Non-Trapping, Non-Invasive, Rapid Surveillance Sampling Using Tracking Tunnels, Trail Cameras, and eDNA to Determine Presence of Pest Predator Species

Aaron B. Shiels
USDA National Wildlife Research Center, Fort Collins, Colorado

Tyler Bogardus
Oahu Army Natural Resources, Schofield Barracks, Hawaii

Claudia D. Lombard
US Fish and Wildlife Service, St Croix, US Virgin Islands

Nicole F. Angeli
Government of the Virgin Islands, Department of Planning and Natural Resources, Division of Fish and Wildlife, St. Croix, U.S. Virgin Islands

Matthew W. Hopken
USDA National Wildlife Research Center, Fort Collins, Colorado and Department of Microbiology, Immunology, and Pathology, Colorado State University, Fort Collins, Colorado

Antoinette J. Piaggio
USDA National Wildlife Research Center, Fort Collins, Colorado

ABSTRACT: A common challenge for land managers is knowing which vertebrate pest species are present in areas they manage, especially if such areas are remote like isolated habitats, rugged terrain, or infrequently traveled islands. Most invasive predator species, such as feral dogs, cats, mongoose, and commensal rodents pose great threat to human health and key resources such as native species. Animal trapping to determine the presence of a pest species can be expensive and dangerous, requiring permits, experienced personnel, multiple days, and multiple trapping methods. Furthermore, many invasive pest species may go unnoticed because they are nocturnal, secretive, or leave little evidence of their presence. Tracking tunnels, trail cameras, and environmental DNA (eDNA) are non-trapping methods that can be used to rapidly assess if vertebrate pest species are present in a given habitat or ecosystem, including before, during, and after pest suppression techniques are implemented. We share tracking tunnel dimensions and specifications so readers can make their own tracking tunnels for rodent and other small mammal sampling, and we provide some common distributors where tracking tunnels can be purchased. A brief overview of trail camera technology and eDNA forensic uses are described, as well as their applications for vertebrate pest identification, surveillance, and damage management. To demonstrate these methods, we share example case studies from the Caribbean, including first time records of house mouse presence at Sandy Point National Wildlife Refuge in St. Croix (US Virgin Islands) and along a rainforest elevation gradient in the Luquillo National Forest, Puerto Rico. Additionally, we describe case studies of trail camera use on Desecheo Island (Puerto Rico) to determine brodifacoum bait consumption, and eDNA use in Wyoming to determine native bird depredation events. Tracking tunnels and trail cameras are recommended as quick and inexpensive ways to reveal the vertebrate pest species that are present at a site or habitat. These non-trapping, non-invasive techniques can provide quick and efficient methods of surveillance, detection, and monitoring of vertebrate pests, and otherwise may be used as effective tools to aid in wildlife damage management.

KEY WORDS: brodifacoum rodenticide, eDNA, island invasive species, isolated habitat sampling, Mus musculus, native biodiversity, pest monitoring, tracking tunnels, trail cameras, Rattus spp.

INTRODUCTION

Pest control companies and land managers often strive to determine, in a relatively quick and safe manner, which vertebrate species are present at a site. Commensal pest species such as invasive rodents (e.g., black rat (Rattus rattus), Norway rat (R. norvegicus), house mouse (Mus musculus)) are frequently sought as culprits of damage, and these species are among the most destructive for urban, agriculture, and natural resources in the U.S. (Witmer and Shiels 2018) and worldwide (Capizzi et al. 2014). Live- or kill-trapping is commonly used to determine vertebrate presence, but trapping may not be the most efficient or otherwise desirable method to accomplish such goals in many regions. For example, when sampling with traps to determine if particular vertebrate pests are present at a site, not all vertebrates that enter a trap are intended to be caught. Additionally, traps are specific to certain animals and therefore some may not be caught; and finally, many institutions and agencies require frequent (e.g., each 24 hour) trap checks, and trapping often requires permits and animal handling experience to maximize safety.

