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Technical Report 203

2019 Kiwikiu Conservation Translocation Report

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Cover photo by Bret Mossman. Translocated female, WILD11, in Kahikinui Hawaiian Homelands.

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The 2019 kiwikiu translocation was a joint operation conducted by member organizations of the Maui Forest Bird Working Group. Organizations that conducted the translocation included American Bird Conservancy, Maui Forest Bird Recovery Project, National Park Service, Pacific Bird Conservation, San Diego Zoo Global, State of Hawai'i Department of Land and Natural Resources – Division of Forestry and Wildlife, The Nature Conservancy of Hawai'i, U.S. Fish & Wildlife Service, and U.S. Geological Survey. In addition to representatives of these organizations, six community volunteers aided in these efforts. This does not include the dozens of volunteers and other organizations involved in planning for the translocation and preparing the release site through restoration and other activities. These efforts were greatly supported by the skilled pilots at Windward Aviation.

Table of Contents

Table of Figures

Table of Tables

1. Summary of 2019 Reintroduction

The U.S. Fish & Wildlife Service (USFWS) Recovery Plan for the kiwikiu (Maui Parrotbill; *Pseudonestor xanthophrys*) (USFWS 2006) recommended establishing a second population within its historical range to protect the species from catastrophic loss in its small current range. In addition to the inherent threats of a small population and small range size, the current kiwikiu population is located on the windward (northeastern) slope of Haleakalā where they are under threat from severe weather events and frequent rainfall that have been shown to reduce reproductive success. The Kahikinui region of Maui on the leeward (southern) slope of Haleakalā was selected as the site of a new population of kiwikiu. Nakula Natural Area Reserve (NAR) was selected as the first release site to begin establishing the species in the Kahikinui region.

The Maui Forest Bird Working Group (MFBWG; hereafter "the working group") wrote a comprehensive Kiwikiu Reintroduction Plan (MFBWG 2018). After many years of preparation, which included building infrastructure, controlling predators, and reducing mosquito densities in Nakula NAR, 14 kiwikiu were transferred to the site: seven wild birds translocated from Hanawī NAR and seven from a conservation breeding facility managed by San Diego Zoo Global. The birds from the conservation breeding facility and the wild were moved to Nakula NAR in mid-October 2019 and releases were completed a few weeks later. After release, birds were monitored using radio telemetry through November 2019 at which point all birds either had died or disappeared (except for one individual that was transferred back to the conservation breeding facility). Necropsies indicated avian malaria as the primary cause of death for all recovered individuals and little hope remains for the few remaining missing birds at the site. Unexpectedly high densities of mosquitoes were later confirmed within the release site. Further investigation revealed that the translocated wild individuals tested positive for the malaria parasite prior to the move to Nakula NAR. In this report, we discuss the strategies that were employed during the kiwikiu translocation, the outcome of those actions, and the steps moving forward for both future release improvements and recovery strategies for the kiwikiu.

2. Background of the Reintroduction

2.1. Natural history of kiwikiu

The kiwikiu (Maui Parrotbill; *Pseudonestor xanthophrys*) is one of the rarest and most critically endangered Hawaiian bird species. Little was known about the species until the late 20th century. We know from subfossil evidence that kiwikiu were once widespread on Maui and Moloka'i in a wide variety of habitats (James and Olson 1991). However, the species can now only be found in a narrow band of rainforest of approximately 30 km^2 on the windward slopes of Haleakalā Volcano (Judge et al. 2019) (Figure 1). The biology of the kiwikiu and the causes of their population decline were intensively studied in the 1990 and between 2006 and 2015. Kiwikiu are highly specialized insectivores that extract their prey from woody tissue and fruits of native Hawaiian plants (Simon et al. 1997). This specialization translates into large home ranges (Warren et al. 2015) and extended parental investment; a single chick is reared for up to 18 months (Simon et al. 1997, Mounce et al. 2013). A classic *K*-selected species, kiwikiu have low natural productivity and rely on high adult survivorship to maintain the species (Mounce et al. 2013, 2014, 2018). Loss of adults to invasive predators or disease is particularly detrimental to this species given its biology. Further, nest failures frequently occur after heavy rainfall

that is common in the windward forests of their current range (USFWS 2006, Becker et al. 2010, Mounce et al. 2013).

Since the late 1970s, fencing and, thus forest protection from invasive ungulates has progressively improved and recovered habitat within the kiwikiu range. Their entire range is now fenced, and most of it is ungulate free. Despite this, the kiwikiu population continues to decline. Surveys in 2017 estimated the total abundance at just 157 individuals (95% CI = 44–312) (Judge et al. 2019). The species in its current range is threatened by invasive mammalian predators and non-native disease spread by mosquitoes. Many of the remaining threats in their current range are extremely difficult to control at a meaningful scale; eradicating or even reducing invasive predators would require a herculean effort to be effective and landscape-scale mosquito control techniques are not currently available. Until recently, disease had also been viewed primarily as a factor limiting the species' elevation range rather than driving declines within the core of their range. Consequently, the small range of the species, threats within that range, and our inability to effectively mitigate these threats supported establishing a second population. Expanding the species' range would help protect against loss in the single extant population, and some of the persisting threats could be more effectively managed in the new range (USFWS 2006).

2.2. Reintroduction Plan

In 2006, the recovery plan published by USFWS highlighted the need to expand the current kiwikiu range by establishing a second population elsewhere in their historical range. This document focused on protecting and restoring habitat on the leeward slopes of Haleakalā noting that the area "holds great potential to provide suitable habitat" for kiwikiu (USFWS 2006). The remnant koa (*Acacia koa*) forest in the Kahikinui region was highlighted as a potential release site on the leeward slope of the mountain. Other areas had been considered as potential release sites, including The Nature Conservancy's (TNC) Waikamoi Preserve and the Manawainui planeze in Haleakalā National Park (NP). However, subsequent research revealed existing kiwikiu populations in these areas and that these populations, especially in Waikamoi, may be at their ecological limits. The fact that the Kahikinui forest was dominated by koa also made the site attractive, as koa is thought to have been a historically favored food source (host plant for *Plagithmysus* beetle larvae among others) in other parts of their former range (Perkins 1903).

Following the creation of Nakula NAR in 2011 in the Kahikinui region, the working group nominated this site for the first kiwikiu releases in the region. Extensive protection and restoration measures began in Nakula NAR shortly after its creation (see 3.2. Restoration in Nakula NAR). Research was conducted to investigate the suitability of the site for a kiwikiu reintroduction, including studies on prey availability (Peck et al. 2015), mammalian predator densities (Maui Forest Bird Recovery Project unpubl. data), and disease prevalence (Warren et al. 2019a). In 2017, the working group produced the Kiwikiu Reintroduction Plan that detailed the procedures that would be followed for the reintroduction (MFBWG 2018). This plan called for several releases over a three-year period. The Reintroduction Plan detailed the source of the birds and how birds would be released and monitored in Nakula NAR. San Diego Zoo Global (SDZG) that managed the kiwikiu conservation breeding program identified eight birds as release candidates. The working group decided that all suitable birds from the conservation breeding program would be released in the first year in conjunction with the release of translocated wild birds from Hanawī NAR, collectively up to 20 birds (Figure 1). Hanawī NAR held a healthy population of kiwikiu and had been the site of over a decade of research on the species. Existing infrastructure (e.g. camps, trails) in Hanawī NAR aided in capture efforts. The Reintroduction Plan called for a soft-release

technique with birds held in aviaries at the release site to acclimate them to the area and introduce them to supplemental feeders. Monitoring efforts would be aided by using radio telemetry and the released birds would be monitored for a minimum of one year following release.

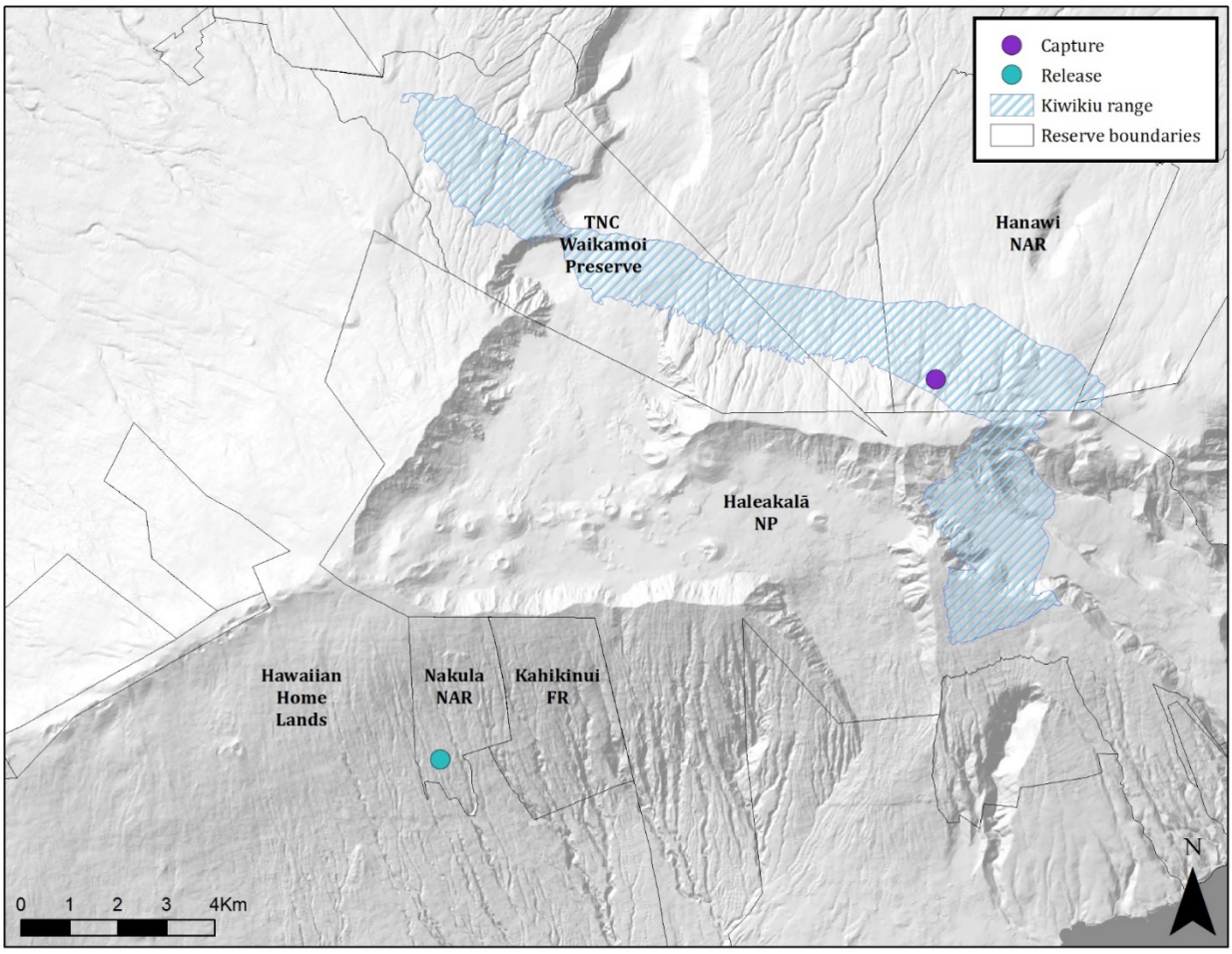


Figure 1. The kiwikiu (Maui Parrotbill; Pseudonestor xanthophrys*) current range showing the capture location, Hanawī Natural Area Reserve (NAR), the release site, Nakula NAR, and relevant land management areas of East Maui including Haleakalā NP (National Park), The Nature Conservancy (TNC) Waikamoi Preserve, State of Hawaii Hawaiian Home Lands, and Kahikinui Forest Reserve (FR).*

3. Reintroduction Site Preparations

3.1. Prey Availability

Among the main questions in selecting a release site is whether the site would provide sufficient prey resources. The primarily prey base of kiwikiu are Coleoptera and Lepidoptera larvae usually extracted from woody tissue, bark, or fruits of native trees (Peck et al. 2015, Simon et al. 1997). In 2012, Peck et al. (2015) conducted research to investigate relative abundance and diversity of arthropods in Nakula NAR and two sites where kiwikiu remain: TNC's Waikamoi Preserve and Hanawī NAR. They found that arthropod biomass per stem was as great (or greater) in Nakula NAR compared to the currently

occupied sites. This finding was consistent among several substrates including koa and ʻōhiʻa (*Metrosideros polymorpha*) bark and foliage. These results were limited to the scale of the individual branch or tree, and vegetation density was almost certainly lower in Nakula NAR compared to the other sites. However, these results were encouraging, suggesting that overall arthropod biomass in Nakula NAR could be increased by increasing the woody biomass within the site through restoration.

3.2. Restoration in Nakula NAR

The forest on the leeward slopes of Haleakalā, including Nakula NAR, generally exists in a deteriorated state owing to over a century of damage caused by invasive ungulates. However, intact forest patches persist, and few ungulates remain throughout much of the region following extensive fencing and eradication efforts. The forest in this area has shown significant recovery as a result of natural regeneration and conservation restoration efforts.

The State of Hawaiʻi created Nakula NAR in 2011 to protect some of the last montane mesic forest on Maui. Nakula NAR is located in the ʻahupuaʻa from which it takes its name in the center of the Kahikinui region. The entire 6.7 km^2 reserve was fenced by the end of 2012 and ungulates were removed shortly after. Much of the habitat within the reserve consists of savanna and open grassland dotted with pockets of intact native forest. Internal fencing and restoration timelines naturally divided Nakula NAR into three management units: Wailaulau, West Pahihi, and Mauka (Figure 2). The Wailaulau unit, ranging from 1100 to 1900 m (3600–6200 ft) above sea level (asl), contains most of the intact forest habitat and was selected as the site of the kiwikiu releases. The forest habitat in this unit is dominated by koa, ʻōhiʻa, and ʻaʻaliʻi (*Dodonaea viscosa*) trees.

Shortly after ungulates were removed from the unit, Maui Forest Bird Recovery Project (MFBRP) and the State of Hawaiʻi Department of Land and Natural Resources (DLNR) – Division of Forestry and Wildlife (DOFAW) Native Ecosystem Protection and Management (NEPM) program began restoration efforts in the Wailaulau unit. During 2013–2016, MFBRP conducted experimental restoration trials to explore techniques that may be employed to increase density and diversity of native vegetation within the reserve (Warren et al. 2019b). The NEPM program began large-scale planting in the West Pahihi unit in 2014. Collectively, NEPM and MFBRP planted over 170,000 native seedlings in Nakula NAR from 2012 to 2019. Overall tree cover and diversity increased throughout the Wailaulau unit between 2012 and 2019, transforming much of the area from savanna to burgeoning forest.

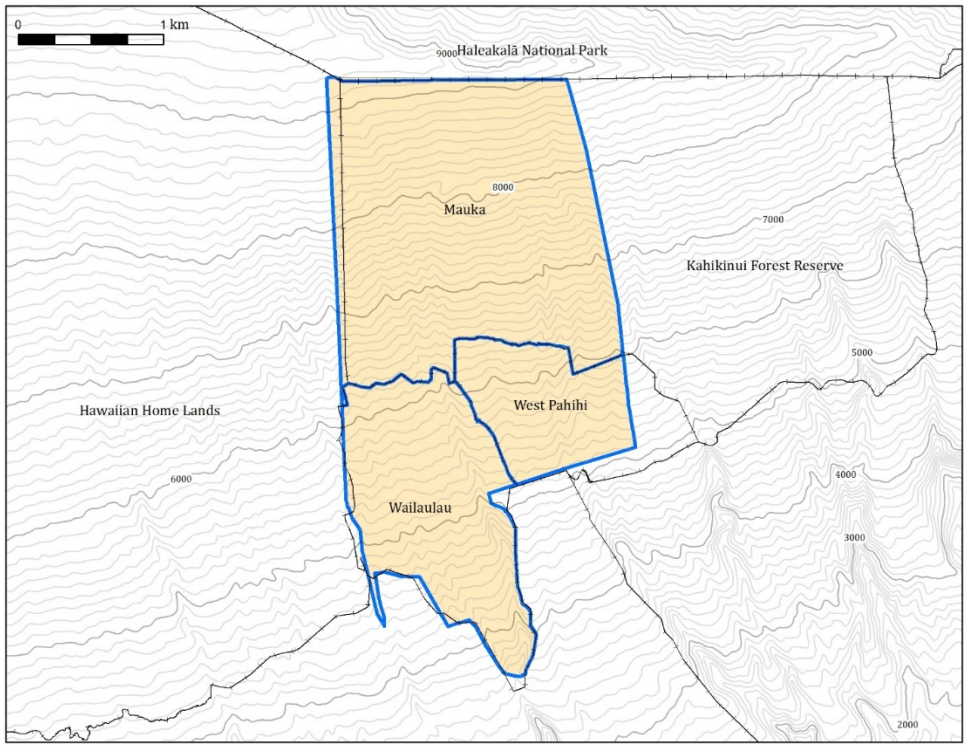


Figure 2. Nakula Natural Area Reserve (yellow) management areas (blue outlines), surrounding conservation areas, and fenced units.

3.3. Predator Management in Nakula NAR

Background research – Small Indian mongooses (*Herpestes javanicus*), feral cats (*Felis sylvestris cattus*), and rats (*Rattus* spp.) were documented in Nakula NAR prior to the kiwikiu reintroduction. MFBRP conducted a predator abundance study in the Wailaulau unit of Nakula NAR in 2014–2015 (MFBRP unpubl. data). The purpose of this research was to determine the relative densities of mammalian predators in Nakula NAR to provide information on what predator control measures might be necessary during the kiwikiu reintroduction. During this study, they captured both Black (*Rattus rattus*) and Polynesian rats (*R. exulans*) and three mongooses. No cats were trapped during this study, although one was seen within the trapping area. In general, the study found rat densities to be low in Nakula NAR compared to Hanawī NAR. MFBRP felt that the low number of mongooses captured may have been the result of trap sensitivity issues and may not have been reflective of overall densities.

Threat mitigation during reintroduction – In order to reduce the threat of predators during the kiwikiu reintroduction, MFBRP established a trapping grid throughout the central Wailaulau unit centered on the release aviaries. Additional traps were placed in the immediate vicinity of the release aviaries. The details of the predator control design, trap placement, monitoring, and results are available from MFBRP (MFBRP unpub. data). Herein we briefly describe the predator control efforts used for this project.

The predator grid consisted of 221 trap stations collectively covering a 64–ha area with stations placed at 50–m intervals (Figure 3). Five trap varieties were used: Goodnature® A24, Belisle® body grip, DOC250, Victor® snap traps, and Oneida Victor® Soft Catch® Wildlife Traps (leg-holds). An A24 was placed at each station (221 total traps). Forty (40) stations also included body grip traps: 20 ground–

style stations, which allow for two traps per/box (40 traps), and 20 elevated–style (modified Steve Allan) stations (20 traps) (60 body grip traps in total). DOC250 traps were placed at 40 stations (40 traps). The body grip and DOC250 traps were spaced ≥100 m apart (approximately every other station) within the center of the grid. Along the edge of the grid (outer lines), a body-grip or DOC250 trap was placed at every station, alternating trap type among stations. Placing these traps at a higher density along the perimeter was intended to reduce animals moving into the release area; the traps in the interior of the grid were thought to be sufficient to remove animals from inside the area. Five Victor® snap traps and one leg-hold were deployed in the immediate vicinity of each release aviary. The snap traps were set in September 2019 and both the snap traps and leg-holds were activated and monitored daily by MFBRP and SDZG while birds were held in the aviaries (October 2019). After birds were released, the elevated body-grip, snap, and leg-hold traps were deactivated.

MFBRP monitored the prevalence of mammalian predators throughout the site using tracking tunnels to monitor rodent density and game cameras to monitor mongooses and cats. Mongooses were observed on cameras inside and outside the grid, but cats were not captured on any of the images (although scat was observed within the trapping grid). MFBRP established tracking tunnel transects within (40 tunnels) and outside (40 tunnels) the trapping grid to evaluate rodent trapping efficiency. The tracking tunnels were monitored prior to trapping and quarterly while traps were active. Pre-trapping tracking tunnel data indicated low densities of rats and high densities of mice. Immediately following the activation of the A24 traps, rodent densities (tunnel prevalence) within the trapping grid dropped sharply. Rat prevalence within the grid dropped to zero after the first few months of trapping and remained zero within the grid throughout the trapping period. However, rat capture rates did not change throughout the trapping period and rats continued to be captured in the center of the grid. Mouse prevalence in the grid continued to drop sharply for the first six months of trapping but densities recovered in the fall.

From October 2018 to November 2019, MFBRP removed 225 rats (*Rattus* spp.), 640 mice (*Mus musculus*), and 26 mongooses. The snap traps and leg-holds did not capture any predators. A steady reduction in capture rates in mongooses indicated that the trapping efforts were effective in reducing the densities of these animals in the release site. The capture data also indicated that mongooses were removed from the site in the first few months and subsequent captures were likely additional animals attempting to recolonize the site. Capture and tracking tunnel data indicate a moderate reduction of rats, but not eradication, within the trapping area and no effect on mouse densities. Trapping was discontinued in November 2019 and traps were removed from the site shortly after.

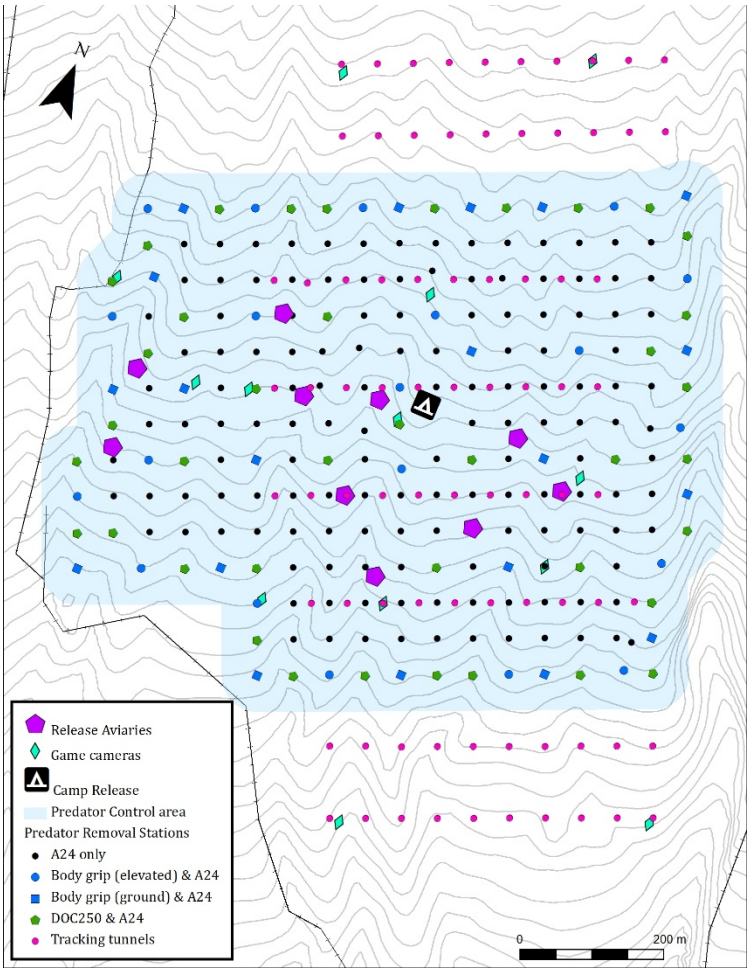


Figure 3. Map of the predator grid within Nakula Natural Area Reserve showing tracking tunnels, A24s, DOC250s, body-grip, and game cameras. Snap traps and leg holds circled the Release Aviaries. The blue shaded area represents the 64–ha "trapping area", including a 50–m buffer around the edge stations.

Recommendations for future predator control – Perhaps the greatest challenge to predator control is employing efforts at an effective scale. As soon as the kiwikiu were released, many individuals quickly dispersed outside the predator control grid (see 10.1. Tracking Kiwikiu in Nakula NAR). Only one of the five translocated wild birds settled inside the grid, while two others settled near the eastern and western edges. Two individuals settled well outside the grid. Most of the released kiwikiu from the conservation breeding facility stayed close to camp near the center of the predator control grid and, thus, the grid offered at least some protection from predation to these individuals, perhaps the most vulnerable of the released birds. Even though these efforts were effective in reducing mammalian predators within the grid, whether the effort and cost of these measures was worthwhile is questionable given how far the wild birds dispersed. The large home range size of kiwikiu in general may mean that the scale of an effective trapping grid for even a few individuals (or pairs) is so large as to not be feasibly maintained. While maintaining the trapping grid for this project was practical with the staffing available (4–7 person days each month), enlarging the grid to encompass all the areas that the kiwikiu dispersed to would likely make maintenance of such a grid impractical. Future translocations

may be able to reduce trapping labor to the areas immediately surrounding each release aviary with densely-spaced A24 and other traps in order to protect individuals within the aviaries and the areas immediately surrounding release aviaries; chemical control efforts broadcast on the landscape-scale may be able to be employed to efficiently increase the predator control area.

The bait in the A24s was presented in both static and automatic lures pump (ALP) styles as different sites in Hawai'i have had varying success with both forms. In ideal conditions, both bait types can last 3–4 months but others have found that local conditions can reduce longevity of both styles. In Nakula NAR, warm temperatures seemed to result in the bait dropping prematurely (< 1 month) in the ALP style. However, while the bait inside the static lures appeared to last longer, the condition of the bait (freshness) may not have been sufficient to attract animals. Thus, we recommend the use of both styles in future trapping efforts with the expectation that the bait will likely last 1–3 months.

