Received: 18 August 2021 | Revised: 25 February 2022 | Accepted: 24 March 2022

DOI: 10.1111/csp2.12689

CONTRIBUTED PAPER

Conservation Science and Practice
A journal of the Society for Conservation Biology
Open Access

WILEY

Genetic analyses are more sensitive than morphological inspection at detecting the presence of threatened Mojave desert tortoise (*Gopherus agassizii*) remains in canid scat and raven pellets

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Funding information
Desert Conservation Program of Clark County, Nevada

Abstract

Subsidization of predator populations increases predation pressure on prey species, which is exacerbated when natural resources are scarce. Estimating the frequency of predation by subsidized predators on vulnerable species, especially low-density, long-lived species such as the federally threatened Mojave desert tortoise (*Gopherus agassizii*), allows landscape managers to evaluate the need for actions like reducing subsidies, discouraging predation by taste aversion, or predator removal. Most studies of Mojave desert tortoise predation have relied on morphological analysis to identify hard parts in predator scat. Here, we developed and validated a qPCR assay to test for the presence of Mojave desert tortoise in DNA extracted from scat and pellets. We used the assay to detect tortoise DNA in scat and pellets collected in a conservation easement adjacent to Boulder City, Nevada, from three Mojave desert tortoise predators: coyotes (*Canis latrans*), desert kit foxes (*Vulpes macrotis arsipus*), and common ravens (*Corvus corax*)—all of which consume anthropogenic resources. We compared the results of our qPCR assay to results from morphological analysis of the same samples and found that the qPCR method is much more sensitive at detecting the presence of tortoise remains. Although neither method can determine whether consumption was the result of predation or scavenging, nor how many individual tortoises were consumed, our findings indicate that conservation managers may benefit from focusing efforts on reducing subsidies that attract and support predators and on reducing tortoise mortality from predation.

KEYWORDS

common raven (*Corvus corax*), conservation genetics, coyote (*Canis latrans*), diet DNA, kit fox (*Vulpes macrotis*), Mojave desert tortoise (*Gopherus agassizii*), noninvasive genetic sampling, subsidized predators

1 | INTRODUCTION

Subsidized predators can have significant impacts on threatened or endangered prey species. Conversion of natural landscapes for human uses, considered one of the greatest threats to biodiversity conservation (Hanski, 2011), not only decreases the total area of natural habitat for native species, but also introduces anthropogenic resource subsidies. These subsidies, including refuse and water, can increase and sustain predator populations at levels above that which would occur without added resources and insulate predators from the effects of declines in prey populations (Gompper & Vanak, 2008). Through increased predation rates, subsidized predators can cause population declines or prevent the recovery of threatened prey species (Boarman, 2003; Sinclair et al., 1998). It is important to obtain accurate estimates of the frequency of predation to evaluate the impact that subsidized predators have on prey species and to assess the efficacy of management actions for mitigation.

In the Mojave Desert, increasing urbanization and large-scale renewable energy projects have resulted in increased human populations and improved access to previously inaccessible areas through the creation of roads (USFWS, 2010). This introduces anthropogenic subsidies that have been shown to increase the population density of predators of the federally threatened Mojave desert tortoise ("tortoise" hereafter), including coyotes (*Canis latrans*) and common ravens (*Corvus corax*). Elevated populations of both species have been shown to exert increased predation pressure on tortoises and have been implicated in population declines (ravens: Kristan & Boarman, 2003; coyotes: Esque et al., 2010).

Desert kit foxes (*Vulpes macrotis arsipus*, "kit foxes" hereafter) are also known predators of tortoises and, like coyotes, they expand their dietary niche breadth during times of resource scarcity (Kelly et al., 2020). Although they consume anthropogenic items less frequently than coyotes, they do increase anthropogenic consumption when their primary prey is less abundant. This anthropogenic subsidization may lead to higher predation on tortoises (Kelly et al., 2019). Previous studies report a low frequency of tortoise remains in kit fox scats (0.57%; Kelly et al., 2019). However, the true rate of predation may be higher, in part because kit foxes are known to consume tortoise eggs (Bjurlin & Bissonette, 2004), which may not be detectable by morphological analysis based on visual evidence of tortoise remains in scat.

Previous estimates of the frequency of predation on Mojave desert tortoises have relied on visual evidence of tortoise remains in scat (canids) or in regurgitated pellets (ravens), in which prey species are identified by inspection of hard, indigestible material such as bones, teeth, or scales (Cypher, 1993; Cypher et al., 2018; Kelly et al., 2019). This method is prone to biases in the analysis of both pellets (Mersmann et al., 1992) and scats (Matejusová et al., 2008) caused by absence of hard parts of prey species (Matejusová et al., 2008). Therefore, it is likely that previous studies may have underestimated true consumption rates. Previous studies have shown that molecular methods are more sensitive for the detection of prey species in scat (Egeter et al., 2015; Matejusová et al., 2008). However, these methods have not been widely adopted (Monterroso et al., 2019), and to our knowledge, predation on Mojave desert tortoises has never been estimated by identifying the presence of tortoise DNA in scat or regurgitated avian pellets. Unfortunately, neither the genetic nor the morphological detection method is capable of differentiating between scavenging and predation.