The non-trapping techniques that we cover here include tracking tunnels (Shiels and Ramirez de Arellano 2018), trail cameras (Shiels et al. 2019), and environmental DNA (eDNA) (Piaggio et al. 2013). These techniques are quick and efficient relative to trapping,
which enables their use in remote or isolated ecosystems (e.g., rugged terrain, islands) or in unfamiliar areas (e.g., new structures or sites) where the vertebrate community is not well known. These three non-trapping techniques do not require live-animal handling, and often do not require permits, or at least not at the level that most live animal sampling requires. Further, these non-invasive techniques are safer for the human observer because animal bites and scratches are avoided, and pathogen transmission is minimized. Non-invasive methods also minimize animal stress (especially compared to live-traps or the momentary stress imposed by kill-traps); also, trap shyness is minimized, as is risk to non-target species. Finally, in areas where pest vertebrate species population suppression is occurring, these non-invasive techniques can provide a method of independent monitoring that allows one to assess the efficacy of target population suppression to help ensure management goals are met. For example, if bait

Figure 1. (Upper picture) A tracking tunnel (black tunnel; by Pest Control Research LP) with a peanut butter baited and inked tracking card ready to be inserted into the tunnel, and (Lower picture) a tracking card collected at Sandy Point National Wildlife Refuge, St. Croix, U.S. Virgin Islands, with tracks of mongoose (largest foot prints, in central and left side), rat (medium foot prints, at far right side, and lowest in center), and house mouse (smallest foot prints, or most abundant tracks of smallest dots visible).
stations are deployed to reduce vertebrate pest populations, evidence of chewing on the bait or bait removal is not an effective index for concomitant population monitoring because it is not an independent method; similarly, using number of trapped animals while suppressing a vertebrate pest by traps is not an independent monitoring method to assess population suppression. Thus, tracking tunnels, trail cameras, and eDNA would provide independence for monitoring population reduction simultaneously with any population suppression technique (Innes et al. 1995, Lindsey et al. 1999, Shiels et al. 2019).

Here we provide descriptions of three non-trapping techniques and their common uses in a variety of ecosystems where the identification of vertebrate pest species presence is sought. We give example case studies of how tracking tunnels, trail cameras, and eDNA are used for monitoring and surveillance for vertebrate pests. Lastly, we provide the specifications and costs associated with each of these tools and, for tracking tunnels, we detail how these monitoring devices can be made, as well as the retailers where they can be purchased.

Tracking Tunnels - Specifications and Costs
Tracking tunnels are baited ink cards placed in tunnels so that foot prints of animal visitors can be captured and identified. Most of the tracking tunnels that are sold for rodent sampling have the following dimensions: ~60 cm long with 10 × 10 cm openings (Figure 1). Two commonly used websites to purchase the rodent-sized tracking tunnels are Pest Control Research LP (www.traps.co.nz) and Gotcha Traps Ltd (gotchatraps.co.nz); both companies operate out of New Zealand and currently there are no distributors in the U.S. Although each website gives the cost per unit, the shipping from New Zealand to the U.S. is quite expensive; quotes that include shipping estimates can be obtained by contacting these companies, but a rough estimate of costs in U.S. dollars with shipping included is approximately $12 for a tracking tunnel, and about $1.30 for the tracking card and ink.

As an alternative to purchasing the tracking tunnel equipment from the New Zealand companies, many ecologists and land managers make their own tracking tunnel equipment (Lindsey et al. 1999). Some of the material for the tunnels include corrugated plastic, paperboard, juice or milk cartons (either plastic or wax-coated paperboard), and PVC pipes. A wire flag or turf staples are often used to secure the tunnels on the ground. Common material used for the tracking cards include construction paper (available at art supply stores) or rite-in-the-rain paper. Paperclips can be used to secure the tracking cards in the base of the tracking tunnel so wind or animals do not displace them. Tracking ink can be made using computer printer ink mixed with mineral or vegetable oil; common shoe polish dispensers that have a foam applicator may be used to distribute the tracking ink. Tracking ink can be applied directly to the center of the tracking cards or applied to a piece of peel-and-stick contact paper in the center of the tracking card such that the slick and waxy surface prevents the ink reservoir from absorbing into the paper prior to animal visits. We recommend an inked area of about 10 × 19 cm in the center of a 49-cm tracking card, like those that Gotcha Traps Ltd sell (Figure 2); use of substantially smaller ink reservoirs risks animals feeding on bait without getting ink on their feet or otherwise leaving no tracks. Typically, the bait of choice (e.g., peanut butter, coconut chunks) is placed in the center of the tracking card with the ink reservoir surrounding it (Figure 1). Depending on study goals, tracking tunnels can be placed on the ground or secured in the canopy (Lindsey et al. 1999). Furthermore, the case studies outlined below provide additional details of tracking tunnel use including common spacing between tracking tunnels along a transect, which is typically about 20-50 m apart for rats and mice (Lindsey et al. 1999, Shiels 2010).

