Effectiveness of Snap-trapping, Goodnature A24 Automated Traps, and Hand-broadcast of Diphacinone Anticoagulant Baits to Suppress Invasive Rats (*Rattus* spp.) and Mice (*Mus musculus*) in Hawaiian Forest

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ABSTRACT: Invasive rodents (rats and mice) commonly occur on islands and often damage natural resources largely by predation of native species. Suppressing invasive rodent populations and their damages is therefore a common practice in many parts of the Hawaiian Islands, and land managers such as the Army Natural Resources Program on Oahu often control rodent populations by using large-scale rat snap-trapping and Goodnature A24 automated rat traps (henceforth A24s). While rat traps can be effective at suppressing rat populations, mouse populations are not generally suppressed and may expand greatly. In an effort to reduce rodent populations to levels below that accomplished with rat traps alone at a 5-ha mesic forest on Oahu (Ohikilolo), we assessed the effectiveness of a one-time (two application) hand-broadcast of anticoagulant (Diphacinone-50) bait pellets applied at 13.8 kg/ha per application while A24s and rat snap-traps were active. We monitored rat and mouse activity during trapping and before, during, and after the bait applications using tracking tunnels, which are baited ink cards placed in tunnels so that foot prints of animal visitors can be identified. We found that rat trapping alone was effective at reducing rat populations but not the mouse population, and that the one-time hand-broadcast of diphacinone bait reduced both rat and mouse activity to 0% tracking for about 1 month. However, rat and mouse populations rebounded 2 months later to 15% rat tracking and 41% mouse tracking, which were roughly pre-treatment levels. Rat suppression using A24s at Ohikilolo appeared much more effective year-round than at a nearby 26 ha site (Kahanahaiki), though mouse suppression was poor at Ohikilolo relative to Kahanahaiki. The hand-broadcast of diphacinone bait at both Ohikilolo and Kahanahaiki was effective but short-lived, so repeated baiting during the seasonal peaks in rodent abundance and increasing the size of the buffer area would more likely protect target natural resources from rats and mice.

KEY WORDS: A24 self-resetting traps, diphacinone rodenticide, hand-broadcast baiting, island invasive pest species, isolated habitat monitoring, native biodiversity, snap-trapping, tracking tunnels, rodent management

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INTRODUCTION

Rodents (*Rattus* spp. and *Mus musculus*) have been introduced to many ecosystems worldwide and are among the most widespread and problematic invasive animals affecting island biota (Towns et al. 2006, Angel et al. 2009, Witmer and Shiels 2018). Through mostly unintentional introductions by humans, invasive rats occupy >80% of the major islands worldwide (Atkinson 1985, Towns 2009). On islands that are too large or complex (i.e., humans occupy them), eradications of rats are generally not possible; therefore, rat control or suppression through trapping and/or poisoning within segments of islands is the most common form of protecting native species from the negative impacts of rats on islands (Duron et al. 2017).

Mesic forests are generally the most diverse ecosystems in Hawaii, and many rare, threatened, or endangered plants, snails, and birds reside in Hawaiian mesic forests. The U.S. Army is required to stabilize populations of endangered species and their habitat as per Biological Opinions issued by the U.S. Fish and Wildlife Service (USFWS). Due to the large negative effects of introduced rats on natural resources in Army-managed mesic forests on the island of Oahu, the Oahu Army Natural Resources Program (OANRP) has been engaged in rodent control since 1995 using various techniques including snap traps, automatic traps, diphacinone rodenticide (the only approved rodenticide for use in conservation areas) applied in bait stations, and physical barriers. OANRP rat-control tools became more limited in 2012, which was when OANRP halted rodenticide use at all of the sites they manage because of a change in the Special Local Needs (SLN) label that made bait-station application unfeasible in the steep, rugged terrain. Due to the high habitat quality and small sizes of army-managed lands, grids of Victor snap-traps were installed in 2009-2011 to protect native species from rats. These rat trapping grids were augmented with bait stations until 2012 and both were re-baited each 6 weeks. Snap-trapping results in an initial knock-down in the rat population (Pender et al. 2013) followed by a fluctuating rat population below pre-trapping levels. Many of the snap-trap grids were then replaced by Goodnature A24 rat + stoat traps (Goodnature Limited, Wellington, NZ; hereafter A24 traps or A24s), which are self-resetting traps that can fire 24 times with one CO_2 cartridge. A24s and rat snap-traps were baited every 4 weeks. Rat populations fluctuated during uses of both snap-traps and A24 grids, and the targeted levels of rat suppression (<20% rat tracking using tracking tunnels, which are baited ink cards placed in tunnels so that foot prints of animal visitors can be identified) were not always being met with the rat trapping grids; this resulted in noticeable losses of native and endangered seeds and predation of native snails by rats. Mouse populations often increased when rat trapping and suppression occurs (Witmer et al. 2007). Due to these shortcomings in rat and mouse control using traps, there is interest, but little experience, in rodenticide baits to assist with rodent suppression so that target natural resources are better protected.