Performance of each trap type varied by species, but some types generally performed better. A24 traps were seemingly a good choice for capturing rodents, although most animals removed were mice. Additionally, it is not clear if the trapping intensity utilized in Nakula was sufficient to reduce population densities of rats and was clearly ineffective for mice. However, the self-resetting feature of these traps is very important in situations such as these with high numbers of mice and relatively low densities of rats. In previous efforts using snap traps in Nakula NAR, triggers by mice drastically reduced the trapping effort possible for rats. The DOC250 traps were also very effective traps, able to capture rats while rarely being triggered by mice. In total, a similar number of rats were captured in A24 traps (50.7%) and DOC250 traps (42.2%). The performance of the DOC250 traps for capturing rats is notable given that there were more than 5× the number of A24 traps versus DOC250. Comparatively few rats (7.1%) were captured in body grip traps. Nearly two-thirds (73.1%) of mongooses were captured in DOC250 traps compared to body grips (26.9%). Thus, the DOC250 traps outperformed the body grip traps by a 2 to 1 margin for mongooses and 7 to 1 margin for rats. We highly recommend this trap design for similar future efforts.

The Victor® snap traps and leg holds surrounding the aviaries ultimately captured no animals and may have been unnecessary. The Victor® snap traps captured no rodents and the bait (peanut butter and raisins) was often removed without setting the traps off, likely by mice. The matrix of traps throughout the area was dense and may have been sufficient in removing predators from around the aviaries. The leg holds could have been used more effectively in locations where cat scat was seen rather than around the aviaries. A similar, targeted design could be used for deployment of body grip traps for cats, as it is not clear how well the DOC250s would perform for (especially larger) cats.

3.4. Mosquito Mitigation in Nakula NAR

Background research – MFBRP conducted a study from 2015 to 2016 to better understand the risk avian malaria and avian pox might pose to a translocated population of kiwikiu in Nakula NAR. This study is detailed in Warren et al. (2019a). This study included surveys for larvae, and adult mosquitoes, and malaria prevalence testing of avian blood samples and captured mosquitoes. To contextualize mosquito densities in Nakula NAR compared to the current kiwikiu range, MFBRP also conducted trapping for adult mosquitoes and tested avian blood samples from TNC's Waikamoi Preserve in 2016.

One of the most concerning findings of Warren et al. (2019) was the presence of a persistent population of the mosquito *Culex quinquefasciatus*, the primary vector for avian malaria, in Nakula NAR. Adult and

larval mosquitoes were captured in every season (although not every trapping session) in both years of study. As is typical for mosquito trapping studies, capture rates were highly variable, ranging from 0 mosquitoes per trap night (mq/TN) to 6.39 mq/TN; an overall rate of 1.22 mq/TN for the entire study. Despite the relatively high capture rates of adult mosquitoes (especially for the elevation, 1530–1620 m asl), malaria prevalence (*Plasmodium relictum* detected via qPCR) was low; 0% in 2015 (0 positives/38 mosquitoes) and 5.5% in 2016 (10/181). No *C. quinquefasciatus* were captured in Waikamoi but avian blood samples exhibited the presence of malaria (Warren et al. 2019a).

The presence of *C. quinquefasciatus* in Nakula NAR appeared to represent a greater risk of infection for kiwikiu than in Waikamoi. However, the low *P. relictum* prevalence rates in captured mosquitoes tempered concerns about the risk of infection. The results of qPCR testing of avian blood samples further ameliorated these concerns. The overall rate of *P. relictum* in avian blood samples in Nakula NAR was 10.4%: 8.2% in Hawai'i 'amakihi (*Chlorodrepanis virens*) and 15.8% in 'apapane (*Himatione sanguinea*). These rates are typical or lower than rates seen in these species at other sites of this elevation. Warren et al. (2019a) concluded that, although capture rates of mosquitoes were elevated, the rates of *P. relictum* in both the vector and host populations were not reciprocally high compared to similar sites. Based on the results of this study, the working group decided to proceed with the translocation of kiwikiu to Nakula NAR.

Threat minimization during reintroduction – Knowing mosquitoes were present and thus represented a risk of malaria infection to released kiwikiu, we applied larvicide to all major drainages in the Wailaulau Unit of Nakula NAR prior to and throughout the release. We applied (sprinkled) VectoMax® fine-granule formula to pools at a rate of 10 g/ft^2. VectoMax® is a biopesticide ingested by mosquito larvae (and very little else), which causes rapid death. VectoMax® purportedly can persist in pools for up to 28 days, unless washed out. Throughout the treatment period, granules regularly persisted through heavy rainfall periods. We conducted six applications in July–November 2019. Between July and September, applications were made monthly. Following the first deaths of the translocated kiwikiu in October (and initial necropsies indicated avian malaria as cause of death) and an apparent increase in mosquito larvae in pools, we increased the application rate to twice per month. At this increased rate, the granules from the previous treatment were usually still visible in treated pools (application totals remained well under the label limit).

We treated 310–644 "pools" each month. The fewest pools were found in July and the greatest number was found in November. This mostly corresponded with overall rainfall patterns. "Pools" are defined here as treatment units for which area was calculated to determine the amount of pesticide to apply. Treatment units were often a collection of multiple pools in close proximity; the total number of water bodies was much higher. Treated water bodies varied between ~7 cm to over 300 cm in diameter. Additionally, we expanded treatment application to include a few more stream sections discovered in September and October.

We sampled for mosquito larvae in every pool treated on a monthly basis (no larvae sampling during the second treatment of October or the first treatment of November) to determine efficacy of the treatments. Larvae numbers declined between the first treatments in July/August and September, seemingly indicating that the treatments were effectively reducing mosquito numbers (Figure 4). However, larval numbers returned to pretreatment levels in October, after the kiwikiu were already moved to Nakula NAR. Adding a second VectoMax® monthly application in October (and thereafter)

appeared to be effective as we subsequently saw a sharp decrease in larvae numbers by November. The proportion of pools with larvae found in November was similar to the low rate in September. Alternatively, several days of heavy rain in early November may have reduced larvae habitat by lowering the pool temperature and/or flushing out pools. As such, we do not know if the reduction in larvae was a result of the increased VectoMax® treatments or natural fluctuations in habitat due to weather patterns. In general, we do not have a good idea of natural fluctuations in larvae densities as we only sampled for larvae in the treatment area and did not have a control site.

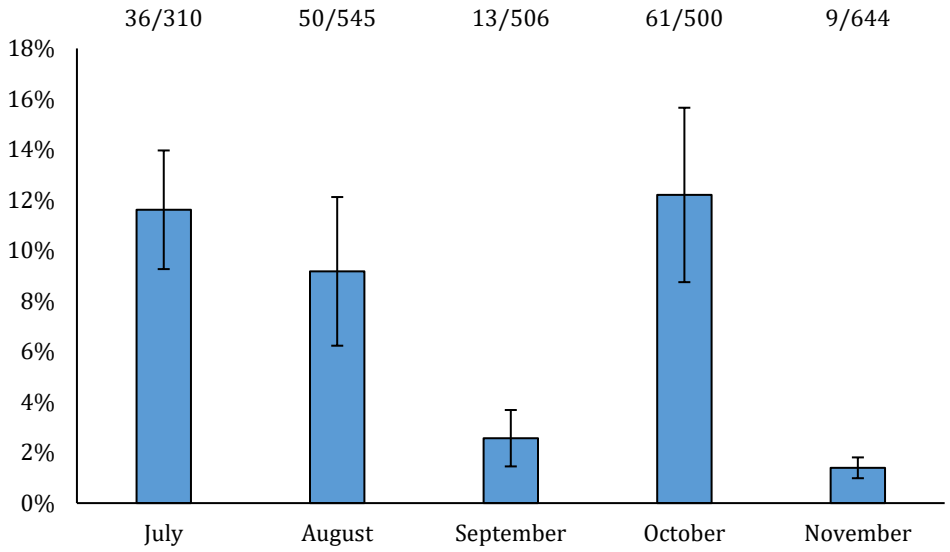


Figure 4. Pools with mosquito larvae during VectoMax FG application between July and November 2019 in Nakula Natural Area Reserve. July was the first treatment thus representing "pre-treatment". Positive-larvae pools compared to the total number of pools treated are shown above each bar.

Recommendations for the future mosquito control – The post–release mosquito trapping found a peak in mosquito densities in the fall of 2019, a pattern not seen during the 2015 and 2016 surveys. With all the information available now, it appears that mosquito densities in Nakula NAR may undergo rapid increases in density under the right weather conditions. Further analyses of these increases in combination with rainfall data would be helpful for interpreting these results. It is possible that the kiwikiu release tragically coincided with a temporary spike in mosquito numbers and baseline mosquito densities may not represent an increased disease risk compared to the current range. Yet, these temporary spikes in vector densities have the potential to determine the fate of a reintroduced population. Judging the suitability of a release site may depend on detecting peaks in mosquito abundance.

The larvicide treatments did not have the desired impact. Applying these treatments at a scale that is meaningful in reducing mosquito populations is challenging. Even if we were successful in reducing

mosquito numbers within the release site, the infection risk may have been too high to support kiwikiu, as evidenced by the mortality rate in released birds (although see

11.6. Further malaria analysis). It is not clear if the larvicide treatments were insufficient in reducing mosquito densities, whether mosquitoes were coming from outside the treatment area, or both. The larvicide treatments were hazardous to implement and required personnel to traverse dangerous terrain in search of every small pool in each drainage. The treatments were also very labor intensive requiring up to 7–10 person days to apply the larvicide in all drainages and we ultimately conducted twice-monthly treatments. It is not reasonable to spend 20+ person days each month treating a meaningfully large area in perpetuity. Especially without an obvious effect on the mosquito population.

Additionally, larvae surveys revealed very spotty distribution among pools, ~3–20% of pools per drainage. It would be very easy for someone to miss or undertreat a few tiny pools containing larvae. Most critically, we saw a resurgence of larvae in October after three months of treatments, suggesting the larvicide had little to no lasting impact on the larval habitat despite the fact that the product was capable of persisting in pools between treatments. In all, these treatments were time consuming, potentially dangerous to staff, and seemingly ineffective at preventing malaria infections. Although it is clear that landscape-level mosquito control will be critical for any future kiwikiu translocations to Nakula NAR, using larvicide in this manner was not effective.

4. Infrastructure in Nakula NAR

4.1. Camp and Transportation

MFBRP established a camp named Camp Release in the center of the Wailaulau unit in 2012 at 1582 m (5194 ft) asl to be used by those conducting restoration and reintroduction work. The camp has a WeatherPort® tent structure on a deck platform that serves as a communal cooking and living space and a water catchment structure with a 1,000–gal water tank. Other structures at camp include an outdoor shower and toilet structure for compostable human waste. A helicopter landing zone is maintained just below the WeatherPort® platform and all access to the site is via helicopter. Personnel sleep in individual tents in designated campsites. In general, Camp Release could support up to ten people for extended periods.

Gear and supplies for the first several weeks of the reintroduction were flown to camp prior to the birds being transported to Nakula NAR to reduce the amount of helicopter activity around the aviaries. We established additional landing zones above and below the main aviary area (1400 m and 1700 m asl) to fly people in and out of the field rather than using the landing zone at the camp, which was close to several aviaries. These landing zones were used while birds were held in aviaries and we started using the camp landing zone again once all birds had been released and could move around freely. The birds did not exhibit any extraordinary signs of stress when the helicopter was in the area. In one example, the helicopter was forced to fly directly over the aviary closest to camp due to low clouds. Both birds in that aviary were seen feeding immediately after the helicopter left. Another bird, a male from the conservation breeding facility, moved to camp on his own after release and even stayed during helicopter operations at camp. As a whole, neither kiwikiu from the conservation breeding facility nor those translocated from the wild showed signs of undue stress related to helicopter noise.

4.2. Aviary Construction

As this was designed to be a soft release, each bird was held in their own separate compartment with each aviary having two adjacent compartments. Some aviaries included a door that could be opened to allow birds from both compartments to interact. Ten aviaries were constructed at ten sites (Figure 5). Locations were chosen to be within 150 m of another aviary, a distance where we felt the birds could hear each other but not feel territorial. The aviary locations were placed in areas with tree canopy cover and intact forest patches nearby. Aviaries were generally on ridgetops overlooking gulches containing some of the best habitat in Nakula NAR. Aviaries were within a 5–10 minute walk from another aviary.

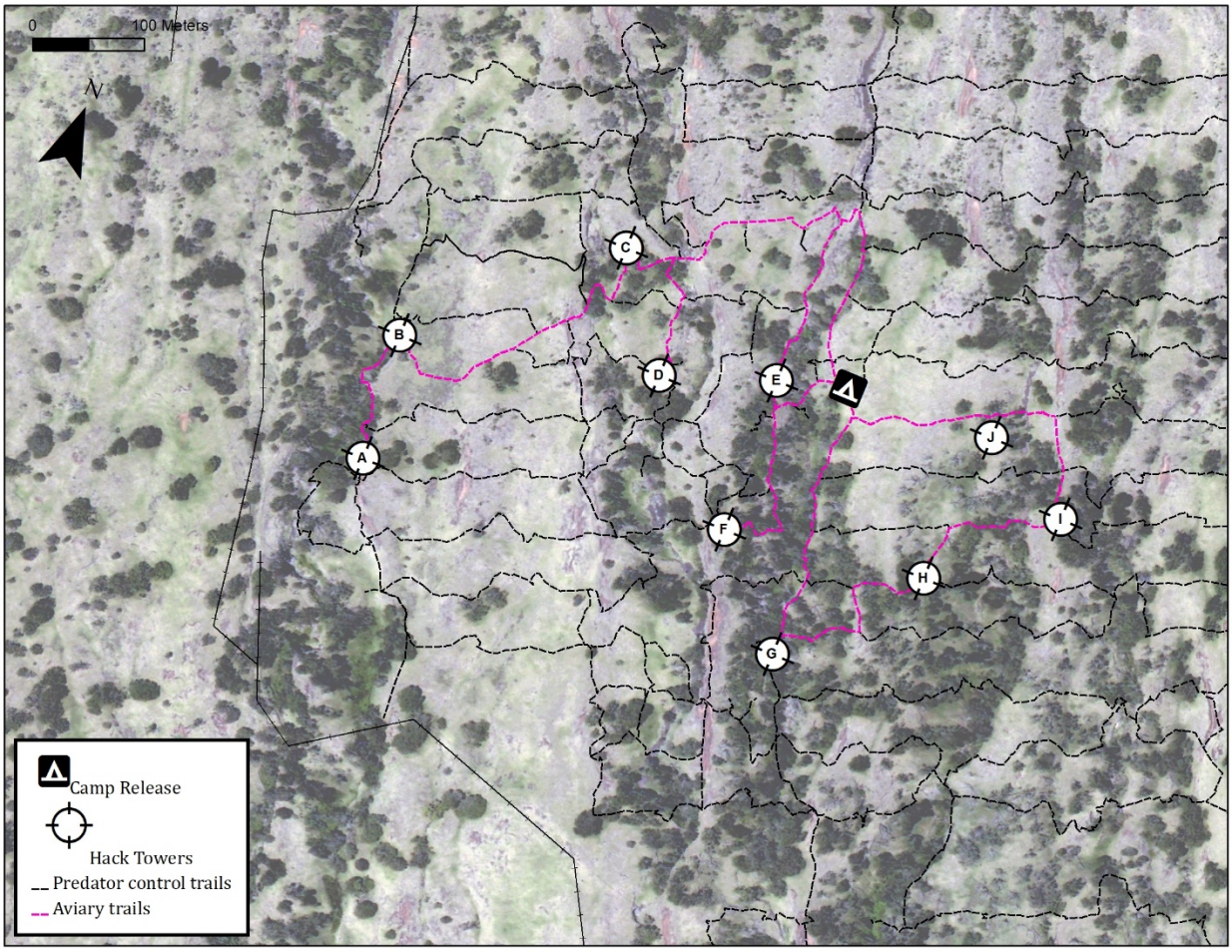


Figure 5. Map of aviary locations in Nakula Natural Area Reserve, Wailaulau Unit.

Aviaries were placed on 2.5 × 2.5 m wooden platforms, constructed using pressure-treated wood, and suspended on 6–cm diameter square steel signposts (Figure 6). MFBRP staff and volunteers constructed the platforms in the fall of 2018. The 3–m signposts supporting the platforms that were driven into the ground a minimum of 1–2 m. The platforms were then suspended level on the signposts. Platforms were suspended at varying distances off the ground depending on the slope. Generally, platforms were suspended ~0.5 m off the ground on the uphill side and a maximum of 1.5 m off the ground on the

downhill site. Prior to the platform construction, the grass was cut with a weed-whacker and thick black plastic sheets were installed under the platforms after construction to reduce the grass regrowth.

The aviaries were constructed off site by SDZG staff at the Maui Bird Conservation Center (MBCC) in Olinda (see Appendix A for full details). The aviaries were approximately 2.44 m long × 2.44 m wide × 2.13 m tall and constructed with 2.67–cm PVC and 1.27–cm welded wire hardware cloth. SDZG designed the aviaries so that the wall and ceiling panels could be easily assembled and disassembled, the panels being held to the frame and to one another by pipe clamps and cable ties. A single large door allowed people to access each side-by-side aviary and a second small door next to each large door was designed to allow people to access the food and water trays without entering the aviary itself. A piece of corrugated plastic was attached to the ceiling panel at the rear of each aviary to provide shade and shelter from rain. The panels were disassembled at MBCC and stacked on top of each other for transportation to the site (Figure 6). The aviary stacks were ratchet-strapped together and flown in bundles of 2–3 aviaries at a time to Camp Release. The bundles were separated and then dispersed one-by-one to each of the ten sites via helicopter.

The aviaries were assembled on site in Nakula NAR in August 2019. Perches and feeders were added, and minor adjustments were made to the aviaries in September. During assembly, the frame was attached to the platform with U-brackets and the entire aviary was ratchet-strapped to the platform in case of high winds. Koa and ʻaʻaliʻi branches were placed in the aviaries to provide perches, shade, and forage. Shade cloth was placed on the door side of each aviary to obscure people approaching the aviaries and allow for discreet observations of the birds. The final inspections of the aviaries were conducted by SDZG staff in early October prior to moving the birds from the conservation breeding facility.

Figure 6. Photos (from left to right, top to bottom): Wooden platform, transport of aviaries in bundles to helicopter pick up site, completed aviaries in Nakula Natural Area Reserve, installing a supplemental feeder, and release of a kiwikiu to an aviary from transfer box.

Recommendations for the future – The spacing of the aviaries was designed in detail to address several concerns, primarily balancing territoriality with conspecific attraction. We wanted the aviaries to be close enough for each bird to be within earshot of another aviary while also not so close as to feel stressed by proximity to a potential rival. Also, we anticipated that a single breeding pair may establish a territory near each aviary due to supplemental food being provided. In practice, however, many of these concerns may not have been as important as other considerations. For one, once the birds were placed in the release aviaries in Nakula NAR, they were quiet and vocalized much less than expected, usually only exhibiting the occasional nonspecific "chip" note. Only one male from the conservation breeding facility was observed by on-the-ground staff to sing a few times. Placing the aviaries as far apart as we did (150 m) meant that it is unlikely they could have heard each other in most circumstances particularly given how quiet the birds were. As such, this distance seemed unnecessarily far, requiring personnel to hike for much longer distances than was necessary. This distance reduced observation times and increased the time it took to address any concerns with individual birds. Additionally, we placed aviaries

near good forest patches with relatively dense understory. However, once released most birds travelled hundreds of meters and were not exclusively observed in areas with intact understory. Furthermore, released birds were not anchored at all by the supplemental food as we expected. In Nakula NAR, it would have been possible (and more ideal) to place aviaries within 50 m of each other and allow all aviaries to be within a 5–10 minute hike from camp.

One element of aviary placement that was debated before the aviaries were utilized related to the amount of vegetation around the aviaries. Many felt that positioning the aviaries in spots with minimal vegetation in the immediate surroundings would reduce the threat of predators (e.g. rats) from getting onto the aviaries. Fewer trees and shrubs around also maximized the distance individuals could see and become familiar with their new surroundings prior to release. On the other hand, others felt that placing the birds in a more open area with fewer surrounding understory plants might have been a source of stress. Kiwikiu typically live in dense forest with a lot of forest structure. By reducing the birds' access to cover, the birds may have felt unnaturally exposed. In the future, it may be possible to achieve all these goals (including those discussed below), particularly if aviary spacing is not limited to a 150-m spacing requirement.

Based on our experiences and observations during the reintroduction effort, some other easy additions to the aviaries could also be utilized in the future reduce the stress of the birds. Adding shade cloth to more of the ceiling would be advisable. By design, we wanted the birds to be able to see the canopy and surrounding vegetation to familiarize themselves with their surroundings and possibly increase the likelihood of returning to supplemental food provided at the aviary after release. Furthermore, providing exposure to the elements was done to help birds from the conservation breeding facility transition to conditions they would experience after release into the wild. However, when many birds, particularly those that were translocated from the wild, were initially placed in the aviaries, they flew towards the ceiling panels in an attempt to escape. Several birds continually flew up to the ceiling when stressed (e.g. when people approached), seemingly in an attempt to escape. Other birds habitually looked up as if searching for aerial predators (e.g. owls). Closing off a visual barrier may reduce these stressors. Second, more vegetation inside the aviaries may allow the birds to feel more relaxed and provide more stimulation (e.g. things to chew). Although branches were added to provide shade and perching, we were forced to minimize this in order to capture birds. Furthermore, adding more vegetation within each aviary will result in less detailed behavioral observations. It is difficult to observe behaviors of birds within aviaries when there is thick vegetation inside the aviary. Also, behavioral observations needed to be conducted from a distance away where the birds could not see the human observers, because wild-translocated kiwikiu increased their stress behaviors when they detected humans near the aviary. In order to be able to conduct adequate behavioral observations of kiwikiu in the aviaries, future release aviary construction sites need to include multiple hidden observation points, such as behind existing thick vegetation. An alternative design for capture (see below) could allow additional branches to be added.

The design of the aviaries worked very well for this project, especially in overall weight and how easily they could be assembled and disassembled. The fact that Nakula NAR is accessible only by helicopter limited materials and the overall weight of the aviaries. The terrain was such that it would not have been safe or feasible to carry large or heavy supplies to each site and the light PVC panels allowed for a minimal number of people to be present at assembly and disassembly. Furthermore, PVC and all other materials used for the release aviaries were chosen because of the low cost. However, PVC is bendable

making it difficult to tighten panels together and firmly attach the aviary to the platforms without deforming the PVC. Transport also bent some of the PVC segments. Attaching the wire mesh using cable ties worked well on each panel but ensuring that there were no gaps between panels was challenging and often required dozens of extra cable ties. Preventing gaps was particularly challenging around the doors and many doors were difficult to operate. It would be possible to construct the doors or the entire front panels out of wood. This would not have compromised the overall weight too much and would have allowed for much better operation of the doors and reduced the chance of accidental escape. This also would help solve the difficulty in operating the doors under the shade cloth. Despite these concerns, however, no birds escaped, and the doors operated well enough.

For future releases, we also recommend a few alterations to the aviary design to increase the ease in capturing birds from the aviaries. Each bird needed to be captured and returned to the aviary at least once to attach the transmitter harness. Some birds needed to be captured in the aviary up to three times, which was more than expected. Capturing birds inside the aviaries presented a major challenge particularly for staff who are not experienced with this capture technique and required a person to enter the aviary and catch the bird in a hand net. Although this technique increased the stress on each bird during capture, it was often utilized in a managed care setting because experienced staff could capture the bird very quickly. However, a system like that of the transfer boxes (see 5.2. Kiwikiu captures and strategies) could be easily incorporated as an additional capture option (see Figure 7). A net could be placed on the outside of a small trap-door panel and the bird could be gently shooed into the net, assuming the bird is mobile and able to fly. Additionally, retractable screens could be incorporated into the design to temporarily reduce the size of the aviary during capture. One person could hold the net on the outside and operate the trap door, while a second person quietly enters the aviary, closes the retractable screen (thereby shutting the bird in a small portion of the aviary), and gently coaxes the bird into the trap door opening near the top of the aviary with the net. The trap door with the net is designed to appear as an escape and the birds are drawn to the opening. In the transfer boxes and holding cages, this design worked well and resulted in very rapid capture with minimal stress.

Releasing the birds from the aviaries could also be enhanced by alterations to the design. As designed, the only openings in the aviaries once assembled were the two human doors. The large door only extended to within 40 cm of the roof panel. This was designed to prevent birds from escaping by flying over the heads of someone entering the aviary. However, when it came time to release the birds, many birds did not immediately leave the release aviary. Many flew back and forth, landing on the panel just above the open door multiple times. It took upwards of two hours for some individuals to leave their aviary once the doors were open. We learned after the first few releases that birds essentially needed to be shooed out of the door by someone walking around the outside or entering the aviary, undoubtedly adding stress to the release process. Allowing birds to leave the aviary on their own accord regardless of the number of hours after the door is open may allow birds to be released without added stress but it was not clear that they would ever fly out through the open door. One key observation was that the birds seemed to stay within the upper 0.5 m of the aviary and to escape, they had to fly downwards to go out the door. Further, the door may represent a frightening element of the aviary to the birds. This is where humans enter the aviary and most birds were reluctant to fly to the door. If the goal in the future is to release birds as quickly as possible, then easily removable panels into the side and/or rear walls should be incorporated into the aviary design. Even though the wall panels were designed to be easily disassembled, it would not have been easy to remove wall panels without causing distress to the bird

inside due to the dozens of cable ties and pipe clamps needing to be removed. However, it would be possible to design some wall panels to be able to be removed easily from the outside, either just by cutting a few strategic cable ties and/or by incorporating latches and hinges, like a large door. This should open up all the way to the ceiling panel so birds could easily fly out of the aviary without having to fly down. These wall panels could be the full length of the aviary and would not be associated with people coming and going (see Figure 7).