Trail Cameras - Specifications and Costs
Trail cameras are digital, motion-activated infra-red (IR) cameras that are typically used by hunters to help establish game trails and behaviors. Each trail camera contains a battery source and SD card, and these items as well as the camera setting functions are contained in a waterproof housing. There are a variety of trail cameras and associated prices. Some of the characteristics that determine the price are picture quality; multi-triggering

Figure 2. A tracking card (~49 cm; by Gotcha Trap Ltd., Auckland, New Zealand) with an inked area of about 10 × 19 cm in the center of the card; these particular cards are purchased with the ink already in place.
feature and timing; video option; reliability (i.e., the likelihood that the camera will trigger when an animal is present); additional flexibility; and image transfer (e.g., cellular communication). Costs for trail cameras range from approximately $100 (basic model) to $1200 (cellular capabilities to send/receive images). The Reconyx Hyperfire HC600 is commonly used for ecological and conservation research with good reliability, and it is about $500-$600.

**eDNA - Specifications and Costs**

Environmental DNA is DNA that is collected from soil, water, or other substrate where DNA has been exuviated. Aquatic eDNA sampling has been common, but terrestrial eDNA sampling has been targeted less frequently (Deiner et al. 2017). Examples of terrestrial vertebrate detection using eDNA include Burmese pythons (Python bivittatus) in waterways in Florida (Piaggio et al. 2013), and feral swine (Sus scrofa) from domestic water troughs or sampling wallows (Williams et al. 2017).

Environmental DNA can be used to identify the predators of deposed bird eggs or carcasses by swabbing saliva from the eggs or carcasses. This technique has proven successful in determining the predators of greater sage grouse (Centrocercus urophasianus) nests: Hopken et al. (2016) used two mitochondrial loci to screen for predator DNA from swabs taken from grouse eggs and carcasses (see case studies below for more details).

Importantly, eDNA is typically DNA of low quality and quantity, and therefore multiple sample replicates from the field and technical replicates in the lab are required to avoid false negatives and confirm positives. Typical costs for eDNA analysis are $25-$75 per sample due to technical replicates. When using eDNA to determine the predators of carcasses or eggs, it is important to consider the following: 1) the sample must be obtained within ~24 hours of the depredation event; 2) it is best to collect the whole carcass or egg material to enable the eDNA laboratory to swab and sample the deposed items meticulously and with replicates, which can be difficult or time-consuming in field situations; 3) the samples must be immediately frozen and then shipped to a genetics laboratory that specializes in eDNA analysis; and 4) utmost care must be taken to prevent sample contamination.

**METHODS**

**Tracking Tunnel Case Studies**

Tracking tunnels were recently (in Summer 2017) used in two Caribbean locations. The first location was in the El Yunque National Forest (ENF), which is in northeastern Puerto Rico (18°18’N, 65°50’W). The ENF is a 19,650 ha tropical evergreen rainforest, spanning elevation of 150-1075 m. The main objective of the research, which is summarized in Shiels and Ramirez de Arellano (2018), was to use tracking tunnels to determine whether non-native (invasive) rodents occur along the 0-1075 m elevation gradient that passes along the main highway through the ENF.

Three tracking tunnels were placed at each 50 m elevation-gain (n = 66 total tunnels), beginning at sea level (1 m elevation) and then passing through the ENF to El Yunque peak (1075 m). At each 50 m elevation point, the three tunnels were spaced ~20 m linear distance from the next closest tunnel; the tunnel spacing was based on average mouse and rat nightly linear movements in Hawaiian forest (Shiels 2010). At each location, a tracking tunnel was placed on the ground and an inked and baited (Skippy creamy peanut butter topped with a 2 × 2 cm coconut chunk) card was placed inside the tunnel. All tunnels, cards, and ink were purchased from Pest Control Research LP (www.traps.co.nz). Rodent activity was measured as the number of tunnels (up to three) for which there were rodent tracks present. Tracking tunnels were set approximately 3-5 m from the road edge along this 1-1070 m elevation gradient on 31 July 2017, and recovered one day later. Although black rats have been the only rats ever recorded in the ENF, it is not possible to unequivocally determine rat species’ tracks present in tracking tunnels; therefore, rodent tracks were scored as either rat or house mouse.