The goal of the current study was to determine if a one-time (two application) hand-broadcast of Diphacinone-50: Conservation rodenticide, applied according to label (Diphacinone 50: Conservation, EPA Reg. No.: 56228-35, State of Hawaii Lic. No. 8600.1) and during rat suppression through constant trapping with A24s and snap-traps would reduce the invasive rodent population (and therefore tracking) at Ohikilolo mesic forest. An acceptable level of rat and mouse activity that promotes stable or increasing native/endangered species is unknown, but Innes et al. (1995) found that if rat tracking (in tracking tunnels) was 10% it protected a native bird species in New Zealand, and Pender et al. (2013) found that tracking tunnel activity of approximately <20% was sufficient for increasing seed production of an endangered plant in mesic Hawaiian forest. Thus, our level for determining effectiveness of rat and mouse suppression was based on tracking tunnel indices of <20%. Achieving such rodent reduction using rodenticides should translate to improved conditions for native and endangered species.

METHODS

Study Site and Rat Impacts

Ohikilolo (158° 11' 35.553"W, 21° 30' 47.459"N) is located at ~900 m elevation in the Waianae Mountain range, within the Makua Military Reservation, on Oahu Island, Hawaii. The rat control area within Ohikilolo is approximately 5 ha and is fenced to exclude ungulates. Non-native rodents are ubiquitous at Ohikilolo, including black rats (*Rattus rattus*), Pacific rats (*R. exulans*), and house mice (*Mus musculus*); black rats numerically dominant these forests in the Waianae Mountains, out-numbering Pacific rats by ~10-fold (Shiels 2010).Negative impacts of each of these three rodent species in mesic forest near Ohikilolo have been reported to span native plants, insects, snails, and birds (Shiels et al. 2013), and the dominant black rat is known as the most damaging rodent to island forests (Shiels et al. 2014). At Ohikilolo, there is a stand of endangered palm, *Pritchardia kaalae*, that is the last remaining large stand (~85 adults) on Oahu and it has seeds that are highly vulnerable to black rat predation (Shiels and Drake 2015). Once goats were removed and rat suppression was in place at Ohikilolo, the juvenile palm numbers went from nearly zero to 1600 individuals in just a few years (OANRP Staff Report 2017). Several additional native plant species receive high rates of predation by black rats in mesic forest on Oahu (Shiels and Drake 2011). Similarly, endangered tree snails (*Achatinella mustelina*) present at Ohikilolo suffer predation by rats.

Rodenticide Application Procedures

At Ohikilolo, there were two bait applications by hand-broadcast, spaced seven days apart (June 7 and June 14, 2016), and each application included ~69 kg of bait applied at a rate of no greater than 13.8 kg/ha. Bait used for this study at Ohikilolo was left-over from a much larger (26 ha) hand-broadcast in nearby Kahanahaiki mesic forest (Shiels 2017). Bait was applied by walking a gridded trail system and evenly distributing (via hand-broadcast) rodenticide bait 10 m to each side of each trail and fenceline. The total amount of bait applied was 138 kg (69 kg for each of the two applications in the 5 ha area). This resulted in approximately one bait pellet per m^2. Because each pellet was approximately 1.1 g, there were approximately 62,727 pellets applied per application. All bait applicators were certified with Hawaii restricted pesticide category 2 (Forest Pest Control) at the time of the operation. For the months before, during, and after the Ohikilolo hand-broadcast, the baiting area had rat traps armed (53 A24s and 127 Victor snap traps). Every 4 weeks, rat snap-traps were baited with Skippy Creamy peanut butter, and A24s were baited with static Goodnature chocolate lure. The labeled bait concentration for Diphacinone-50 is 0.0050% (50 ppm), and we verified the diphacinone concentration of our applied bait by sampling (i.e., making n = 9 samples of ~30 pellets each) from the entire batch of bait received and then having the National Wildlife Research Center (NWRC) chemistry unit analyze them; this batch was (mean ± SE) 0.00526 ± 0.6% diphacinone.

