Short Title.—Differential Predation and Chemical Signature Differences

DIFFERENTIAL CANID PREDATION OF TRANSLOCATED JUVENILE DESERT TORTOISES (*GOPHERUS AGASSIZII*) AND CHEMICAL SIGNATURE DIFFERENCES BETWEEN FEMALE AND MALE ADULT AND JUVENILE DESERT TORTOISES

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***Abstract.*—Differential predation was observed in a population of 59 translocated juvenile Desert Tortoises (*Gopherus agassizii*) of known sex during a juvenile translocation survival study between September 2012 and November 2017. The main source of mortality (77%; 24 of 31 deaths) was attributed to Coyote (*Canis latrans*) and Kit Fox (*Vulpes macrotis*) predation. Predation was skewed with 71% (*n* = 17) female mortality versus 29% (*n* = 7) male mortality. We tested the hypothesis that juvenile females smell different than males which leads to increased canid predation. We also explored differences in chemical signatures of resident adult female and male Desert Tortoises. We collected oral, cloacal and chin/forelimb swabs from translocated juvenile and resident adult female and male tortoises during fall 2015 and fall 2017 and analyzed them using headspace gas chromatography/mass spectrometry to determine potential differences in the chemical signatures among the four groups. Standardized chromatographic peak responses were subjected to repeated analyses of variance (ANOVA). For development of artificial scents, mean responses were calculated for each juvenile tortoise from standardized responses representing all collections (i.e., oral, cloacal, and chin/forelimb swabs) and grand means were determined for males and females. Repeated measures ANOVAs clearly demonstrated that the collections of volatiles differed according to age and/or sex depending on the body location of collection. Among the plausibly endogenous volatiles that differed by age, many of them are alcohols. Two field trials using captive Coyotes and one field trial partially within the translocation area were conducted to test if Coyotes showed a preference for female or bias against male synthesized scent. No consistent**

preference or bias was shown, suggesting that no innate preference for female odor was evident.

Key Words.—mortality; predation ecology; sex bias; survival; translocation

INTRODUCTION

The Mojave population of the Desert Tortoise (*Gopherus agassizii*) that occurs north and west of the Colorado River in the United States is protected as a threatened species under the United States Endangered Species Act due to declining populations (United States Fish and Wildlife Service [USFWS] 1990). Predation is one of the main factors contributing to the continued population decline (USFWS 2011; Berry and Murphy 2019). While predation by a wide variety of predators has been summarized on Desert Tortoises at different life stages (Berry and Murphy 2019), information on predation ecology (e.g., how do predators find Desert Tortoises, how do Desert Tortoises respond to or interact with predators) is lacking.

A major source of mortality documented in several studies has been from canid predation, primarily Coyotes (*Canis latrans*) (Peterson 1994; Esque et al. 2010; Lovich et al. 2014; Nagy et al. 2015), Kit Foxes (*Vulpes macrotis*) (Kelly et al. 2019) or both (Nussear et al. 2012; Germano et al. 2017). Luckenbach (1982) suggests that Coyotes are the major predator of adult Desert Tortoises. Similarly, the main source of mortality (77%; 24 of 31 mortalities) after five years of a long-term survival study of 59 translocated juvenile Desert Tortoises of known sex was attributed to Coyote and Kit Fox predation (Hall and Perry 2018). Surprisingly, results indicated a large difference in predation between sexes with nearly 2.5 times more females being depredated than males (17 versus 7). Germano et al. (2017) reported findings from the first year of this study and Hall and Perry (2018) summarized study findings related to survival after five

years. To our knowledge this was the first study that documented differential predation between sexes in juvenile Desert Tortoises. Nagy et al. (2015) evaluated survival of translocated juveniles of unknown sex over a three-year period (2005-2008) and documented 32% survival with most mortalities caused by predation with Common Ravens (*Corvus corax*) the dominant predator of smaller Desert Tortoises (<110 mm midline carapace length [MCL]) and Coyotes the main predator of larger juveniles (>110 mm MCL).

Differential predation in adult Desert Tortoises was recorded by Esque et al. (2010) who found that females were more likely than males to be killed by Coyotes. Riedle et al. (2010) reported higher female mortality than male mortality from Mountain Lion (*Puma concolor*) predation which may be attributed to females being active earlier in the season than males in the Sonoran Desert where their study took place.

Reasons for higher canid predation on either adult or juvenile female Desert Tortoises have not been investigated before. Esque et al. (2010) mention that higher adult female predation by Coyotes was counter to what might be expected given that adult male Desert Tortoises have larger home ranges and generally move greater distances and concluded, "We are not aware of any other behaviors that are gender specific that would afford greater survival in a confrontation with a Coyote."

We suggest that other possible explanations for higher canid predation on female juvenile Desert Tortoises exist. Among these are that female juveniles spend more time aboveground or travel farther which makes them more susceptible to predation. We tested this hypothesis using radio telemetry tracking observations of our translocated juveniles from March to October 2012-2017. Results showed that females actually were observed spending more time in their burrows and less time in the open than males, and based on calculated straight-line distances between tracking

locations, females and males traveled similar distances. (Hall and Perry 2018). Another possible explanation is that females smell differently than males and this difference may attract or repel canid predators or that canids have the ability to associate an odor with a prey item with some advantage to prey on females rather than males. Because canids use olfaction as one of their main senses to find prey (Wells and Lehner 1978) we determined that this concept was worth investigating.