A second example of a case study where tracking tunnels were used was at Sandy Point National Wildlife Refuge (NWR), St. Croix, U.S. Virgin Islands (17°40’N, 64°54’W), which is a 146 ha peninsula on the southwest corner of the island of St. Croix with elevation that spans from sea level to ~4 m. A predator-proof fence (Young et al. 2013; Angeli and Fitzgerald, in press) has been proposed to extend the width of the peninsula at the eastern end; such a fence would provide protection for sea turtles, as the NWR is one of the most populous sea turtle nesting grounds in the Caribbean. If the fence was to be established, all vertebrate predators would be removed from the interior and thus maintained as predator-free. It is necessary to know the whole suite of invasive predators present at the NWR to establish strategies for both the needed aperture of the fencing material so it keeps the smallest vertebrates from passing through, and to determine the best predator-removal strategies (e.g., traps and poison bait, and the amounts and types needed to be effective). Prior to our recent study using tracking tunnels, the known vertebrate predators at the NWR were feral dogs (Canis familiaris), cats (Felis catus), mongoose (Herpestes auropunctatus), and black rats.

Tracking tunnels were placed along the access road that travels the length of the peninsula at the NWR. A total of 34 tracking tunnels were set on July 27, 2017, and they were recovered 24 hours later. Each tracking card was baited with approximately a tablespoon of Skippy creamy peanut butter (Hormel Foods, Austin, MN) topped with a 2 × 2 cm coconut chunk. Tracking tunnels were spaced approximately 25 m apart and placed inside the vegetation about 1-3 m from the edge of road.

**Trail Camera Case Study**

During a rat eradication on Desecheo Island, Puerto Rico in 2016, trail cameras were used to determine the animals responsible for eating rodenticide bait pellets. The target species being removed was the black rat; however, there were several endemic lizards and native birds on the island that could potentially become exposed to the toxicant (brodifacoum) that was mixed into the bait pellets. Desecheo (18°23’14”N, 67°28’19”W) is a small (1.2 km²
or 116 ha) and dry island approximately 21 km from the western shore of the main island of Puerto Rico with a peak elevation of 218 m. Further details about Desecheo, the rat eradication operation and monitoring, and biotic responses to the rat removal are summarized elsewhere (Shiels et al. 2017a, Shiels et al. 2017b, Shiels et al. 2019).

Trail cameras were established at 11 sites on Desecheo in areas near established trails. A total of 15 cameras were used simultaneously for this study; all 11 sites had trail cameras monitoring bait pellets and some sites had multiple cameras. There were 12 Reconyx HyperFire trail cameras (models HC500 and HC600) and three Browning (model BTC-6HD) trail cameras used. Each camera was secured to the lower 30-70 cm of a tree or rock and aimed at two bait pellets placed 40-90 cm away from the camera. We ensured that the cameras were pointed at the two bait pellets by using the ‘walk test’ function on the cameras where an indicator light would provide evidence that our motion at the two pellets would successfully trigger the camera. The Browning trail cameras did not have a ‘walk test’ function, so we triggered the camera and viewed the pictures to better position the cameras to monitor the bait pellets. The cameras were set to be triggered by motion, but also were programmed to take a picture each hour (on the hour), and sometimes more frequently (15 or 30 min) at set intervals to help account for periods where bait disappeared or was visited without an animal triggering the camera (e.g., insects rarely trigger these cameras). Once a Reconyx camera was triggered by motion, it would take 10 consecutive pictures over 20 seconds; Browning cameras would take one picture each time triggered.

Upon activating the cameras on the day of each bait application, the baits and cameras were checked daily for at least seven consecutive days, which was the duration that field staff were on the island. For analysis, we scored the number of incidences where an animal was observed contacting the bait (i.e., touching, eating, removing), which included consuming it (i.e., evidence of gnawing or swallowing bait). An incidence ended when the animal left the camera’s field of view, and a series of pictures produced by one triggering event was also considered one incidence. Here we share the first six days of trail camera activity following the first application of bait on Desecheo; the additional 1.5 months are summarized in Shiels et al. (2019).

cDNA Case Study
Environmental or non-invasive DNA sampling was used in Wyoming sagebrush country in attempt to identify the nest predators of greater sage grouse by swabbing for predator saliva on depredated eggs and carcasses. The optimized method could be applied to identifying mammalian predators of any ground nesting bird eggs and adult carcasses. Briefly, there were 14 sage grouse nests identified as depredated, and seven sage grouse carcasses found. Carcasses and egg shells were swabbed for mammalian predator saliva and cDNA analysis was completed at the USDA NWRC Genetics Laboratory. This analysis included sequencing two partial fragments of two mitochondrial loci (Cytochrome-b and Control Region) and genotyping eight canid-specific microsatellite loci for canid predator species identification. Additional details for this study are reported in Hopken et al. (2016).