Our objectives were to (1) develop and validate a qPCR assay to test for the presence of Mojave desert tortoise DNA in noninvasively collected canid scat and raven pellet samples, (2) compare the rate of detection between morphological analysis and the qPCR assay, and (3) use the qPCR assay to estimate the frequency of occurrence of tortoise DNA in the scats or pellets of three known predators, coyotes, kit foxes, and common ravens, collected in and around a Mojave Desert conservation easement adjacent to Boulder City, Nevada. For all canid samples, we confirmed species of origin using a molecular assay (Bozarth et al., 2010). We then performed both qPCR and conventional morphological analyses on the same scat and pellet samples and compared the detection of tortoise remains in each method. Finally, we discuss the implications of our results for the conservation management of tortoises in this landscape.

2 | MATERIALS AND METHODS

2.1 | Sample collection

We collected 341 putative canid scat samples and 34 raven pellets in and around the Boulder City Conservation Easement Area (BCCEA) between September 2015 and

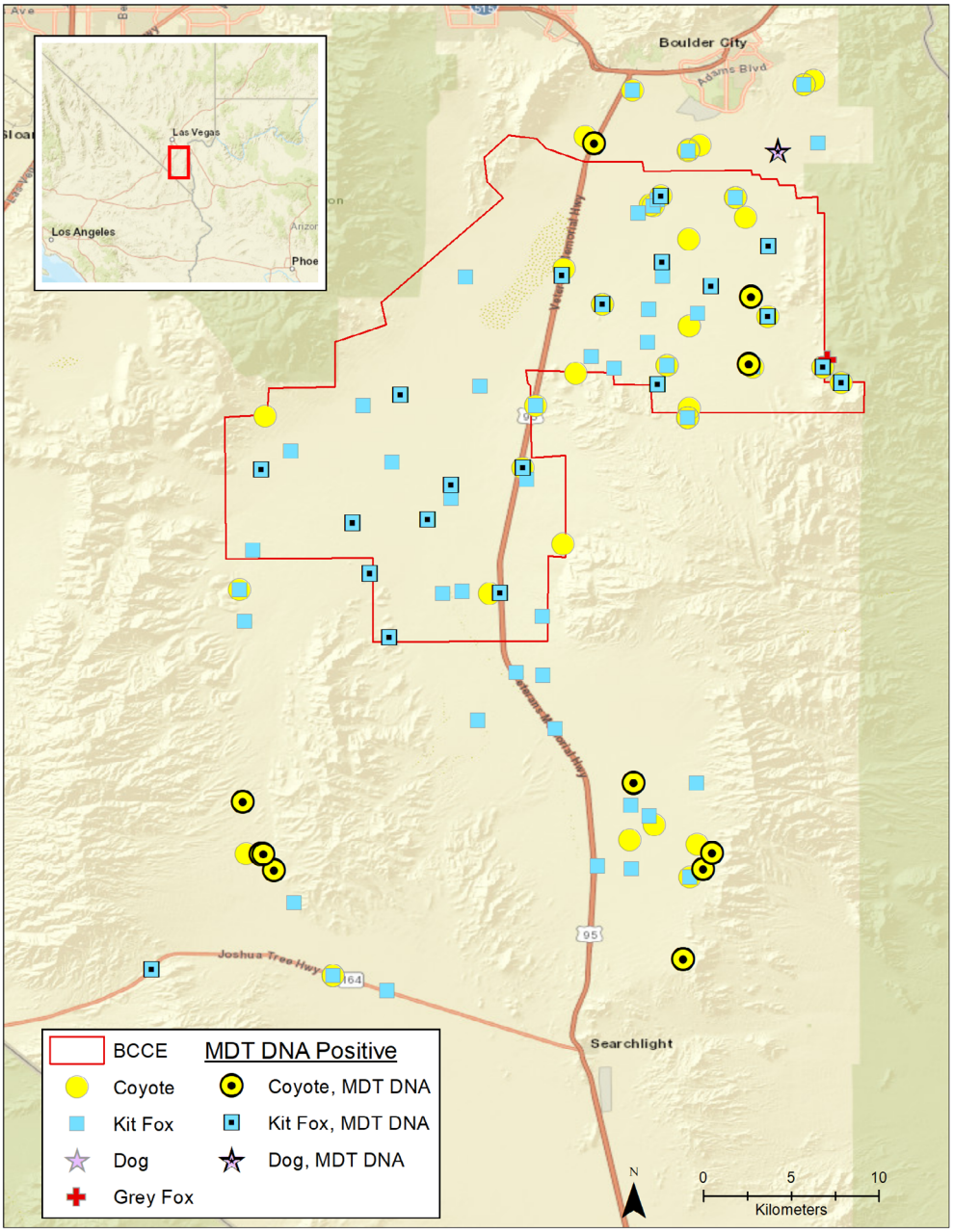

FIGURE 1 Collection localities of canid scat indicating tortoise positive samples. (MDT, Mojave desert tortoise. Data organized and map generated using Esri ArcMap 10.4.1. Base map source: Esri© OpenStreetMap contributors 2021)

April 2018 (Parker et al., 2021; Figure 1). The BCCEA is a 34,800-hectare area of public land south of Boulder City, Nevada, in the northeastern Mojave Desert within the Eldorado Valley. It was established in 1995 for the conservation of the Mojave desert tortoise and other desert species. Although it has no recreational facilities, the area includes multiple off-highway vehicle (OHV) trails.

