20 cm), with most precipitation occurring during winter and the summer monsoon (peak in July and August; Global Historical Climatology Network station USC00267369, Searchlight, Nevada, USA). Soil types vary from silt/clay to sand/loam, with *G. agassizii* typically occupying the relatively low-lying alluvial fans, plains, and colluvial/bedrock slopes. Additional details on the Ivanpah Valley are specified in Turner et al. (1984) and Sieg et al. (2015).

Tortoise monitoring.—We captured, quarantined, and translocated 73 *G. agassizii* at the ISEGS. We captured the tortoises close to the project boundary and released them < 500 m from the centroid of their original home range. To determine the approximate location of their original home range, we radio-tracked them once per week for up to 2 y prior to moving them. We equipped all 73 tortoises with radio transmitters, recaptured them on a biannual basis for health assessment, and regularly monitored them to detect movements and mortality events (translocation group). We followed the same procedure for two other groups of tortoises that were

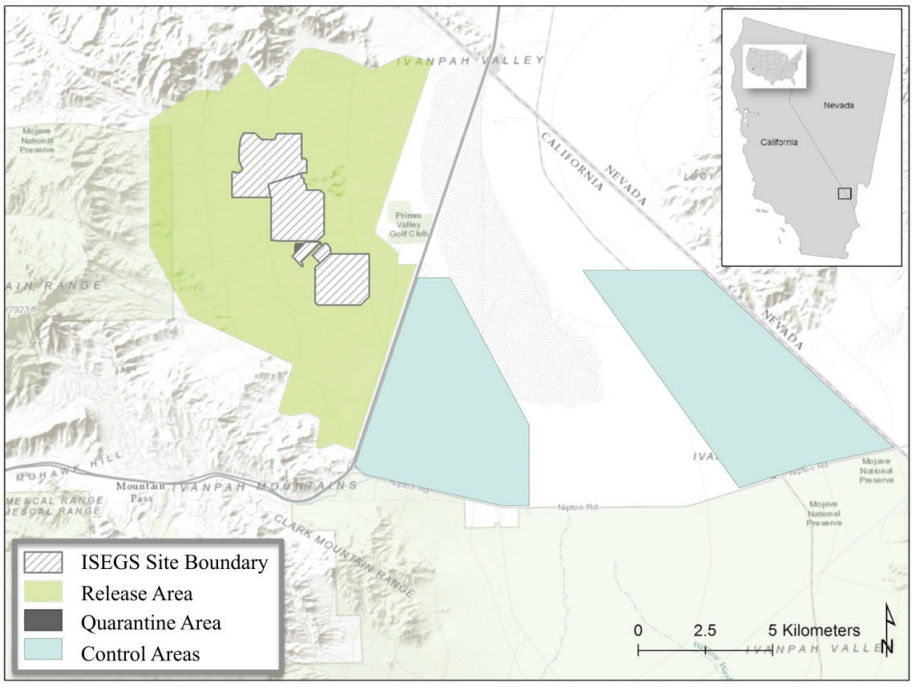

Figure 1. Ivanpah Solar Electric Generating System (ISEGS) project footprint within the Ivanpah Valley study area in southern California, USA, described in the Effectiveness Monitoring Plan. (Taken from ISEGS, Ivanpah Solar Electric Generating System; EMP, Effectiveness Monitoring Plan).

not subject to translocation: a control group of 149 individuals with home ranges in a comparable habitat within the Ivanpah Valley (< 20 km of the ISEGS site; control areas are shown in Fig. 1), and a resident group of 112 individuals with home ranges within the release area of translocated tortoises. Evaluation of the control and resident groups allowed us to isolate the effects, if any, of translocation from other potentially confounding variables, e.g., precipitation, soil, and vegetation characteristics. See Dickson et al. (2019) for a detailed description of the study design and execution.