RESULTS
Tracking Tunnel Case Studies
Of the 66 tracking tunnels set along the Highway 191 elevation gradient in the ENF, most contained tracks from rats (83%) and house mice (50%). It should be noted that each tracking card can be tracked by multiple animal species. House mice had not been previously known to occur in this forest. There was just one tunnel (2%) with mongoose tracks, which was at 700 m elevation, and one tunnel at 1000 m elevation that had the tracking card removed from the tunnel with tracks left by a house cat. Several tunnels showed evidence of ants and unidentifiable insects, over one-third had evidence of Caracolus caracoll snails, and some had lizard tracks and frog (probably Eleutherodactylus spp.) tracks. Conclusions from this study included 1) house mice had not been previously reported in the ENF, and were found only at the forest edge along Highway 191 at some elevations between 50-150 m and 300-1070 m, whereas rats were found at all elevations and in all habitat types sampled, and 2) logistic regressions revealed that mice and rat presence increases with elevation (Shiels and Ramirez de Arellano 2018).

Of the 34 tracking tunnels set at the Sandy Point NWR on St. Croix, 21% were tracked by mongoose, 18% by house mice, 9% by rats, and 3% by cat (Table 1). House mice had not been known to the NWR before this study. Further details for this study are reported in Hopken et al. (2016).

Table 1. Results of tracking tunnels that were set at Sandy Point National Wildlife Refuge, St. Croix, U.S. Virgin Islands. There were 34 tracking tunnels set, and each was checked after 1 day to identify animal tracks. Note that a single tracking card can have multiple animal tracks present.

| Animal Tracking | No. of Cards Tracked (out of 34) | % of Cards Tracked |
|-----------------|---------------------------------|-------------------|
| Mongoose        | 7                               | 21                |
| House mouse     | 6                               | 18                |
| Rat             | 3                               | 9                 |
| Cat             | 1                               | 3                 |
| Lizard          | 1                               | 3                 |
| Ant             | 5                               | 14                |
| Total insect    | 18                              | 53                |
sampling. Of the 34 tracking tunnels, there were just two that did not contain any tracks, as most tracking cards at least had insect tracks (53%), some of which were ants (15%) (Table 1). The tracking tunnels that did receive visits from mammals did not have any bait remaining; those that had no tracks or only had insect tracks still had bait remaining.

**Trail Camera Case Study**

During the first five days after the first bait application on Desecheo, there were 40 animals pictured in contact with the bait: twenty black rats, 13 hermit crabs (*Coenobita clypeatus*), five ameiva lizards (*Ameiva desechensis*), and two insects. Rats and hermit crabs ate the bait pellets in place or removed them from the field of view. The ameivas did not consume the bait pellets, but instead they touched the pellets with their tails or other parts of their body as they passed by the pellets. Additionally, there were no rats pictured after six days on any of the trail cameras for the remainder of the study (Shiels et al. 2019).

eDNA Case Study

Of the sage grouse nests and carcasses that were identified as depredated and swabbed for mammalian DNA, there were 11 of 14 nests (79% success rate), and three of the seven carcasses (47%), where the family or species of predator could be identified. The main nest predator was coyote (*Canis latrans*; five of the 11), followed by domestic dogs (*C. lupus familiaris*), and the remaining of the predator identifications included cattle (*Bos taurus*), striped skunks (*Mephitis mephitis*), a deer mouse (*Peromyscus maniculatus*), and a wild canid (*Canis* sp.) that could not be confidently identified to a lower taxonomic level (Hopken et al. 2016).

**DISCUSSION**

The three non-trapping, non-invasive techniques highlighted can provide quick and efficient methods of surveillance, detection, and monitoring of vertebrate pests, and otherwise be used as effective tools to aid in wildlife damage management. Tracking tunnels and trail cameras do not require handling live or dead animals, and only in certain cases such as swabbing eggs or carcasses to determine what animal was responsible for depredation does eDNA require (dead) animal handling. Tracking tunnels and trail cameras are particularly useful for sampling new, remote, or infrequently visited locations; eDNA often requires visitation and sampling 24-48 hours following a depredation event (which can be monitored by cameras at nests) for successful predator determination. Costs of such sampling equipment ranges from relatively inexpensive tracking tunnels (~$12 each) to several hundred dollars for a trail camera. Tracking tunnels also have been used to identify invertebrates, such as rare insects (Watts et al. 2008), or common insects as found in our case studies. Each of these three non-invasive techniques may be valuable components of pest management plans, including those in commensal and natural area settings.