Reptiles rely more on their chemical senses than any other vertebrate class and many behavioral studies and anecdotal observations suggest that chemical cues (sex pheromones) are important in the communication and reproduction of many reptiles (Martin and Lopez 2011). Other researchers have suggested that chemical cues from conspecifics play a role in influencing Desert Tortoise movement and burrow use patterns (Patterson 1971; Berry 1986; Bulova 1997). Terrestrial tortoises (Testudinidae) appear to have two primary sources of pheromones and include the cloacal glands and the mental or chin glands (Mason 1992; Bulova 1997). The cloaca has been shown to be a source of conspecific chemical cues in many vertebrate species (references in Birch 1974) and male tortoises smell the cloacal area of females during courtship (Weaver 1970; Auffenberg 1977). Martin and Lopez (2011) note that the chemical composition of cloacal secretions and feces remain undescribed. In contrast, Rose et al. (1969) studied chin gland secretions of four species of *Gopherus* and found they contained phospholipids, triglycerides, free fatty acids, and cholesterol. They concluded that although the functions of the chin glands are not known completely, they are undoubtedly involved in sex recognition, may have been involved in species recognition in the past, and the enlarged glands during mating season may serve as an indicator of sexual readiness to females and/or as a challenge to other males in the area. They also theorized that although it is not known which of the gland

components elicit an olfactory response, the lipids are likely involved and further explain that fatty acids have characteristic odors and these compounds warrant further investigation. They also determined that chin glands are functional in females of all four species of *Gopherus* and that glandular secretions of females contained a cathodal migrating protein not present in males.

Rose (1970) in an effort to further isolate and characterize the fatty acids from chin gland secretions of male Texas Tortoises (*Gopherus berlandieri*) found the presence of caprylic, capric, lauric, myristic, palmitic, palmitoleic, stearic, oleic, and linoleic fatty acids in the secretions. They also studied male and female behavior of tortoises in response to a fatty acid solution painted on a plaster model of a tortoise and found the fatty acid composition served as an olfactory cue which elicited combat behavior (i.e., ramming) and not courtship in other males and a male-female attraction from females. Based on these observations, Rose (1970) concluded that there may be sexual differences in either the fatty acid composition or percentage composition on individual acids but all attempts to secure sufficient amounts of female secretions for fatty acid analyses failed due to the extremely small size of the female chin glands. Similarly, Alberts et al. (1994) were unable to collect secretion samples from females due to small glands.

Weaver (1970) studied courtship and combat behavior in the Texas Tortoise and found that males and females had different responses to cloacal scent and chin gland secretions with females but not males being able to recognize sex of a conspecific by cloacal scent alone and males recognizing sex of a conspecific from chin gland secretions but not cloacal scent. Galeotti et al. (2007) studied Hermann's Tortoise (*Testudo hermanni*) using choice experiments to determine if they could detect and distinguish the odor of conspecifics from that of another species and an odorless control, as well as determine if they were able to discriminate sex and

sexual maturity of individuals by chemical cues. They found that both sexes correctly discriminated between their own species and another species but only males could distinguish sex and sexual maturity of potential mates by olfactory cues. These results indicated a sexual dimorphism in olfactory sensitivity in this species and the authors suggest that males and females could rely on different communication channels during social interactions.

Alberts et al. (1994) studied the social significance and chemistry of chin gland secretions in the Desert Tortoise. They concluded that both males and females discriminated between the chin gland secretions of familiar and unfamiliar male conspecifics and revealed the presence of 12-17 protein components ranging in size from 25,000 to 115,000 Daltons with slight individual differences in the number and size of high molecular weight components. They suggest that, "It is possible that the protein components of chin gland secretions function primarily as a matrix to retard evaporation of lipids," and further suggest that the adaptive significance of differences in protein composition between sexes and among individuals needs further investigation.

Collectively, these studies reveal differences in chemical signatures generally (i.e., no specific chemical compounds listed) between male and female chin gland secretions and different responses of males and females to chemical cues from chin glands and the cloaca as related to species and sex recognition for courtship and combat and infer that differences in chemical signatures between males and females and their adaptive significance needs further investigation. Studies using domestic detection dogs (*Canis familiaris*) to find Desert Tortoises further emphasize that Desert Tortoise odor is a chemically un-described odor signature that should be studied more (Cablk et al. 2008; Cablk and Heaton 2011). In one study detection dogs detected Desert Tortoises of all sizes with no preference for female or male Desert Tortoises (Cablk et al. 2008). Cablk and Heaton (2006) found that wiping the tortoise neck and front legs with gauze

was sufficient to capture enough scent to be able to train the dogs to identify a tortoise. Cablk et al. (2008) noted that another source of tortoise odor could be from the breath.

In an effort to better define specific differences between female and male chemical signatures in both translocated juvenile and resident adult Desert Tortoises, we collected oral, cloacal, and chin/forelimb samples and analyzed them for specific chemical signatures. During three separate field trials, we tested the hypothesis that different chemical signatures between female and male translocated juveniles may have contributed to increased canid predation.

MATERIALS AND METHODS

Study Area—Sample Collection and Field Trial 3.—The NNSS is located in south-central Nevada, approximately 105 km northwest of Las Vegas, and encompasses approximately 3,561 km^2 (Figure 1). It is located in an area of southern Nevada that lies between the Great Basin Desert and the Mojave Desert as defined by Jaeger (1957). NNSS land has been withdrawn from public use since the 1950's as a U.S. Department of Energy Reservation, and a majority of the site (90%) has remained undisturbed. Our study area encompasses the southern one-third of the NNSS which coincides with the known Desert Tortoise habitat on the site (Figure 1). Relative Desert Tortoise abundance is low (3.9-17.4 tortoises per km^2) based on multiple surveys over several decades (EG&G/Energy Measurements 1991; Mueller and Zander 1994; Woodward et al. 1998; USFWS 2019). Within the study area, three sites were selected in the western portion of Area 22 for the release of juvenile Desert Tortoises which then dispersed up to 6 km (Figure 1). The resident adult Desert Tortoises were opportunistically captured at various locations in the study area during a separate but concurrent study (Figure 1). Field Trial 3 was conducted along an obscure, two-track dirt road that was located partially through release Site 2 (Figure 1). Dominant vegetation consists of creosote bush (*Larrea tridentata*) and white bursage (*Ambrosia*

dumosa) in the valleys, lower bajadas and broad drainages with blackbrush (*Coleogyne ramosissima*) in the upper bajadas and upland areas. Elevation ranges from 823 to 1,488 m. Average annual precipitation for the study area is around 12 cm (Soule 2006) and the climate is characterized by hot, dry summers and cool, dry winters with most of the precipitation coming during the winter and some during the summer monsoon season.