Another helpful addition for the aviary design could be to improve the mechanism to remotely weigh the birds in the aviaries. We successfully collected weights on each bird within the release aviary at least once per week, which was the agreed upon goal prior to the reintroduction. However, in the future, we could improve the design to make the data collection easier. For example, some of the wild birds were averse to and took longer than expected to become comfortable with eating from the supplemental feeder in the aviaries. Also, after kiwikiu began to succumb to avian malaria, the desire to monitor weights increased. Given the weight of kiwikiu (16–30 g), remotely weighing these birds more frequently would be best achieved by not using the supplemental feeder. In general, the wild-translocated birds only approached food pans if people were not present or were hidden in the distance. SDZG staff placed a digital scale with a smartphone video recording the digital scale display at the supplemental feeder. Then, SDZG staff left the aviary area and waited for the bird to feed. Using this technique, weights were obtained for most birds at least once per week, as planned. However, a perch scale that would allow observers to obtain weights without utilizing the supplemental feeder would be preferable to record weights more frequently. Alternatively, scales could be programmed to log weights to be reviewed later. Because the birds did not use the supplemental feeders after release into the wild, we recommend no longer using the supplemental feeders within the release aviary. Instead, small tray feeders attached to the sides of the aviaries could be used to remotely weigh birds. By providing a single perch for these sidewall food trays, the birds could be enticed to sitting on the scale for a longer period, thereby increasing the accuracy of the weights recorded.

With this species, though, soft-release techniques (holding in aviary at release site) may not be needed in the future as discussed in the next sections below.

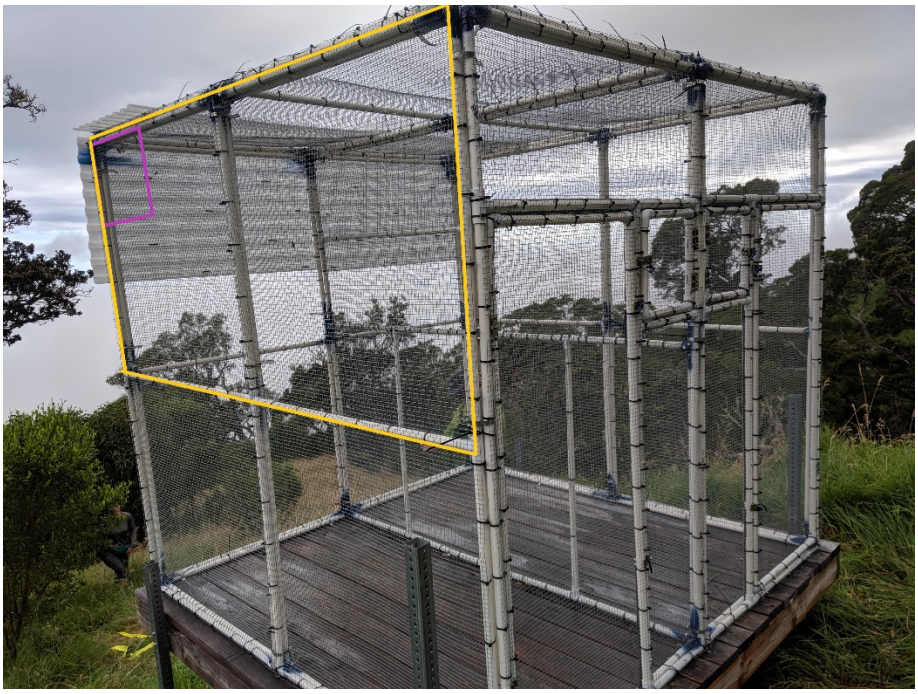


Figure 7. Release aviary in Nakula Natural Area Reserve showing suggested changes; an easily removable panel for releases is shown in yellow, and an additional door to aide in capturing birds from aviary in purple.

4.3. Feeder Design

Food was continuously provided to each bird while in the release aviaries. This diet consisted primarily of dry pelleted food (Mazuri® Softbill Diet for Iron-Sensitive Birds) and mealworms. The food was offered in two ways, a combined gravity-fed and automated feeder, termed the "supplemental feeder", and small food trays attached to the sidewalls of the aviaries. The same combination of pellets and mealworms were provided in both feeder designs. The supplemental feeder combined two feeder types in a five-gallon bucket suspended over a large tray (Figure 8). Mealworms were placed in the top of a series of trays each with numerous holes. The mealworms then fell through the holes to successive trays until they fell out onto the feeding tray. In this way, the natural movements of the mealworms acted as a time delayed dispersal of the food. Below these trays inside the bucket was a battery-operated time-delayed feeder that dispensed pelleted insectivore diet food. This feeder design was intended to be used as a food source for the birds while being held and after they were released. Using this feeder inside the aviary was an attempt to acclimate the birds to the feeder and encourage the birds to return after release. The sidewall trays were primarily used as an alternative to the supplemental feeders particularly in cases where birds showed aversion to the larger feeder (a scenario that occurred with the wild translocated birds).

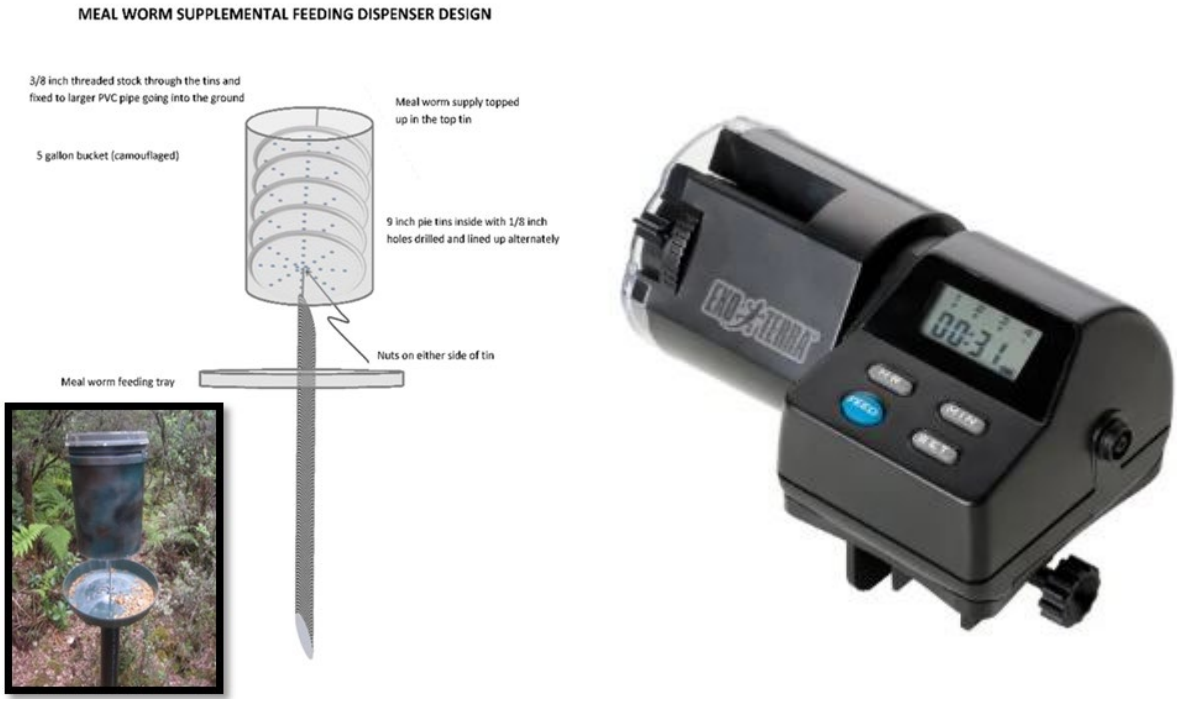


Figure 8. Supplemental feeder design (left) and Exo Terra® Turtle Feeder/Automatic Feeder Unit used to dispense dry pelleted food that was placed inside the bucket of the supplemental feeder.

After the kiwikiu were released and the birds had moved away from the aviary, we moved the supplemental feeders outside of the aviary and closed the aviary doors. However, no kiwikiu were known to feed from these feeders after the birds were released. Two birds from the conservation breeding facility fed off small trays offered at camp or on the outside of their aviary after they were released, but neither approached any of the supplemental feeders. Game cameras monitored the feeders after they were moved out of the aviaries. The only animals that used the supplemental feeders were Red-billed Leiothrix (*Leiothrix lutea*). These feeders were maintained for two weeks after birds were released but shut down after it was clear kiwikiu were not using them. In 2013–2014, MFBRP placed several of the same supplemental feeders (without the pellet feeders) throughout TNC's Waikamoi Preserve including near active wild kiwikiu nests and territories and monitored activity using game cameras. Just as in Nakula NAR, the only birds to utilize the feeders were Red-billed Leiothrix. The refusal of the wild kiwikiu to use the supplemental feeders even after being acclimated to them could be seen as a good sign in that the birds were able to find enough food by foraging on their own. However, it is also possible that given the choice between feeding in a natural manner, even without success, is preferable to the birds rather than returning to such an unnatural (and possibly frightening) source of food. The fact that even the birds from the conservation breeding facility that had fed on these feeders for over a year did not return to the supplemental feeders after release is also intriguing and defies an easy explanation. The small sidewall feeders were a ceramic orange color that may have provided a better visual cue than the green camouflage-painted supplemental feeders. The supplemental feeder design involves a bucket placed above the feeder tray. Having something above the birds' heads might have been enough to deter birds of either origin from this feeder type.

Recommendations for the future – The supplemental feeders operated as designed and showed that they were capable of automatically dispensing food for up to one week (unless food was spoiled by rain). However, the wholesale failure of the supplemental feeders to attract kiwikiu of either origin (wild or from the conservation breeding facility) strongly suggests that this design should not be used in the future. It is possible that modifications could be made that enhance the attraction of this type of feeder, but it is questionable if this is even needed for wild translocated birds. The post-release behavior of the birds from the conservation breeding facility suggested that supplemental feeding may be required if birds of this type are released in the future. In this case, additional research and development of a new supplemental feeder design would be required. The small food trays were more attractive to the birds, but these would need to be replenished daily. The original supplemental feeder design would have allowed for weekly replenishment. It is unclear if staff visiting the release aviaries less often would have resulted in the wild translocated birds acclimating to the supplemental feeder faster and/or adjusting to being housed in the release aviaries faster. While birds were held in the aviaries and after release, staff was able to check feeders daily. As such, daily food replenishment is possible especially if the number of birds released is similar to that in 2019. Feeder placement should also be carefully considered as we had some issues with rain collecting on the feeder trays that spoiled the food.

Another concern with the supplemental feeder design was that the lid collected water. The kiwikiu were often observed using this water to bathe and providing some alternative bathing source should be considered. However, this standing water is a potential source of mosquito larval habitat. Artificial water sources of this kind have been found to harbor mosquito larvae. In Nakula NAR, we did not observe mosquito larvae in the feeder lids, but great care should always be given to avoid attracting mosquitoes with this type of water source. Similar considerations should be made concerning roofing materials of the aviaries as the roofs also collected water on occasion.

The above suggestions for both aviary and feeder design are meant to improve future releases using a similar delayed release. However, there are multiple reasons why a soft release may not be necessary for future releases. Delayed or “soft” releases have resulted in lower mortality and dispersal in other species, particularly for captive-reared animals (e.g. Mitchell et al. 2011, Moseby et al. 2014). However, this is often not the case for wild translocated animals and a hard release is often recommended in this case (e.g. Castro et al. 1994, Richardson et al. 2015). Thus, the composition of future releases (wild or from conservation breeding facilities) may dictate what methods are used (Atkinson and Seddon 2007). In 2019, we were attempting to incorporate birds of both managed care and wild origin and the soft release technique was selected to accomplish this. This was designed to hold birds of mixed origin near each other to potentially form pairs and/or share important behaviors. It was thought that birds from the conservation breeding facility could show wild birds how to feed from artificial feeders and the wild birds could share wild foraging techniques. We also hoped that the birds from the conservation breeding facility might help “anchor” wild birds to the site, assuming the birds from the conservation breeding facility were less likely to leave the site and may serve as conspecific attraction for any wild birds that may be tempted to return to their capture site. Previous translocations of wild palila (*Loxoides bailleuri*) were ultimately unsuccessful in establishing a sustaining population in part because many birds returned to their capture site (Banko et al. 2014). Future kiwikiu releases are likely to be based heavily on wild translocation and hard releases (possibly from holding cages like those discussed in 5.3. Holding birds in Hanawī NAR) would likely be used in this case. Although kiwikiu from the conservation breeding facility, when paired with wild translocated birds in side-by-side aviaries, may have helped wild birds

acclimate to the artificial feeders, none of the wild birds returned to the feeders after they were released. Moreover, most wild birds that were not paired with birds from the conservation breeding facility fed from the supplemental feeders while in the aviaries on their own. Wild kiwikiu captured and brought to the conservation breeding facility in the past were observed to learn to eat from food pans quickly. However, open food pans, not the supplemental feeder, were used in those cases. Finally, none of the wild birds released in Nakula NAR attempted to immediately leave the area to return to their capture sites in Hanawī NAR. Translocated po'ouli (*Melamprosops phaeosoma*) and Maui 'alauahio (*Paroreomyza montana*) all returned to their capture site within just a few days. Additionally, 67% of the translocated palila that returned to their capture site did so within the first two weeks. Although we have no way to know if the soft release or the presence of the birds from the conservation breeding facility aided in kiwikiu remaining in Kahikinui for as long as they did, it seems unlikely that this element would be necessary for future wild bird releases. We also do not know whether these birds would have persisted in the area in the long-term, given the relatively short period birds survived in Nakula NAR.

5. Obtaining Wild Kiwikiu for Translocation

5.1. Capture site preparation and personnel

Several sites were proposed as the source of wild kiwikiu for translocation including TNC's Waikamoi Preserve and Hanawī NAR (MFBWG 2018). Known genetic and cultural variation between these sites made it clear that incorporating as much of this variation into a new leeward Haleakalā population would be desirable in the long term (Mounce et al. 2015). Ultimately, the working group decided to capture and translocate birds from a single site in Hanawī NAR in the first year. It would not have been logistically feasible to source birds from multiple sites in a single year. Subsequent captures would have been proposed at additional sites, including TNC's Waikamoi Preserve, in the following years if the kiwikiu release had been successful at establishing birds in Nakula NAR.

The Frisbee Meadows study site in Hanawī NAR had been used by MFBRP for over a decade to study the biology and population dynamics of kiwikiu. The site contains a network of trails and a camp, although the site had not been used regularly in several years. In August 2019, MFBRP prepared and repaired the camp and NEPM began clearing trails. In September, about two weeks prior to the capture mission, six MFBRP team members went to Frisbee Meadows to clear trails, search for kiwikiu, and map locations for potential captures. This proved valuable as all the birds captured came from these pre-identified areas. Having the trails cleared also helped with navigating safely back to camp while carrying a bird in a transfer box.

The capture trip occurred on 9–17 October 2019 (7.5 days of capture attempts). There were 11 people at a time at camp, but 13 individuals took part in the captures in total. Some team members were switched out on 14 October. Personnel from six organizations participated in the capture trip: American Bird Conservancy, MFBRP, Pacific Bird Conservation, SDZG, DOFAW, and USFWS. Media coverage of the event was conducted by DLNR and Honolulu Civil Beat.

5.2. Kiwikiu captures and strategies

Capture teams were made up of 2–3 people per team and each of three teams was equipped with mist-nets, banding poles, banding gear, playback (speakers and player with kiwikiu calls), and two transfer boxes. Each team focused on separate areas based on the kiwikiu detections made in September and

previous years. The weather was generally good for mist netting, especially for Hanawī NAR, and we were able to attempt captures for at least part of every day. There were some high winds, especially the first couple of nights, but little rain fell during the capture trip.

Captures were attempted systematically throughout the study site but within areas where we could get kiwikiu back to camp in a safe and timely manner. Our goal was for a maximum of two hours between placing the kiwikiu in the transfer box and arriving to camp. With this goal in mind, we attempted captures within all areas within a 2-hour hike during the October trip. Additionally, we attempted to get kiwikiu back to camp no later than 15:00–16:00. Ideally, kiwikiu would also have at least a day to calm down prior to translocation; thus attempts were made for kiwikiu only during the morning of the last day with the expectation that kiwikiu would be in holding by noon.

The first captures were made on 9 October and at least one bird was captured on each subsequent day (Table 1). Most captures occurred before noon even though attempts were made in the afternoon. The first nine captures were males leading to concerns that the targeted playback method was deterring females. We then diversified strategies incorporating two arrays of passive mist nets in areas where males had been captured. The hope was to passively capture the mate of one of the captured males. However, the passive netting did not result in additional kiwikiu captures. The remaining captures, including females, were made using target netting with playback.

We captured 13 total kiwikiu: 10 males and 3 females (Table 1, Figure 10). The Reintroduction Plan (MFBWG 2018) laid out criteria for whether individuals could be transferred to holding prior to translocation. Among these criteria was that females in breeding condition (e.g. brood patch) must be released at the capture location, as they were likely to be caring for offspring. The first female captured was in breeding condition and was released after banding. One male was released at the net because there were enough males already in holding. If an individual met the criteria for holding, they were placed in a transfer box (Figure 9) and carefully carried back to camp. Individuals transferred to holding at camp were given a sequential number ID based on the order in which they were captured (Table 1).

Figure 9. Boxes used to transfer captured kiwikiu to camp for holding as well as from Hanawī Natural Area Reserve (NAR) to Nakula NAR. Photo by Nathan Eagle – Honolulu Civil Beat.

The skewed sex ratio in captured individuals led to lengthy discussions among the capture team, particularly after the fourth day at which point all nine birds captured were male. The Kiwikiu Reintroduction Plan (MFBWG 2018) expected an uneven sex ratio in translocated individuals in a given year and predicted that fewer females would likely be part of the first year's release cohort, especially given the ratio among the birds from the conservation breeding facility (6 males to 1 18-year old female). However, no one anticipated capturing so few (or no) females. The team discussed how to move forward if we continued to catch only males. Likely all birds in holding would have been released if no females were captured. Thankfully, two females were captured on 13 October (one was released) and a third was captured the following day. The team decided to target a 2:1 male–female ratio in the first translocated group as long as more capture attempts were made in January 2020. We attempted additional captures on 15–16 October but did not capture any additional kiwikiu. As a result, the team decided to release three males still in holding (a fourth male had already been released) to achieve the desired 2:1 sex ratio among adult birds. This left four adult males, one second-year (SY) male and two females to be translocated to Nakula NAR.

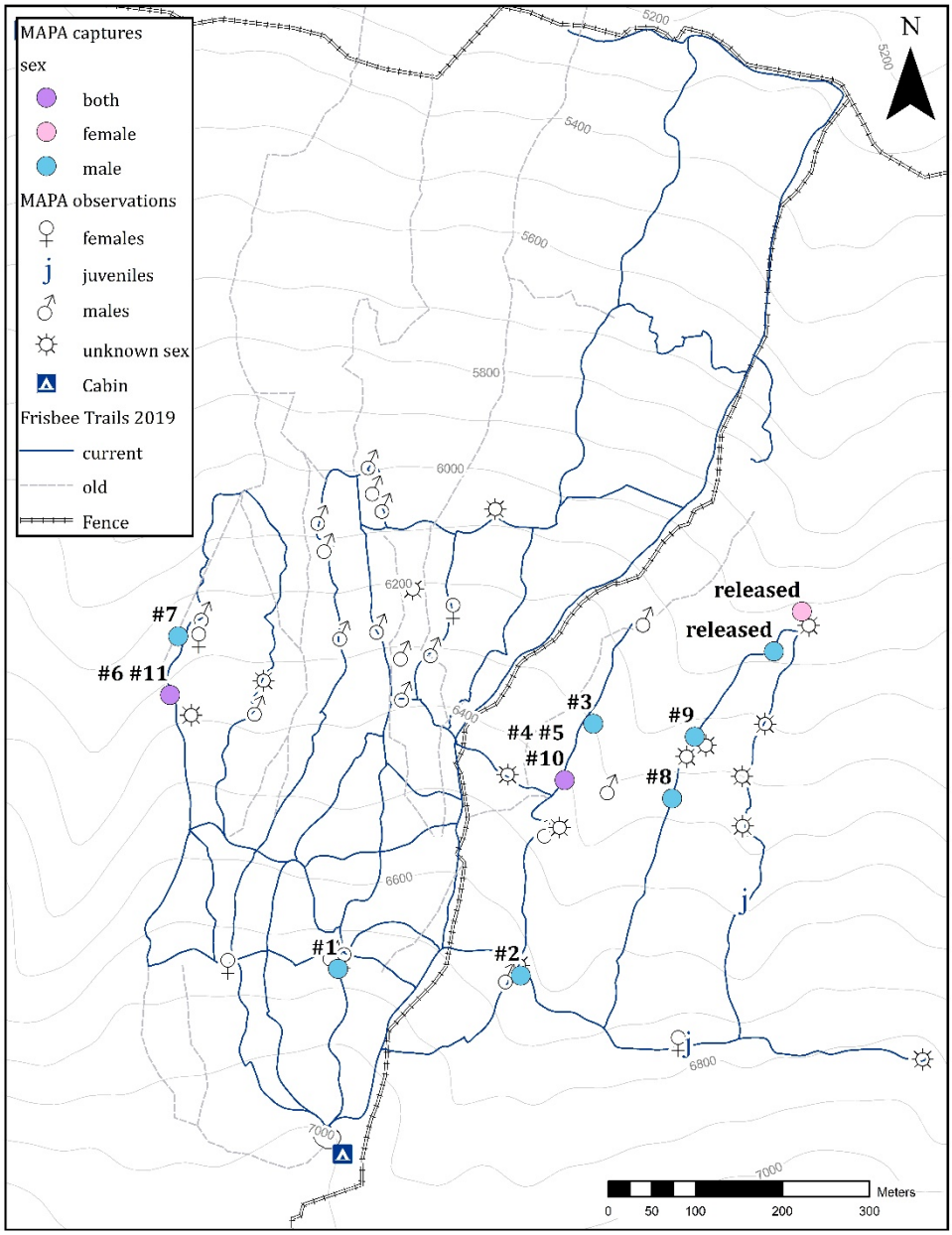


Figure 10. Map of kiwikiu capture (with Capture ID number) and detections within Hanawī Natural Area Reserve. Detections were recorded in September and October 2019. An observation point represents a separate encounter with a kiwikiu; the sum of these observation points does not reflect the number of individual birds at the site.

*Table 1. Kiwikiu (*Pseudonestor xanthophrys*) captured in Hanawī Natural Area Reserve (NAR) 9–14 October 2019. The decision to translocate to Nakula NAR or release back into Hanawī NAR was made based on individual condition and the desired sex ratio of translocated individuals. Color Bands = black (BK), blue (BL), green (GR), yellow (YE), white (WH), no-band (NB), federal band (AL). Age= After hatch-year (AHY), After second-year (ASY), and Second-year (SY).*

Bird ID	Band #	Color Bands	Capture Date	Age	Sex	Capture Weight (g)	Notes
WILD1	1971-12180	BK/YE, NB/AL	10/9/2019	ASY	male	24.3	Translocated to Nakula NAR
WILD2	1971-12177	n/a	10/9/2019	ASY	male	23.4	Released on 10/16/2019 after holding
WILD3	1971-12178	n/a	10/10/2019	ASY	male	22.9	Released on 10/16/2019 after holding
WILD4	1971-12179	n/a	10/10/2019	ASY	male	23.3	Released on 10/16/2019 after holding
WILD5	1971-12181	WH/BK, NB/AL	10/11/2019	ASY	male	23.3	Translocated to Nakula NAR
WILD6	1971-12162	n/a	10/12/2019	ASY	male	21.8	Released on 10/12/2019 after transfer to camp, displaying stress, sufficient males captured already
WILD7	1971-12176	YE/GR, NB/AL	10/12/2019	ASY	male	23.7	Translocated to Nakula NAR
WILD8	1971-12183	NB/AL, BK/GR	10/12/2019	ASY	male	27.5	Translocated to Nakula NAR
WILD9	1971-12182	GR/BL, NB/AL	10/12/2019	SY	male	21.4	Translocated to Nakula NAR
n/a	1401-47600	n/a	10/13/2019	ASY	female	20.0	Released on 10/13/2019 at net, brood patch
n/a	1971-12155	Al/RD, YE/YE	10/13/2019	ASY	male	24.0	Released on 10/13/2019 at net, too many males
WILD10	1971-12174	NB/BL, NB/AL	10/13/2019	AHY	female	17.3	Translocated to Nakula NAR. Possibly a third-year based on plumage.
WILD11	1971-12175	NB/YE, NB/AL	10/14/2019	ASY	female	18.5	Translocated to Nakula NAR

Kiwikiu observations – Altogether, 13 male and seven (7) female kiwikiu were either captured or approached nets during playback (Figure 10). While the sex ratio among captured individuals was heavily skewed toward males, the ratio among observed individuals was less disproportionate. We captured comparatively more birds on the edges of the study site compared to the center and 70% were captured in the greater Hanawī Gulch area on the eastern edge of the site (Figure 10). Several observations were made between 1800 and 1950 m asl in the center of the site but no captures were made in this area. Three males and one female approached nets in this area, and it is possible additional captures would have been made in the center of the site with more effort.