Health assessment and pathogen transmission.— Consistent with USFWS protocols (Berry and Christopher 2001; USFWS 2016) to quantify general patterns in the health status of the tortoises, we conducted biannual (May and September) health assessments beginning in spring 2012. We conducted visual health assessments that included but were not limited to documenting clinical signs of URTD (i.e., discharge from nares and/or eyes, swelling and/or redness of eyes, lethargy, and poor body condition), CD (i.e., lesions typical of CD, peeling laminae or scutes, lesions on the bone or scutes, mold, and fungus), and trauma (i.e., missing or other trauma to the limbs and shell). To evaluate body condition scores and test whether the tortoise condition varied among the three groups and over time, we used protocols developed by the USFWS (2016). We also collected and tested blood samples via enzyme-linked immunosorbent assay (ELISA) at the University of Florida to detect exposure to the bacteria *Mycoplasma agassizii* and *M. testudineum*. Between April 2012 and June 2017, we collected 3,673 samples from within the three groups for the purpose of ELISA testing.

Blood sampling and metal concentration analysis.— We collected blood samples from tortoises for the purpose of heavy metal analysis during each biannual health assessment. The study area collectively contained highly developed or disturbed areas (proximate to the I-15), as well as less affected areas (e.g., upper bajada of the recipient site and eastern portions of the control site). By measuring metal concentrations in the treatment groups and their habitats seasonally, we quantified natural and anthropogenic toxicant concentrations to be used in further analyses to determine whether they affected health and disease.

Between fall 2013 and spring 2015, we collected 191 samples from a subset of *G. agassizii* in the translocation study (41 translocated tortoises, 63 residents, and 86 controls; Table 1). We sampled tortoise blood using a novel Dried Blood Spot (DBS) method, which was validated by Lehner et al. (2013). We collected 50 µL of whole blood from the subcarapacial vein (Hernandez-Divers et al. 2002), and placed blood samples on Whatman 903® Filter Card filter paper (Whatman; GE Healthcare, Buckinghamshire, UK) to measure concentrations of heavy metals (e.g., iron, arsenic, cadmium, lead, mercury, nickel, lead, selenium, thallium, titanium, and uranium). We followed the

Table 1. Heavy metal concentrations (Fe = iron, Se = Selenium, Pb = Lead, As = Arsenic, Cd = Cadmium) in blood samples of Mojave Desert Tortoises (*Gopherus agassizii*). Data are shown as mean ± standard deviation (sample size for individual heavy metals). Abbreviations are F = female, M = male, Unk. = unknown, ND = no data, MCL = midline-carapace length, ppm = parts per million, and ppb = parts per billion.

Study Group	Sex (No.)	MCL (mm)	Fe (ppm)	Se (ppb)	Pb (ppb)	As (ppb)	Cd (ppb)
Control	F (33)	219 ± 16	266 ± 98 (33)	106 ± 43 (33)	53 ± 47 (33)	29 ± 22 (14)	19 ± 11 (4)
	Male (47)	247 ± 25	312 ± 91 (47)	140 ± 53 (47)	54 ± 43 (43)	22 ± 2 (2)	13 ± 3 (3)
	Unk. (6)	138 ±19	236 ± 79 (6)	99 ± 47 (6)	50 ± 26 (5)	40 ± 14 (2)	10 (1)
	All (86)		289 ± 96 (86)	124 ± 51 (86)	53 ± 44 (81)	27 ± 18 (28)	16 ± 8 (8)
Resident	F (30)	230 ± 15	246 ± 79 (0)	105 ± 31 (30)	33 ± 22 (29)	13 ± 4 (2)	ND
	M (33)	258 ± 23	321 ± 96 (33)	149 ± 52 (33)	41 ± 36 (29)	16± 8 (2)	ND
	Unk. (1)	174	294 (1)	149 (1)	51 (1)	13 (1)	ND
	All (64)		286 ± 95 (64)	128 ± 48 (64)	38 ± 30 (59)	14 ± 5 (5)	ND
Translocated	F (20)	222 ± 17	258 ± 76 (20)	111 ± 31 (20)	32 ± 40 (18)	15 ± 4 (5)	13 ± 4 (2)
	M (20)	252 ± 22	313 ± 65 (20)	158 ± 56 (20)	38 ± 35 (20)	17 ± 8 (5)	15 (1)
	Unk. (1)	168	247 (1)	95 (1)	51 (1)	15 (1)	ND
	All (41)		285 ± 75 (41)	134 ± 50 (41)	36 ± 37 (39)	16 ± 6 (11)	15 ± 3 (3)
ALL	F (83)	224 ± 16	257 ± 86 (83)	107 ± 36 (83)	41 ± 39 (80)	24 ± 19 (21)	17 ± 9 (6)
	M (100)	251 ± 24	315 ± 88 (100)	147 ± 53 (100)	46 ± 40 (92)	20 ± 11 (19)	14 ± 2 (4)
	Unk. (8)	146 ± 22	245 ± 70 (8)	105 ± 43 (8)	51 ± 21 (7)	27 ± 17 (4)	10 (1)
	All (191)	235 ± 31	287 ± 91 (191)	128 ± 50 (191)	44 ± 39 (179)	23 ± 16 (44)	15 ± 7 (11)