Study Area—Field Trials 1 and 2.—Behavioral assays were conducted with captive Coyotes at the United States Department of Agriculture, Animal and Plant Health Inspection Service, National Wildlife Research Center, Millville Predator Research Facility, near Millville, Utah. Coyotes were housed in 0.1 ha pens and provided a daily ration of 650 g of commercial mink food (Fur Breeders Cooperative, Logan, Utah) with water provided ad libitum.

Study Animals—Desert Tortoises.—On 21 September 2012, 59 juvenile Desert Tortoises of known sex (29 female, 30 male) estimated to be less than 10 years old and ranging from 99-151 mm MCL were translocated from the Desert Tortoise Conservation Center (DTCC) in Las Vegas, Nevada to three release sites near the southern NNSS boundary as part of a long-term survival study. Juveniles were randomly assigned to each release site (20 each to Sites 1 and 2, 19 to Site 3) with nearly equal numbers of males and females placed at each site. Sex was determined by measuring plasma testosterone levels using a protocol modified by Rostal et al. (1994) pre-release. Their histories and origins were variable with some tortoises hatched at the DTCC and others acquired through a hotline that accepted tortoises from the general public. Tortoises were clinically healthy with no signs of nasal exudate for 90 days, negative for *Mycoplasma agassizii* and *M. testudineum* antibodies, and able to pass an official DTCC translocation screen that assessed for disease indicators, body condition scores, and other indices of health. Very high frequency (VHF) radio transmitters (Model PD-2 [6-month] or RI-2B [12-

month], Holohil Systems Ltd., Carp, Ontario, Canada) that were less than 10 percent body mass, were affixed to the first costal scute of each tortoise. Tortoises were tracked via radio telemetry at least weekly during March to October and monthly during November to February using a three-element Yagi antenna and receiver (MODEL R1000, Communications Specialists, Inc., Orange, California, USA). Transmitters were changed each spring and/or fall through fall 2017 and were rotated between the left and right side of the carapace.

Thirty resident adult Desert Tortoises ranging from 180-306 mm MCL were opportunistically captured between 10 May 2012 and 7 October 2015. VHF transmitters (Model RI-2B [24-month], Holohil Systems Ltd., Carp, Ontario, Canada) and Global Positioning System (GPS) loggers (Model G30L, Advanced Telemetry Systems, Inc., Isanti, Minnesota, USA; Model GT-120 i-GotU USB GPS Travel and Sports Logger, Mobile Action, New Taipei City, Taiwan) were affixed to the carapace. Adults were tracked similarly to the juveniles. All juvenile and adult Desert Tortoises were handled according to USFWS guidelines (USFWS 2009b) by USFWS approved Desert Tortoise biologists.

Study Animals—Captive Coyotes.—We used 10 Coyotes in 2018 (5 females, 5 males) and 12 Coyotes in 2019 (6 females, 6 males). These animals ranged in age from two to seven years old. All animals were of reproductive age, and had similar history of vaccinations, feeding, and animal care. Handling and study protocols were reviewed and approved by the Institutional Animal Care and Use Committee at the USDA-National Wildlife Research Center (protocol QA-2994).

Sample Collection.—Cohort 1: Oral, cloacal, and chin/forelimb swabs were collected from 27 juvenile Desert Tortoises (19 males, 8 females) between 24 September and 14 October 2015 and

27 adult Desert Tortoises (10 females, 16 males, 1 unknown) between 23 September and 21 October 2015.

Cohort 2: Additional samples were taken from 26 juveniles (18 males, 8 females) between 18 September and 10 October 2017 and 12 adults (9 males, 2 females, 1 unknown) between 6 September and 3 October 2017.

Oral samples were collected with a sterile, cotton-tip applicator on a wooden stick. The applicator was gently swabbed multiple times around the inside of the tortoise's mouth. The last 50 mm of the stick containing the cotton tip was broken off and placed in a 10-mL vacutainer and sealed with a rubber stopper. Cloacal samples were collected with the same type of cotton-tip applicator which was inserted gently into the cloacal opening and swabbed multiple times around the inside of the cloaca. It was then broken off and placed in a 10-mL vacutainer similar to the oral sample. Chin/forelimb samples were taken by rubbing a circular cotton patch (about 50 mm diameter) under the chin where the chin glands are located and then on the front of the forelimbs. The cotton patch was then inserted into a 10-mL vacutainer and sealed with a rubber stopper. Samples were placed in a freezer until shipped. Samples were shipped frozen with ice packs overnight to the Monell Chemical Senses Center in Philadelphia, Pennsylvania, USA for analysis. Sterile cotton-tip applicators, empty 10-mL vacutainers with rubber stoppers, and new round cotton patches were also provided for quality control.