Rodent Monitoring

Twenty-six tracking tunnels, which are baited and inked cards placed inside a plastic tunnel so that foot tracks of animal visitors can be identified (Shiels and Ramírez de Arellano 2018), were active during a 24 hour period each 1-2 months from January to December 2016, including on the same day as the first broadcast (June 7-8, 2016) and five weeks post-broadcast (July 19-20, 2016). Each tracking tunnel was spaced ~25 m apart in a grid-like fashion, and baited with Skippy Creamy peanut butter. After 24 hours of deploying tracking tunnel cards, each card was removed, inspected, and tallied for evidence of rat and/or mouse foot-prints; tunnels were left in place for subsequent monitoring events.

RESULTS

Tracking tunnels revealed that mouse tracking ranged from 15-33% during the 6 months prior to diphacinone bait application (when rat traps were continuously active), then reduced to 0% tracking on the day of the first bait application (June 7, 2016) and the subsequent sampling on July 19, 2016. Mouse tracking increased to 40% on September 6, 2016, persisting above 20% for the remainder of 2016 (Figure 1).

Rat tracking was <15% during the whole year, averaging about 5% tracking during the prior 6 months to diphacinone bait application. Rat tracking was 7% on the day of the first bait application (June 7, 2016), 0% on the subsequent sampling on July 19, 2016, and then was 15% by September 6, 2016 (Figure 1).

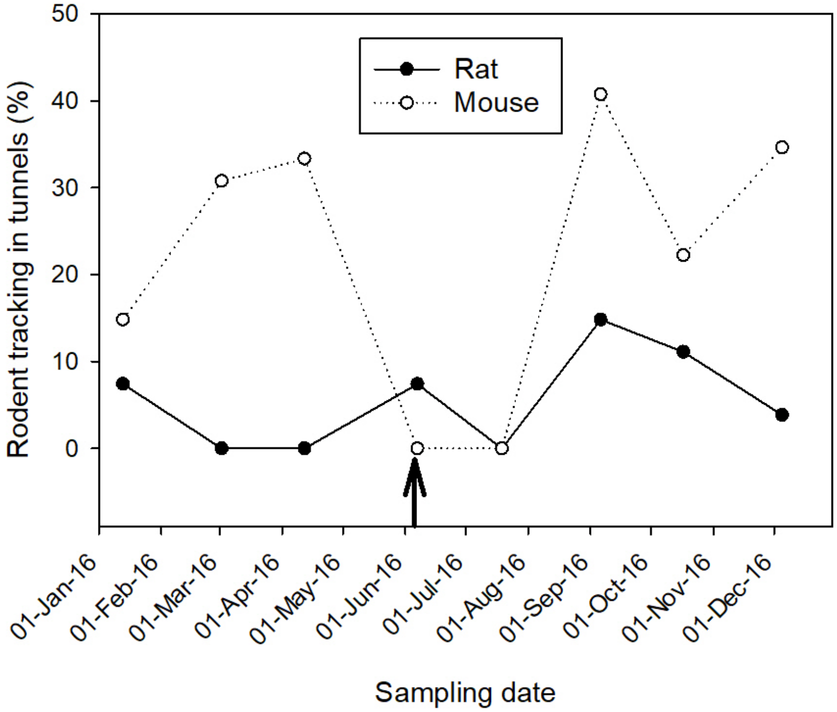

Figure 1. Tracking tunnel results, which indicates rodent activity and population status, for invasive rats (Rattus spp.) and mice (Mus musculus) at Ohikilolo mesic forest, Waianae Mountains, Oahu, Hawaii. The arrow represents the date (June 7, 2016) of the first hand-broadcast application of Diphacinone-50 rodenticide bait, which was also when tracking tunnels were activated for a 1-night assessment (recovered on June 8, 2018). A second hand-broadcast occurred 7 days after the first, on June 14, 2016.