methods described by Lehner et al. (2013), which involved overnight digestion of each blood sample and measurement of heavy metal concentrations using inductively coupled plasma mass spectrometry (ICP-MS) at the Michigan State University Diagnostic Center for Population and Animal Health, Lansing, USA. The limits of quantification below which we could not reliably detect concentrations was 10 parts per billion (ppb) for arsenic, cadmium, selenium, uranium, titanium, and thallium, 20 ppb for mercury and lead, and 500 ppb for nickel.

Soil sampling.—We collected soil samples close to the locations where we captured *G. agassizii* for health assessments. Soil toxicology monitoring followed the protocols described by Chaffee and Berry (2006). We used soil cores to collect soil samples from within the home range of the translocated, resident, and control groups at a depth of about 2–8 cm, a distance shown to contain most of the roots of annual and perennial plant species and Aeolian transported contaminants (Chaffee and Berry 2006). We prepared all homogenized soil samples for laboratory analyses at the Applied Science, Engineering, and Technology Laboratory (ASET) at the University of Alaska, Anchorage, Alaska, USA, using ICP-MS, and quantified 29 elements.

Data analyses.—We used heavy metal concentration data generated from blood samples collected in spring 2014 and 2015 to model annual survival probability from May 2014 to May 2015 and from May 2015 to May 2016 for *G. agassizii* individuals with a midline-carapace length (MCL) of ≥ 160 mm. The concentrations of most heavy metals were below detection limits; however, iron and selenium concentrations were above the detection limit in all tortoises, whereas the lead concentration was above the detection limit in > 96% of individuals in 2014 and > 91% of individuals in 2015. For survival analysis, we assigned a value of 0 to all tortoises in whom the lead concentration was below the detection limit.

To evaluate the influence of individual covariates and heavy metal concentrations on estimates of annual and cumulative survival probabilities (probability of survival for the duration of the study) for all three groups, we used tracking data and a known-fate model (White and Garrott 1990). We used the data collected during annual spring health assessments as the focal sampling period; therefore, estimates of survival probability were from May of one year to May of the following year (i.e., May 2014 to May 2015 and May 2015 to May 2016). Next, we developed a set of candidate models representing competing hypotheses regarding the causes of variation in survival probability and used an information-theoretic approach (Burnham and Anderson 2002) to evaluate the relative levels of support for competing models. We calculated Akaike's Information Criterion adjusted for a small sample size (AIC_C), and prior to modeling we centered and standardized values for all covariates (Schielzeth 2010). Subsequently, we diagnosed correlations between covariates using Pearson's

Correlation Matrix, but we excluded those that had a Pearson's Correlation Coefficient > |0.70| in the same model. When interpreting a set of candidate model results, we considered the model with the lowest AIC_C to have the most support, although other models (e.g., those within 8 AIC_C units of the highest-ranked model; Anderson 2008) might also be supported. In addition, we examined 95% confidence intervals (CIs) around estimates of regression coefficients, and if the 95% CIs around estimates of regression coefficients included 0, we concluded that the effect was negligible. Finally, we used model-averaged estimates of coefficients to explore the relationship between individual covariates and survival (Burnham and Anderson 2002).