Sampling details are described in detail in Parker et al. (2021). Briefly, we used stratified random sampling (Ratti & Garton, 1994) to select 84 sample localities that were at least 1 km from any other sampling point. In addition to these points, we collected regurgitated pellet and scat samples opportunistically under power towers and fences near raven nests. At each collection locality, we searched 10-m diameter circles for pellets and scat and collected all relevant material within the perimeter. No attempt was made to select only fresh samples. Collectors visually estimated the species that produced each sample and recorded this information as well as the date and collection location waypoint using a Garmin Montana 650 GPS device (Garmin Ltd., Olathe, KS). Samples were individually stored dry in sealable plastic bags except scats that were found as part of a latrine (Ralls & Smith, 2004), in which case multiple samples were placed in a single bag. Bags were marked with the date and waypoint and shipped to the Center for Conservation Genomics (CCG), Smithsonian Institution, Washington, D.C. At CCG, subsamples of approximately 1.5 cm^3 were taken and stored dry in 15 ml conical tubes until DNA extraction. In

cases where multiple scats were collected per locality during the same sampling session (i.e., multiple samples were taken from a latrine), we took one subsample per individual piece of scat. After subsampling for genetic analysis, the remaining scat was sent to the Endangered Species Recovery Program (ESRP) for morphological dietary analysis.

2.2 | Species identification

We extracted DNA from scats and pellets using a Mag-Bind® Stool DNA kit in 96-sample plate format (Omega Bio-Tek, Norcross, CA) following the manufacturer's "Standard Protocol" with modifications described in Parker et al. (2021). We then performed a species identification assay (Bozarth et al., 2010) by amplifying a small fragment of the control region (CR) of the mitochondrial genome that is a different length in each sympatric species in the study area. In each PCR reaction, we included a positive (DNA from a kit fox tissue) and a negative (no DNA added) control. Fragment length was determined by running PCR products on an Applied Biosystems DNA Analyzer 3130xl (ABI 3130xl, ThermoFisher, Waltham, MA) at CCG.

As part of a previous study (Parker et al., 2021), we used in-solution DNA hybridization capture followed by high-throughput sequencing to determine multi-locus single-nucleotide polymorphism (SNP) canid genotypes using the confirmed kit fox and coyote canid scat samples. We identified individual canids by calculating pairwise identity-by-state (IBS) and identity-by-descent (IBD). Allowing for stochasticity in genotyping methods, as well as allelic dropout, our cutoff to consider two samples the same individual was IBD >0.4 (IBS >0.95).

2.3 | Genetic analysis to detect Mojave desert tortoise in scat and pellets

To test for the presence of tortoise DNA in the scat- and pellet-derived DNA samples, we developed a qPCR assay. First, we designed a set of primers to amplify a short region of the Mojave desert tortoise CR. Using Geneious v9.1.2 (Biomatters, Ltd, San Diego, CA), we aligned published Mojave desert tortoise CR sequences (GenBank accessions U22812.1 and U22811.1) with the MAFFT v7.450 plugin (Katoh, 2005) and selected primers to amplify an 83 bp sequence with the Primer3 plugin v2.3.4 (Untergasser et al., 2012; forward primer, 5′-ACTGGTGATATGCTAGYGGT-3′, reverse, 5′-TGCCGYGCACAGTAGAGAAA-3′). We tested the specificity of the primers in silico by performing a PrimerBLAST search (Ye et al., 2012) on the nonredundant nucleotide database in GenBank (NCBI, accessed July 25, 2018).

We used DNA extracted from three Mojave desert tortoise tissue samples collected in a previous study (Mulder et al., 2017) as positive controls to test the sensitivity of the primer pair. We diluted the DNA extracts 1:50, 1:100, 1:500, 1:1000, and 1:5000 (with starting concentrations of 24, 19.3, and 18.7 ng/μl) and performed qPCR on a Bio-Rad CFX96 Touch Real Time System (Bio-Rad, Hercules, CA). Each 20 μl qPCR reaction contained 1 × KAPA SYBR FAST qPCR Master Mix (KAPA Biosystems, Wilmington, MA), 0.2 μM of each primer, and 2 μl DNA. Cycling conditions were as follows: 95°C for 3 min, followed by 37 cycles of 95°C for 3 s, 60°C for 20 s, and 72°C for 20 s, with a final extension at 72°C for 5 min. We used a high-resolution melting step to determine the melting point for the tortoise sequence in order to validate tortoise positives in our scat samples. We ran qPCR on each scat-derived DNA sample in triplicate following the protocol above, including a negative (no DNA added) and positive (tissue-derived DNA sample diluted 1:100) control in each reaction batch. Each sample that amplified by 40 PCR cycles or fewer and had the same melting point as the positive controls was considered positive for Mojave desert tortoise DNA.

To confirm amplicon sequence identity, we Sanger sequenced the positive controls as well as a subset of six scat positive qPCR reactions. We cleaned the qPCR reactions with Exo-sapIT (Affymetrix, Santa Clara, CA) and prepared the sequencing reaction with Big Dye Terminator v3.1 (ThermoFisher, Waltham, MA) following the manufacturers' protocols. Sequencing reactions were cleaned with Sephadex G50 (GE Healthcare Life Sciences, Marlborough, MA) and analyzed on an ABI 3130xl (ThermoFisher, Waltham, MA) at CCG. We visualized and quality-controlled the sequence chromatograms using Sequencher 5.2.4 (Gene Codes, Ann Arbor, MI). Cleaned sequences were compared to the nonredundant nucleotide database in GenBank using BLASTn v2.7.1 (database accessed August 20, 2018) to confirm species identification.