Chemical Analysis.—Samples from the two collection cohorts were analyzed separately using headspace gas chromatography/mass spectrometry to determine if any chemical differences were detected. Cotton patches and applicator swabs were individually placed in 20-mL sample vials with septa crimp-seals and subjected to dynamic headspace analysis using a HT3 dynamic headspace analyzer (Teledyne Tekmar, Mason, Ohio, USA) outfitted with Supelco Trap K

Vocarb 3000 trap (Sigma-Aldrich Co., St. Louis, Missouri, USA). The sample vial was maintained at 40 ˚ C, swept with helium for 10 minutes (min) (flow rate of 75 mL/min), and the volatiles collected on the thermal desorption trap. Trap contents were desorbed at 265 ˚C directly into a Thermo Scientific ISQ single-quadrapole gas chromatograph-mass spectrometer (Thermo Scientific, Waltham, Massachusetts, USA) equipped with a 30 m x 0.25 mm id Stabiliwax®-DA fused-silica capillary column (Restek, Bellefonte, Pennsylvania, USA). The GC oven program had an initial temperature of 40 ˚C (held for 3.0 min) followed by a ramp of 7.0 ˚C/min to a final temperature of 230 ˚C (held for 6.0 min). The mass spectrometer was used in scan mode from 33 to 400 m/z.

Baseline correction, noise elimination, and peak alignment of the chromatographic data were achieved using MetalignTM (Lommen 2009). Resulting multivariate data (consisting of all mass spectrometric responses exceeding a defined threshold at each scan event) were further processed with the MSClust tool for mass spectra extraction and generation of individual selected ion chromatogram peak responses (Tikunov et al. 2012). The resultant dataset consisted of a single response for all peaks identified in the chromatograms and was suitable for statistical analyses. All peaks were tentatively identified by their spectra in comparison to the National Institute of Standards and Technology standard mass spectral database.

Field Trials 1 and 2.—In order to test if differences in chemical signature influenced differential canid predation on female and male juvenile Desert Tortoises, we conducted a field trial (Field Trial 1) 27-29 September 2018, at the Millville Predator Research Facility. Synthesized juvenile female and male Desert Tortoise scent (Table 1) diluted with ethanol to make it less concentrated was infused into standard scent tabs (Pocatello Supply Depot, Pocatello, Idaho) made of plaster

of Paris and a control tab diluted with ethanol with no Desert Tortoise scent added were presented to 10 captive Coyotes (5 female, 5 male) in a choice trial to determine if they showed any preference. One male scent tab, one female scent tab, and one control tab were randomly assigned to a location about 20 m apart inside a clover pen (0.1 ha in size) containing a single Coyote. The scent tab was set on the ground within 0.5 m of the fence. Tabs were left in place for about 24 hours. Motion-activated cameras (Bushnell Trophy Cam HD Trail Camera) were secured to the fence approximately 3 m off the ground and oriented so the scent tab was within the camera's field of view. Cameras were set to record a 20-second video clip each time the camera was triggered and a minimum 1-minute time lapse between video recordings. Video clips were viewed and the number of visits, number of investigations, and duration of investigations to the nearest second were tallied for each Desert Tortoise scent (female, male) and control for each Coyote. A visit was defined as each time a Coyote entered the field of view and an investigation was when a Coyote directed its attention to the scent tab (e.g., sniffing, scent marking). Field Trial 2 was conducted 16-19 September 2019 at the Millville Predator Research Facility using the same methods as Field Trial 1, except the synthesized juvenile Desert Tortoise scent was not diluted with ethanol before it was infused into the scent tabs and 12 different captive Coyotes (6 female, 6 male) were used.

Field Trial 3.—The captive Coyotes at the Millville Predator Research Facility were naïve to Desert Tortoises having never encountered one; therefore, we conducted a field trial (Field Trial 3) at the NNSS in Desert Tortoise habitat under the assumption that Coyotes and Kit Foxes in this area had encountered Desert Tortoises or their scent. The study was conducted from 30 October to 7 November 2019 using a protocol used to census Coyotes adapted from Linhart and Knowlton (1975) and Roughton and Sweeny (1979, 1982). The same formulation of female and

male Desert Tortoise scent tabs that were used in Field Trial 2 were used in this trial. Paired stations with female and male scent tabs randomly placed on opposite sides of a dirt road were set up at 15 locations, spaced about 500 m apart. A 1-m^2 area was cleared to make animal tracks more visible in the dirt, and the scent tab was placed in the middle of this cleared area. Sites were checked daily for 9 days, except for one two-day check over the weekend. During each check, cleared areas were inspected for canid tracks and then cleared of all tracks. Tracks were identified to species using expert knowledge and Murie (1975).

Data Analysis--Chemical Analysis.—Sixty-three chromatographic peaks were identified among the samples of cohort 1 that could be attributed to the biological collections by comparison to chromatograms of quality control blank patches and applicators. Similarly, 61 peaks were uncovered during analyses of cohort 2 samples. Examination of age and sex differences as well as artificial scent development focused on the 33 compounds common to both cohorts. Peak responses were standardized by dividing each individual peak response by the total of 33 peak responses in each sample.

Standardized peak responses were subjected to repeated analyses of variance (ANOVA) using the MIXED procedure in SAS (SAS 2008). Age (adult or juvenile) and sex (male or female) were fixed effects and "volatile" was the repeated measure. Responses from the three body locations (oral, cloacal, chin/forelimb) were analyzed separately. Where appropriate, univariate ANOVAs were conducted to determine which individual volatiles were subject to age or sex effects. The false discover rate controlling procedure was used to account for conducting 33 univariate tests of individual volatiles (Benjamini and Hochberg 1995). For development of artificial scents, mean responses were calculated for each juvenile Desert Tortoise from

standardized responses representing all collections (i.e. oral, cloacal, and chin/forelimb swabs) and grand means were determined for males and females.