Data for the development of covariates were unavailable for all *G.agassizii* individuals for all years. For instance, we analyzed blood samples for heavy metal concentrations in a subset of all radio-telemetered tortoises. In these cases, we censored animals for the intervals where data were missing. The overall dataset used in survival analysis included 106 individuals in the control group, 78 in the resident group, and 54 in the translocated group; heavy metal concentration data were available from 86 control, 63 resident, and 41 translocated animals (Table 1).

Candidate models included the effects of group (control, resident, and translocated), body size (MCL), the concentration of each of the three heavy metals for which we had sufficient data (selenium, iron, and lead), and two-way interactions between group and heavy metal concentrations and between group and size. As described by Dickson et al. (2019), preliminary analysis indicated no apparent relationship between survival probabilities and sex; we did not include the effects of sex in the final set of candidate models. To contextualize the results of the survival analysis, we also examined possible relationships between heavy metal concentrations and health parameters (e.g., body condition documented during health assessments) and the characteristics of home ranges (e.g., proximity to the I-15 or to non-operational mine sites and the estimated area of roads and fences within home ranges). To evaluate the effects of five heavy metals (iron, selenium, arsenic, lead, and cadmium) on the body condition score of tortoises, we used a Generalized Linear Model with a binomial link at $\alpha = 0.05$ (Zuur et al. 2009) using the statistical program R (R core development team 2018). The proportion of body condition scores < 4 (average body condition score) from the health assessment data was our response variable, whereas sex, study group, and the maximum heavy metal concentration were covariates (running a separate model for each heavy metal). For cadmium, we did not include sex and group as covariates because of the small sample size (n = 11). We also investigated potential relationships between heavy metals and other health parameters, such as signs of URTD or CD, and ELISA-positive samples, despite the extremely infrequent occurrence of negative health indicators (< 5% of all observations; Dickson, B.G., B.P. Wallace, R.D. Scherer, M.E. Gray, and A. Kissel. 2017. *Op. cit.*). To further examine the relationship between anthropogenic features and heavy metal concentrations, we quantified home range sizes, distance to the I-15, distance to non-operational mine sites, and total distance of roads and fences within each tortoise home range and compared these factors with heavy metal concentrations in blood and soil samples (See Farnsworth et al. 2015 and Dickson et al. 2019 for detailed descriptions on home range determination). We calculated these values on the basis of an interannual average home range for each tortoise.

Results

We found no between-group differences in heavy metal concentrations (Tukey HSD post-hoc analyses; all $P > 0.05$ for metals shown in Table 1). Concentrations of several heavy metals in blood samples (e.g., mercury, titanium, thorium, and uranium) never or rarely exceeded minimum levels of detection (i.e., typically 0–7% of samples in a given season for cadmium and arsenic). Blood concentrations of lead (Fig. 2; Supplemental Information Fig. S1), selenium (Fig. 2; Supplemental Information Fig. S2), iron (Fig. 2; Supplemental Information Fig. S3), and arsenic (Fig. 2; Supplemental Information Fig. S4) were typically lower than or within published ranges for turtles (including tortoises; Nagle et al. 2001; Burger 2002; Martínez-López et al. 2010; Yu et al. 2011). Other vertebrate (including reptiles) concentrations were also generally higher (Eisler 1988; Burger et al. 2007; Hamilton 2004; Buekers et al. 2009; Grillitsch and Schiesari 2010).