Some of the key benefits for tracking tunnel use were demonstrated by the case studies presented. There is no animal handling or associated permits when using tracking tunnels, enabling sampling in remote locations, such as infrequently traveled island habitats where little is known about the small mammal community. Because the tracking tunnels are inexpensive, a large number of replicates can be included, as demonstrated across the Highway 191 elevation gradient in Puerto Rico (n = 66 tunnels) and the linear distance of Sandy Point NWR in St. Croix (n = 34 tunnels). An additional benefit of the tracking tunnels over traps is the lack of metal, as small mammals such as rodents are often shy to enter metal traps; additionally, some traps have large enough apertures that small vertebrates, such as house mice, may not be caught or can escape through the metal mesh material used for traps, which is not the case with tracking tunnels (Shiels 2010, Shiels et al. 2019). Metal traps and large apertures may be possible reasons why house mice were not captured or detected in previous rat-trapping attempts in the ENF (Engeman et al. 2006). The few shortcomings with using tracking tunnels include 1) it is not always possible to determine the animal species visiting a tunnel (e.g., the different species of *Rattus* are not always distinguishable based on tracks); 2) the tracking tunnels provide an index, not a density of animal populations (e.g., a single individual could circulate multiple tracking tunnels, despite attempts to account for much of this by spacing the tunnels according to the animal’s biology/ecology); and 3) the tracking tunnels need to be retrieved before inked tracks become covered or cards degrade, which can occur within 48 hours in rainforest (Shiels and Ramirez de Arellano 2018), but generally the tracking cards should be collected within ~1 week following deployment.

House mice had not been previously discovered at ENF or NWR despite attempts of live-trapping using Tomahawk traps at both locations and using tracking plates, which is similar to tracking tunnels but without the tunnel, in the ENF (Engeman et al. 2006). The presence of house mice along ENF Highway 191 gradient, and their absence at other interior forest habitats in the ENF that were sampled (Shiels and Ramirez de Arellano 2018), may be due to their highly commensal behavior (Witmer and Jojola 2006), small home ranges (Shiels 2010), and likely points of introduction (i.e., via cargo transported up the highway). Alternatively, invasive rats were found at all elevations and habitats sampled in the ENF (Shiels and Ramirez de Arellano 2018), have much larger home ranges than house mice (Shiels 2010), and have been documented in the ENF for over 60 years (Weinbren et al. 1970). At Sandy Point NWR where house mice were tracked along the access road that spans the entire length of the refuge peninsula, mice probably dispersed from cargo moved along the access road or from the dwellings adjacent to the refuge property lines. If mice had not been detected at Sandy Point NWR, strategies for rodent elimination would have likely been different because some baits and traps are less effective on mice than on rats, and the size aperture of the predator proof fencing would not need to be as small if mice were not present in this region of St. Croix. Knowing the rodent community in the ENF and NWR will help generate successful future strategies of rodent management or elimination.
Trail cameras have a variety of uses within wildlife damage management, and are commonly used for surveillance, detection, and monitoring. For large mammals and birds that cannot pass through the small mammal tracking tunnels that we have described above, trail cameras can be used in their place. In Hawaii, trail cameras are used to monitor bird nests (VanderWerf 2001), feral pig traps and game trails, and feral dog and cat populations (T. Bogardus, unpubl.). Additionally, trail cameras may be implemented to assess particular prey (e.g., fruit and seed) that are most attractive or vulnerable to rodent predation (e.g., Shiels and Drake 2011), and to document biological change after rodent removal by quantifying before and after native prey survival (e.g., Pender et al. 2013).

On Desecheo Island, trail cameras worked well to document the community of animals that visited and contacted the rodenticide bait pellets. As expected, black rats (the target pest) were the animal species documented the first five days with the most number of contacts with bait pellets. However, much of the bait was collected and consumed by the native hermit crabs, which are not affected by the brodifacoum toxicant (the action of this toxicant is vertebrate-specific). Furthermore, after six days following the first application of bait, and during the next 1.5 months when the cameras were monitoring, there were no rats observed by the trail cameras, which was likely due to successful rodenticide elimination of the rat population (Shiels et al. 2017a, Shiels et al. 2019). Thus, trail cameras provide temporal and spatial information regarding the effectiveness of rodent removal; help inform rodenticide hazards; and easily incorporate with rodent removal operations. Furthermore, trail cameras can be placed across a variety of habitats, installed to monitor bait or resources of interest for extensive periods (days to months), and reliably record diurnal and nocturnal visitation of target and non-target animals while not substantially altering behaviours or harming resident animals.