All birds were captured or observed above 1800 m asl, mostly above 1920 m. Survey effort in previous years has always been skewed toward higher elevations but detections were routinely made down to 1615 m asl. During the scouting trip, no kiwikiu were detected below 1800 m and sufficient numbers of birds were captured above this elevation during the capture trip such that little effort was expended in trying to locate birds below this elevation (especially since it would have been too long of a hike back to camp with a captured bird). Nonetheless, the lack of observations below 1800 m raises concerns about the occurrence of potential range loss for the species.

The capture trip was designed to be outside of the typical breeding season for kiwikiu to reduce the chance of removing adults with dependent juveniles. Although most breeding occurs February–June, kiwikiu are known to attempt breeding throughout the year (Simon et al. 1997). The long juvenile dependency (>6 months) would also mean that adults caring for juveniles could be observed at any time of the year. We observed at least one hatch-year with a female during the capture trip and a juvenile was heard in the same area in previous weeks. Additionally, one female was released because she had a brood patch, indicating that she was either nesting or caring for a juvenile. The SY male (WILD9) that was captured made juvenile begging calls prior to capture, likely in response to the playback. Despite these observations, few other indications of breeding were noted. In general, males were not singing in the rates usually observed in later months and the other captured females were not in breeding condition. Thus, October proved to be a good month in terms of avoiding the breeding season. However, attempting to capture birds outside of the breeding season may have caused fewer females to be attracted to the playback than might be typical.

Recommendations for future captures – The general plan for captures, having multiple teams spread out throughout the site, worked well. Each team set up 1–2 nets and conducted playback at each net for about an hour before moving to another site at least 100 m from the previous location. This maximized coverage of an area and avoided netting efforts in unproductive areas. In terms of the number of kiwikiu captured, this method exceeded many expectations and was an achievement in itself. With additional time, we would undoubtedly have been able to capture more individuals. The only obstacle we ran into was the fact that comparatively few females were captured.

In the past, MFBRP has relied on a combination of passive and targeted mist netting to capture and band kiwikiu. Most individuals in the past were captured using targeted netting with playback and we felt that this method would be sufficient for the translocation. However, every year MFBRP found some individuals that avoided capture using the targeted method and MFBRP relied on passive netting to capture these individuals. For passive netting to be successful, an area needs to be blanketed with mist nets, more than we planned to have on the October capture trip. In the past, MFBRP often ran arrays of up to 30 passive mist nets in an area and this effort usually produced 1–2 kiwikiu captures after a few

days (while also occasionally using playback on some of the nets). During the capture trip in 2019, we were only able to devote 6–8 nets per passive net array in each of two areas, while maintaining a few nets for target netting. This low-level effort may be why the passive netting did not result in additional captures. Future efforts could consider a combined approach with staffing for one large passive array as well as a few teams for mobile target netting. Playback can also be used on nets set up for passive banding especially if kiwikiu are observed in the area and are not being captured by the passive nets.

Although the October trip resulted in a large number of captures, future efforts should plan for the possibility that multiple trips might be necessary to capture the desired number and sex ratio for translocations. We discussed this contingency in 2019 especially in regard to capturing additional females. The team ultimately decided to translocate more males than females in October under the expectation that additional capture attempts would be made for females in the near future. These additional efforts were ultimately cancelled given the mortality rate among the first cohort. Additionally, the weather conditions within Hanawī NAR often limit banding efforts and weather delays are likely during future attempts.

The Kiwikiu Reintroduction Plan (MFBWG 2018) expressed a desire to capture breeding pairs and subadult (second-year) birds. We expected that capturing enough subadults to make up the entire cohort would be extremely difficult. We ultimately captured one SY male (WILD9) and a second- or third-year female (WILD10). Given the predicted difficulty in capturing sub-adult birds, this was an excellent result and should guide future expectations as to the number of subadults that may be captured with similar effort. Although we did not expect to capture many pairs, it remained a stated goal; however, this may have been unrealistic. The major challenge in this goal is knowing whether two birds are indeed a mated pair without prior observations of color-banded individuals. Past captures have shown that capturing birds in the same net, even at the same time, is not sufficient to determine if two birds are paired (MFBRP unpub. data). Additionally, once released in the new habitat there is no guarantee that birds will remain together. Both females translocated to Nakula NAR were captured in the same nets as males. However, there were no other indications that either was paired with the males captured in those nets or in the vicinity. WILD11 (female) was captured in the same net as WILD6 (male) although not on the same day; WILD6 was ultimately released in Hanawī NAR (Table 1). WILD7 (male) was captured in a net <150 m from where WILD11 was captured and they were placed in side-by-side aviaries once in Nakula NAR in the hope that they were (or would form) a pair. However, once released neither bird associated with the another. Future capture efforts should be focused on capturing suitable numbers of both males and females with the hope that pairs will be formed in the new habitat. If pairs were captured in the source range, this would be ideal, but is likely to be an extremely rare event.

As discussed above, October appeared to be outside of the main breeding season, as was the goal. However, as most birds were not in breeding condition perhaps they did not respond to the playback in the ways we were previously accustomed to during the breeding season. One possible scenario is that during this time of year males are seeking out females with which to mate and spending less time defending territories. This could have been the reason why we captured so many males relatively easily, including three males in two nets <150 m apart in two consecutive days. Females during this time of year also may not have been feeling territorial and would be less attracted to playback as they would have been during the breeding season. Thus, future capture attempts may be more successful if conducted slightly later in the year, targeting the time of year when birds have paired up and are

settling in for the breeding season, such as December–January. Multiple capture trips planned between September and January would be ideal.

5.3. Holding birds in Hanawī NAR

Bird room setup and cage design – As soon as the team arrived at Frisbee Meadows, the team set up the "bird room" in one of the WeatherPorts® (Figure 11). The bird room was staffed by Peter Luscomb of Pacific Bird Conservation and one of two veterinarians with SDZG. Inside the bird room, we assembled the shelving unit for the holding cages constructed from EZ Corners pipefittings (http://ezcorners.com/index.asp) and 2.5 cm conduit pipe EMT. The shelving unit had two shelves and was able to hold 13 cages. The bird room also contained a few collapsible camp tables, feeding trays, food, and veterinary supplies.

Figure 11. The "bird room" WeatherPort® where the birds were held between capture and translocation. Tables inside helped to organize food, banding and veterinary equipment, and bird boxes. The holding cages are on the right side of the photo on the shelving unit. Photo by Nathan Eagle—Honolulu Civil Beat.

Once the teams captured a bird in the field, the bird was transported back to camp in a hand-carried transfer box (Figure 9). Minimal information on the bird was taken in the field to get the bird back to camp quickly and to reduce stress. We recorded weight and general body and breeding condition at the capture site and recorded these along with location, team name, weight, and sex of bird on the box. One to two team members carried the bird back to camp while one person stayed to either try to catch another bird or shut down the nets (depending on the number in the team, distance from camp, and difficulty of trail). Sometimes two team members were needed to bring the bird back to camp because there were difficult gulch or fence crossings. The ability to pass the carrying box to another team

member proved useful in many situations. When the bird arrived at the bird room, it was taken inside and placed on the table until it was transferred to a holding cage.

The transfer box (Figure 9) and the holding cage (Figure 12) both included “trap-door openings” on one side that aided in transferring the bird between carriers or capturing the bird with minimal handling. With the trap doors lined up on both the transfer box and holding cage, one person removed (slid up) both doors, allowing the bird to hop over into the new cage. Thus, the transfer can be accomplished with no handling of the bird. Similarly, these trap doors can be used to capture birds from the transfer box or holding cage with minimal stress to the bird. To do this, one person stood to the side of the cage and placed a small hand net over the trap-door opening (Figure 13). A second person then slid open the trap door to allow the bird to fly into the net. Holding the net bag extended out (taut) from the cage made the trap door opening appear to be an opening from which they could escape. Often a bird would not immediately fly into the net and someone would slide their fingers under the service door in the front. This usually encouraged the birds to go into the net quickly. After the bird flew into the net, it could be secured and extracted from the net safely.

Each bird was held in a single-compartment holding cage within the bird room until release or translocation. Once each bird arrived at camp, it was immediately transferred into the holding cage. We did not handle the birds until a veterinary exam a few days after capture to give the bird time to adjust. We placed a paper liner along the bottom and food and water containers in the cage prior to transferring a bird to a holding cage. The information tag from the transfer box was placed on the holding cage. After a bird was placed in a holding cage, we briefly checked on the bird to ensure they were on a perch and then left the room to allow the bird to calm down. Generally, the captured kiwikiu calmed down quickly (but see below: “Bird Behavior in Holding”).

The holding cages were approximately 23 cm wide, 20 cm high, and 40.5 cm deep and were constructed out of 6-mm polyvinyl chloride (PVC) sheet material (Figure 12). The cages were collapsible and able to be stored flat. The two sidewalls were solid and had a series of 2.5-cm ventilation holes along the upper portion of each wall. The right sidewall had ventilation holes along the upper portions of the wall plus a trap door measuring 7.5 cm wide by 10 cm tall in the upper rear area. This opening was covered with a removable sliding door. The back wall had an opening covered with mosquito-proof pet screen to facilitate ventilation of the cage. The cages had two perches, one secured to the sides of the cage in the back and a hanging perch in the front used to weigh the birds. The front perch was held by two vertical support dowels that extended up through the top of the cage as part of a remote weighing system. The support dowels for the front perch slid through two holes (14 mm) in the top of the cage and attached to a separate PVC sheet. To weigh the birds, someone simply slid an Ohaus® HH120 digital scale under the piece of PVC supporting the front perch. Once a bird landed on the front perch, an observer recorded the weight displayed by the scale.

The bottom of the holding cage included a removable floor, a “service tray”, made of the same PVC sheet material as the rest of the cage. The rear of the service tray included a lip that sat flush with the rear wall of the cage when fully inserted. On this lip, we hooked two hanging “d-cups” with water and food. Other food and enrichment materials (e.g. berries of *Coprosma montana*, *Broussasia arguta* and *Cheirodendron trigynum*) were offered on a flat 15 cm tray along the floor. The entire service tray, including the food and water trays could be slid in and out of the cage without lifting the door more

than a small opening. In this way, food and water could be refreshed and the paper liner could be changed. The paper liners also allowed those caring for the birds to monitor fecal output.

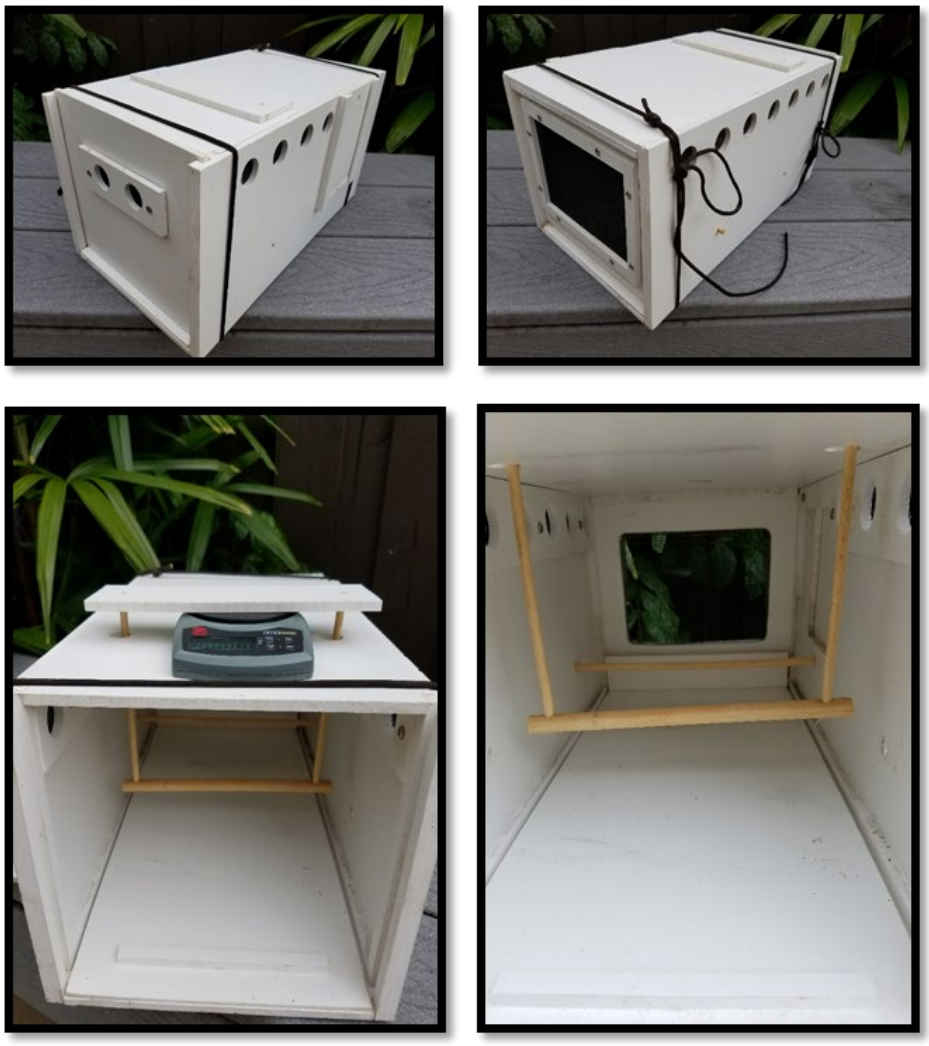


Figure 12. Single compartment holding cages used at the capture site camp with remote weighing system and removable perches and floor.

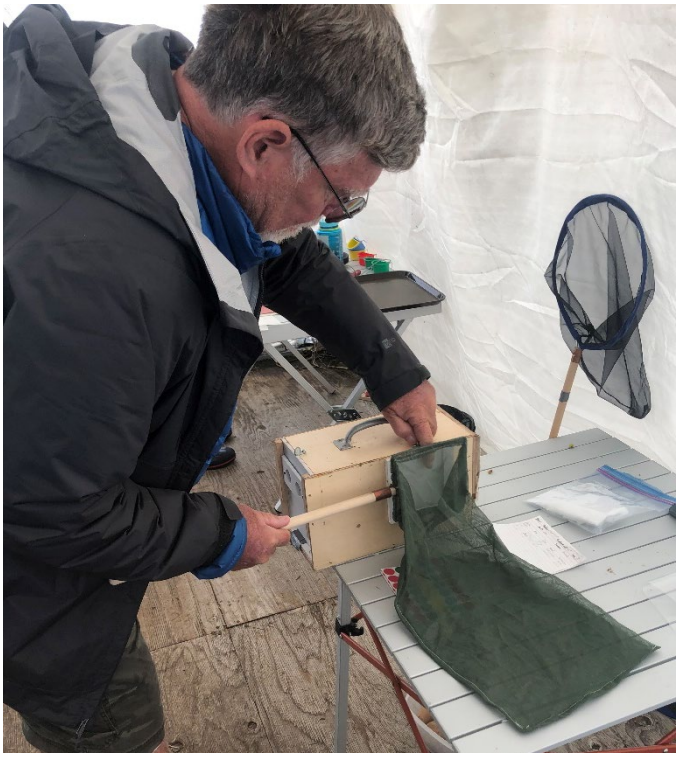


Figure 13. Peter Luscomb demonstrating how to recapture a bird from a transfer box using the trap door and net.

Bird diet in holding – We fed the kiwikiu held in Hanawī NAR a diet consisting of 1 tablespoon of a dry mix (1/3 part Mazuri softbill pellets, 1/3 part dried fly larvae and 1/3 part Quicko egg food) provided by SDZG and 20 large mealworms. The birds ate the mealworms readily but ate very little of the dry mix. We offered scrambled egg, but the birds did not respond to it. We also experimented with supplemental food and enrichment from the wild, such as pilo (*Coprosma montana*) and kanawao (*Broussaisia arguta*) berries attached to branches. The birds responded strongly to these. They did not eat the berries but rather ripped them open, likely looking for insect larvae. We also provided ʻōlapa (*Cheirodendron trigynum*) berries but the birds showed no interest.

We misted the birds with water at 12:00 each day with a 3-cc syringe and 27-gauge needle. The birds showed some indication that they would bath and spent some time preening after being misted. Two males, WILD2 and WILD7, used their water bowls to bath. We tried placing the 15 cm tray feeders in the cage with water at the 12:00 check but no birds showed interest in using these to bathe during the 30 minutes that these trays were offered.

Management of birds in holding –We fed and observed the birds three times per day at 07:00, 11:00, and 15:00. At the start of each feeding and observation period, the bird room staff (1–2 people) looked at each bird and determined their basic status. We then recorded the weight of each bird using the remote weighing system (front perch). All birds were weighed twice daily, and the weights were logged to track daily fluctuations and carefully monitor for any changes. We used the first morning weight as the target weight to maintain and assess the birds' condition. After weights were recorded, we began food preparation and removed the service trays from each holding cage.

The service trays were designed to be removed from the cage with minimal disturbance to the bird and to minimize the risk of escape. To remove the service tray, we lifted the front door up off the service tray by no more than 1 cm and slowly pulled the tray out, lifting the door only high enough to allow the floor tray to pass underneath. When the d-cups hit the back of the door, we lifted the door straight up and over the food and water cups without exposing more than a 1-cm opening. We had two sets of food and water containers to allow one set to be cleaned while the other was filled and offered to the birds. This made it possible to change out food and water quickly without having to wait to replace the cups after cleaning. We cleaned the service trays during the first feeding after replacing the paper liners and food and water trays. We disposed of all unconsumed food and paper liners. During the 11:00 and 15:00 feedings, we pulled the service tray out just enough to check the food and add more food and water if needed.

Once all birds were fed, we briefly observed each bird's behavior, recording their location in the cage, activity, and posture. We paid close attention to any changes in a bird's behavior, as deviations from an individual's baseline behavior can be critical in early detection of problems. If we noticed anything out of the ordinary, we made further observations. In addition to behavioral observations, we recorded food consumption and fecal output.

Bird behavior in holding – While held in Hanawī NAR, most of birds appeared stable and transitioned to managed care quickly. There was individual variation, especially in how active individuals were in the holding cages. Some birds sat still, while others moved around a lot. Several of the birds were very flighty and bashed into the sides of their boxes; one bird so much so that he was released the same day he was captured (WILD6). Others took a few days to fully calm down. At first, these birds spent a lot of time jumping/flying up to the side of the holding cages and holding on to the side air vents and banging into the tops of the boxes. The birds did this every 5–10 seconds and in the process caused some lost feathers from the tops of their heads. Veterinary staff reviewed the birds and determined that there was no damage done to birds' skin or skull and the damage was restricted to feather loss. This behavior slowed or stopped after the first few days for the birds that remained in holding.

After the first few hours, every bird began eating, consuming the provided food throughout the holding period. The birds primarily ate the mealworms and left the dry mix untouched. Bird weight fluctuated throughout the day; the birds lost weight overnight and gained weight throughout the day. The birds averaged 1.65 ± 0.48 g lighter in the morning than the previous afternoon (Table 2). This is a common observation among many passerines and has been observed in Hawai'i 'amakihi held in managed care (Work et al. 1999). Weights (am) fluctuated among days but all individuals stayed within 8% of the previous day's weight and the biggest fluctuations were weight gains. Bird weight was fairly consistent throughout the holding period in Hanawī NAR and were between 95.8% and 109.8% of their starting weight when released back into Hanawī NAR or translocated to Nakula NAR. This is encouraging given weight losses recorded in managed care prior to translocations of other honeycreepers. Some translocated palila lost up to 22.6% of their body weight in just 36 hours of holding during one translocation (Fancy et al 1997) and generally lost 1.5% to 6.8% of body weight on average during subsequent translocations (Banko et al. 2014). The fact that the kiwikiu gained 1.3% of their body weight on average while in holding speaks to how well the species took to holding in general (Table 2). Although 50% of the individuals lost some weight while held in Hanawī NAR, all translocated birds gained weight after being moved to Nakula NAR, gaining 10.3 ± 8.4% on average prior to release.

Table 2. Weight fluctuations of translocation candidate kiwikiu on a morning/afternoon, daily, and overall basis while in managed care in Hanawī NAR. Weights were recorded each morning (am) and afternoon (pm). Daily changes reflect variation in the am weight from one day to the next. Overall weight change compares the first am weight and final am weight prior to translocation or release. Negative values are presented in parentheses.

Bird ID	No. am weights	Mean am/pm fluctuation (g)	Mean am/pm fluctuation (%)	Mean daily change (g)	Mean daily change (%)	Overall weight change (%)
WILD1	7	1.62	6.9	0.53 ± 0.32	(2.2) ± 1.4	(2.9)
WILD2	7	1.23	5.5	0.37 ± 0.24	(1.5) ± 1.3	(2.2)
WILD3	6	1.58	7.2	1.32 ± 0.7	6.3 ± 3.4	6.7
WILD4	6	1.37	5.7	0.33 ± 0.27	1.1 ± 1.5	(0.8)
WILD5	5	1.48	6.4	0.74 ± 0.25	3.3 ± 1.2	3.6
WILD7	3	2.28	10.1	1.63 ± 0.45	7.6 ± 2.1	9.8
WILD8	3	2.75	10.6	0.57 ± 0.15	2.2 ± 0.6	2.7
WILD9	3	1.35	6.6	0.5 ± 0.3	(2.4) ± 1.5	(3.9)
WILD10	3	1.47	9.0	1.03 ± 0.76	(3.6) ± 8.8	(4.2)
WILD11	1	1.35	7.7	0.70	4	4.0
	Σ	1.65 ± 0.48	8 ± 2%			1.3 ± 4.8%

Eligibility for translocation – Each bird was evaluated several times as to whether they were eligible for translocation to Nakula NAR. The Kiwikiu Reintroduction Plan (MFBWG 2018) included a dichotomous key to determine eligibility for translocation starting immediately after capture. The first part of the evaluation was to determine breeding status, particularly of females. Those in breeding condition with a brood patch were not eligible for translocation and were released at the capture site. As mentioned above, this occurred with one female (see 5.2. Kiwikiu captures and strategies). Other considerations included age (second-years were highly desired), overall condition (signs of excessive stress), and the age and sex composition of the group of birds being prepared for release. One male was released at the capture site because there were already eight males in holding at the time of his capture.

Once a bird was determined to be suitable at the capture site and transferred to holding, they were evaluated regularly for signs of stress, unsettling behaviors, or declining health. One male transferred to holding, WILD6, would not settle once in the holding cage. He repeatedly flew around the cage, hitting the sides and ceiling. These behaviors were somewhat typical of birds after they were first transferred to a holding cage, but these behaviors slowed down or stopping after 10–20 minutes in all other cases. In

the case of WILD6, these behaviors continued for more than an hour and showed no signs of lessening. The bird room staff, including the SDZG veterinarian, decided that this individual should be released. At the time, we were holding five other male kiwikiu and the group decided there was no need to risk the wellbeing of this individual, especially given that we were so close to meeting our goal for males.

Given the skewed sex ratio among captured kiwikiu, the group decided to release three additional males (see 5.2. Kiwikiu captures and strategies). All birds passed the final veterinary examination and none of the males in holding showed signs of undue stress or failing health as of 14 October. As such, there were no obvious candidates to release based on health or behavior. Instead, the group chose the candidates that were most ideal for translocation based on several characteristics and released the three lowest ranked individuals. The subadult (SY) WILD9 male was selected for translocation because of his age, likely being highly philopatric to the release site and adaptable (Greenwood and Harvey 1982). Two males were chosen for translocation based on their overall large body size and weight, WILD1 and 8, indicating overall vigor and could also support the largest transmitter size, allowing for maximum monitoring time after release (Table 2). The two other selected males, WILD5 and WILD7, showed some of the most consistent weight gain and were calmer in the holding cages than most. This left WILD2, WILD3, and WILD4, to be released, which were not ranked as highly as others based on weight fluctuation patterns, overall signs of stress, and similar capture locations as other individuals (not taking too many males from a similar location; Figure 10). We translocated only one of the four birds (WILD7) that rubbed the feathers from the tops of their heads from jumping up onto the sides of the cage, a behavior we interpreted as a sign of stress. However, all three of the birds released in Hanawī NAR would have made good candidates for translocation under different circumstances (i.e. if more females had been captured).

Recommendations for future temporary holding – Overall, the process for capturing, transporting, and caring for birds at the capture site went extremely well. We were able to care for ten birds at a time and we likely would have been able to care for more if needed. This species proved fairly easy to care for and most birds quickly learned how the cages and feeding routine worked. Transportation from the capture net to the bird room took less than two hours (usually less than an hour) and all birds arrived in good condition and transferred to the holding cages easily with minimal stress. The birds were handled only 1–2 times, one or two veterinary examinations (depending on when they were captured) and one of these was combined with banding and blood sample collection. One individual (WILD1) was the first bird to be captured during the trip on 9 October, was the last be released in Nakula NAR on 30 October (22 days in holding/aviary), and was also the last individual seen surviving in Nakula NAR on 24 November, suggesting this type of temporary holding has little effect on his survival.