We detected lead, iron, and arsenic in soil samples; however, only approximately one-third (67/205) of soil samples had detectable levels of selenium. Soil concentrations of lead (Supplemental Information Fig. S2), selenium (Supplemental Information Fig. S3), and iron (Supplemental Information Fig. S4) were not significantly related to blood concentrations of the same elements; however, soil and tortoise blood concentrations of arsenic were positively related ($r^2 = 0.30$, $F_{1,43} = 17.60$, $P < 0.001$) in the same locations (Supplemental Information Fig. S4). Soil concentrations of lead and arsenic were similar to or lower than those reported in other locations in the Mojave Desert (Chaffee and Berry 2006).

The highest-ranked candidate model included the effects of selenium, and the second-highest candidate model included the effects of iron; we found no effects of body size or group (Table 2). The estimated

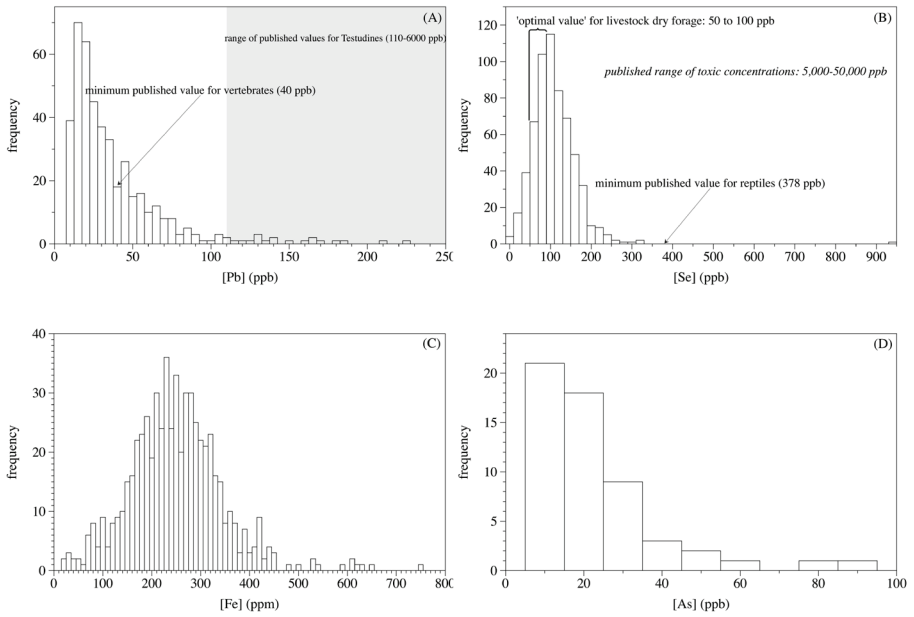

Figure 2. Frequency distributions of (A) lead, Pb, (B) selenium, Se, (C) iron, Fe, and (D) arsenic, As, concentrations in blood samples collected from Desert Tortoises (*Gopherus agassizii*) in the Ivanpah Valley study area of California, USA.

regression coefficient for selenium from the top-ranked model was 0.99 (95% CI = 0.04–1.94), indicating that tortoises with high selenium concentrations in their blood have higher annual survival (Fig. 3). Although we estimated a positive effect of iron on survival (Fig. 3), the CI around the estimated regression coefficient from the second-ranked model included 0 (0.58, 95% CI = -0.02–1.86). We found no evidence of survival probability being affected by lead concentrations (Fig. 3).

Table 2. Candidate model selection results for evaluation of the effects of iron, selenium, and lead concentrations and size (MCL) using the large-tortoise data set. A similar analysis was not conducted on the small-tortoise (120–160 mm MCL) data set because of insufficient sample size. Acronyms are –2LL = –2 times the log of the likelihood function at its maximum, k = number of parameters in the model, AIC_C = Akaike's information criterion adjusted for the small sample size, ΔAIC_C = difference between AIC_C of a given model and AIC_C of the highest-ranked model, = w_i, Akaike weight, and MCL = midline-carapace length.