DISCUSSION

Our study uncovered rodent activity levels during a year-long period when rat traps (both snap-traps and A24s) were continually active, as well as the effectiveness of diphacinone rodenticide bait as applied via a one-time (two application) hand-broadcast during rat trapping. Rat trapping alone, which was a combination of snap-traps and A24 traps, was effective at reducing rat populations as they were continuously held below 15% tracking. In contrast, rat trapping did not suppress mouse populations to the target levels of <20%, with the exception of the first sampling period in 2016. Due to the already low levels of rats at Ohikilolo resulting from constant trapping, the additional reduction to the rat population by applying Diphacinone-50 was minimal, decreasing it from 7% to 0% for just one sampling period (~1 month). Reduction in mouse activity from Diphacinone-50 was also short-lived at Ohikilolo, but the reduction was much more dramatic than for rats, as mouse tracking reduced from 33% to 0% upon bait application. The short reduction time in rodent populations by the one-time rodenticide application is probably due to the small-sized area treated, as larger buffers are needed to account for the typically rapid ingress that occurs when doing rodent control rather than whole-island rodent eradication.

The one-time hand-broadcast of diphacinone bait reduced both rat and mouse activity to 0% tracking for about 1 month. Approximately 2.5 months after bait application had finished, rat and mouse populations had rebounded to pre-treatment levels of 15% rat tracking and 41% mouse tracking. Diphacinone-50 bait pellets generally last 2-3 weeks when applied by hand-broadcast in a mesic forest like Ohikilolo (Shiels 2017), and there were some visible bait pellets 7 days after the first hand-broadcast at Ohikilolo and no visible bait pellets at the subsequent visit to the site 2.5 months later. At Kahanahaiki, which is a 26-ha mesic forest near Ohikilolo where the same hand-broadcast methods were used to treat the site 6 months prior to Ohikilolo, 50% of the applied bait had disappeared after 1 week, and the remaining had disappeared within 2-3 weeks (Shiels 2017). One week of bait exposure should have been ample time for all rodents in the treatment area to gain a lethal dose of diphacinone poison, and our findings at Ohikilolo reflect this for both rats and mice. Typically, diphacinone bait should be available to rodents for at least 3-4 nights to allow for the multiple feedings needed to obtain a lethal dose (Pitt et al. 2011). In cages, Swift (1998) exposed wild black rats to diphacinone bait (50 ppm) for seven days and obtained >80% rat mortality, and six days for Pacific rats and obtained 90% rat mortality. Therefore, for the two rat species at Ohikilolo, seven days of bait availability should have been sufficient to obtain high levels of rat control/suppression at the site. Less is known about the effectiveness of diphacinone bait on house mice in field conditions, but there was similar bait palatability and effectiveness for house mice offered diphacinone baits in the laboratory in Hawaii as found for Pacific rats and black rats offered the same diphacinone bait (Pitt et al. 2011).

Using one night to estimate rodent activity has its limitations, and one possibility for the rat tracking, but not mouse tracking, on the first night that bait was available

(June 7, 2016) may have been due to mice immediately shifting to eating the Diphacinone-50 bait, and therefore they did not go through the tracking tunnels to access the peanut butter bait. By contrast, some rats apparently visited the tracking tunnels even when the newly present diphacinone bait was available. Black rats are competitively dominant over house mice and Pacific rats in these forests (Shiels 2010, Shiels et al. 2013), and therefore the desirable foods and premium microhabitats will typically be exploited by black rats first. Because we cannot easily decipher black rat tracks from Pacific rat tracks in the tracking tunnels, it is unknown which rat species was utilizing the tracking tunnels on June 7, 2016 or other days sampled.

A key difference between rodent population control and rodent eradication on islands is that rapid ingress of rodents often occurs when control methods are used, and this is likely the reason that the one-time hand-broadcast of rodenticide resulted in such short population reductions of the target rodents. Invasive rodents are ubiquitous across most ecosystems in Hawaii, including the areas surrounding Ohikilolo (Shiels 2010). Rodent control at Ohikilolo and other sites is assumed to be constant when using A24 traps, snap-traps, and rodenticide bait stations, as long as these devices are regularly checked and serviced. However, rodents from outside the treatment plot migrate into the treatment area, and this process is rapid when control devices are not baited and active. Because the ingress is potentially constant even when these control devices are in place, resources at the edges of a treatment area receive less protection than the core, and therefore rodent control plots need to include appropriate buffers (see Shiels 2010 for daily movement patterns of these rodents) to ensure the management goals and protection of natural resources are realized.