Environmental DNA is a non-invasive technique that can be used to detect or monitor predators in all (terrestrial and aquatic) environments. Applications of this technique spans predator detection from recently depredated bird nests or animal carcasses, to follow-up surveillance to determine if predator removals (e.g., island-wide eradication) were successful. Although eDNA is low quantity and quality DNA, increased technology is allowing for detections of animal species (and possibly individuals) from regular behaviors of rubbing and shedding of cells and through bodily fluids such as saliva, urine, and scat. Although potentially a widely applicable tool in wildlife management, users must be aware that 1) eDNA sampling must generally occur within 24-48 hours of the visit or event (e.g., depredation), and 2) samples must be frozen or have a preservation buffer added upon collection and shipped to a molecular ecology laboratory (Williams et al. 2016).

Ground- and tree-nesting birds can suffer predation by a suite of species including invasive or native pest species. In New Zealand and Hawaii, predator-proof fencing has been used to maintain predator-free zones such as within bird nesting grounds where dogs, cats, and rats are common predators (Young et al. 2013). Similarly, tree-nesting birds like the endangered ‘elepaio (Chasiempis sandwicensis ibidis) are depredated by invasive rats in Hawaii, as evidenced by trail cameras (VanderWerf 2001). Environmental DNA provides another tool for identifying nest predators, which in some cases may be more applicable to certain field situations than other methods (Hopken et al. 2016).

ACKNOWLEDGMENTS
This research was funded by USDA and approved by the USDA NWRC Institutional Animal Use and Care Committee (IACUC) as QA-2805. Mention of a company or commercial product does not mean endorsement by the U.S. government.

LITERATURE CITED
Angeli, N. F., and L. A. Fitzgerald. In press. Reintroducing species when threats still exist: suitability of contemporary landscapes for island endemics. Oryx.
Capizzii, D., S. Bertolino, and A. Mortelliti. 2014. Rating the rat: global patterns and research priorities in impacts and management of rodent pests. Mammal Review 44:148-162.
Deiner, K., H. M. Bik, E. Mächler, M. Seymour, A. Lacoursière-Roussel, F. Altermatt, S. Cree, I. Bista, D. M. Lodge, N. de Vere, M. E. Pfrender, and L. Bernatchez. 2017. Environmental DNA metabarcoding: transforming how we survey animal and plant communities. Molecular Ecology 26:5872-5895.
Engeman, R., D. Whisson, J. Quinn, F. Cano, P. Quinnones, and T. H. White, Jr. 2006. Monitoring invasive mammalian predator populations sharing habitat with critically endangered Puerto Rican parrot Amazona vittata. Oryx 40:95-102.
Hopken, M. W., E. K. Orning, J. K. Young, and A. J. Piaggio. 2016. Molecular forensics in avian conservation: a DNA based approach for identifying mammalian predators of ground-nesting birds and eggs. BMC Research Notes 9:14.
Innes, J., B. Warburton, D. Williams, H. Speed, and P. Bradford. 1995. Large-scale poisoning of ship rats (Rattus rattus) in indigenous forests of North Island, New Zealand. NZ Journal of Ecology 19:5-17.
Lindsey, G. D., S. M. Mosher, S. G. Fancy, and T. D. Smucker. 1999. Population structure and movement of introduced rats in an Hawaiian rainforest. Pacific Conservation Biology 5:94-102.
Pender, R. J., A. B. Shiels, L. Bialic-Murphy, and S. M. Mosher. 2013. Large-scale rodent control reduces pre- and post-dispersal seed predation of the endangered Hawaiian lobeliad, Cyanola superba subsp. superba (Campanulaceae). Biological Invasions 15:213-223.
Piaggio, A. J., R. M. Engeman, M. W. Hopken, J. S. Humphrey, K. L. Keacher, W. E. Bruce, and M. L. Avery. 2013. Detecting an elusive invasive species: a diagnostic PCR to detect Burmese python in Florida waters and an assessment of persistence of environmental DNA. Molecular Ecological Restoration 14:374-380.
Shiels, A. B. 2010. Ecology and impacts of introduced rodents (Rattus spp. and Mus musculus) in the Hawaiian Islands. Ph.D. dissertation, University of Hawai‘i at Mānoa, Honolulu, HI.
Shiels, A. B., and D. R. Drake. 2011. Are introduced rats (Rattus rattus) both seed predators and dispersers in Hawaii? Biological Invasions 13:883-894.