Model	-2LL	k	AIC_C	ΔAIC_C	w_i
Selenium	110.3	2	114.3	0.0	0.34
Iron	111.3	2	115.3	1.0	0.20
group*iron	104.4	6	116.7	2.4	0.10
no predictor variables	115.2	1	117.2	2.9	0.08
group + selenium	109.2	4	117.4	3.1	0.07
Size	113.8	2	117.9	3.6	0.06
group + iron	110.3	4	118.4	4.1	0.04
Lead	114.6	2	118.6	4.3	0.04
group*selenium	107.5	6	119.8	5.5	0.02
Group	114.2	3	120.3	6.0	0.02
group + size	113.2	4	121.4	7.0	0.01
group + lead	113.5	4	121.6	7.3	0.01
group*lead	110.6	6	122.9	8.6	0.00
group*size	112.7	6	124.9	10.6	0.00

Body condition scores were not significantly related to any of the five heavy metals we analyzed (iron, selenium, arsenic, lead, and cadmium; Table 2). We also found no relationship between high heavy metal concentration and other negative health indicators, likely because such indicators were extremely rare overall. Even when focusing only on tortoises with the highest heavy metal concentrations, we found no discernible pattern between increased heavy metal concentrations and negative health indicators (e.g., body condition, URTD, and CD; data not shown). Although overall, heavy metal concentrations in blood and soil samples were relatively low, higher concentrations of lead (Fig. 2; Supplemental Information Figs. S1 and S5) and arsenic (Fig. 2; Supplemental Information Figs. S4 and S8) were more frequent in the control area southeast of the I-15 (more proximate to the spill sites from the rare earth element mine 1997 pipeline breach); however, we found no significant relationships between distance of home ranges from the I-15 and heavy metal concentrations in blood (Supplemental Information Fig. S9). Similarly, we found no relationship between heavy metal concentration and other anthropogenic features of home ranges (e.g., selenium vs. proximity to known toxic spill sites, $F_{1,181} = 0.74$, $P = 0.390$; abandoned mine sites. $F_{1,181} = 0.850$, $P = 0.360$; and areas of roads within home ranges, $F_{1,181} = 0.450$, $P = 0.500$).