To our knowledge, and in addition to a larger mesic forest site (Kahanahaiki) that we treated with hand-broadcast 6 months prior to Ohikilolo, there have been just two other hand-broadcast application in Hawaii of a similar bait product as used at Ohikilolo, and these are reported in Dunlevy et al. (2000) and Spurr et al. (2013). Dunlevy et al. (2000) investigated the optimal bait application rate to maximize exposure to rats while minimizing the amount of bait used; the bait was the same matrix as used at Ohikilolo and Kahanahaiki (i.e., Ramik Green fish-flavored cereal grain bait pellets; manufactured by Hacco Inc., Randolph, WI) but it was inert bait pellets that contained a biomarker instead of the anticoagulant compound diphacinone, and the pellets were 6 g each instead of 1.1 g like those at Ohikilolo. The Dunlevy et al. (2000) study was completed at Waiakea Forest Reserve, a wet forest outside of Hilo, on Hawaii Island. The key results were that all captured Pacific rats had eaten the bait at all application rates (11.25, 22.5, and 33.75 kg/ha), whereas the optimal sowage rate for black rats was determined to be 22.5 kg/ha. Spurr et al. (2013) conducted a field trial at Hawaii Volcano National Park (Hawaii Island) by hand-broadcasting pelleted (6 g each) Ramik Green, which is the same formulation as Diphacinone-50, for purposes of registering the product with the EPA for hand-broadcast for rat control. The treatments were effective in both forest types, resulting in 100% reduction in the 4 ha plots 1-4 weeks after an application event. Similar to our study at Ohikilolo, Spurr et al. (2013) reported that rat recolonization into the treatment area occurred, and the rat abundances recovered, within about two months after bait application.

Rat control at Ohikilolo by use of traps has occurred for >10 years, and the last several years have had A24s added into rat-control grids to supplement the snap-traps. It has been decades since Ohikilolo has not had a rat control program, and rat traps and toxic baits placed in bait stations had been the long-standing rat control method used by the land managers. Although we did not have reference plots in our study where nearby patches of similar forest would not have had rodent control so that efficacy of the rat-control (treatment) plot of Ohikilolo could be better judged (but see Shiels et al. 2019), we feel that the combined methods of using snap-traps with A24s is effective and has maintained rat populations below our established threshold of 20% tracking. Interestingly at additional mesic forest sites managed by OANRP that have A24s as the sole rat suppression technique or in combination with snap-traps, the rat tracking cannot be maintained below 20% tracking for the entire year. For example, at Kahanahaiki, rat tracking ranges from 20-40% for half of the year, and the other half it is <20% (Shiels 2017). Additional studies outside of Hawaii have also found that A24s may have variable success in rat population reduction and maintaining the rat populations below target levels (Gillies et al. 2012, Carter et al. 2016). However, the new modifications to A24s are promising, and deserve future testing at a variety of sites, including those where A24s have not reduced rats below target levels.

House mice do not appear to be sufficiently suppressed with using grids of rat snap-traps and A24s. The A24s were designed for rats and stoats, not mice, and the efficacy of A24s on suppressing house mice has not been previously tested to our knowledge. Rat snap-traps do not always reliably function for house mice because mice typically do not produce enough downward force on the snap-trap's treadle to trigger the trap (Shiels et al. 2017). The inability of house mice to consistently trigger a rat snap-trap is therefore in part due to the large difference in average weight of a mouse (~12 g) relative to a Pacific rat (~52 g) or black rat (~124 g) in Hawaiian forest (Shiels et al. 2013).

Invasive rodent control rather than island-wide eradication is the current best practice for protecting resources in ecosystems too large or complex to eliminate all individuals of the rodent species. Unfortunately, rodent control is not a one-time effort but is instead indefinite (Duron et al. 2007). In areas where rodenticide use is unwanted or infeasible (e.g., too expensive for long-term rodent control), automatic trapping using A24s alone or in combination with snap-trapping can maintain rat populations at desired levels at some sites (e.g., Ohikilolo) but not others (e.g., Kahanahaiki). Hand-broadcast or aerial-broadcast of bait pellets should therefore be considered for some sites where invasive rodents threaten resources. The hand-broadcast of diphacinone bait at both Ohikilolo and Kahanahaiki was effective but short-lived, so repeated baiting during the seasonal peaks in rodent abundance and increasing the size of the buffer area would more likely protect target natural resources from invasive rats and mice.

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