In the future, we would make a few alterations to the holding cages that could further reduce stress on the birds, increase resiliency of the cages, and aide the bird room staff in monitoring the birds. As mentioned previously, several birds rubbed feathers off the crowns of their heads by flying to the sides, and hitting the sides and ceiling, holding onto the screen covering the ventilation holes, and in the process brushed the tops of their heads on a corner of the ventilation screen support. To prevent this, we would remove the side ventilation holes entirely and make sure all exposed PVC corners on the interior of the cages were rounded over. As this behavior was only exhibited by several birds, we could also have a few solid-sided cages on hand should birds exhibit this behavior and keep the side ventilation holes for most cages.

Kiwikiu have very strong mandibles designed to cut into wood. As such, it is not a surprise that they damaged the wooden dowels serving as perches inside the holding cages. While the damage caused to the horizontal perches was not critical, several birds seriously damaged the vertical supports that suspended the front perch as part of the remote weighing system. It may be possible to replace the vertical supports with metal rods or other materials the birds cannot damage. We also may attempt to replace one or more perches with those from natural wood materials. Regardless of materials, it will be important to have extra perches on hand to replace damaged pieces.

The kiwikiu in holding were very messy eaters and typically threw food material around the cage while eating. When feeding from the d-cups, the birds were able to feed from the perch and were less messy than when feeding from the flat tray on the bottom of the cage. When feeding from the flat tray, the birds often stepped in and scattered food and enrichment material around the cage in search of food. We eventually stopped putting the dry mix food in the bottom tray, as this seemed to be the source of a lot of the mess. We also reduced the amount of dry mix in their diet (from 1 tbsp to 1 tsp) as the dry mix provided a place for the mealworms to burrow into, making them harder for the birds to find and causing the birds to flick the material around the cages. Upon examination, all birds had caked-on food material on their feet and mandibles. In the wild, kiwikiu routinely rub or wipe their bills on branches to remove excess food material. Replacing the perching surface with a natural wood material may provide a better surface for which to clean their bills and may rub off some of the food material from their feet. Switching birds to new cages may also aid in keeping the birds in a clean environment, providing them with clean perches.

The diet provided to the kiwikiu in holding was sufficient to support the birds for this length of time, but is possibly not nutritionally ideal for longer term. Since the wild birds did not appear to consume much of the dry food, additional food items could be considered for future efforts to increase the nutritional qualities of the managed care diet, particularly to ensure the birds are obtaining sufficient Vitamin A and E. Future efforts could consider black fly larvae, fruit fly larvae, bee larvae, ground termite larvae, or silk worms in addition to mealworms. We used Fluker® gut loading for the mealworms. However, Repashy® formula is thinner, making it easier to sift through and is held in high regard by reptile keepers. Further, the flat tray placed on the floor of the cage was not deep enough to contain the mealworms, allowing them to escape and hide under the paper liner or food tray. A different design for this tray is warranted.

Visual inspection of the birds was done minimally but required peering through the small window opening in the door or the larger window at the back. This was clearly a source of stress for the birds. Using a small camera, such as a GoPro®, with a remote viewing window would greatly enhance our ability to observe the birds without peering into the cage. While someone peered through the window, many birds froze in place but when a camera was held up to the window, the birds resumed their normal behavior. A simple bracket could be fitted onto one of the window openings to allow a GroPro® to be held in place. It would be advisable to have a dummy camera in place at all times so the addition of the camera is not frightening to the birds.

The setup inside the bird room worked relatively well but the small folding camp tables were not ideal. A larger sturdy table, allowing more trays to be laid out at a time, would have been helpful. Having ample sanitizing wipes on hand would also have made cleaning materials and surfaces easier in this situation. The addition of a lighting system would also improve the working conditions of the bird room. Most feedings and cleanings took place after sunrise, but the final feeding and preparations for transportation

occurred early in the morning. Headlamps placed at the back of the cages provided some light to wake birds up and allow them to feed, but a more substantial lighting system would have been ideal. Finally, we did not see signs of rodent activity in the bird room throughout the capture trip but we captured two rats on the final night after setting traps in preparation for departing camp. Having consistent rodent control before and throughout the holding period is highly advisable. This is more of a concern regarding loss or contamination of food or veterinary materials as opposed to the safety of the birds; rats are unlikely to be able to climb the cage rack. However, reducing this risk is critical.

6. Preparing Kiwikiu from the Conservation Breeding Facility for Release

6.1. Background on conservation breeding

Conservation breeding efforts for kiwikiu in captivity were initiated in 1997, and the genetic founders of the current flock were collected from the Hanawī NAR in 1999, 2001, and 2005 (see Mounce *et al.* 2015). The kiwikiu that made up the conservation breeding population managed by SDZG were derived from six genetic founders. SDZG managed kiwikiu at facilities on Maui (MBCC) and Hawai'i (Keauhou Bird Conservation Center) Islands. The conservation breeding flock has changed over time, but overall managers has never been very successful at pairing or producing viable eggs from this species. It was determined that this population of conservation breeding birds managed at the SDZG facilities would be phased out. Thus, all kiwikiu at the conservation breeding facility were evaluated for release eligibility.

6.2. Preparations prior to release

Preparations prior to Nakula NAR – In preparation for the reintroduction, all kiwikiu held at the Keauhou Bird Conservation Center were moved to MBCC in 2018. Starting in April 2018, each bird was fed from the supplemental feeders that would be used in Nakula NAR. This also meant a change in their overall diet to strictly mealworms and dry pellets, as would be provided in Nakula NAR. Besides the changes to their diet and feeder, all care including veterinary checks occurred normally through March 2019.

In April 2019, we conducted trials applying transmitters to several kiwikiu at MBCC (see 8.1. Determining best practices for transmitters). Transmitters had never been used with kiwikiu and several potential alterations were tested to ensure safe and effective operation of the transmitters and harnesses. These trials lasted through May 2019, although most transmitters were only on the birds for a few days. We learned several valuable lessons from these trials and gained crucial experience in attaching the harnesses to kiwikiu in a controlled setting.

Eight kiwikiu at the conservation breeding facility were considered as release candidates (Table 3). One female, MP015, previously became egg-bound and was deemed to be unreleasable. SDZG conducted a final veterinary check on 9 October, the day before the birds were transported to Nakula NAR, and MFBRP banded all of the release candidates immediately following the veterinarian check. Some individuals were given one fewer color band than is typical to account for the weight of a transmitter that would be attached just prior to release. During the checkup, the veterinarian determined that one of the older males, MP017, was likely suffering from arthritis in his wings and this bird was deemed unreleasable. All other birds were determined to be in good health. One male, MP024, was considerably lighter in weight than the other males and had a more prominent keel. However, there were no

underlying medical concerns beyond being slightly thinner to raise concern and this male was allowed to be sent to Nakula NAR for holding.

One concern when releasing animals held under managed care into the wild is whether they will demonstrate appropriate responses to predators. In Nakula NAR, the only native avian predators the released kiwikiu were likely to encounter were pueo (Hawaiian Short-eared Owls, *Asio flammeus sandwichensis*). The release candidates from the conservation breeding facility were all tested for their response to this predator. This was not conducted as part of predator-aversion training, as is sometimes done in these cases, but rather this exercise allowed us to assess each individual's natural behavior when presented with a predator. Each individual was presented with a taxidermied pueo mount and a model nēnē (*Branta sandvichensis*), a non-predator control, separately. Each time the kiwikiu were presented with these models, we played vocalizations of the predator or non-predator. We then observed each kiwikiu's behavioral responses to the models. Little is known about the natural response of wild kiwikiu to pueo. The only behavior that has been noted was kiwikiu becoming quiet and still if they perceive a threat. As such, managers were not necessarily looking for any particular behavior, but rather a different response to the predator versus the non-predator. Two individuals, MP024 and MP027, did not show a negative response to the pueo and even approached the pueo model. As a result, both were deemed unreleasable. However, given the potential benefit of these birds acting as "anchors" for released kiwikiu, the working group decided to transport both of these males to Nakula NAR and hold them in the release aviaries. We also hoped that these individuals might show a more appropriate response to wild pueo in Nakula NAR.

*Table 3. Kiwikiu held at the SDZG conservation breeding facility as of October 2019. *Wild captures, exact hatch date is unknown.*

Studbook ID	Sex	Founder/ Descendant	Hatch Date	Age (yrs)	Releasable / Unreleasable	Reproductive history/Notes
MP009	female	founder	6/12/2001	18	releasable	has never laid an egg
MP015	female	descendant	3/5/2005	14	unreleasable	no descendants, becomes egg bound
MP017	male	founder	1/1/2005*	14	unreleasable	no descendants, arthritis in wings
MP018	male	founder	1/1/2005*	14	releasable	sire to MP022, 24, 26, and 27
MP022	male	descendant	3/2/2012	7	releasable	no descendants
MP023	male	descendant	3/2/2012	7	releasable	no descendants
MP024	male	descendant	4/2/2012	7	unreleasable	no descendants
MP026	male	descendant	4/15/2013	6	releasable	no descendants
MP027	male	descendant	3/23/2014	5	unreleasable	no descendants

Holding birds from the conservation breeding facility in Nakula NAR – Seven kiwikiu were moved from MBCC to Nakula NAR on 10 October 2019, one week prior to the arrival of the wild birds. The seven birds were transported in transfer boxes by vehicle from Olinda to the Windward Aviation hangar in Kahului and flown via helicopter to Camp Release in Nakula NAR. The transfer boxes had two separate compartments to carry two individuals per box. Two people held all five transfer boxes on their laps in the rear of helicopter. Once the birds arrived in Nakula NAR, they were placed in the WeatherPort® while the team (5 people) placed fresh branches and food for the birds in the aviaries. Personnel then hiked the transfer boxes to the aviaries and released the birds into them (Table 4, Figure 5). SDZG staff monitored the birds in the release aviaries to make sure they were adjusting to their new surroundings. Each bird was observed until they were seen eating. Some birds flew in repeated circular routes, flying and hopping between the same series of perches, i.e. “pace flying”, immediately after release into the aviaries. Many appeared preoccupied by the surrounding vegetation, a novel sight for these animals. Most settled down and returned to normal activity within a few hours after being placed in the aviaries. Over the next seven days, SDZG staff fed and monitored the kiwikiu from the conservation breeding facility before the wild translocated birds arrived. Daily monitoring also included 20-minute focal observations where staff recorded detailed observations of all behaviors exhibited. Throughout the time that the birds were in the aviaries, there were typically two SDZG staff members on site and each member had the same set of aviaries to look after each day to detect any behavioral changes between days. As much as possible, there was at least a day of overlap with SDZG incoming staff so the previous staff members could convey what had occurred with the birds over the week and review the tasks for the upcoming week.

Table 4. Individual kiwikiu by aviary location and release/removal date. Birds noted as "not released" died prior to release. "Unreleaseable" birds were not planned to be released because they did not pass the predator aversion trials.

Aviary	Bird ID	Origin	Sex	Color bands	Date released (R)/ removed (M)	Notes
A	-	-	-	-	-	unused
	-	-	-	-	-	unused
B	MP027	managed care	male	YE/AL, RD/YE	10/23/19 (M)	unreleasable
	WILD1	wild	male	BK/AL, NB/AL	10/30/19 (R)	released
C	WILD11	wild	female	NB/YE, NB/AL	10/28/19 (R)	released
	WILD7	wild	male	YE/GR, NB/AL	10/28/19 (R)	released
D	MP024	managed care	male	BK/AL, NB/RD	10/19/19 (M)	unreleasable
	-	-	-	-	-	unused
E	WILD9	wild	male	GR/BL, NB/AL	10/29/19 (M)	not released
	MP022	managed care	male	GR/AL, YE/BK	10/29/19 (R)	released
F	MP026	managed care	male	BL/AL, GR/GR	10/29/19 (R)	released
	-	-	-	-	-	unused
G	WILD10	wild	female	NB/BL, NB/AL	10/29/19 (M)	not released
	MP023	managed care	male	BK/AL, WH/GR	10/28/19 (R)	released
H	WILD5	wild	male	WH/BK, NB/AL	10/28/19 (R)	released
	MP009	managed care	female	RD/AL, YE/BL	10/23/19 (M)	not released
I	MP018	managed care	male	YE/AL, BL/BL	10/18/19 (M)	not released
	-	-	-	-	-	unused
J	WILD8	wild	male	NB/AL, BK/GR	10/27/19 (R)	released
	WILD9	wild	male	GR/BL, NB/AL	10/17/19 (M)	moved to E

7. Wild Kiwikiu Translocation

Transportation to *Nakula NAR* – The seven wild kiwikiu were transported via helicopter from Hanawī NAR to Nakula NAR on the morning of 17 October. The birds were moved to transfer boxes in the bird room in Hanawī NAR on the morning of the 17th after one final feeding. We then placed the transfer boxes into large Rubbermaid® plastic bins with holes drilled in the sides (Figure 14). We placed towels

and cardboard underneath and around each transfer box to lessen the noise and vibrations from the helicopter while not compromising air circulation. The birds were transported in two double-sided and three single transfer boxes (Figure 9) dispersed within two of the plastic bins to transport the birds to Nakula NAR. The birds were transported in two flights; only one of the plastic bins can fit in the back seat of the helicopter with a single MFBRP staff member in each flight.

Figure 14. Transfer boxes holding the kiwikiu were placed into large Rubbermaid® plastic bins with holes drilled in the sides to protect birds from the vibrations and sounds of the helicopter. One plastic bin was loaded in the helicopter with one staff member and transported to the reintroduction site.

The helicopter landed at Camp Release and was met by MFBRP, NEPM, and SDZG staff to help carry the boxes from the helicopter to the WeatherPort®. Once at camp, the transfer boxes were placed on the table inside of the WeatherPort®. The team (n=9) waited a few hours before transferring the birds to the aviaries because a helicopter tour was expected in the area. The transfer boxes were then hiked to their assigned aviaries by teams (Table 4, Figure 15). Birds were placed in specific aviaries in the hopes of forming pairs, to foster managed care/wild interactions, or to serve as conspecific “anchoring”. After each bird was released into their aviary, one person stayed behind to observe the bird from afar.

The general procedure to release a bird into an aviary was to place the transfer box on the platform of the aviary and open the front door of the box. If the bird did not immediately fly out of the box, someone placed their hand on the back screen of the box and/or slipped their fingers under the trap door on the side. This was sufficient to stimulate the birds to fly out of their transfer boxes. The boxes were placed facing perches so the bird would fly to the perches rather than the mesh of the aviary. Once the bird was out of the transfer box, the person left the aviary and moved to the observation point at a distance. As with the managed care birds, the goal was to observe each bird until they settled down and ate. None of the birds ate right away, and most paced around the upper portion of their aviaries. After a couple of hours of the birds not approaching the supplemental feeders, wall-mounted feeder trays (with mealworms) were moved to locations along the route which the birds were pacing. The birds ate more quickly from this food presentation and all birds were observed feeding before the observer left.

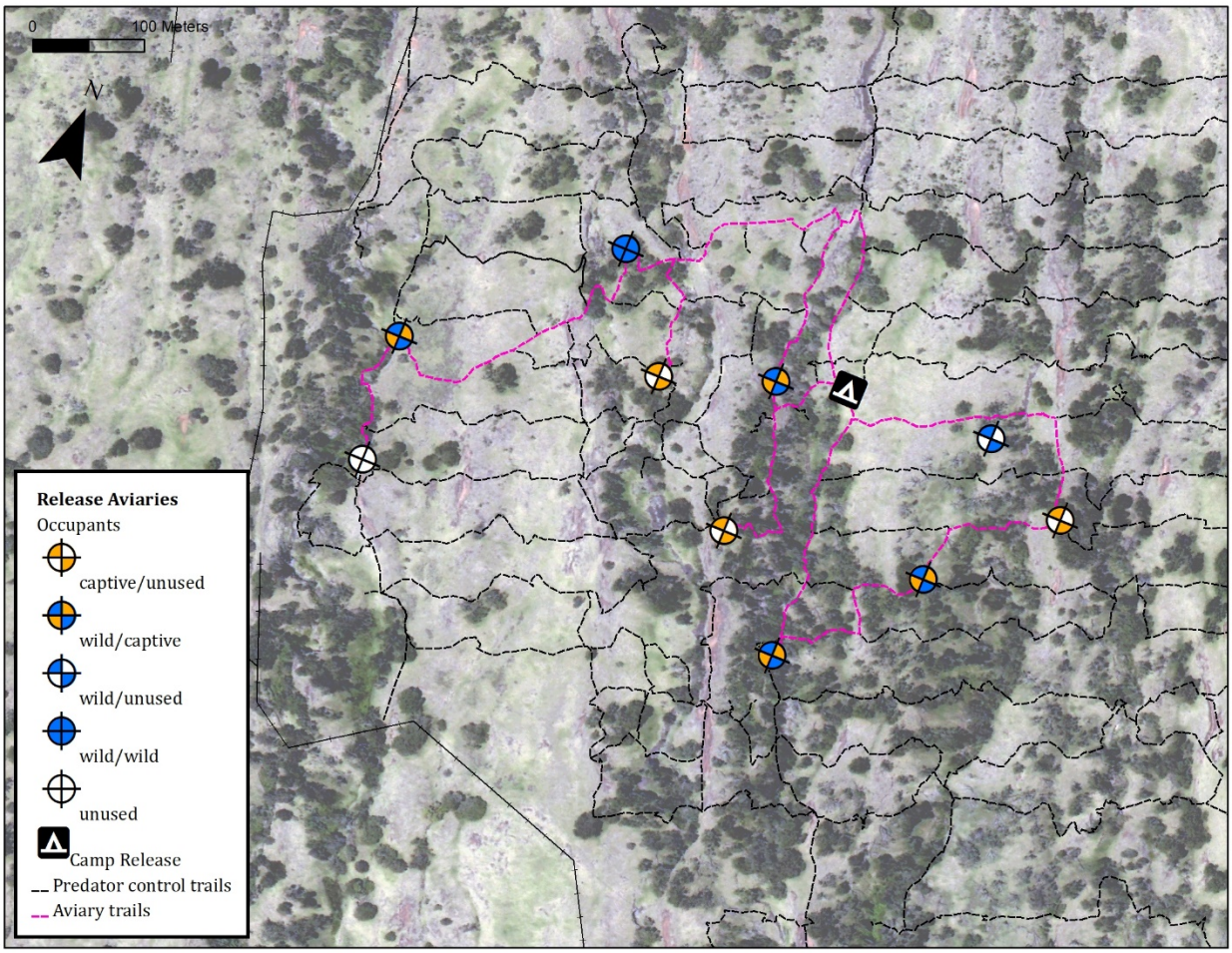


Figure 15. Release aviaries in Nakula Natural Area Reserve showing placement of managed care and wild translocated kiwikiu.

Holding wild birds in Nakula NAR – The birds had a variety of responses to being held in the release aviaries (Figure 16). Most of the managed care birds returned to normal behavior quickly, as was expected. The wild birds exhibited more behaviors indicating stress that lasted between a few hours and a few days. Most wild birds engaged in pace flying for some time. After they were released into the aviaries, the birds frequently looked up at the canopy seemingly trying to figure out how to escape or survey for danger. The birds appeared anxious and stayed near top of the aviary. With a few exceptions, the stress behaviors in the wild birds were reduced or ceased after a few hours. The birds hopped and foraged among branches, visited the feeders, preened, and chipped occasionally.

Figure 16. A male kiwikiu in a release aviary.

We observed some signs that placing birds from the two different sources, managed care and wild translocated, in side-by-side aviaries, the “buddy system”, helped the wild birds adjust to the release aviaries (Figure 15). The young female, WILD10, was placed side-by-side with a managed care male, MP023, and she calmed and began eating from the feeder faster than other wild birds. She appeared to mimic the managed care male as he fed from the supplemental feeder. The managed care male appeared very interested in the female and observed her movements closely. The subadult male, WILD9, also adjusted quickly after being re-paired with a managed care bird in the side-by-side aviary. Other mixed pairings did not show obvious signs that this arrangement had a calming impact on the wild birds but even those in these pairings calmed within a few hours.

A few wild birds took noticeably longer to adjust to the release aviaries and some changes were made to aid in their transition. Initially we paired WILD8 and WILD9, as both were captured in the same general area in Hanawī NAR and WILD9 was potentially the offspring of WILD8. After being placed in side-by-side aviaries, WILD8 showed signs of being highly stressed, pace flying and panting, for several hours. Meanwhile WILD9, a subadult male, constantly exhibited begging calls and mirrored the movements of the adult male. We grew concerned that the constant begging from the younger bird was adding to the stress of the adult male and decided to remove the young bird. He was placed overnight in a transfer box at camp to settle. The next morning, he was released in a separate aviary aside MP022 from the conservation breeding facility. WILD9 initially begged from MP022 but ceased this behavior quickly and began eating from the trays. Unfortunately, the adult male, WILD8, did not calm down after the removal of WILD9 from the other side of his aviary. He only calmed down the following morning after we opened the center divider between the side-by-side aviaries. This was the only bird allowed to roam in both halves of an aviary. The effect of opening the center panel was nearly immediate and WILD8 began

feeding soon after this. This suggests that wild translocated kiwikiu might benefit from larger holding aviaries.

We placed one wild female, WILD11, and one wild male, WILD7, in side-by-side aviaries. This pair was the most wary of people throughout their time in the release aviary and took the longest time to adjust. Neither bird approached or fed from the supplemental feeder for the first few days and the female never fed from this feeder type. We placed additional sidewall feeders in both birds' aviaries, and both were observed feeding from these. The relatively long adjus^{tm}ent period in this wild-wild placement may also serve as supporting evidence for the managed care-wild "buddy system" placement. These birds also showed more fear of humans approaching or being near the aviary than others and would pace for long periods after people fed the birds or entered the aviaries. As a result, neither bird was successfully weighed (digital scale with a smartphone recording the display) while in the release aviaries until they were captured for transmitter attachment.

While in the release aviaries, the wild birds appeared to be highly attuned to noises outside of the aviaries, including calling Ring-necked Pheasants (*Phasianus colchicus*) and pueo. Their general reaction to these sounds was to freeze and slowly look around for the source of the sound. Several times, pueo flew over or near the aviaries causing the kiwikiu to remain still for 5–6 minutes. Encouragingly, one of the males (MP024) that previously "failed" the predator test at MBCC exhibited similar behavior to that of the wild birds when a pueo flew over his aviary. Had this bird survived the holding period (this bird died after only 9 days in Nakula NAR), we might have decided to release this bird.

Although not an overly vocal species, the limited number of vocalizations exhibited by kiwikiu from both sources (wild or conservation breeding) in Nakula NAR was notable. One bird, MP018, a wild-captured male that had been in the conservation breeding program, sang several times while in his aviary. With this one exception, none of the males were heard singing at any time in Nakula NAR before or after release. Few of the wild birds produced whistle notes in the aviaries and most vocalizations from the wild birds were restricted to chip notes, generally considered nonspecific call notes. Several of the managed care birds produced whistle calls before and after release that clearly resembled the palila calls that the kiwikiu would have heard at the SDZG facilities.

Recommendations for future soft releases – In general, the wild birds were slow to start feeding from the supplemental feeders, although all but the one female eventually did use this feeder type. To get the birds to eat, several sidewall tray feeders were moved to the birds' flight path and they fed from these trays within 10–20 minutes. As discussed in 4.3. Feeder Design, the supplemental feeders may have been somewhat frightening possibly due to the bucket held above the food tray. Since none of the birds, managed care or wild, used the supplemental feeders after release these feeders may be unnecessary or some alterations should be made to the design. Two of the released managed care birds continued to eat off smaller tray feeders and future wild birds may do the same.

Several of the aviaries included a panel in the center wall separating the two side-by-side aviaries that could be opened allowing birds to move between the two sides. This was intended to allow two birds to associate with one another if courtship or other behaviors were observed (this was briefly done in Aviary C). However, this panel proved useful to calm WILD8 who was having a hard time transitioning to the release aviary. Additionally, because we did not translocate as many birds as we intended, several aviaries or half-aviaries were left unused (Figure 15). In two cases, wild males (WILD1 and WILD5) were eventually alone in their aviaries after their managed care "buddies" were removed. However, because

their aviaries did not include this center panel, we could not allow them to access the larger area. We suggest the future aviaries of this design all have the removable central panel to allow the possibility for individuals have additional room and potentially reduce stress.

It is unclear how much a soft release approach will play a part in future kiwikiu releases. However, we learned quite a bit about how kiwikiu respond to various stressors and how we can mitigate those sources of stress. As we saw when in holding birds in Hanawī NAR (see 5.3. Holding birds in Hanawī NAR), kiwikiu have proven to be an adaptable species that takes to eating provisioned food sources quickly in a small holding box. The release aviaries, however, appeared to be more stressful for some individuals than the holding cages used in Hanawī NAR. This may be because the birds have more space and visibility in a release aviary compared to a holding box. Many individuals acted quite fearful, possibly due to how exposed they felt given mesh walls and ceiling. The addition of some solid sides and/or roofs could reduce some stress (see 4.2. Aviary Construction).

8. Transmitter attachment

8.1. Determining best practices for transmitters

Transmitter specifications – We used Lotek PicoPip® radio transmitters with leg-loop harnesses to track the released kiwikiu in Nakula NAR. The PicoPip® line of transmitters are designed for attachment to very light animals and are commonly used for small songbirds. Several other companies produce transmitters for songbirds, but few produce transmitters in the weight-range required for the smallest kiwikiu. We also decided to use the leg-loop harness attachment as opposed to a glue-on attachment because this method is common practice and least likely to cause injury to the birds. We also felt that kiwikiu were capable of removing glue-on transmitters.