Shiels, A. B., G. W. Witmer, C. Samra, R. S. Moulton, E. W. Ruel, J. R. O’Hare, J. D. Eismann, S. F. Volker, and D. A. Goldade. 2017a. Assessment of bait density, bait availability, and non-target impacts during an aerial application of rodenticide to eliminate invasive rats on Desecho Island, Puerto Rico. Final Report QA 2588. USDA, APHIS, WS, NWRC, Ft. Collins, CO.

Shiels, A. B., W. P. Haines, K. J. Swinnerton, S. Silander, C. Figuerola- Hernández, D. Will, J. G. García-Cancel, and C. W. Torres-Santana. 2017b. Sudden appearance and outbreak of Eunica monima (Lepidoptera: Nymphalidae) on Desecho Island, Puerto Rico. Florida Entomologist100:176-179.

Shiels, A. B., and G. E. Ramírez de Arellano. 2018. Invasive rats (Rattus sp.), but not always mice (Mus musculus), are ubiquitous at all elevations and habitats within the Caribbean National Forest, Puerto Rico. Caribbean Naturalist 48:1-14.

Shiels, A. B., D. Will, C. Figuerola- Hernández, K. J. Swinnerton, S. Silander, C. Samra, and G. W. Witmer. 2019. Trail cameras are a key monitoring tool for determining target and non-target bait-take during rodenticide removal operations: evidence from Desecho Island rat eradication. Pages 223-230 in C. R. Veitch, M. N. Clout, A. R. Martin, J. C. Russell and C. J. West, editors. Island invasives: scaling up to meet the challenge. Occasional Paper SSC no. 62. Gland, Switzerland: IUCN.

VanderWerf, E. A. 2001. Rodent control decreases predation on artificial nests in O’ahu ‘elepaio habitat. Journal of Field Ornithology 72:448-457.

Watts, C. H., D. Thornburrow, C. J. Green, and W. R. Agnew. 2008. Tracking tunnels: a novel method for detecting a threatened New Zealand giant weta (Orthoptera: Anostostomatidae). NZ Journal of Ecology 32:92-97.

Weinbren, M. P., B. M. Weinbren, W. B. Jackson, and J. B. Villella. 1970. Studies on the roof rat (Rattus rattus) in El Verde Forest. Pp. E169-E182 in H. T. Odum and R. F. Pigeon, editors. A tropical rain forest: a study of irradiation and ecology at El Verde, Puerto Rico, US. Atomic Energy Commission Division of Technical Information, National Technical Information Service, Springfield, VA.

Williams, K. E., K. P. Huyvaert, and A. J. Piaggio. 2016. No filters, no fridges: a method for preservation of water samples for eDNA analysis. BMC Research Notes 9:298.

Williams, K. E., K. P. Huyvaert, and A. J. Piaggio. 2017. Clearing muddied waters: capture of environmental DNA from turbid waters. PLoS ONE 12:e0179282.

Williams, K. E., K. P. Huyvaert, C. VerCauteren, A. J. Davis, A. J. Piaggio. 2018. Detection and persistence of environmental DNA from an invasive, terrestrial mammal. Ecology and Evolution 8:688-695.

Witmer, G., and S. Jojola. 2006. What’s up with house mice? A review. Proceedings of the Vertebrate Pest Conference 22:124-130.

Witmer, G. W., and A. B. Shiels. 2018. Ecology, impacts, and management of invasive rodents in the United States. Pp. 193-219 in W. C. Pitt, J. C. Beasley, and G. W. Witmer, editors. Ecology and Management of Terrestrial Vertebrate Invasive Species in the United States. Taylor and Francis Publishing, New York, NY.

Young, L. C., E. A. VanderWerf, M. T. Lohr, C. J. Miller, A. J. Titmus, D. Peters, and L. Wilson. 2013. Multi-species predator eradication within a predator-proof fence at Ka‘ena Point, Hawai‘i. Biological Invasions 15:2627-2638.