2.4 | Morphological analysis to detect Mojave desert tortoise in scats

We performed morphological analysis on the scats and pellets to determine whether visible Mojave desert tortoise remains were present and to compare the sensitivity of this method to our qPCR assay. Scat samples were dried at 60°C for at least 24 h to kill any parasites present. Samples were then placed in nylon pouches, washed in a clothes washing machine to remove soluble material, and dried in a tumble dryer. The remaining material was examined for the presence of tortoise scales or bones following the methods of Cypher et al. (2018).

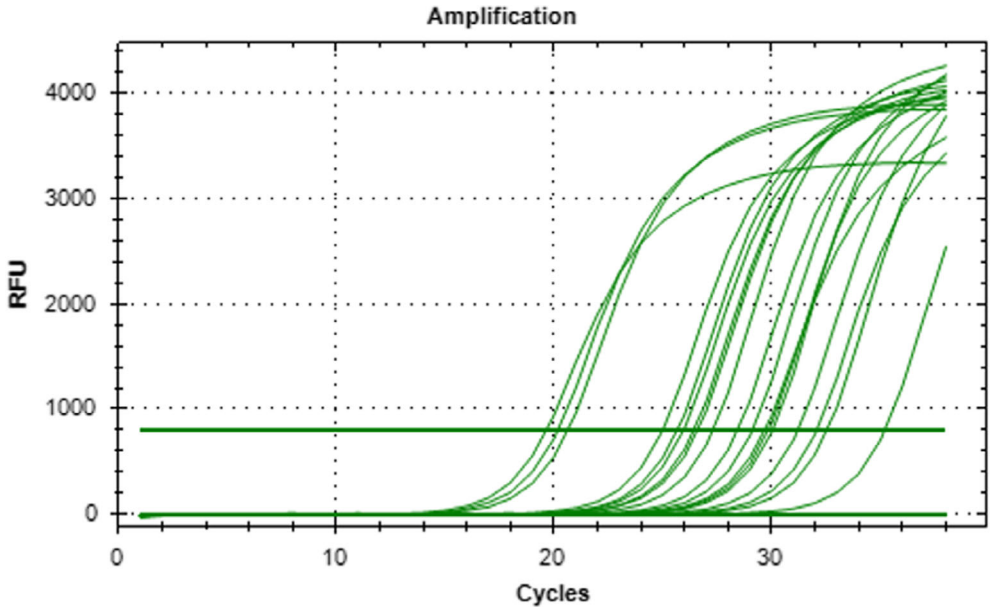

FIGURE 2 qPCR amplification plot of positive controls dilution series, with samples diluted 1:1 reaching threshold after 20 cycles of PCR (RFU, relative fluorescence units)

3 | RESULTS

3.1 | Species identification

We extracted DNA from 341 putative canid scat samples. Of those, the PCR-based species identification assay following Bozarth et al. (2010) identified 85 coyotes, 212 kit foxes, five gray foxes, and one dog (Figure 1). We were unable to conclusively identify nine scat samples that had genetic signatures for multiple canid species. One of these nine samples had DNA fragment sizes characteristic of both gray fox and coyote, and eight had fragment sizes characteristic of both coyote and kit fox. Mixed species scat samples may indicate sample contamination during collection or laboratory processing, environmental contamination due to defecation in mixed-species latrines (Ralls & Smith, 2004), or instances of coyotes killing and consuming foxes, which has been documented (Cypher, 1993; Ralls & White, 1995). Finally, the species identification PCR failed on 29 samples. It is possible that these samples were not from canids, the DNA was too degraded for the PCR reaction to proceed, or the presence of PCR inhibitors prevented the reaction from occurring (Ramón-Laca et al., 2015). Because all raven pellets ($n = 34$) were collected near active raven nests by experienced collectors, we did not perform species identification on the DNA extracted from pellets.

Sample collectors correctly identified the species of origin for 246 of the 312 putative canid scat samples for which the species identification reaction worked. That is, the species of canid indicated by the PCR methods in the lab matched 79% of the visual identifications made by collectors in the field.

3.2 | Detection of Mojave desert tortoise DNA by qPCR and morphological analysis

All tested dilutions of tissue-derived tortoise DNA amplified prior to 40 PCR cycles (Figure 2). All scat and pellet DNA samples were screened for tortoise DNA. Of the 375 samples, 65 amplified in our qPCR assay. The six scat-derived qPCR-positive samples and the two positive controls from tissue produced identical Sanger sequences. The BLAST search on the nonredundant nucleotide database in GenBank confirmed the sequence identity as *Gopherus agasizzii*; the sequence fragment matched 100% with published Mojave desert tortoise GenBank sequence U22811.

Of the 65 total tortoise positive samples, 14 were identified as coyote (16% of coyote scats), 27 were kit fox (13% of kit fox scats), one was dog, two were mixed coyote and kit fox samples (Figure 1), and nine failed in the species identification reaction (in the field, three of these were visually identified as kit foxes and six were identified as coyote). Of the 34 raven pellet DNA samples screened, 12 samples tested positive for tortoise DNA (35% of pellets, Figure 3). The number of qPCR-positive samples was correlated with our sampling effort: we collected most of our samples in the spring (58%), and most of the positives were collected in spring (72%). We collected the fewest samples in the summer (1%), and found the fewest qPCR-positives in summer (3%, Table S1). This pattern is also consistent with tortoise activity, which decreases as the temperature increases in the summer (Franks et al., 2011).