U.S. Geological Survey (USGS) granted us permission to attach transmitters to kiwikiu and allowed us to attach auxiliary markers (e.g. bands, transmitter) up to 5% of a birds' body weight, rather than the typical 3%. Given the small size of kiwikiu, a 3% limit would have essentially precluded the use of transmitters on a large number of kiwikiu. Part of the difficulty meeting a weight limit using transmitters is that kiwikiu require a comparatively heavy set of bands including a steel federal band (they can remove aluminum) and wrap-around color bands melted closed (they can remove butt-end bands). With a 3% weight limit, we estimated that a bird with a minimum number of bands (one federal and one color) would have to weigh a minimum of 22.7 g for us to attach the smallest Lotek® transmitter (0.22 g lasting ~6 days). However, being allowed to attach up to 5% of their body weight allowed us to use transmitters capable of lasting up to 33 days on birds as small as 17.6 g. The 5% weight limit is commonly used on honeycreepers in Hawai‘i under an experimental authorization by USGS (Permittee: Lainie Berry [Hawai‘i DOFAW], #08487).

Our goal was to monitor released birds for the maximum time possible and we wanted to attach transmitters with the longest battery life within the weight restrictions. Generally, battery life strongly correlates with transmitter weight, the heavier the transmitter/battery the longer the battery life. Kiwikiu are an especially dimorphic species in overall mass between the sexes. The MFBRP database contains 279 banded kiwikiu, including 149 males, 118 females, and 12 individuals of unknown sex. Among these, males ranged from 21.5 to 30 g and females ranged 16.5 to 26 g in weight. With the sizes

of Lotek PicoPip® transmitters available, the variation in kiwikiu size required us to order several sizes of transmitter. We purchased 24 transmitters of three models accounting for variation in mass of the birds (Table 5).

Table 5. Lotek PicoPip® transmitters used of the kiwikiu translocation with average weights (g) of all auxiliary markers attached to kiwikiu: the transmitter, harness, neoprene (for padding underneath the transmitter), a steel federal band, and a single color band. Also shown are the minimum weight of a bird that is approved to hold these auxiliary markers (5%; additional color bands would increase this number) and the estimated battery lifespan for each transmitter model.

PicoPip model	transmitter (g)	harness & neoprene (g)	steel federal band (g)	one color band (g)	total aux. marker wt (g)	min. wt. of bird (g)	est. lifespan (days)
Ag317	0.42	0.18	0.22	0.06	0.88	17.6	33
Ag379	0.45	0.18	0.22	0.06	0.91	18.2	42
Ag376	0.69	0.18	0.22	0.06	1.15	23	75

Transmitter trials at the conservation breeding facility – No one had previously attempted to attach transmitters to kiwikiu and we were not sure if any special modifications would be needed for this species. The kiwikiu bill is incredibly strong and sharp and the birds are extremely dexterous when feeding. As such, most of our initial concerns about the transmitters were based on the possibility that the birds could damage or remove the transmitters. Previous transmitter attachments on palila found that the birds were capable of kinking and curling the antenna wire, which led to birds becoming tangled in trees and some even died as a result (Dougill et al. 2000, Banko et al. 2014). Managers solved this problem by increasing the thickness of the antenna wire to the point that the birds were not capable of kinking the antenna (L. Berry pers. obs.). Given all of these concerns, we discussed a variety of potential alterations to the harness material, harness arrangement, potting (plastic coating) on the transmitters, antenna thickness, and antenna length.

In April–May 2019, MFBRP, SDZG, and USGS conducted trials to test various transmitters and harnesses on several of the kiwikiu at MBCC (Table 6). Lotek designed several "dummy" (inactive) PicoPip transmitters in the Ag376 and Ag379 size with three antenna weights, the typical Teflon®-coated NiTi wire, 14 kg–test wire, and 21 kg–test wire. We also tried to use two harness materials, Spectra® webbing and jewelry elastic. The elastic material is typical for these harnesses, but we felt that kiwikiu could cut this material. Spectra® webbing is used in harnesses for larger birds and is very difficult to cut. We also constructed the harnesses with a typical "weak link" of rubber band material. The weak link is designed to be a time-delayed release mechanism where the rubber band will fail over time after exposure to UV radiation and weather. However, we were also concerned that the weak link could be weak point that the kiwikiu could exploit to damage and remove the harness.

While trying to use the Spectra® webbing harnesses, we immediately recognized that this material would be very challenging to use on kiwikiu. The lack of elastic in the webbing made proper placement and tightness of both leg loops extremely difficult to the point that we decided to halt the first attempt. We tried a second attachment on another bird with a similar poor result and removed the harness. At

this point, we decided to attach elastic harnesses to several birds to determine if it was possible for them to remove these harnesses before we made future attempts using Spectra® webbing. Attachment of any of these harnesses is made more difficult by the frequent attempts by the kiwikiu to bite the person attaching the harness. Bites from kiwikiu easily draw blood and can be quite painful. The harness attachment required a combination of patience, time, and careful handling of the bird to manage this.

During the first session, we attached transmitters to four kiwikiu that all had varying responses to the transmitter. Two birds had no issues and were moving around their enclosures within a few hours. SDZG staff observed that these two individuals took up to three days to return to full activity level. A third bird, MP027, reacted poorly to the transmitter attachment. The attachment itself went smoothly and quickly. However, after release into his enclosure, the bird refused to move onto a perch and laid on his back on the floor of the cage. We removed his harness after a few minutes as it became clear this bird was not improving. The fourth bird, MP018, moved onto a perch after the transmitter was attached but sat very still for a long time. We decided that SDZG staff should observe his behavior for longer period and remove the harness later if he did not improve, which they did on the following day because the bird had badly damaged the antenna. We attached a transmitter to another individual the following week with no issues. In both cases where the birds reacted poorly to the transmitter attachment, the birds returned to normal activity immediately after the transmitters were removed. Overall, we felt that the transmitter attachment process with the elastic harness went well, but there was clearly variation among individual birds in their tolerance of having the transmitter attached.

SDZG and MFBRP removed the transmitters after 24 days from the two successful attachments in the first session (Table 6). Both birds were in excellent condition besides small abrasions on their backs where the transmitter sat. Inspection of the transmitters revealed patches of dried skin and feathers stuck to the ventral side of the potting. Much more concerning was the abrasion seen on MP017 after 17 days with the transmitter attached. In this case, the abrasion was more severe affecting deeper layers of skin and the patch of tissue and feathers adhering to the transmitter was much larger (Figure 17). These wounds were troubling and none of us had seen similar injuries in other transmittered birds. We reached out to additional people with transmitter experience and none had seen this type of injury before. However, we recognize that most transmitters deployed among wild birds are not retrieved and it is possible that these types of abrasions are more typical than is known. It is also possible that some of the damage to the birds' backs was self-inflicted as they attempted to itch or relieve the discomfort of having the transmitter against their backs. Because of these tests, we decided to glue a small layer of 3-mm neoprene to the entire ventral side of the transmitters' potting to allow the transmitter to slide more easily over the skin and feathers with the potting having no contact with the skin. We also discussed the tightness of the harness as a possible cause and decided to use a tongue depressor to gauge tightness, as discussed below in 8.2. Transmitter attachment in Nakula NAR. We used the tongue depressor to prevent the harness from becoming too tight but also not too loose as to allow the birds to remove the harness and/or the harness getting snagged on something. On September 23rd, 2019, MFBRP and DOFAW—NEPM implemented these changes to the harness and applied them to two kiwikiu at MBCC, MP027 and MP015 (an unreleasable female). Unlike the earlier trial, MP027 did not lie still on his back, but perched, flew around, and ate. Thus, we were confident that these were the appropriate changes. The two harnesses were not on either of the birds for very long as the release date was near (MP027 only had his on for a brief observation), but when the harness was taken off of MP015

on October 2nd, no abrasions were observed. During these trials, we also implemented a pre- and post-holding period inside the carrier boxes, discussed in 8.2. Transmitter attachment in Nakula NAR.

In most cases during the first trial, the kiwikiu did not harm the transmitter and the only damage was done to the antenna. In contrast to palila, we found the thinnest antenna wire to be the most ideal. Within the first day of attachment one male, MP018, badly kinked (nearly 90°) and curled the thickest antenna wire tested, 21-kg test (Figure 17). Other birds were able to put a slight curve in the 14-kg test antenna wires but none of the others bent their antennas quite so much as MP018. None of the NiTi wires were damaged or kinked. Thus, it became clear that because kiwikiu were capable of kinking even the thickest antenna wire, we should use the thinnest.

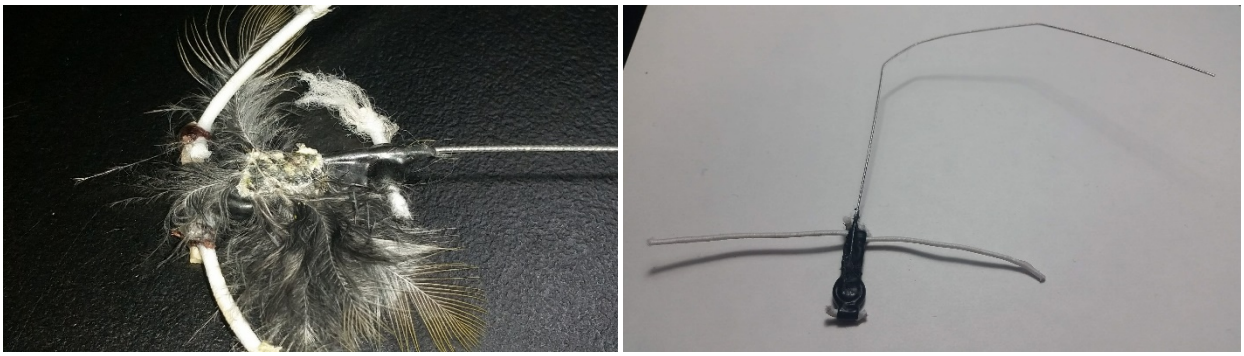


Figure 17. Photos of transmitters after they were removed from kiwikiu at Maui Bird Conservation Center. These photos show the most extreme examples of dead skin and feathers stuck to the ventral surface of the transmitter (left) and bent antenna (21-kg test wire) (right).

To verify that the thin NiTi wire antenna could not be cut, we attached a transmitter with this antenna weight to the same individual, MP018, that damaged the heavy-weight wire. He was not able to damage the antenna more than a slight bend during the two days the transmitter was attached. On the same day, we also attached a transmitter to an individual that had not been used in these trials, MP022. This individual managed to remove the harness within two days. This bird either cut the weak link rubber band or cut the thread knot attaching the rubber band to the elastic. This was the only case in which a kiwikiu removed a harness on their own in these trials.

These trials were a critical part in our understanding of how best to use transmitters on this species. It was fortunate that the managed care birds were available for this activity and the modifications made because of these trials made for safer transmitter attachments for the released birds. The final transmitters were essentially off-the-shelf typical versions with the NiTi antenna wire and light potting (none of the birds bit into the potting) from Lotek. We attached a small piece of neoprene (cut from a diving wet suit sock) to the underside of each transmitter. The harnesses were made using the elastic material with a rubber band weak link. Even though one individual damaged the weak link, we felt this was still an important element and we could remove this element later if needed. We also found that constructing the harness so the knotted end is at the top of the transmitter, rather than the bottom as in Figure 17, allowed for better control in tightening the harness.

Table 6. Transmitter trials with kiwikiu at Maui Bird Conservation Center with various harness materials, weak links, and antenna types (Teflon®-coated NiTi, 14 kg-test wire, or 21 kg-test wire). The total weight of all auxiliary markers (bands, transmitters, and harnesses) were < 5% of the birds' body weights.

Studbook ID	Bird wt (g)	Harness material	Weak link?	Antenna type	Transmitter & Harness (g)	% Body wt (all aux markers)	Date attached	Date removed	Days attached	Damage to transmitter	Notes
MP015		elastic	Y				9/23/19	10/02/19	9	none	
MP017	24.9	Spectra®	Y	14kg	-	-	4/8/19	4/8/19	0	-	attempted, removed
		elastic	Y	14kg	0.9	4.4%	4/16/19	5/2/19	16	bent antenna	
MP018	26.3	elastic	N	21kg	0.95	4.4%	4/8/19	4/9/19	1	bent antenna	
		elastic	Y	NiTi	1.06	4.8%	5/21/19	5/23/19	2	none	
MP022	24	elastic	Y	NiTi	0.79	4.1%	5/21/19	5/23/19	2	cut weak link	
MP023	21.3	elastic	N	NiTi	0.8	4.3%	4/8/19	5/2/19	24	none	
MP024	21.1	elastic	N	14kg	0.93	4.9%	4/8/19	5/2/19	24	none	
MP026	23.4	Spectra®	Y	14kg	-	-	4/8/19	4/8/19	0	-	attempted, removed
MP027	22.3	elastic	N	14kg	0.93	4.7%	4/8/19	4/8/19	0	none	did not tolerate
MP027	22.3	elastic	Y				9/23/19	9/23/19	0	none	tolerated well, we removed transmitter since it was close to translocation.

8.2. Transmitter attachment in Nakula NAR

Transmitter selection per individual – By the time we were preparing to attach transmitters and begin releases, several of the managed care birds had fallen ill and/or were removed from the site. This left ten individuals (three from the conservation breeding facility and seven wild) to be fitted with transmitters. Transmitter type (i.e. size) was chosen based on the weight of each bird. After the harnesses were attached to the transmitters, we estimated that the smallest type (Ag317) could be attached to kiwikiu weighing a minimum of 17–19 g if the birds were only fitted with one USFWS steel band and one color band. Knowing that both wild females fell into this range, we banded these females (in Hanawī NAR prior to translocation) with single color bands and selected two transmitters of appropriate size. We estimated that birds would have to weigh at least 22–23 g to be fitted with the largest transmitters (Ag376). We banded the remaining wild birds with only two color bands to reduce the overall weight of the auxiliary markers and allow us to put the largest size transmitter on as many individuals as possible. However, only two males were of the size to attach the largest size transmitter, WILD1 and 8 (Table 7). The remaining five birds were fitted with the medium size (Ag379) transmitters. Based on Lotek's estimated battery life, we expected these transmitters would last 33 days for the females and 42–75 days for the males, depending on which transmitter they were assigned.

Harness attachment procedure – The transmitter trials indicated that it might take the birds up to three days to return to fully normal behavior after transmitter placement. As such, we planned to attach the transmitters on kiwikiu in the release aviaries three days prior to their planned release (although this timeline changed, see 9. Release into Nakula NAR). All ten individuals were fitted with transmitters over the course of five days, 26–30 October.

Prior to attachment, each transmitter was started by soldering the activation wires, completing the circuit to the battery. After soldering, we placed a drop of UV glue on the solder and covered the glue with additional potting. Once activated in this manner, it was not possible to deactivate a transmitter. We continually verified that the transmitter was active with a receiver throughout the process. We also verified that each transmitter was operating immediately before and after attaching to the bird.

The procedure to attach the transmitters involved one SDZG person capturing the bird (after an observation period) and placing them in a transfer box inside the aviary. We then allowed the bird to calm down in the box for 30 minutes, given the stress involved in capture (see 4.2. Aviary Construction). After this, three MFBRP team members entered the aviary and removed the bird from the transfer box (see 5.3. Holding birds in Hanawī NAR). One person acted as the primary handler, fitting the harness loops over the legs and positioning them and the transmitter into the correct placement (Figure 18). A second person helped position and tighten the harness loops and tie the knot (double overhand knot). We found tweezers to be helpful in arranging feathers and pulling the harness elastic through the loops on either end of the transmitter. The third person applied and cured the glue on the knot (Damn Good® UV Glue Plastic Weld).

The transmitter attachment took 6–9 minutes per bird and went smoothly for all birds. We found having the same people in the same roles improved efficiency, although we did use only two people a few times. We focused on the fit and tightness of the harness in particular. We found pushing the transmitter high on the back allowed for the best fit and tightness, as well as a proper angle for the antenna. We tightened the transmitters to the point that one wooden tongue depressor could be passed underneath the transmitter. Passing the tongue depressor under the transmitter also smoothed

feathers below it. After the transmitters were attached, we returned the birds to the transfer box for 20 minutes to allow them time to calm down. We then released them back into the aviaries and observed their behavior from a distance.

Figure 18. Transmitter attachment procedure in a release aviary at Nakula Natural Area Reserve. The team positioned the leg loops on both legs (top left), tightened the harness and tied the knot (top right), and glued the knot (bottom left). The final fit is shown on the bottom right.

Table 7. Transmitters attached to kiwikiu showing weights (g) of the transmitter and harness, bands, and total auxiliary markers (transmitter, harness, and bands) in comparison to the body weight of each bird. Deployed date refers to the date at which the transmitters were activated; birds were released on various days after this date.

Bird ID	Color Bands	Battery	Deployed	Transmitter & Harness (g)	Bands (g)	Total Aux Markers (g)	Release wt (g)	% Body wt	Notes
MP022	GR/AL, YE/BK	Ag379	10/27/19	0.67	0.4	1.01	24.8	4.1%	negative response (mandible stuck in top knot), removed and released without it.
MP023	BK/AL, WH/GR	Ag379	10/26/19	0.59	0.4	0.93	22.5	4.1%	suspected malfunctioned post-release
MP026	BL/AL, GR/GR	Ag379	10/27/19	0.67	0.4	1.01	23.4	4.3%	malfunctioned post-release, recaptured, and replaced
MP026	BL/AL, GR/GR	Ag379	10/27/19	0.68	0.4	1.02	22.7	4.5%	second transmitter
WILD1	BK/YE, NB/AL	Ag376	10/30/19	0.84	0.34	1.12	24.0	4.7%	removed first harness via weak link while in aviary, applied second harness without weak link, malfunctioned post release
WILD5	WH/BK, NB/AL	Ag379	10/27/19	0.61	0.34	0.89	25.1	3.5%	no issues
WILD7	YE/GR, NB/AL	Ag379	10/26/19	0.63	0.34	0.91	26.5	3.4%	bird removed, breaking weak link post release. Weak signal at times, likely due to gulch or angle of antenna.
WILD8	NB/AL, BK/GR	Ag376	10/26/19	0.84	0.34	1.12	27.4	4.1%	malfunctioned while in aviary & replaced
WILD8	NB/AL, BK/GR	Ag376	10/27/19	0.85	0.34	1.13	27.4	4.1%	second transmitter

Table 6 (cont). Transmitters attached to kiwikiu showing weights (g) of the transmitter and harness, bands, and total auxiliary markers (transmitter, harness, and bands) in comparison to the body weight of each bird. Deployed date refers to the date at which the transmitters were activated; birds were released on various days after this date.

WILD9	GR/BL, NB/AL	Ag379	10/27/19	0.68	0.4	1.02	21.8	4.7%	not released, transmitter reused
WILD10	NB/BL, NB/AL	Ag317	10/26/19	0.6	0.28	0.86	18.4	4.7%	bird removed first harness via weak link while in aviary, not released
WILD11	NB/YE, NB/AL	Ag317	10/26/19	0.61	0.28	0.87	19.0	4.6%	top knot undone post release, glue held together, likely would have fallen off

Response of birds to transmitters – Just as in the transmitter trials, the birds being released responded in a variety of ways to having the transmitters attached. As a whole, the birds responded more positively to the transmitters compared to the trials. The first six transmitter attachments went smoothly, and the birds flew strongly out of the transfer boxes back into the aviaries. Most birds immediately began preening and biting the transmitter, focusing on the knot. The amount of time each bird spent biting and worrying the transmitter varied per individual. All birds were observed until they began eating, which occurred between 10 and 30 minutes after transmitter attachment. There was also variation in the ferocity with which individuals bit and chewed on the transmitters. Some wrenched on the knot or transmitter so hard, they nearly fell off their perches.

All birds, from managed care and wild, were harder on the transmitters in terms of biting than we saw in the trials. The birds were primarily focused on the glued knot and the additional potting concealing the activation wires, two spots that stuck out from the body of the transmitter. Damage to either one of these parts could have resulted in loss or deactivation of the transmitter. We became concerned that prolonged biting of the transmitter could result in permanent damage to the transmitter as well as distracting the birds from feeding or other behaviors. Although we had planned to wait three days before releasing the birds, we worried that holding them in the aviaries with the transmitters attached could potentially be more detrimental than releasing them sooner. We hoped that by releasing them, the birds would be distracted by their new environment and stop worrying the transmitters. Furthermore, all of the first batch of birds to have the transmitters attached were able to fly and feed normally within 30 minutes.

The following five cases highlight some of the issues arose after transmitter placement. The first transmitter was attached to the largest wild male, WILD8, and he was particularly aggressive toward the transmitter. The morning after the attachment, we observed a pronounced drop in the signal strength from his transmitter. An observer had to be within 3–4 feet of the bird to detect a signal, making future monitoring nearly impossible. As such, we again captured this bird, replaced his transmitter and decided to release him the same day, 27 October. After release, WILD8 was not observed biting the transmitter and was focused on foraging and exploring the release site.

The first troubling transmitter attachment from the perspective of the birds' behavior came on 29 October (the fourth day of transmitter placement) with both birds in Aviary E, WILD9 and MP022. Unlike all previous attachments, neither bird flew out of their transfer boxes and instead hopped out onto the ground and stayed on the floor of the aviary. MP022 was very focused on biting the transmitter even rolling onto his side on the ground attempting to bite the transmitter. In the process of biting the knot, MP022 got his mandible stuck between the harness knot and the potting beneath. Staff entered the aviary, freed his mandible, and removed the transmitter. This individual was the only bird to remove a transmitter during the previously mentioned trials at MBCC. We decided that given this individual's history with transmitters, he would be released without a transmitter, the only individual for which this occurred.

The issues with WILD9 after the transmitter attachment we now believe may have been related to the first signs of a previously undetected malaria infection. Throughout the transmitter attachment, this bird was somewhat lethargic, and he did not fly to a perch after the transmitter was attached. This bird showed little sign of noticing the transmitter but simply sat still and quiet. After several minutes, staff checked that the harness was not too tight and placed him on a perch. Over the next 30 minutes, staff

watched him, and after he tucked his head under his wing, staff removed the transmitter. Unlike MP022 who immediately flew to a perch and returned to normal behavior after the transmitter was removed, WILD9 continued to sit quietly on a perch. At this point, staff grew more concerned about the health of this individual and transferred him to a holding cage for further evaluation and veterinary care at camp (unfortunately, he died on the evening of 29 October). It is possible that the stress of the transmitter attachment heightened some of the early symptoms of malaria, which was the ultimate cause of death as determined via necropsy, or if the transmitter attachment just happened to coincide with the first symptoms. The speed of progression between the first symptoms in WILD9 and death, 1–2 days, was typical for all individuals, released or not (see 11.4. Necropsy results).

The last bird to receive a transmitter, WILD1, acted similarly to WILD8 in that he immediately attacked and bit the transmitter ferociously after release back into his aviary. Within twenty minutes, he cut the weak link of his harness and removed it. This bird had also almost removed one of his wrap-around color bands, which was replaced. We replaced his transmitter the following day with a new harness without a weak link. One of the two females, WILD10, also cut the weak link of her harness after the first day (while still in the aviary) and we replaced her transmitter, including a weak link, the following day. Similar to WILD9, this female showed the first symptoms of malaria following the second transmitter attachment. Also, like WILD9, we do not know if stress may have contributed to an immune response that ultimately led to her death. She was also eventually placed in a transfer box and brought back to camp for care, ultimately dying the evening of 30 October.

Transmitter lifespans – A host of factors may contribute to the ultimate lifespan of a transmitter including battery size, manufacturing defects, soldering errors, damage by the bird, weather, and temperature. Throughout the 2019 reintroduction, transmitters were active on kiwikiu for 1–21 days, 7.5 ± 6.7 (SD) days on average (see Table 9). This includes two transmitters that were replaced on two separate birds due to malfunction and two transmitters that stopped functioning while attached to birds but were not recovered (see Table 7). Another bird, WILD7, held his transmitter for 13 days and then shed the harness. This transmitter was recovered (Figure 19), and the bird was later observed without the transmitter.

Figure 19. The recovered transmitter of WILD7 showing that he had untied the string in the weak link (rubber band), which eventually led to him to dropping the transmitter.

In all, we deployed eleven transmitters on kiwikiu, four of which malfunctioned 1–3 days after deployment and the remaining six functioned normally. We were able to replace two malfunctioning transmitters by recapturing the birds before or after release and were able to monitor both birds until their deaths. One malfunction was confirmed from a released bird (WILD1); the bird was observed after the malfunction and we confirmed that a signal was not being generated. We suspect another transmitter (MP023) failed while on a bird but we were not able to observe this bird after the transmitter failure and did not recover this bird or its transmitter.

We continued to record the battery lifespan of the recovered transmitters out of the field. The functioning transmitters recovered from deceased individuals (n = 7) continued to transmit well beyond the estimated battery life (Table 8). The recovered medium-sized transmitters (Ag379) continued to transmit for an average of 24.75 ± 19.8 days beyond their projected 42-day lifespans. Both recovered small-sized (Ag317) transmitters ultimately lasted as long as the medium-sized transmitters, 54 days, compared to an average of 56.25 ± 6.5 days for the medium size. The one recovered functioning Ag376 lasted at least 128 days, a full 53 days beyond its expected 75-day lifespan. We do not know how exposure to the elements (or the birds' behavior) might have influenced the battery life of these transmitters, but it seems likely that if these birds had lived, we would have been able to track them for much longer than planned. Given the propensity for individuals to chew on the harness, it is possible more birds would have been able to remove their harness before their transmitters stopped functioning. In addition to the three birds previous mentioned, when we recovered the body of WILD11 (wild female), we discovered that she had successfully untied the knot (only dots of glue on the elastic prevented its removal) and we expect the transmitter would likely to have fallen off within a week or two (Figure 20).