Data Analysis--Field Trials 1 and 2.—Video clips were analyzed and the number of visits, number of investigations, and duration of investigations were recorded and summed for each scent choice-Coyote combination. Relative percent frequency was calculated by dividing the raw number for each scent choice by the total number for each Coyote. Goodness-of-fit and Chi-square analysis was used to test for differences among the female scent, male scent, and control for number of visits and investigations. Analysis-of-variance (ANOVA) was used to test for differences among the female scent, male scent, and control for duration of investigations. Statistical significance was set at $P = 0.05$.

Data Analysis--Field Trial 3.—Due to low numbers, the results are limited to summaries rather than statistical analysis. This includes the number of visits to each scent choice and total number of canid tracks within the 1-m^2 area by species.

RESULTS

Chemical Analysis.—Of the 33 identified compounds shared between samples from both cohorts, 14 were considered exogenous in nature, were unavailable commercially, or unknown (Table 1). Recipes employing 19 odorants listed in Table 1 were determined for female and male scents through exploration of peak responses produced by neat sources of each.

The volatiles collected from the chin/forelimb location were subject to age effects ($P < 0.0001$), regardless of sex ($P = 0.989$) or the interaction ($P = 0.288$). The standardized responses of several individual volatiles listed in Table 2 were statistically different between adults and

juveniles when accounting for multiple comparisons (i.e., using the false discover rate controlling procedure). Similar to the chin/forelimb volatiles, oral volatiles were subject to age effects ($P = 0.0053$) and neither sex ($P = 0.908$) nor the interaction ($P = 0.990$). However, no individual oral volatiles were significant when accounting for multiple comparisons. Cloacal volatiles were subject to both age ($P < 0.0001$) and sex ($P = 0.0082$) effects, but not the interaction ($P = 0.992$). Only one individual cloacal volatile, styrene, demonstrated a significant age effect when accounting for multiple comparisons (Table 2).

Field Trials 1 and 2.—Results from Field Trial 1 are shown in Table 3. Coyotes visited female scent and the control significantly more than male scent ($\chi^2 = 17.081$, df = 2, $P = 0.0002$). No significant differences were detected among choices in the relative frequency of investigations ($\chi^2 = 0.978$, df = 2, $P = 0.613$) or duration of investigations ($F = 0.26$, df = 2, 27, $P = 0.770$).

Table 4 contains the results from Field Trial 2. Coyotes visited female scent significantly more than male scent or the control ($\chi^2 = 5.988$, df = 2, $P = 0.0501$). No significant differences were detected among choices in the relative frequency of investigations ($\chi^2 = 0.218$, df = 2, $P = 0.896$) or duration of investigations ($F = 1.60$, df = 2, 33, $P = 0.217$).

Field Trial 3.—Results showed two Kit Fox visits to female Desert Tortoise scent, both at Station 2 on days 1 and 2 of the trial and two visits to male Desert Tortoise scent, both at Station 5 on days 3 and 5 of the trial. Stations were not checked on day 4 so it remains unknown if the Kit Fox visit was on day 4 or day 5. Total number of Kit Fox tracks detected was 24 at the female scent station and 9 at the male Desert Tortoise scent station. No Coyote tracks were detected.

DISCUSSION

Chemical Analysis.—Prey seeking involves multiple sensory cues. Predators may detect the prey item from great distances via olfactory cues and investigate it. Investigative and consummatory behaviors may incorporate multiple sensory inputs (e.g., taste, odor, visual). In general, this is performed at a very short distance from the food item. The analytical tools employed in this study were capable of identifying only those highly volatile chemicals that are detectable by olfaction and not phospholipids, triglycerides, cholesterol, or protein components found in other studies. Importantly, chemical signals need not be volatile to be detected by canids. For example, non-volatile compounds on the surface of particulates (such as shed skin cells) can be presented to the main olfactory epithelium (Craven and Settles 2006). In addition, many other non-volatile compounds such as proteins and triglycerides are detected by the accessory olfactory, or vomeronasal (VNS), system. Although the VNS was primarily thought to participate solely in social and reproductive communication, the main and accessory system are now known to be highly overlapping (Ma 2007) and the role of the VNS in prey seeking by reptiles is explicitly known (Miller and Gutzke 1999).

Using headspace analyses, many highly volatile compounds were observed in the samples collected from juvenile and adult Desert Tortoises. This was a complex suite of volatiles that differed by sex, age, and body location. These odorants, singly or in some combination, may very likely serve as cues to foraging predators – the odor memory capabilities of canids being quite excellent in comparison to other mammals (Lo et al. 2020). Because we were interested in differential predation between female and male juvenile Desert Tortoises, we used chromatographic data to prepare synthetic scents of juvenile male and female Desert Tortoises for bioassays with captive and free-ranging mammalian predators. We did not test predator response to synthesized scent from adult Desert Tortoises.

Repeated measures ANOVAs clearly demonstrated that the collections of volatiles differed according to age and/or sex depending on the location of collection. The lack of significant individual volatiles suggests that the "volatile" effect is complex. That is, there are distinct patterns of volatiles which correspond to age or sex, but these patterns are not well-described by examination of individual volatiles. Among the plausibly endogenous volatiles that differed by age, many of them are alcohols (Table 2). Many alcohols are products of lipid and fatty acid metabolism (Wishart et al. 2018). However, the role of the microbiome in production of these volatiles should not be ignored (Rojo et al. 2017).

Field Trials 1 and 2.—Overall, the captive Coyotes showed little to no preference for female Desert Tortoise scent, male Desert Tortoise scent, or the control scent tabs. While more visits were made to the female scent tab during Field Trial 2, visits to the control tabs were about equal to this during Field Trial 1 suggesting there may be a slight preference for the female scent or weak bias against male scent. However, this is based on number of visits which was when a Coyote passed through the camera's field of view. If a true preference for female Desert Tortoise scent or bias against male Desert Tortoise scent exists, this pattern should be exhibited even more strongly in the number of investigations (actual interaction with the scent tab) or duration of investigations. This was not the case because there were no significant differences in number of investigations or duration of investigations to female versus male versus control scent tabs in either Field Trial 1 or 2.