We ranked samples by their relative quantity of tortoise DNA based on the mean Cq value across replicates (Table S1). Because we did not include synthetic target DNA standards, we cannot quantify the absolute number of

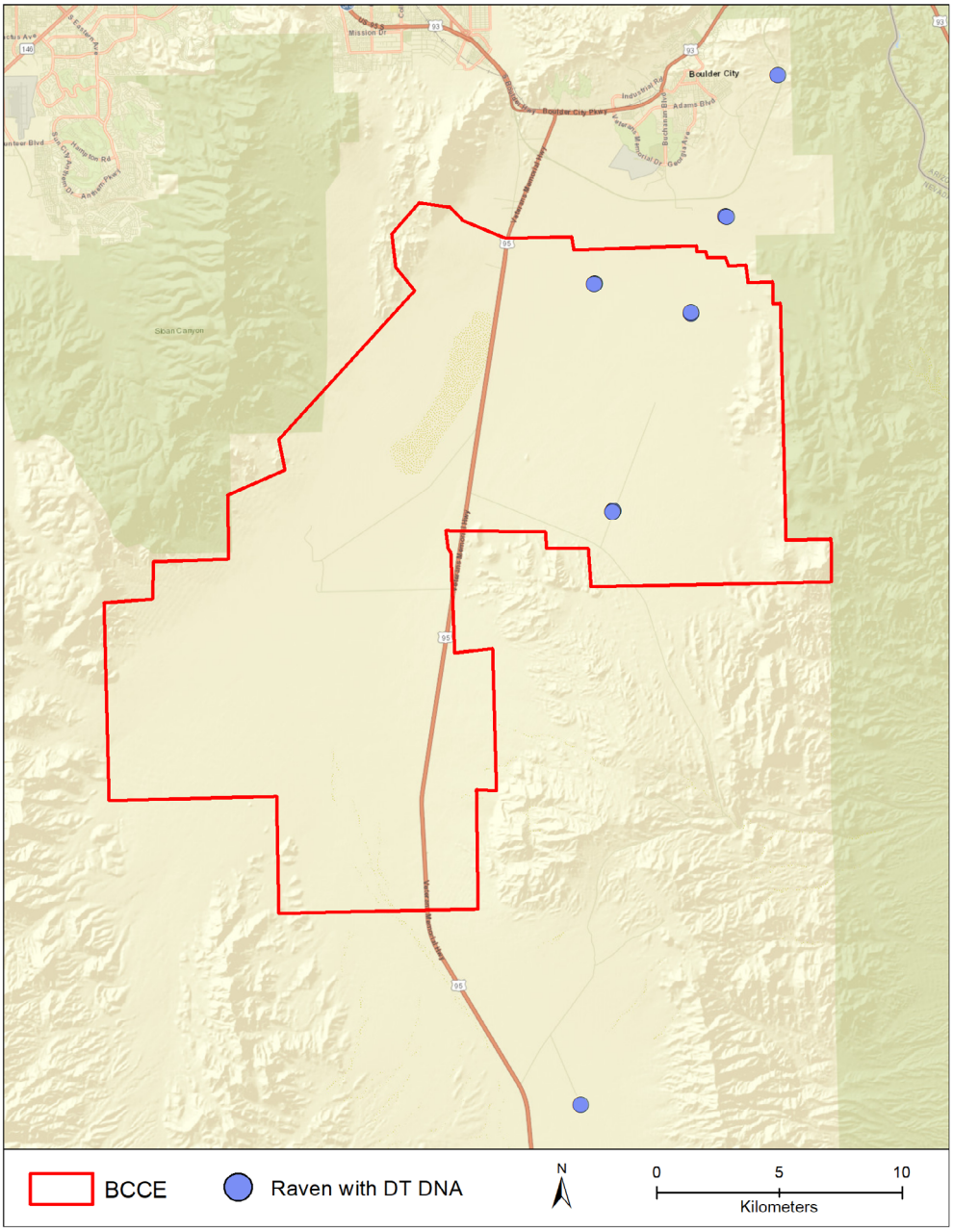

FIGURE 3 Collection localities of raven pellets (data organized and map generated using Esri ArcMap 10.4.1. Base map source: Esri© OpenStreetMap contributors 2021)

copies of tortoise mitochondrial DNA in our samples and we cannot account for the possible effect of PCR inhibition which would artificially increase the Cq value for a given copy number of starting DNA (Jane et al., 2015).

We determined multi-locus SNP genotypes for 17 canids using the scat samples, including 10 of the 14 coyote samples that tested positive for the presence of tortoise DNA. All of these were unique coyote individuals, including four males and six females. We obtained kit fox genotypes for seven of the 27 kit fox scat samples that tested positive for tortoise DNA; all of these were also unique fox individuals, including four females, two males, and one for which sex could not be determined. We did not determine raven genotypes.