Table 8. Average battery lifespans (±SD) of Lotek PicoPip transmitters deployed on kiwikiu by size. ‡The average number of days a transmitter was active on a bird (Days of bird) does not include active transmitters removed by managers (e.g. bird died) but does include malfunctioning transmitters remaining on or removed from birds.

Battery type	Weight (g)	Weight w/ harness (avg)	No. deployed	No. malfunct-ioned	Est. lifespan (days)	Days on bird‡	Days active	Max days active
Ag317	0.42	0.71	2	0	33	21	54 ± 0	54
Ag379	0.45	0.79	6	1	42	8.3 ± 6.3	38.3 ± 28.2	66
Ag376	0.69	0.96	3	2	75	6 ± 5.7	57.7 ± 63.7	128

Recommendations for future transmitter use – The functioning transmitters performed their primary purpose, allowing us to determine the birds' fate. Unfortunately, most of these birds died, but the transmitters allowed us to recover their bodies and determine cause of death. Without the use of transmitters, we likely would have only recovered two individuals after release (managed care birds found under an aviary or at camp). Two individuals moved well outside of Nakula NAR and we likely would not have been able to locate them without telemetry. If we had not recovered the deceased individuals, the full scope of the malaria threat in Nakula NAR would not have been realized. Of the fourteen birds held in Nakula NAR, we know the fate of eleven individuals. Ten of these individuals died from malaria, as such we hypothesize that the remaining three birds met a similar fate; only one of the fourteen birds survives currently at MBCC.

Figure 20. The recovered body and transmitter of WILD11 showing that she had untied the harness knot, likely to have resulted in removal of the harness within a week if she had survived.

The importance of the transmitters to this project cannot be overstated. However, attaching transmitters to kiwikiu was not without challenges or risks. We feel confident that the alterations we made to the transmitters and the process we used to attach the transmitters were appropriate and functioned well. Nevertheless, we discovered that some individuals may never tolerate transmitters and future efforts should consider the possibility of releasing such individuals without transmitters. We discussed the possibility of gluing on transmitters instead of using a harness, but we never had occasion to use this approach. This may be an option of last resort for individuals intolerant of a harness. However, we suspect that this species would rip off a glued-on transmitter.

At the outset, we were concerned that kiwikiu would cut the elastic material of the harness. While they are certainly capable, none of the individuals in the trials or in the release cut the elastic. Instead, most individuals targeted the knot, presumably because it stuck out above the transmitter. The UV glue held well against their biting but eventually the elastic around the glue began to unravel. Obscuring the knot, making a second "dummy knot" for the bird to focus on instead, or securing the harness in a different way may help reduce this damage.

Although several birds cut the weak link in the harness, we strongly advise future efforts retain this feature. We feel it is better to have a bird in the wild without a transmitter than to have a bird with a non-functioning transmitter attached. We released WILD1 without a weak link after he cut the weak link of the first harness attached. This bird's transmitter subsequently failed one day after his release, possibly due to damage inflicted by the bird and/or soldering error. We were able to locate this male on two occasions without the use of telemetry. The first instance occurred when an MFBRP staff member located WILD1 in an ʻōhiʻa unable to fly from a branch, possibly caught on something by the harness, but

the bird was able to free himself. Fortunately, this male was observed unharmed 18 days after this episode and the harness did not appear to be troubling him, but it was still attached. Unfortunately, this bird was released with a transmitter that immediately malfunctioned attached by a harness that may remain on his body for a long time.

The cause(s) of the transmitter malfunctions cannot be determined in all cases. We sent two recovered transmitters back to Lotek to analyze and attempt to determine the cause of failure. One transmitter was recovered from WILD8, after the signal strength decreased. The second transmitter was recovered from MP026 after we received intermittent and then no signals from his transmitter. In both cases, Lotek determined that there was an excessive amount of solder used in activating the transmitters and hypothesized that the heat from the soldering may have damaged the crystal connection on the board beneath the wires. The intermittent signal received from MP026's original tag is highly suggestive of this kind of heat damage. We indeed had difficulties with the soldering in the field and had to resort to thicker solder wire to activate the tags. Using the thin solder (such as that provided by Lotek with the tags) is highly advisable and applied with an iron that puts out the appropriate amount of heat. Adding too much solder and using a butane-fueled soldering iron may have transferred too much heat to the tags. Adding the UV glue to the wire connection after soldering was suggested to us from a colleague and appeared to strengthen the connection. However, Lotek felt this was unnecessary and potentially harmful to the connection. In the spring of 2019, we tested the Lotek transmitters and tracking methods on Hawai'i 'amakihi in Nakula NAR (see Fukunaga et al. 2019 for a summary on these trials). We did not use the UV glue on the wire connection during the 'amakihi trials and only one out of the nine transmitters deployed failed unlike four of 11 activated transmitters during the kiwikiu release. This further suggests that using glue on the connection wires may be unnecessary and/or ill-advised. Neither analyzed transmitter indicated that damage by the bird affected the functioning of the tag. However, if the connection was already poor or compromised due to incorrect soldering, a bite from a bird could conceivably sever the connection between the tag and the battery. In conclusion, it seems that most of the malfunctions were due to issues with soldering and that the birds' biting the tags may simply have loosened an already poor battery connection.

Some of our other concerns during the transmitter trials at MBCC were harness tightness and skin lesions from the potting. We did not observe any wounds on the backs of recovered kiwikiu bodies and did not see any excessive feathers or skin in collected transmitters. Regarding tightness, it helped to have the same person applying the transmitters across all the birds to maintain consistent tightness. We did recapture one bird, MP026, post-release, in order to replace his malfunctioning transmitter. It appeared he was able to loosen the transmitter harness quite a bit which had not been previously observed in the dummy transmitter trials. As a result, the new transmitter was placed a little tighter (while still using the tongue depressor) than previously to allow for this loosening (this should be considered in future transmitter placement). MP026 was released back into Nakula NAR after a 30-minute holding period in the transfer box. He released well, flying into a koa tree next to camp, where he had been occupying prior to recapture. Releasing birds directly from a transfer box could be an option in the future, although if there were any issues like MP022, these could be difficult to address. Since MP026 was next to camp and did not venture far, we were able to observe him closely for long periods. He was not seen excessively chewing on the transmitter post release. He fed regularly from the mealworm tray on the camp deck and perched in the koa trees near camp.

In addition to MP026, it appeared that WILD7 was also able to loosen his transmitter harness. Prior to release, we recaptured this individual in his aviary to check on the transmitter placement, since it appeared he had pulled it lower and the antenna was angled down. Once inspected, we decided the transmitter was still placed correctly. Post-release, we often had issues picking up his signal, which could have been due to topography, but we also usually had to be close to him to pick up a signal. This could have been the result of the bird loosening the harness, thus affecting the angle of the antenna. This bird also was able to remove the harness entirely thirteen days post-release. Overall, this is a challenging species to put transmitters on and while additional modifications may be possible to keep the harnesses tighter and keep the transmitters on longer, these may be detrimental to the health and well-being of the birds. There may be a certain percentage of the kiwikiu that simply do not tolerate a transmitter well and future release efforts will have to accept that not all individuals will be trackable in this manner.

9. Release into Nakula NAR

Release timeline – The Kiwikiu Reintroduction Plan (MFBWG 2018) laid out a scenario in which all birds would be released over the course of 10 days. However, this was based on the assumption of releasing 20 birds, and the birds would be released after a three-day waiting period following transmitter attachment. As previously discussed, 14 individuals were moved to Nakula NAR but several were removed from the site due to failing health and/or died on site prior to release. We also decided to release birds sooner than planned after the transmitters were attached (see 8.2. Transmitter attachment in Nakula NAR). This decision was based on two factors. One, there was a (unconfirmed) hypothesis that the aviaries themselves may have been artificially increasing the birds' exposure to mosquitoes and avian malaria. Secondly, the birds returned to normal behavior after the transmitter attachment much sooner than the birds in the trials.

We released eight kiwikiu (three from the conservation breeding facility and five wild) in Nakula NAR 27–30 October 2019 (Table 9 and Table 10). The process for release involved transmitter attachment (see 8.2. Transmitter attachment in Nakula NAR), a brief observation period following transmitter attachment, and then opening the doors to allow the birds to fly out of the aviary. As discussed in 4.2. Aviary Construction, several design elements of the aviaries impeded birds leaving the release aviary quickly on their own accord and most individuals had to be coaxed out of the aviaries. We discovered that adding perches to the door and/or leading out of the door from the supplemental feeder aided in the release. The birds in general were reluctant to fly straight out the door into nearby trees. Instead, all birds flew to the perches leading out of the door before flying to nearby vegetation. A few birds flew in and out of the aviary before finally flying free.

Monitoring began immediately after birds were free flying. As soon as the bird left the immediate area, we closed the aviary doors and moved one supplemental feeder out of the aviary. The feeders were secured to the ground with rebar at a relatively flat spot within 5 m of the aviary. We also installed a game camera to monitor activity at the feeders (see 4.3. Feeder Design).

Recommendations for future releases – Many of the recommendations for future releases are covered in other sections (e.g. 4.2. Aviary Construction, 8.2. Transmitter attachment in Nakula NAR).

The time between the doors opening to the birds flying free varied from 20 minutes to 2 hours. Although, we began shooing birds out after 30 minutes following a lengthy release period during the

first few attempts. We felt that the relatively small door opening was largely to blame for the lengthy release period rather than birds themselves being reluctant to leave. Most birds flew back and forth to the panel directly above the door, the sidewalls, and ceiling, presumably in an attempt to escape. A larger release opening, such as the removal of sidewall panels, would likely have resulted in the birds flying free from the aviaries almost immediately (see Figure 7).

Adding perches leading out of the aviary are also advisable even with a larger opening. Once the doors were open, most birds did not realize they were free and were not accustomed to flying out into the open. Without exception, birds hopped out onto the perches leading out of the door before flying up into the canopy.

Weather should also be considered when releasing birds. High winds and rain could be harmful to the released birds. We were fortunate to have good weather throughout the releases, except for a brief rain period around the time that WILD7 and WILD11 were released. Fortunately, this did not appear to have an effect on these individuals.

Table 9. Outcomes of translocated kiwikiu from Hanawī Natural Area Reserve to Nakula Natural Area Reserve detailing important dates for the translocation including capture, transportation, and release dates. Also shown are the dates of death (mortality) or last observation and final status of each bird.

Bird ID	Color Bands	Capture Date	Moved to Nakula	Sex	Release Date	Transmitter tracking end date	Mortality/ Last Observation‡	Days in Nakula	Days after release	Status	Notes
WILD1	BK/YE, NB/AL	10/9/19	10/17/19	M	10/30/19	10/31/19	11/24/19‡	38	25	unknown	transmitter malfunction
WILD5	WH/BK, NB/AL	10/11/19	10/17/19	M	10/28/19	11/11/19	11/11/19	25	14	deceased	recovered carcass
WILD7	YE/GR, NB/AL	10/12/19	10/17/19	M	10/28/19	11/8/19	11/12/19‡	26	15	unknown	transmitter fell off after he cut weak link
WILD8	NB/AL, BK/GR	10/12/19	10/17/19	M	10/27/19	11/6/19	11/5/19	19	9	deceased	recovered carcass
WILD9	GR/BL, NB/AL	10/12/19	10/17/19	M	n/a	n/a	10/30/19	13	n/a	deceased	died before release
WILD10	NB/BL, NB/AL	10/13/19	10/17/19	F	n/a	n/a	10/31/19	14	n/a	deceased	died before release
WILD11	NB/YE, NB/AL	10/14/19	10/17/19	F	10/28/19	11/16/19	11/16/19	30	19	deceased	recovered carcass

Table 10. Managed care kiwikiu held and/or released in Nakula Natural Area Reserve (NAR) detailing important dates for the translocation including when the birds were transported to and from Nakula NAR and release dates. Also shown are the dates of death (mortality) or last observation and final status of each bird.

Bird ID	Color Bands	Moved to Nakula	Returned to MBCC	Sex	Release Date	Transmitter end date	Mortality/ Last Observation‡	Days in Nakula	Days after release	Status	Notes
MP009	RD/AL, NB/YE	10/10/19	10/23/19	F	n/a	n/a	10/24/19	14	n/a	deceased	transferred back to MBCC, died off site
MP018	YE/AL, BL/BL	10/10/19	10/18/19	M	n/a	n/a	10/20/19	10	n/a	deceased	transferred back to MBCC, died off site
MP022	GR/AL, YE/BK	10/10/19	n/a	M	10/29/19	n/a	11/2/19	23	4	deceased	recovered carcass
MP023	BK/AL, WH/GR	10/10/19	n/a	M	10/28/19	10/29/19	10/29/19‡	19	1	unknown	transmitter malfunction
MP024	BK/AL, NB/RD	10/10/19	n/a	M	n/a	n/a	10/19/19	9	n/a	deceased	died in transfer to camp in Nakula
MP026	BL/AL, GR/GR	10/10/19	11/5/19	M	10/29/19	11/5/19	11/5/19	26	7	deceased	transferred back to MBCC, died off site
MP027	YE/AL, RD/YE	10/10/19	10/23/19	M	n/a	n/a	alive	13	n/a	alive	non-releasable, transferred back to MBCC

10. Post-release Monitoring

10.1. Tracking Kiwikiu in Nakula NAR

Monitoring protocols – We began tracking birds immediately after they flew away from the aviaries. All released birds had radio transmitters except for one managed care male (MP022) and we primarily located and tracked the birds using radio telemetry. We used Siritrack Yagi antennas (165–173 MHz) with Lotek Biotracker digital receivers to track them. A single individual or team of observers targeted an individual bird, narrowing in on the bird's location using telemetry, before visually scanning and/or listening for the bird. Once a bird was visually located, observers attempted to confirm the individual's identity through its color band combination, also known as "resighting". Observers took a GPS waypoint for each resight and recorded habitat, behavior, and any associations with other birds. We paid close attention to the plant species each bird foraged on and recorded if we observed them successfully finding prey items. Subsequent resights were recorded every 30 minutes and/or every 50 meters from a previous location.

In addition to hand-held receivers, we used Automated Radio Telemetry Systems (ARTS) to track individuals following release. To do this we set up three 7-m towers, each with six fixed Yagi antennas oriented 60° apart, and an automated receiver (manufactured by JDJC Corp./Sparrow Systems, Fisher, IL, USA) programmed to listen for each frequency every 5 minutes. The original intent was to use these data to enhance the movement data collected using handheld receivers. Unfortunately, unresolved issues arose with the bearings recorded per observation in the tower data and these data were not reliable. However, the relative non-directional signal strength was reliable and could be used to investigate relative activity of each individual. The relative signal strength between successive readings vary based on movement (no movement would result in the same signal strength). We used the standard deviation (SD) in signal strength for a 1-hour moving window to look at variance in signal strength over time. The higher the SD values, the greater the amount of movement in the birds. These activity data per bird allowed us to see changes in their activity levels and pin down exactly when the birds stopped moving due to illness or death.

We attempted to observe every bird daily and to record a minimum of five resight points. Daily observations were only possible for those individuals with active transmitters. However, regular searches were conducted for birds without active transmitters especially in areas where they were last observed or where they were suspected to have moved. The one bird released without a transmitter, MP022, was found every day because this bird stayed near his release aviary near camp and vocalized regularly. One bird, WILD11, eventually moved to an area more than an hour hike away. As time went on and she became more regular in this area, we did not track her every day but observed her at least every other day. At times, observers recorded fewer than five resight locations if the birds did not move outside of a 100-m radius during the observation period.

MFBRP staff was organized into two teams of three that alternated weekly monitoring trips to Nakula NAR. They were aided by a series of volunteers and/or partner agency personnel, usually at least one per week. By alternating teams, MFBRP maintained constant monitoring effort in Nakula NAR from 27 October (when the first bird was released) to 24 November. At this point, we believed only a single male kiwikiu with a non-functioning transmitter remained in Nakula NAR and future monitoring trips were suspended.

Tracking challenges – We encountered few challenges tracking kiwikiu with the handheld antennas and receivers. The ‘amakihi trial mentioned earlier greatly assisted with our preparation for tracking kiwikiu in Nakula NAR (Fukunaga et al. 2019). The greatest challenge was the relatively short detection range and the effect topography and vegetation had on this range. At times, we were able to receive a signal from > 1 km across open country if the bird was also near a ridgetop. Birds down in gulch bottoms, on the other hand, were often only detectable within 30 m. Gulches also often led to signal interference. The signals seemed to “bounce” off rocks and cliff faces, making narrowing in on a bird’s location challenging. An observer could be getting a strong signal from a particular bearing, and the bird would subsequently be located > 90° from this bearing. This signal bouncing at times seemed to “split” the signal indicating two equally strong signals coming from two separate locations. Fences also interfered with the signal at times.

In trying to locate birds we had not observed recently, we expanded our search range to the west onto Hawaiian Home Lands. In doing so, we discovered a source of interference coming from far to the west. We subsequently learned that a microwave tower on this property has caused interference with other radio telemetry projects and was likely the source of the interference we discovered. This interference was read by the receivers as a constant signal, rather than the intermittent signals issued by the transmitters.

In general, a combination of using hand-held telemetry and tracking vocalizations allowed observers to locate individuals fairly quickly. This was aided by the fact that most birds settled into an area after about a week and largely stayed in these areas throughout the project (Figure 22). Once this occurred, staff could hike to the location that a bird was last observed and locate the bird within a few minutes, sometimes without the aid of telemetry. After the first week, most observations involved checking to make sure an individual remained in the same area and tracking small movements within and outside of these “core areas” (see below).

The hand-held telemetry proved invaluable in locating each bird’s core area and any movements outside these areas. Without telemetry, our ability to track these birds would have been extremely limited. For example, WILD1’s transmitter failed within 24 hours of the bird’s release. Following this, we were unable to locate this bird for over two weeks at which point he was found on the opposite side of the NAR from where he was released (870 m; Figure 22). This bird was observed one additional time over two weeks after this first sighting also in the same area. Numerous searches in the area between these sightings failed to locate this bird. Without the use of telemetry, these kinds of detection histories may have been more typical, yielding little behavioral information. Furthermore, we were unable to confirm the ultimate fate of WILD1 due to the lack of a working transmitter; in other words, we do not know the cause or time of death for him (Table 9). Additionally, two birds eventually settled outside of Nakula NAR; 250 m and 1 km. Without the radio transmitters, there is little chance we would have been able to find these birds.

Movement patterns – All of the wild birds exhibited a similar pattern of movement following release in Nakula NAR. This pattern included a brief period (1–3 days) in which the birds travelled around the site, presumably exploring their new environment. After this period, most birds “settled” into areas where they were routinely found thereafter, i.e. “core areas” (Figure 21). Once settled in these areas, the released kiwikiu were strongly philopatric. The three males with active transmitters (WILD5, 8, and 7) spent little more than a single day exploring the site before settling into what we would later recognize

as their “core areas”. In all three cases, the site of their last observation (or demise) was within 200 m of where they were observed the day after release. Although he did not have an active transmitter, WILD1 was observed in the same area (within 20 m) 25 days after release as he had been observed seven days after release. The female, WILD11, wandered more slowly and eventually settled farther away from the release site than the males. In the first four days, WILD11, stayed within 300 m of her release aviary. On day five, she began moving west, away from Nakula NAR. By day eleven, she settled in an approximately 350 m diameter area where she remained until her death more than two weeks later.

The distance that the wild birds travelled and settled from their release aviary varied from 284 m to 1.8 km. In the first few days following release, two males travelled a total of over 2 km before either returning to their release location (WILD8) or moving to their eventual “core area” (WILD5). In both cases, the males travelled to the edges of the forest habitat in Nakula NAR at the highest and lowest elevations and returned to more heavily forested areas. Two males (WILD7 and 8) settled and were last observed less than 400 m from their release aviary 10–15 days after release. The other three wild birds were last observed between 840 m and 1.8 km from their release aviaries. Given their demonstrated ability to cover over 2 km in a day, the fact that all birds stayed within 2 km (most within 1 km) of their release location is notable and may speak to the suitability of the habitat in the Kahikinui region.

These movements did not indicate any attempts to return to their capture location (i.e. Hanawī NAR), as was observed in other honeycreeper species (Groombridge et al. 2004a, 2004b, Banko et al. 2014). However, translocated palila that ultimately returned to their capture site persisted at the translocation site for an average of 11 days and some stayed for up to 55 days. In contrast, the single translocated poʻouli and 13 of 18 translocated Maui ʻalauahio returned to their capture locations within the first few days (Groombridge et al. 2004a, 2004b). However, these individuals were translocated a much shorter distance compared to palila and across continuous habitat. As such, because the wild translocated kiwikiu only survived for an average of 16 days and a maximum of 25 days after release (see below) we cannot conclude that they would not have made an attempt to return to Hanawī NAR given more time.

Three released kiwikiu travelled outside the boundaries of Nakula NAR and two of these birds settled well outside of the reserve (Figure 21). Both individuals that left Nakula NAR permanently, settled on Kahikinui Hawaiian Home Lands to the west of Nakula NAR. One individual, WILD5, settled in a relatively small forest patch in a newly fenced area ≥ 200 m west of the Nakula NAR boundary. The other bird, WILD11, moved much farther west, > 1 km from the Nakula NAR boundary. Of the five individuals that remained in Nakula NAR, three of them (WILD8, MP022, MP026) were within the predator control grid (this does not include MP023 since we only have the release day as observations), whereas the two others moved to the edges of the Wailaulau Unit (WILD1, WILD7) just outside the predator control grid.

The released kiwikiu explored areas ranging from 1283 m to 1872 m asl (4212–6144 ft); 1613 m (5292 ft) on average among all observations (Figure 21). The lowest elevations, < 1300 m were visited by one individual during a brief period after the bird was released. However, this individual, WILD5, also settled in an area lower in elevation than any other bird, as low as 1373 m (4507 ft). In their windward range, kiwikiu are found down to approximately 1500 m (4921 ft) asl. In Nakula NAR, five individuals were observed below 1500 m in elevation, although one of these was because their release aviary was below this elevation and this individual moved higher after release. Two individuals spent significant time below this elevation, WILD7 and WILD5, in which 15% and 100% of observations were made below 1500 m asl, respectively. In the Kahikinui region, the treeline is generally 1800–1900 m (5905–6234 ft) and

tree cover noticeably thins above 1770 m (5800 ft). Only WILD11 spent significant time above 1800 m asl; 43% of the observations of this individual occurred above this elevation. This bird was often observed in the highest-elevation trees around. Only two other birds went to areas this high in elevation, including WILD8 that ultimately settled in an area much lower, and perhaps the missing MP023 (see below).

The released birds from the conservation breeding facility behaved differently following release compared to their wild counterparts. One male, MP023, stayed close to his release aviary for the first 24 hours before moving on a direct path out of Nakula NAR on the second day. The final observation of this bird was a signal detected in the extreme northwestern corner of the Wailaulau Unit of Nakula NAR as the bird seemingly travelled farther uphill. This final observation was above treeline and the area that the bird was headed toward would have increasingly become more scattered native shrubland with few trees, not typical kiwikiu habitat. No additional observations were made for this bird and no further radio signals were received. We suspect that this bird's transmitter may have malfunctioned, as we saw in other cases (see 8.2. Transmitter attachment in Nakula NAR), or we were receiving some sort of interference signal as mentioned above. We also discussed the possibility that this bird was preyed upon by a pueo as the movements we observed on the final day seemed very rapid and direct, possibly owing to the movements of a predator rather than the kiwikiu itself.

The other two released kiwikiu from the conservation breeding facility acted similarly to each other. One of these males, MP026, moved from his aviary to camp within 24 hours of being released. He stayed near camp until his death seven days after release. The other male, MP022, was released from an aviary close to camp (65 m) and moved between the aviary and camp the first day of his release, but mostly stayed near his aviary. MP026 fed from food trays placed on the camp deck (Figure 22) and MP022 fed primarily from food trays hung on the outside of his aviary. Neither approached the supplemental feeders placed near their aviaries or near camp (two supplemental feeders were set up at camp). Little can be learned from the movements of these two males besides the indication that they were habituated to humans, which is concerning behavior from the perspective of their ability to live on their own over the long-term. Had either lived longer than a week after release, these birds might have begun exploring areas beyond camp and foraging more on their own; unfortunately, these birds did not have that opportunity.