We suspect that the captive Coyotes may have simply been responding to novel items (i.e., scent tabs) placed in their environment rather than showing a real preference or bias for different Desert Tortoise scent. Across all scent choices, male Coyotes tended to investigate more and for longer periods of time than female Coyotes which suggests male Coyotes may be more curious

and react more strongly to novel objects than female Coyotes. Heffernan et al. (2007) found male Coyotes investigated a large novel object (traffic cone) at a higher rate than female Coyotes, but time investigating the object was similar between sexes. Harris and Knowlton (2001) found males spent a greater amount of time within 5 m of a novel object and made more approaches towards the novel object than female Coyotes.

Field Trial 3.—Canids did not show a preference for female or male Desert Tortoise scent with equal visitation by Kit Foxes to both scents. More individual Kit Fox tracks were found at the female scent which may mean it spent more time investigating the area than at the male scent. Low canid visitation was documented at all stations and no sign from other Desert Tortoise predators (e.g., Bobcat [*Lynx rufus*], Badger [*Taxidea taxus*]) was observed. Perhaps, running the trial for a longer time period would have resulted in increased canid visitation.

Summary.—We collected our samples during September and October which coincides with the latter part of the mating season. Chin gland activity and testosterone levels peak in late summer when courtship, mating, and combat behavior usually occur (Alberts et al. 1994). Chin glands were swollen on several of the adult male Desert Tortoises during collection but not on the adult females or juveniles of either sex. Based on behavioral studies, males are more aggressive especially during mating season. Weaver (1970) cited Francis Rose (pers comm) who reported spray from chin glands of a male Texas Tortoise copulating with a female. This raises the question, do male tortoises spray or exude chemicals from their chin glands during a predator attack? If so, could this repel the predator? With other species utilizing this type of antipredation technique (e.g., some horned lizard species [genus *Phrynosoma*] shoot blood mixed with a foul-tasting chemical from ocular sinuses when threatened by a predator, toads [genus *Bufo*] secrete toxin from mucous glands when threatened by a predator), this idea warrants

further investigation. This might explain lower mortality in adult males with active chin glands but chin glands in juvenile males are typically not actively secreting or enlarged. Another untested idea is that male tortoises may exhibit more aggression than females during a predator attack which could deter the predator. Clearly, more work is needed to understand both juvenile and adult Desert Tortoise response to predator attacks.

Five years post-release, 46% (27 of 59) of translocated juvenile Desert Tortoises in our study were still alive. Of the documented mortalities, 24 of these were attributed to Coyote or Kit Fox predation. Predation was assumed based on evidence found at or near the mortality site such as the carcass broken in pieces, canine puncture wounds in the shell, canid tracks and scat, disturbed soil and vegetation, and/or dug up burrows. Over 90% (22 of 24) of the mortalities occurred within the first two years after release. Precipitation during these two years and the year prior (2012-2014) was below normal, likely influencing canid predation during those years. Canid predation on Desert Tortoises has been shown to increase during times of drought due to having fewer mammalian prey (e.g., lagomorphs and rodents) available (Woodbury and Hardy 1948; Berry 1974; Peterson 1994; Esque et al. 2010).

If higher juvenile female mortality is occurring in natural populations this could lead to a decline in Desert Tortoise populations given the importance of females surviving to adulthood and recruiting into the reproducing population. Juveniles in natural populations should be studied to determine if differential predation is occurring and to document sex ratios for comparison with our results. Berry and Murphy (2019) summarize sex ratios from multiple studies and conclude that in studies between the 1930's and early 1980's, there were equal numbers of female and male adult Desert Tortoises but since the 1990's, results varied by location. In a large epidemiological study of more than 1,000 adults in the central Mojave Desert, the female to male

sex ratio was 1:1.58 (Berry et al. 2015). Of 233 Desert Tortoises for which gender was determined in a multi-year study (1989-1995) at Yucca Mountain (southwest corner of NNSS) the female to male sex ratio was 1:1.16 (Lederle et al. 1997).

Results from our study highlight the importance of documenting the sex of Desert Tortoises, notably juveniles, during translocation studies, as well as studies in natural populations. Based on our findings we recommend that in future juvenile translocations, more females be released than males, perhaps up to twice the number of females to account for potential increased mortality of translocated juvenile females. Conducting translocations during years of normal or above normal precipitation may also decrease the likelihood of canid predation.

Data from the synthesized chemical scents and observations from all three trials suggest that although there are chemical differences between female and male juvenile Desert Tortoises, this does not account for increased predator attraction or curiosity toward female Desert Tortoises and therefore, would not account for increased predation of female Desert Tortoises observed in our study. Further research on canid predation ecology of Desert Tortoises is warranted.

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and required federal and state permits (USFWS #TE-08592A-2 and #TE-83414C-0 and NDOW #506446 and #261454). Adult Desert Tortoises were handled under authority of the Programmatic Biological Opinion (USFWS 2009a) and state permits NDOW #506446 and #261454. This work was done by Mission Support and Test Services, LLC, under Contract No. DE-NA0003624, with the U.S. Department of Energy. DOE/NV/03624—1253.

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Table 1. List of 33 chemicals common to both sets of collection cohorts including 14 considered exogenous in nature, unavailable commercially, or unknown (*) and 19 used to synthesize female and male juvenile Desert Tortoise (*Gopherus agassizii*) scent and their respective concentrations (mL) used in canid bioassay trials. Ethanol was used to dilute the tortoise scent concentration in Field Trial 1 and as the control stimulus in Field Trials 1 and 2.