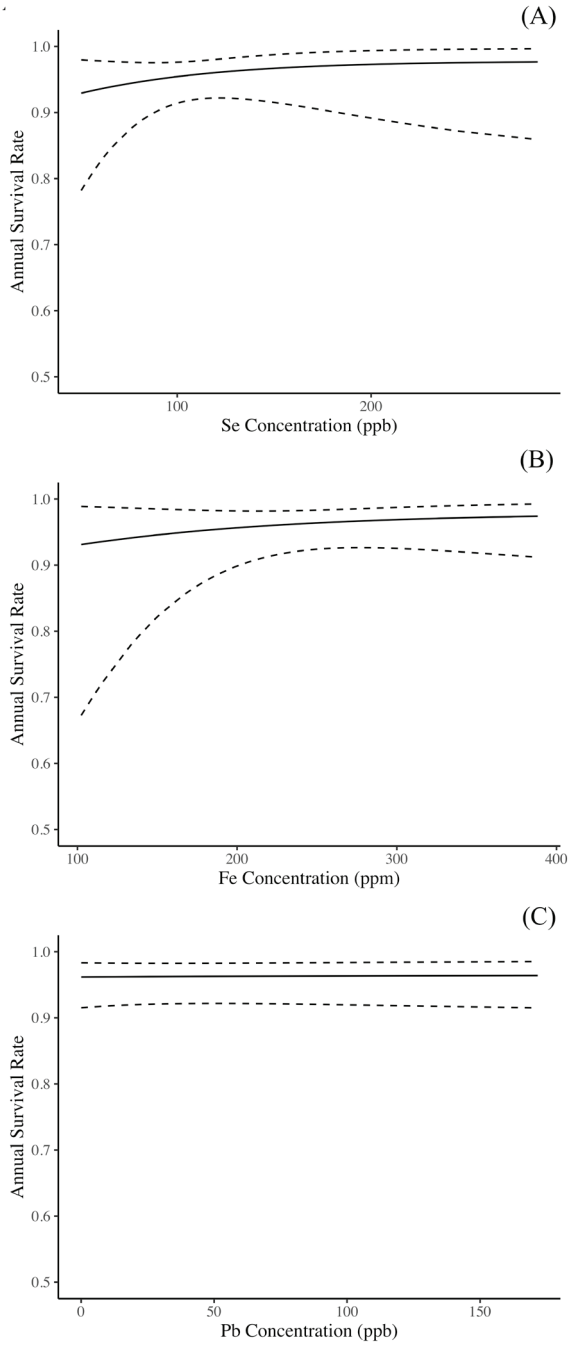

Figure 3. Relationships between estimated annual survival rates of Mojave Desert Tortoises (*Gopherus agassizii*) and concentrations of (A) selenium, Se, (B) iron, Fe, and (C) lead, Pb. The solid line is the estimated trend line between heavy metal concentrations and survival rates, and the dashed lines are the 95% confidence intervals.

Discussion

After being absorbed from the environment and having entered the bloodstream, heavy metals are rapidly distributed throughout the body, most often bound to plasma proteins and blood cells (Kenyon et al. 2001; Grillitsch and Schiesari 2010; Perrault et al. 2011. Once bound, they might be subject to reversible elimination, transferred to different tissues where they might be sequestered, and if not remobilized, stored in a form unavailable to the body. The paucity of data available on exposure of *G. agassizii* to heavy metals or contaminants was our starting point, however, heavy metal concentrations in blood are ephemeral and, when present, reflect recent exposure or remobilization from storage in the liver for reproduction. We acknowledge that our measurements might differ from what would have been detected with more invasive procedures such as liver tissue sampling (Grillitsch and Schiesari 2010). Therefore, using blood as the tissue of analysis in this study was a tradeoff between sampling efficiency and reduced tortoise handling and the ambiguous temporal characteristic of heavy metal concentrations in blood relative to that in other tissues, such as liver, muscle, bone, and scutes (Grillitsch and Schiesari 2010). In the case of an acute or chronic condition, however, increased heavy metal concentrations in blood would be expected. Considering the increased, ongoing development and resultant habitat fragmentation in the Ivanpah Valley, there is a potential for long-term consequences of toxic exposures in *G. agassizii*. With respect only to exposure to toxic concentrations of heavy metals, we suggest that the values we reported here be used as reference values. Samples collected from tortoises in other anthropogenically altered or fragmented habitats could potentially be compared in the future. Given the ongoing, range-wide decline in the *G. agassizii* populations, it is critical to identify and protect high-quality habitat areas, including those with low levels of toxic substances, to meet recovery goals for this federally listed threatened species (Berry et al. 2014).

Our survival analysis results suggested a positive relationship of survival with selenium and iron. Selenium is a necessary detoxifying nutrient but is toxic at high doses (Naganuma et al. 1983). Perrault et al. (2011) reported concentrations of selenium in blood of Leatherback Sea Turtles (*Dermochelys coriacea*) that might physiologically harm hatchlings; however, selenium toxicity in aquatic ecosystems typically occurs at levels > 5,000 ppb (Hamilton 2004), more than an order of magnitude above the concentrations we detected in blood samples. The positive relationship between survival and selenium concentrations in blood supports the notion that selenium concentration does not approach toxic levels. Conversely, low levels of selenium might somehow be related to an increased probability of mortality. Selenium deficiency is recognized as a potentially serious health issue for both humans and livestock because it can lead to

cardiac myopathy (Lenz and Lens 2009). If livestock forage contains selenium levels of < 50–100 μg kg^{-1} (an optimal level), animals can develop white muscle disease (mineralization of the heart muscle ultimately causing chronic heart failure; Walsh and Burch 1963; Beytut et al. 2002). White muscle disease has also been observed in reptiles suffering from deficiency of dietary vitamin E and selenium (Boyer and Scott 2019). It remains unclear whether tortoises with relatively low selenium concentrations experience pathological effects of selenium deficiency. A possible link between low selenium concentration and survival needs to be further investigated. Future studies could quantify selenium availability in forage plants of tortoises, selenium concentrations in various tortoise tissues, and possible relationships between selenium concentrations and tortoise health and other factors such as growth rate.