Morphological analysis identified tortoise remains in two coyote scat samples; no tortoise remains were detected in kit fox samples (Figure 4). Tortoise remains were identified in one of the 34 raven pellets. All three of these samples also tested positive for tortoise DNA in our qPCR assay. Similar to previous studies of coyotes and kit foxes in the Mojave Desert in CA (Cypher et al., 2018; Kelly et al., 2019), the most common items identified in coyote scats were rabbits (62.3%) and rodents (19.8%) while the most common items in kit fox scats were rodents (74.3%), invertebrates (20.2%), and rabbits (14.4%). Anthropogenic items, that is, domestic animal remains or man-made materials, were found in 7.6% of coyote scats, 1.4% of kit fox scats, and 17.9% of raven pellets.

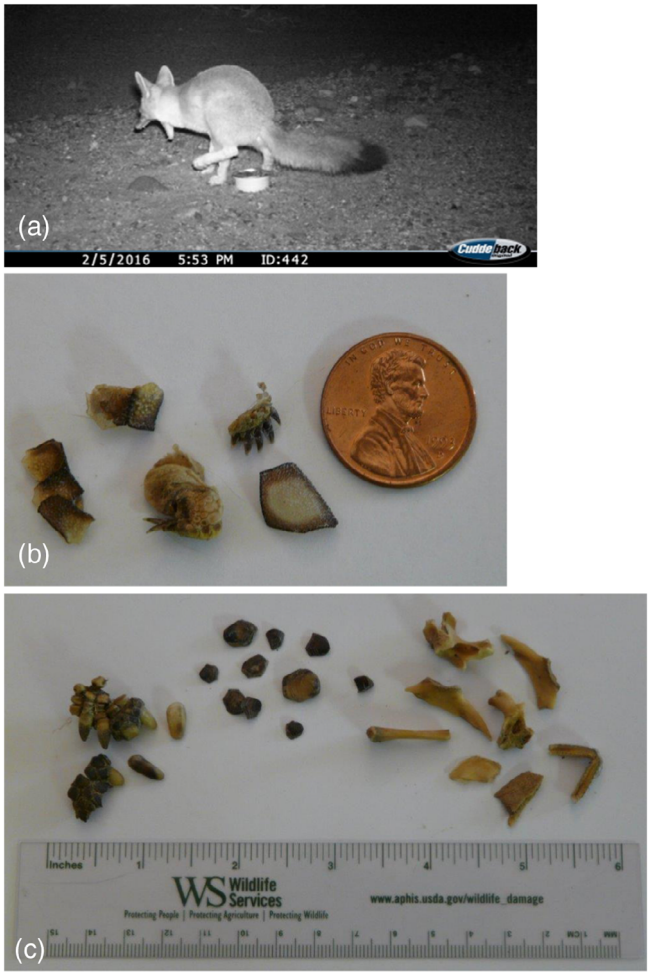

FIGURE 4 (a) Kit fox defecating; (b) and (c) Mojave desert tortoise remains found in scats and pellets

4 | DISCUSSION

4.1 | qPCR is more sensitive than morphological analysis in the detection of tortoise remains in scats and pellets

We found a higher rate (21 times more) of Mojave desert tortoise detection from scats and pellets with our qPCR method than with conventional morphological analysis—of the 65 qPCR positive samples, only three (5%) had identifiable tortoise hard parts, including bones, scales, and shell (Figure 4). This may be due to known biases inherent in morphological analysis that are largely caused by the absence or partial digestion and unidentifiability of hard parts in excreted matter (Matejusová et al., 2008). Our results suggest that previous studies that estimated the frequency of occurrence of tortoise in scats using morphological analysis may have underestimated the true occurrence rate.

Previous studies relied on collector expertise and visual inspection to determine the species of origin for canid scat samples, and it is possible that some samples were misidentified (Smith et al., 2003). In our dataset, collectors correctly assigned species to scat for 79% of the samples. The incidence of misidentification occurred equally for scats visually identified as coyotes that were determined to be kit foxes by species identification PCR and scats visually identified as kit foxes that were determined to be coyotes by PCR.

Although more sensitive than morphological analysis at detecting the presence of tortoise remains, our qPCR method is subject to many of the same caveats. First, the presence of either tortoise DNA or hard parts in scats and pellets is only indicative of consumption; neither method is able to rule out the possibility that the tortoise was consumed post-mortem, that is, the result of scavenging rather than predation. Tortoise mortality could be due to predation by another species, disease, drought (Cypher et al., 2018), or road mortality (Boarman & Sazaki, 2006). Second, neither our qPCR method nor our morphological method can conclusively determine either the number of individual tortoises consumed or the biomass of tissue that was consumed.

To address these caveats in the future, controlled feeding experiments in which tortoises of different age classes are fed to each predator species would be necessary to determine how biomass consumed is related to biomass excreted as identifiable hard parts because conversion factors are unique to each predator–prey pair (Rühe et al., 2008). Controlled feeding experiments would also be necessary to determine how the proportion of biomass consumed is related to the proportion of DNA excreted (e.g., Bowles et al., 2011), how long detectable amounts of DNA are present in excretions post-consumption (e.g., Thalinger et al., 2017), and the rate of false negatives. Because the Mojave desert tortoise is a threatened species with a long generation length of approximately 25 years (USFWS, 1994), it would not be possible to conduct such feeding trials with live Mojave desert tortoises. However, in the future, researchers could utilize opportunistically discovered Mojave desert tortoises that died from other causes, or a tortoise species that is not of conservation concern, for this purpose.

To estimate the number of individual tortoises consumed, future studies could utilize shotgun DNA sequencing (Srivathsan et al., 2015) or in-solution hybridization capture methods to determine multi-locus SNP genotypes for tortoises using DNA extracted from scats or pellets.