Compound	**Female Odor (mL)**	**Male Odor (mL)**	**Control (mL)**
Acetic acid	4.000	3.700	
Acetophenone	0.019	0.014	
Benzaldehyde	0.047	0.047	
Butanol	0.148	0.125	
2-n-Butyl furan	0.020	0.027	
p-Cymene	0.009	0.014	
Decanal	0.179	0.118	
2-Decenal	0.013	0.010	
Ethanol	18.4	18.9	25.0
3,5-Heptadien-2-one, 6-methyl	0.007	0.006	
2-Heptenal	0.035	0.039	
5-Hepten-2-one, 6-methyl	0.028	0.022	
Hexanal	0.385	0.408	
Hexanol	0.203	0.173	
Nonanal	0.170	0.147	
2-Octenal	0.027	0.030	
Octanal	0.114	0.081	
Pentanol	0.788	0.814	
2-Pentyl furan	0.070	0.075	

Phenol	0.330	0.226	
Caprolactone*			
2-Chloroethanol*			
Dodecane*			
Ethyl benzene*			
2-Methyl-1-pentanol*			
2-Methyl-2-propanol*			
3-Methyl-2-butenal*			
Naphthalene*			
1-Octanol*			
o-Xylene*			
1-Penten-3-ol*			
Styrene*			
Toluene*			
Unknown*			

Table 2. Individual Desert Tortoise (*Gopherus agassizii*) volatiles that differ by age from different body location collections.

Compound	**Description**	**Chin/Forelimb**	**Cloaca**
1-Penten-3-ol	Alcohol	Juvenile > Adult ($P = 0.0023$)	
2-Methyl-2-propanol	Alcohol	Juvenile > Adult ($P = 0.0011$)	
1-Pentanol	Alcohol	Adult > Juvenile ($P = 0.0048$)	
Styrene	Exogenous		Adult > Juvenile ($P < 0.0001$)
1-Hexanol	Alcohol	Adult > Juvenile ($P < 0.0001$)	
2-Chloroethanol	Exogenous	Adult > Juvenile ($P = 0.0003$)	
2-Octenal	Aldehyde	Adult > Juvenile ($P = 0.0082$)	

Table 3. Results from Field Trial 1 including number and relative frequency of visits and investigations and average duration of investigations by scent choice-Coyote (*Canis latrans*) combination, September 2018.

			Number and (Relative Frequency) of visits			Number and (Relative Frequency) of investigations			Average Duration of Investigations (seconds)		
Pen No.	**Coyote**	**Date**	**Female**	**Male**	**Control**	**Female**	**Male**	**Control**	**Female**	**Male**	**Control**
NI1	M1413	27 Sep 2018	0 (0.000)	0 (0.000)	0 (0.000)	0 (0.000)	0 (0.000)	0 (0.000)	0	0	0
SI2	F1060	27 Sep 2018	7 (0.280)	7 (0.280)	11 (0.440)	3 (0.25)	4 (0.333)	5 (0.417)	13	13	10
NI2	F1422	27 Sep 2018	4 (0.364)	0 (0.000)	7 (0.636)	3 (0.333)	0 (0.000)	6 (0.667)	14	0	6
SI1	M1383	27 Sep 2018	10 (0.172)	11 (0.190)	37 (0.638)	4 (0.154)	8 (0.308)	14 (.538)	6	6	7
SI4	F1360	28 Sep 2018	0 (0.000)	0 (0.000)	0 (0.000)	0 (0.000)	0 (0.000)	0 (0.000)	0	0	0
SI3	F1372	28 Sep 2018	47 (0.887)	0 (0.000)	6 (0.113)	12 (0.706)	0 (0.000)	5 (0.294)	6	0	9
NI4	F1450	28 Sep 2018	2 (0.250)	5 (0.625)	1 (0.125)	2 (0.250)	5 (0.625)	1 (0.125)	6	12	11
NI5	M1423	28 Sep 2018	19 (0.373)	12 (0.235)	20 (0.392)	13 (0.351)	11 (0.297)	13 (0.351)	15	13	12
NI6	M1331	29 Sep 2018	11 (0.407)	8 (0.296)	8 (0.296)	6 (0.429)	6 (0.429)	2 (0.143)	7	12	4
SI6	M1311	29 Sep 2018	1 (0.038)	12 (0.462)	13 (0.500)	1 (0.071)	8 (0.571)	5 (0.357)	20	11	17

Total (Ave.)	**101 (0.390)**	**55 (0.212)**	**103 (0.398)**	**44 (0.321)**	**42 (0.307)**	**51 (0.372)**	**9**	**7**	**8**

Table 4. Results from Field Trial 2 including number and relative frequency of visits and investigations and average duration of investigations by scent choice-Coyote (*Canis latrans*) combination, September 2019.