Jacobson et al. (1991) reported that the iron concentration in livers of tortoises diagnosed with URTD is significantly higher than in livers of healthy tortoises. The iron concentration in the healthy tortoises was similar to those in our study. Jacobson et al. (1991) also reported no between-group differences in selenium or lead concentrations, which we also found. Regardless, evidence of a positive relationship between survival and blood iron concentration was weak. Furthermore, this apparent statistical relationship is unlikely to be biologically significant.

Thus far, investigation of the role of contaminants in the health of *G. agassizii* has been impeded by a lack of clinical data, limited access to appropriate samples, and the absence of a proven method of assessing the contaminant status of live animals that might or might not exhibit disease symptoms. Nondestructive sampling techniques, particularly blood analyses, might be more easily applied in the evaluation of contaminant exposure in the field to prevent excessive destructive sampling, especially in the case of threatened reptile species. Nondestructive sampling via blood (using the DBS method, for example), toenail clips, or biopsy is an important aspect of bioindicator development (Hopkins et al. 2001; Burger et al. 2007). Although the concentrations of heavy metals are most often higher in the somatic tissues of chelonians and most, if not all, wildlife species, the development of a sustainable, noninvasive methodology through blood sampling provides an alternative for monitoring a threatened species. Opportunistic sampling over time could provide a baseline relationship between the concentration of contaminants in somatic tissues such as organs, muscle, and bone as well as in blood.

In this context, the DBS method is an effective, rapid, minimally invasive procedure for collecting, transporting, and storing whole blood samples. In this instance we used DBS to study heavy metals although the methodology can be applied to a variety of toxicological or epidemiological health concerns or questions (Stove et al. 2012). Our inference about the potential adverse effects of exposure to toxic heavy metals, however, is constrained by the ephemeral nature of heavy metal concentrations in blood; that is, blood samples can reflect either recent exposure via multiple potential pathways or remobilization from storage in the liver for reproduction (Grillitsch and Schiesari 2010). Therefore, to establish a relationship between heavy metal concentrations in blood and other tissues, we recommend that future studies that use the DBS method simultaneously sample multiple tissues (Burger et al. 2007).

We established a baseline for the relative presence of heavy metals in a population of *G. agassizii* blood and soil samples in the Ivanpah Valley and correlated these values to relevant health parameters and mortality estimates used to ascertain the multiyear effects of translocation on *G. agassizii* at the ISEGS. There is an increasing need for biomonitoring, particularly with continued human land-use patterns that encroach on and alter habitats for natural resources (Hunter et al. 2003). Despite the apparent relationships between survival and selenium and iron concentrations, the survival rates across the study area and over more than 5 y of monitoring were among the highest published values for turtles, including desert tortoises (Iverson 1991; Shine and Iverson 1995; Agha et al. 2015; Dickson et al. 2019). For these reasons, our data suggest that *G. agassizii* in our Ivanpah Valley study area were not exposed to toxic levels of metals, based on our method of blood sampling and analysis. There remains a need to further examine how specific toxicants or groups of toxicants affect health, immune system, susceptibility to diseases, and mortality of tortoises. To develop reference intervals for heavy metal and toxicological data from wild free-ranging tortoises, samples must be obtained and analyzed across various habitats, seasons, and climate patterns such as drought or an increase in temperature. This information can support translocation and management strategies by evaluating factors that take into account health tolerances (e.g., acute or chronic toxicity of arsenic) and potential health benefits (e.g., selenium nutrition) within a given habitat.

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