By using in-solution hybridization capture, we were able to successfully determine multi-locus SNP genotypes for 17 out of 41 qPCR-positive kit fox and coyote scats, all of which were unique individuals (Parker et al., 2021). For the remaining 24 samples, we cannot rule out the possibility that some of these represent multiple scats produced by a single individual after consumption of a single tortoise. Due to the sensitivity of the qPCR, it is possible that after consumption of a single tortoise, a

canid could continue to produce scats with detectable tortoise DNA for several days following consumption. For example, Thalinger et al. (2017) showed that cormorants fed large fish meals can produce feces with identifiable prey DNA up to 76 h post-consumption. This would result in a higher number of scats containing detectable tortoise remains than individual consumption events.

There are 14 instances in our dataset of multiple tortoise-positive scats (13 pairs and one trio) collected on the same date less than 10 m apart—for example, two samples from two individual coyotes collected at a single locality on 1/06/2017 (Table S1). This is likely the result of latrine use, which is common for coyotes and kit foxes (Ralls & Smith, 2004). In these cases, multiple canids may have consumed the same tortoise—this could be the result of scavenging or, in the case of coyotes, hunting in pairs or family groups (Bekoff, 1977). By counting only one qPCR-positive sample collected in each one-month period—longer than either scats (Sanchez et al., 2004) or carcasses (Beck et al., 2015) are likely to persist in the desert—we can estimate a minimum number of consumption events. By doing this, the number of tortoise-positive samples remaining is nine—three times more than detected with morphological methods.

As mentioned above, future studies could estimate the number of tortoises consumed and the number of predators consuming them by designing and executing protocols capable of genotyping both the predator (canid or raven) and prey (tortoise) using in-solution hybridization capture to identify individuals. For example, Parker et al. (2021) demonstrated the high degree of flexibility and efficiency of using in-solution capture protocols to simultaneously genotype coyotes and kit foxes using noninvasive samples. The availability of published Mojave desert tortoise genomic data (Scott et al., 2020; Tollis et al., 2017) makes it possible to identify a set of informative tortoise SNP loci. The protocols used in Parker et al. (2021) could be modified to include probes targeting polymorphic tortoise SNPs that are capable of identifying individual tortoises. Being able to estimate the number of individual tortoises consumed would give the best estimate of the effects of canid and raven consumption on the Mojave desert tortoise population using noninvasive methods. However, only through morphological analysis would it be possible to determine the age class of the tortoises consumed (e.g., juvenile or adult, Kelly et al., 2021).

Given that the true frequency of tortoise consumption may be higher than previously reported, it will also be important for future studies to investigate whether tortoise consumption is a result of predation or scavenging on carcasses of tortoises killed by disease, drought, road mortality, or other causes (Boarman, 2002). Recent research has shown that it is possible to determine whether consumption occurred on live or dead animals using bacterial biomarkers associated with tissue decay (Muletz-Wolz et al., 2021). Using a similar method could help determine if, for example, multiple predators are feeding on a single carcass, and allow for a more accurate count of the number of tortoise mortality events that are caused by canids and ravens.

In general, qPCR methods are relatively fast and inexpensive, and we found them to be much more sensitive than conventional morphological analyses at detecting the presence of tortoise in scats and pellets, even in material that was not fresh when collected. Therefore, we conclude that qPCR methods offer a suitable, sensitive complement to morphological analysis for identifying tortoise consumption. In the future, our qPCR method could be used to screen large sets of samples for the presence of tortoise DNA. Because enrichment and high-throughput sequencing methods are more expensive than qPCR, only the qPCR-positive DNA samples could then be enriched for predator and tortoise SNP markers to identify the number of individual tortoises consumed and the number of individual predators that consumed them. Alternatively, metagenomics, that is, shotgun sequencing of all DNA present in a fecal sample (Srivathsan et al., 2015), or metabarcoding, that is, PCR amplification of multiple prey species of interest with universal vertebrate barcoding primer sets followed by high-throughput sequencing, could be used to estimate whole diet composition (Paula et al., 2015).

Implications for the conservation of Mojave desert tortoises: canids.

Coyote populations located near human population centers in the Mojave Desert can be subsidized by anthropogenic resources (Esque et al., 2010; Fedriani, Fuller, & Sauvajot, 2001). Kit foxes also increase consumption of anthropogenic resources during times of resource shortages, suggesting some level of subsidization (Kelly et al., 2019). Previous studies have suggested that subsidized, higher density populations can exert higher predation pressure on tortoises than unsubsidized populations, particularly when preferred prey sources become scarce (Esque et al., 2010). However, other studies have found that the increased use of anthropogenic food items is not positively correlated with tortoise predation. For example, Cypher et al. (2018) found that the presence of anthropogenic items in scats increased substantially (from a low of 4.9% scats containing anthropogenic items to a high of 28.7%) after a period of below-average precipitation; yet, they did not detect a concurrent increase in the incidence of tortoise predation. Rather, the occurrence of tortoise remains in scats was positively correlated with tortoise abundance and activity, suggesting opportunistic consumption. In the same area during the same time period, Kelly et al. (2019) found that only

0.57% of 1230 total kit fox scats collected contained identifiable tortoise remains (hard parts). They also showed that while anthropogenic food consumption increased after the drought period, tortoise consumption did not. These two studies relied on morphological identification of tortoise remains in scat to estimate consumption, and therefore may have underestimated the true rate of consumption. Future studies could test if this pattern is observed when using molecular detection methods. If, for example, consumption of tortoise eggs increases during a period of resource scarcity, this is unlikely to be detected with morphological methods.