			Number and (Relative Frequency) of visits			Number and (Relative Frequency) of investigations			Average Duration of Investigations (seconds)		
Pen No.	**Coyote**	**Date**	**Female**	**Male**	**Control**	**Female**	**Male**	**Control**	**Female**	**Male**	**Control**
NI2	M1221	16-Sep-19	1 (0.250)	2(0.500)	1 (0.250)	1 (0.250)	2 (0.500)	1 (0.250)	6	3	13
NI4	M1703	16-Sep-19	9 (0.563)	4 (0.250)	3 (0.188)	5 (0.556)	2 (0.222)	2 (0.222)	16	6	6
NI6	F1200	16-Sep-19	2 (0.333)	0 (0.000)	4 (0.667)	0 (0.000)	0 (0.000)	1 (1.000)	0	0	14
SI2	M1351	17-Sep-19	3 (0.333)	3 (0.333)	3 (0.333)	1 (0.143)	3 (0.429)	3 (0.429)	13	18	10
SI4	F1620	17-Sep-19	61 (0.670)	11 (0.121)	19 (0.209)	10 (0.455)	7 (0.318)	5 (0.227)	8	9	11
SI6	F1370	17-Sep-19	19 (0.455)	4 (0.182)	8 (0.364)	2 (0.400)	1 (0.200)	2 (0.400)	5	7	20
NI1	F1610	18-Sep-19	2 (1.000)	0 (0.000)	0 (0.000)	2 (1.000)	0 (0.000)	0 (0.000)	7	0	0
NI5	F1250	18-Sep-19	9 (0.375)	7 (0.292)	8 (0.333)	2 (0.167)	5 (0.417)	5 (0.417)	13	12	11
NI3	M1611	18-Sep-19	17 (0.500)	7 (0.206)	10 (0.294)	5 (0.250)	7 (0.350)	8 (0.400)	12	9	14
SI1	F1600	19-Sep-19	6 (0.102)	25 (0.424)	28 (0.475)	2 (0.200)	4 (0.400)	4 (0.400)	19	12	10

SI3	M1623	19-Sep-19	10 (0.435)	7 (0.304)	6 (0.261)	5 (0.417)	4 (0.333)	3 (0.250)	13	11	13
SI5	M1615	19-Sep-19	3 (0.064)	36 (0.766)	8 (0.170)	3 (0.200)	4 (0.267)	8 (0.533)	17	9	18
		Total (Ave.)	**133 (0.395)**	**106 (0.315)**	**98 (0.291)**	**38 (0.319)**	**39 (0.328)**	**42 (0.353)**	**11**	**8**	**12**

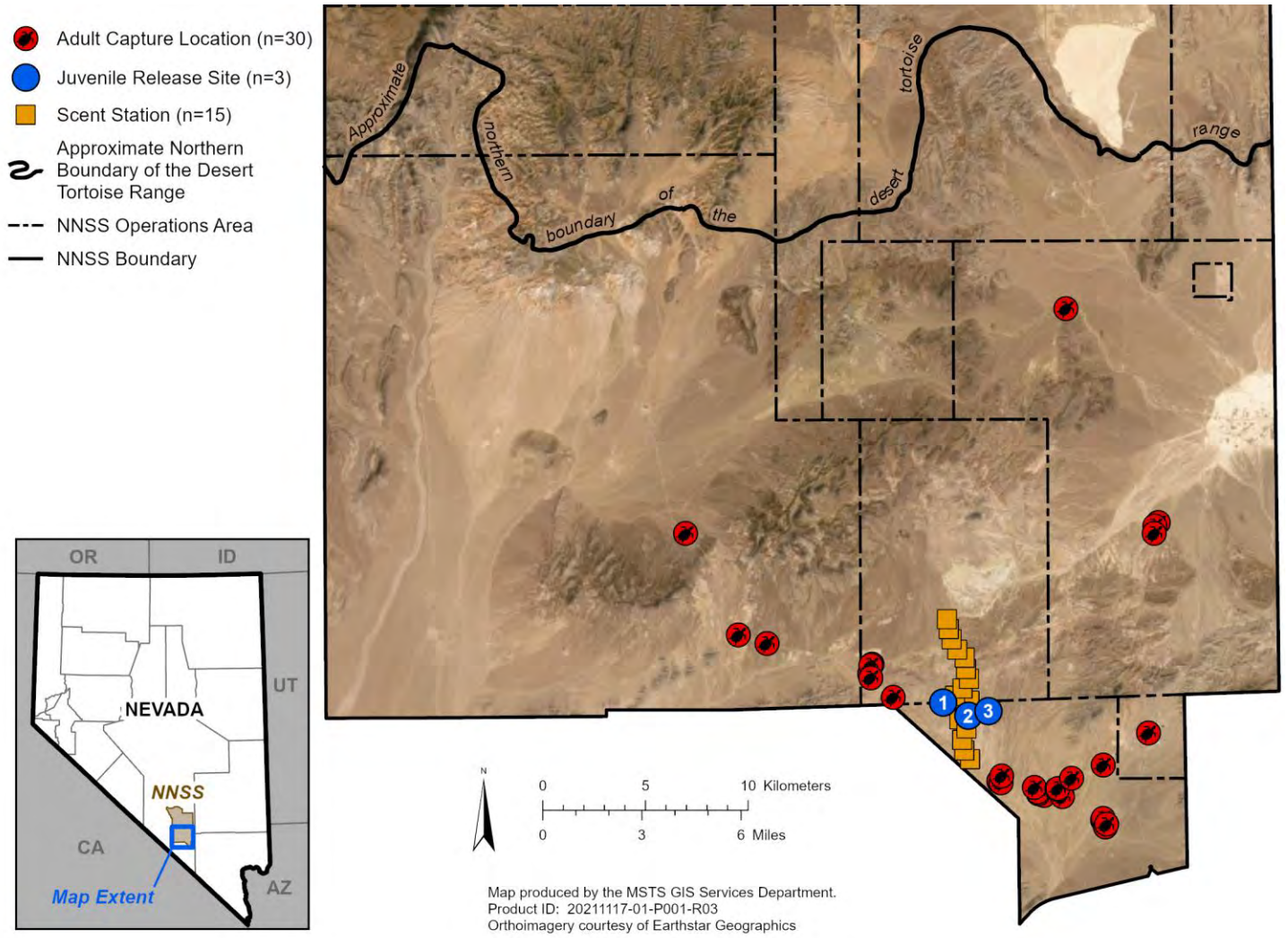

Figure 1. Map of study area including Desert Tortoise habitat, release sites of translocated juveniles, capture locations of resident adults, and scent station locations on the Nevada National Security Site.