Over time, anthropogenic subsidization could support larger canid populations than would be present without subsidization, and both coyotes and kit foxes expand their dietary breadth during times of resource shortages (Kelly et al., 2020), which could result in higher predation on tortoises and other prey species. This suggests that it is important to accurately track the rates of canid predation on Mojave desert tortoises over time to determine whether there is increased consumption following droughts when preferred prey like rabbit and rodent populations decline (Kelly et al., 2019). It is also important for land managers to adopt policies to limit anthropogenic resources and monitor canid population sizes over time to determine the effectiveness of these policies.

In addition to decreasing anthropogenic resources to avoid predator subsidization, managers may also use conditioned taste aversion methods (Maguire, Stojanovic, & Weston, 2009) to deter canids from preying on tortoises by baiting canids with artificial tortoise or egg models treated with taste aversion chemicals (Boarman, W.I. *pers. comm*; Maguire, Stojanovic, & Weston, 2009). Our rate of tortoise detection in kit fox scats was much higher than previous estimates. This could be in part because kit foxes prey on tortoise eggs (Bjurlin & Bissonette, 2004), which may not be detectable from morphological analysis if mostly egg yolks are consumed. This could be tested through feeding trials, for example, by feeding shelled chicken eggs to captive foxes followed by qPCR analysis with chicken-specific primers. Preventing egg predation may be particularly important for the survival of Mojave desert tortoises because kit foxes are the primary predators of tortoise nests (Bjurlin & Bissonette, 2004).

4.2 | Common raven populations in the BCCEA and implications for the conservation of Mojave desert tortoises

Common ravens nest throughout a large portion of the study area in relatively low densities (Boarman et al., 2018). We demonstrated that ravens consume tortoises at fairly high rates; 12 of 34 (35.3%) raven pellets contained tortoise DNA. This proportion closely aligns with previous estimates of raven predation derived from studies using attack rates on highly realistic three-dimensional printed models of juvenile tortoises as surrogates for actual juveniles that are very difficult to find in the field, either dead or alive, except directly beneath raven nests (Boarman, 2003). In one study, nearly 44% of the artificial models were visited by ravens and 18% of them were physically attacked within 1 week (Boarman, W.I. *pers. comm*). These frequent attacks on juveniles may lead to unsustainably high rates of mortality in declining tortoise populations (Congdon et al., 1993). This indicates that strong measures may be needed to reduce raven predation. Because raven populations increase in the presence of anthropogenic resources, and as a result increase predation pressure on tortoises (Boarman, 2003; Kristan & Boarman, 2003), reduction of anthropogenic resources should be a part of management actions in the landscape around the BCCEA.

5 | CONCLUSION

We described a qPCR assay that can be efficiently implemented to screen canid scat and raven pellets to detect the presence of federally threatened Mojave desert tortoise remains. We found that our qPCR method is a fast and sensitive complement to morphological analyses to detect traces of tortoise in canid scats and raven regurgitated pellets, even using samples that were not always fresh when collected. We documented that the frequency of occurrence of tortoise remains in predator scats is likely higher than previously reported in the study area and should be further evaluated for active management. Conservation managers may consider actions to better mitigate resource subsidizes and activities that attract and support predator populations and to discourage canids from consuming tortoises using conditioned taste aversion methods. Our methods may be replicated and extended in the future to assess the impact of management strategies aiming to decrease predation rates.

ACKNOWLEDGMENTS

This work was supported by the Desert Conservation Program of Clark County Nevada. The authors thank Scott Cambrin and Larry Mata of the Desert Conservation Program, and Nancy Rotzel McInerney, Natalí Chavez, and Margad-Erdene Ochirbat of the Center for Conservation Genomics for logistical support and help with lab work.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

William I. Boarman conceived the project, and William I. Boarman, Robert C. Fleischer, and Jesús E. Maldonado secured funding; Lillian D. Parker, Michael G. Campana, Robert C. Fleischer, William I. Boarman, and Jesús E. Maldonado designed the study, William I. Boarman and Ryan Boarman conducted field work and collected samples; Lillian D. Parker, Jessica D. Quinta, and Isabel Rivera carried out laboratory work; Brian L. Cypher and Erica C. Kelly performed morphological hard-parts analysis and provided photographs; Lillian D. Parker analyzed and archived the data and wrote the manuscript with input from all authors; Lillian D. Parker, William I. Boarman, and Ryan Boarman drafted figures and maps; all authors participated in revisions and acceptance of the manuscript.

DATA AVAILABILITY STATEMENT

Locations and dates of sample collection of tortoise qPCR-positive raven pellets and species-identified canid scats are provided in Table S1; primer sequences are provided in the methods section.

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How to cite this article: Parker, L. D., Quinta, J. D., Rivera, I., Cypher, B. L., Kelly, E. C., Campana, M. G., Fleischer, R. C., Boarman, R., Boarman, W. I., & Maldonado, J. E. (2022). Genetic analyses are more sensitive than morphological inspection at detecting the presence of threatened Mojave desert tortoise (*Gopherus agassizii*) remains in canid scat and raven pellets. *Conservation Science and Practice*, e12689. https://doi.org/10.1111/csp2.12689