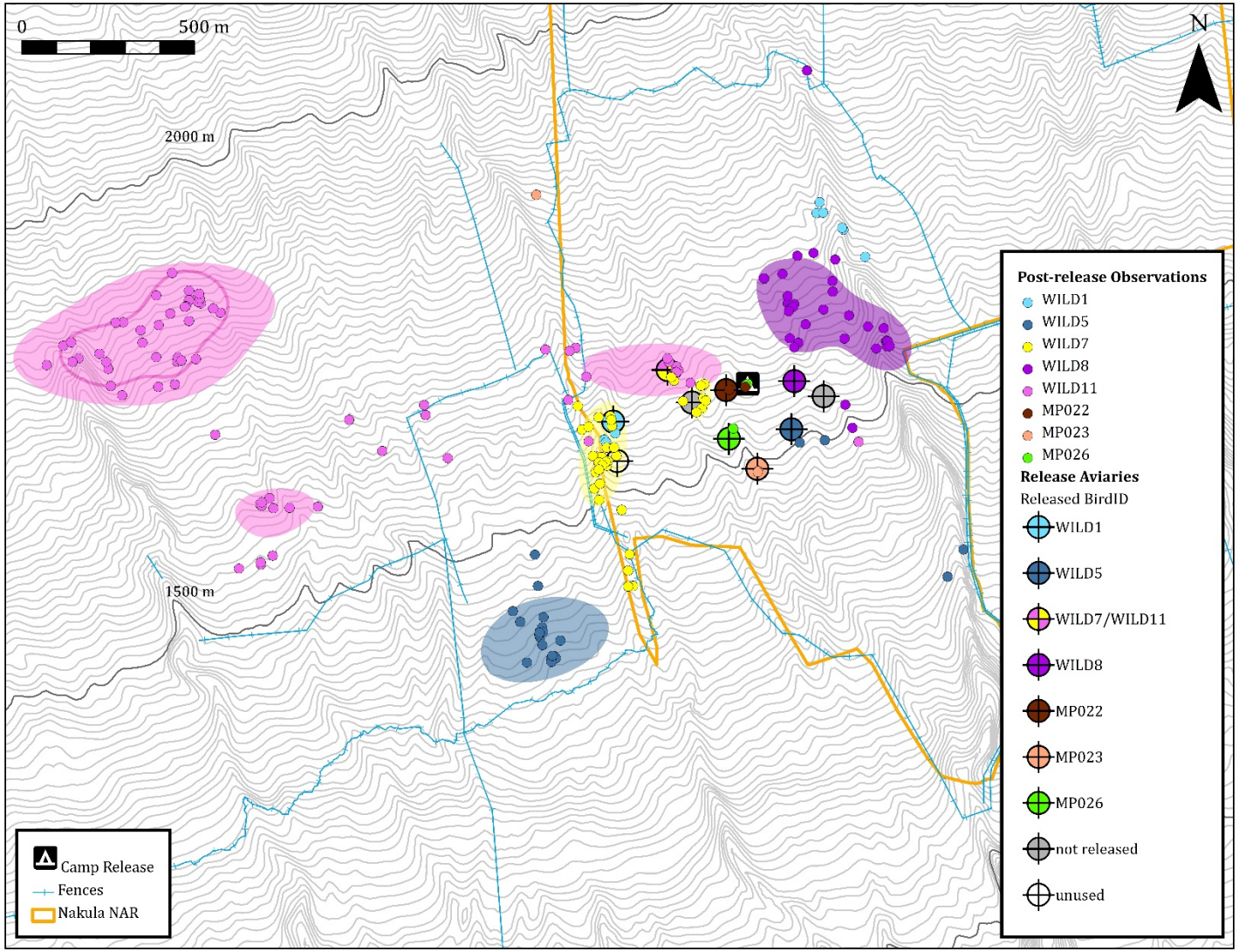
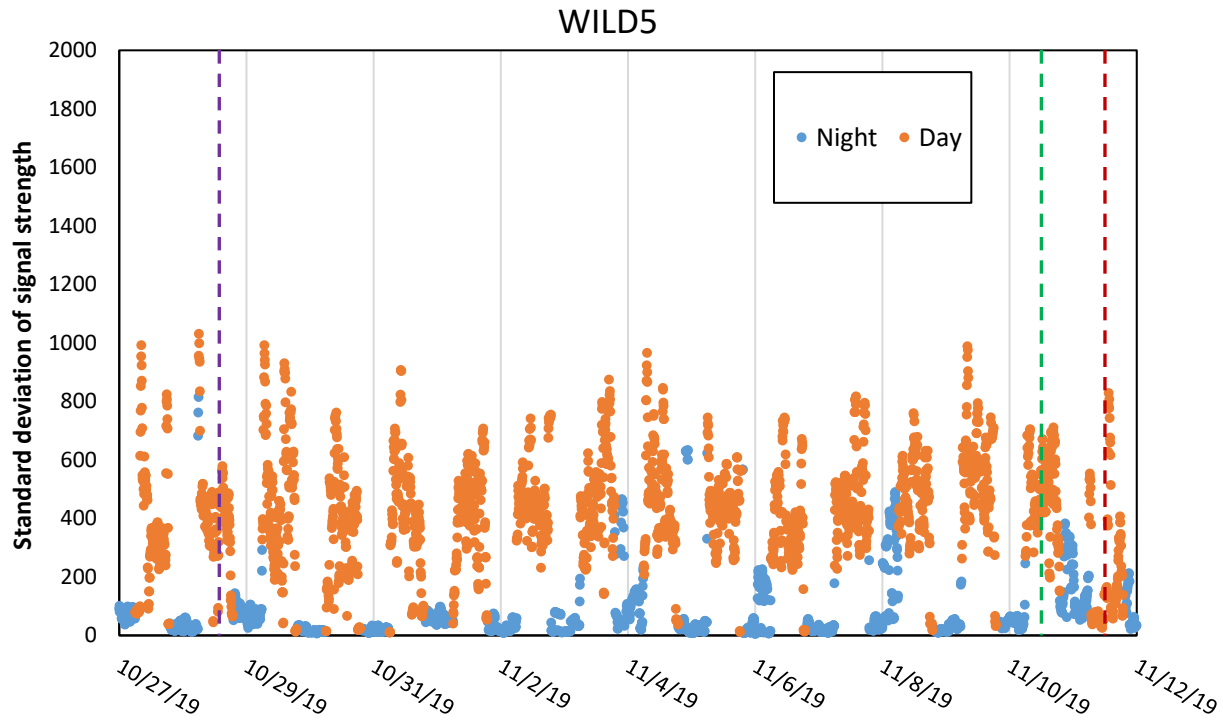


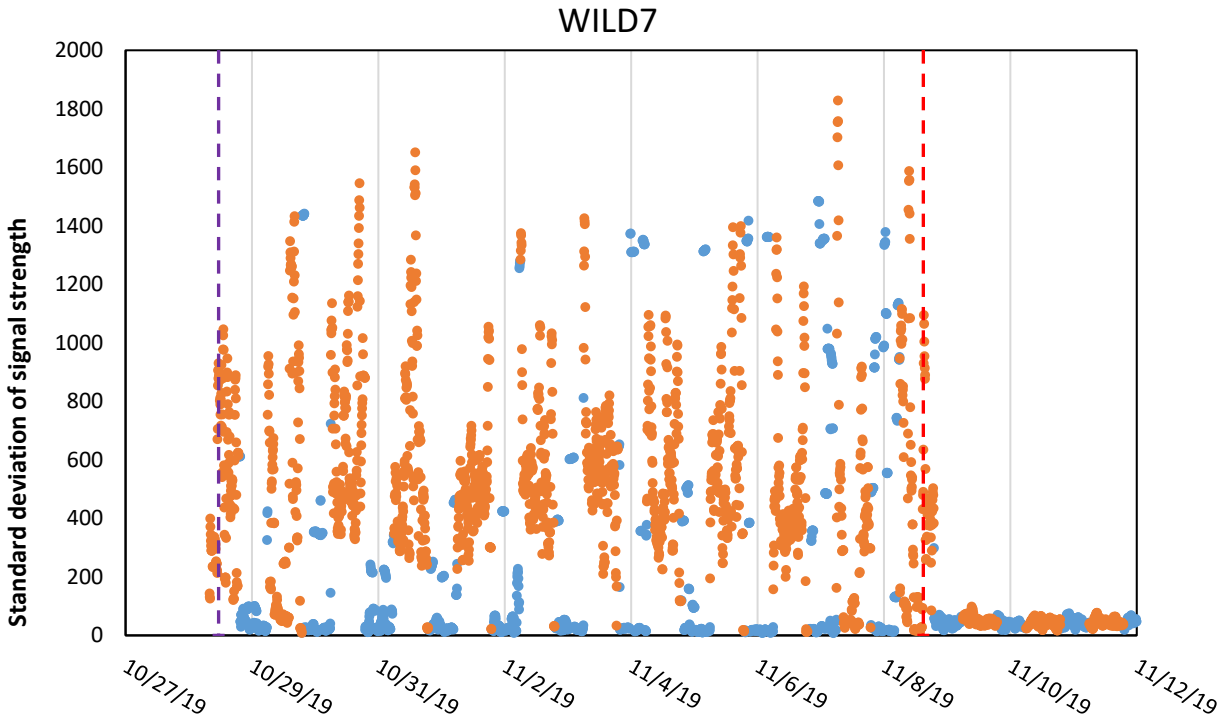
Figure 21. Observations ("resights") of kiwikiu released in Nakula Natural Area Reserve. Colored polygons indicate the "core areas" for individuals observed ≥ 10 times. The dark pink outlined polygon represents the core area of WILD11 excluding the first seven days of observations. The observation of MP023 at 1826 m asl is an extrapolation of a transmitter signal and not a direct observation of the bird.

Figure 22. Released kiwikiu, MP026, feeding off a tray feeder on the camp deck after release. Photo by Bret Mossman.

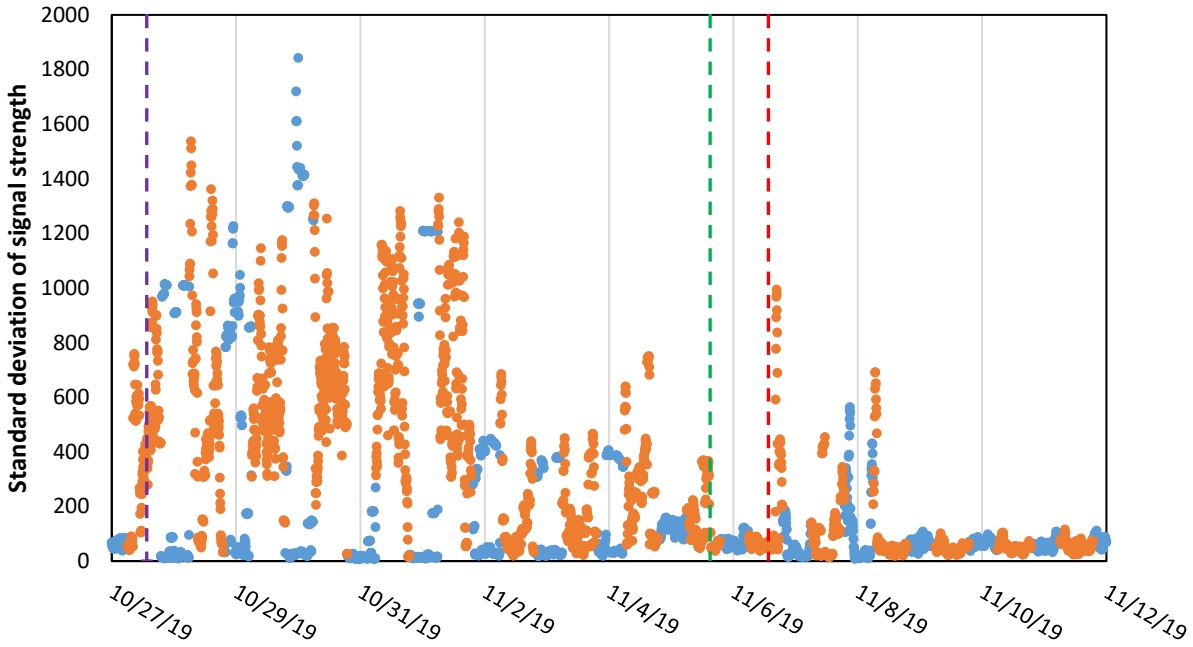
Activity budgets – Of the eight birds released, five kiwikiu had automated tracking data from the towers for more than two days. These birds showed a general pattern of high activity (i.e., high standard deviation values) in the day, and low SD values at night, with some birds showing activity on some nights. Activity at night does not mean that birds were flying around, but could be turning on their perch, preening, or other restless behaviors. Most birds showed signs of high activity through the time until believed died, with very little indication of subdued movement behavior due to acute malaria infection.

WILD8 may have shown some reduced activity prior to his death. This individual was observed nearly every day up to his death on 6 November, 2019. The ARTS data showed a drop in activity between the 1st and 2nd of November and reduced relative activity until the 6th. This apparent reduction in activity corresponds to this individual moving into the Wailaulau Gulch, a very deep drainage that may have interfered with the signal strength received by the towers. This bird returned to an area much closer to the towers on the 4th, which is somewhat represented by a brief increase in activity or signal strength that day. However, this bird had made previous movements in and out of Wailaulau that are not shown in the tower data and this reduction in activity may indicate that WILD8 was beginning to show symptoms of the malaria infection that ultimately resulted in his death on 5 November.

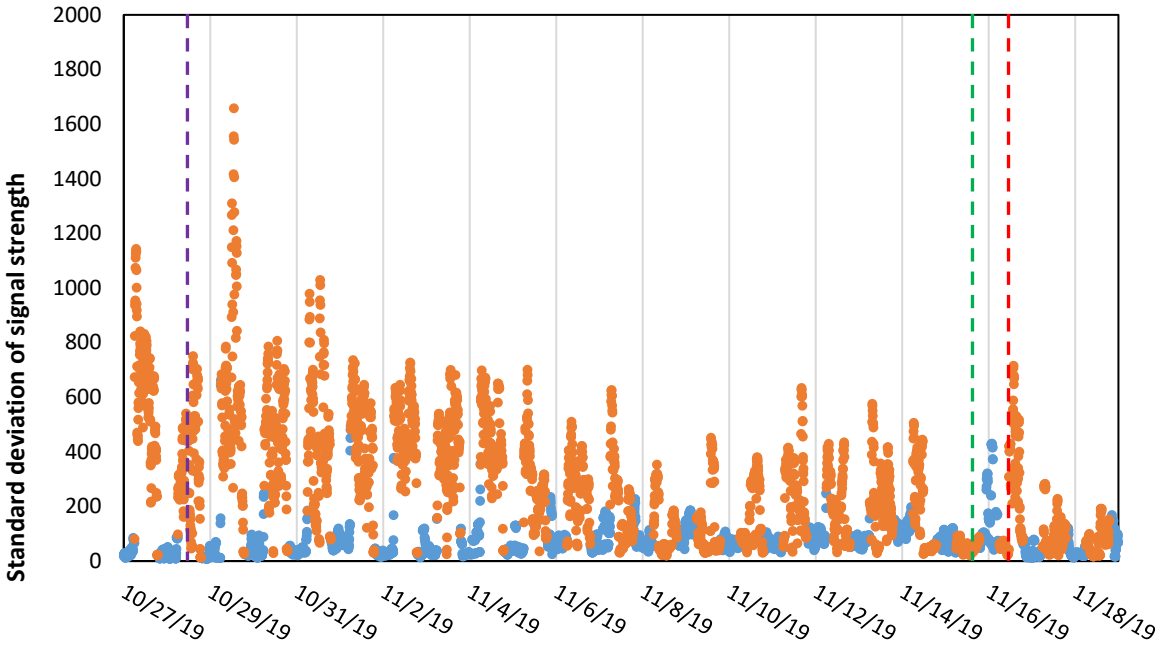

WILD5
Standard deviation of signal strength
2000
1800
1600
1400
1200
1000
800
600
400
200
0
Night
Day
10/27/19
10/29/19
10/31/19
11/2/19
11/4/19
11/6/19
11/8/19
11/10/19
11/12/19


WILD7
Standard deviation of signal strength
2000
1800
1600
1400
1200
1000
800
600
400
200
0
10/27/19
10/29/19
10/31/19
11/2/19
11/4/19
11/6/19
11/8/19
11/10/19
11/12/19

WILD8



WILD11



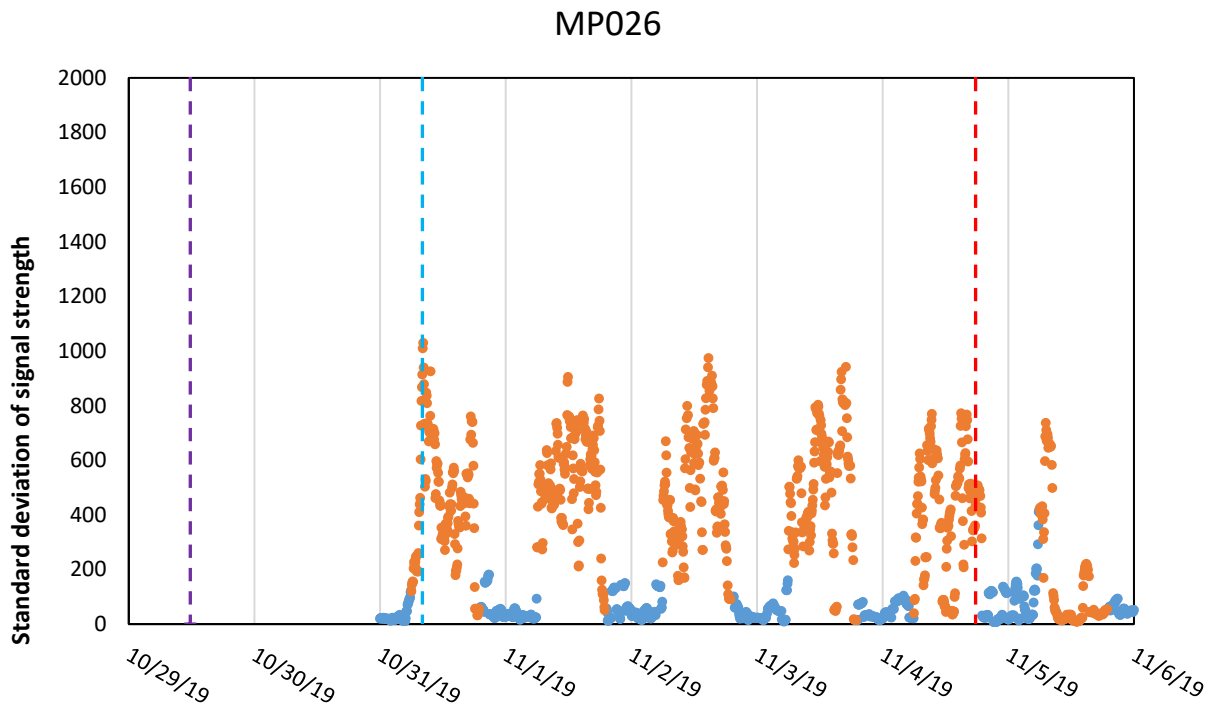


Figure 23. Signal activity recorded by the Automated Radio Telemetry Systems in Nakula Natural Area Reserve (NAR) shown as the standard deviation in signal strength per hour for five released kiwikiu. The purple dashed line indicates when the bird left the release aviary. The green dashed line indicates the final antemortem observation by the field crew. The red dashed line indicates the time the carcass was discovered (WILD5, WILD8, and WILD11) or when the transmitter was recovered (WILD7 and MP026). Morbidity occurred between the green and red lines. WILD7 does not have a green line because it was known to survive after removing his transmitter. MP026 was released with a faulty transmitter that was replaced on 31 October (blue dashed line) and was removed (alive) from Nakula NAR on 5 November. Activity after the recovery of the carcass or transmitter indicates movement by the field crew. X-axis values vary among panels.

The ARTS data indicated a more specific time of death for three individuals than was determined from field observations. There was confusion about whether WILD8 died overnight on 5 October or during the morning of the 6th. Some signal changes near the edge of the Wailaulau Gulch, where the bird was ultimately discovered, made it appear as though the bird may have moved during the morning of the 6th. However, the tower data shows no variance in the signal strength on the morning of the 6th, which supports the idea that the bird died overnight on the 5th shortly after the last antemortem observations of this bird. In fact, the last activity detected by the towers was at 14:40 and the bird was confirmed alive until 15:00. It seems likely the bird died just after it was last seen by the field crew.

The activity of WILD11 was very low throughout the day of 15 November, even though the field crew confirmed that she was alive on this day. The crew observed that she sat very still on a branch of a tree on this day. Her body was recovered > 100 m downhill below another grove of trees and the ARTS data indicates that this movement occurred around 02:00 on 11/16/19 and some minimal activity just before 05:00. Nighttime movement of this kind was relatively unusual, and it seems likely it was related to her illness, such as falling off her perch and fluttering downhill. Given that we recovered this individual's

body at 11:00, we can confirm that she likely died just after 05:00. Thus, her body was recovered within six hours of death. Similarly, WILD5 showed some activity up to 06:46 on 11 November and his body was discovered at 12:00 the same day; also, within six hours of death.

Core area metrics – We calculated the area within which the released birds moved using kernel density estimators summarized in 50% isopleth contours. To do this we only used data from hand-held telemetry equipment and actual observations because the ARTS triangulation data was unreliable. We followed the same methods as Warren et al. (2015), used to estimate home range size of wild kiwikiu. To do this we analyzed the movements of individuals for which we observed at a minimum of 10 locations (resights) using the "kde" tool in Geospatial Modelling Environment version 0.7.2.0 (Beyer 2012). This tool creates a contour or "heat" map of observations based on proximity and density of observation points. We then delineated the region(s) that contained 50% of the contour area (i.e. 50% isopleths) for each individual that we termed their "core area".

Average core area among the translocated birds was 12.34 ± 11.67 ha (n = 4) and ranged from 4.05 to 29.6 ha (Figure 21). These core area polygons contained 75 ± 2% of the observations of each individual. The largest core area was occupied by the female, WILD11. This individual was a clear outlier from the other three birds and much of this area was a result of the bird shifting westward over the first ten days. We also analyzed this individual's core area excluding all observations from the first week (7 days) following release. After excluding the first week of observations, WILD11's core area measured 9.3 ha, still larger than the other released birds, but no longer an outlier. This polygon encompassed 45% of the observations of WILD11.

In the released birds, we do not consider the core areas to be equivalent to home ranges because so little time passed between release and last observations (including death). We also do not expect the movements of these birds after translocation to be the same as those of birds in the wild. As such, comparison of the metrics between the released birds and wild birds is challenging. However, home range size is among the only metrics we have to compare typical kiwikiu movements to those of the translocated birds. Warren et al. (2015) estimated an average kiwikiu home range size of 5.28 ± 0.89 ha (50% isopleths). Thus, the translocated birds moved over a larger area than is typical for kiwikiu on the windward side of Haleakalā. Whether this difference is indicative of habitat suitability/density or just the circumstances of releasing birds to a new area is unknown given the small sample size and no prior translocation knowledge for this species. However, Warren et al. (2015) estimated home range sizes between 0.76 and 22.58 ha in the current range, demonstrating high variability among individuals. Additionally, if you consider the female's core area as 9.3 ha (after excluding the first week), the average among the Nakula NAR birds was 7.26 ± 2.38 ha, much more similar to average home range size in the current range.

The philopatry exhibited by the translocated birds after the first few days may indicate that these individuals were beginning to establish home ranges. We did not observe any singing from any of the males after release and recorded no other indications of territory establishment. Had the birds survived, it seems likely some birds might have begun to sing and establish territories within their home ranges.

10.2. Foraging and other behaviors

Foraging behavior – One of the major unknowns in the reintroduction of kiwikiu to leeward Haleakalā is whether the habitat in its current state could support the species. Kiwikiu likely last existed in Kahikinui

over a century prior to this reintroduction, and during this absence the quantity and diversity of the forest habitat declined due to feral ungulate damage (Medeiros et al. 1986, DOFAW 2015). The reason for the kiwikiu extirpation from this region is unknown but the reduced habitat quality likely played a large part in their disappearance. The restoration that occurred in Nakula NAR and surrounding reserves since 2012 unquestionably increased both the diversity and quantity of native Hawaiian plants in the area, including many known kiwikiu food plants. However, many of the planted seedlings will remain too small to be utilized by the released kiwikiu for many years. Prior to the release, we did not know whether sufficient food resources were present or if the right habitat cues remained to allow the released kiwikiu to survive. Peck et al. (2015) demonstrated that the right prey items existed in Nakula NAR but could not speak to whether the overall abundance within the reserve was sufficient to support a population of kiwikiu. The working group decided that, despite these unknowns, the current population trajectory of the wild kiwikiu population necessitated rapid action to preserve the species, namely increasing the species' range by proceeding with the reintroduction.

The working group also recognized that the only way to know if the species could persist in Kahikinui would be to release kiwikiu in the area and observe their behavior. As such, one of our primary monitoring goals for the released kiwikiu was to observe their foraging behavior to determine if they were able to find sufficient food in the wild. The movement patterns of the released birds might also indicate whether the birds were finding food. For example, a bird ranging widely on a daily basis may indicate that an individual is unable to find enough food resources in one area, requiring them to continually search for food over a large area. We also expected that reliance on supplemental feeders might indicate that birds were not able to find sufficient wild food resources.

Immediately upon release, all individuals (from both sources) began exploring their environment, chewing on branches of numerous plant species. The largest and most abundant trees throughout the site are koa and most individuals were drawn to these trees first. Some of the earliest written observations of the species noted an affinity for koa (Henshaw 1902, Perkins 1903). Interestingly, very little koa exists in Hanawī NAR and the translocated birds likely had never seen this tree species. Nonetheless, many individuals began foraging in koa immediately upon release—possibly due to the tree's abundance in Nakula NAR. We also included koa as foraging materials within the aviaries which they also foraged on. These individuals spent long periods exploring the dead or dying branches and the kiwikiu were often observed flaking off large pieces of bark and other woody tissue. The kiwikiu from the conservation breeding facility also foraged in koa, but spent more time in younger trees foraging on small-diameter branches (Figure 24).

Figure 24. Released kiwikiu from the conservation breeding facility foraging in smaller diameter koa branches.

The wild kiwikiu mostly foraged in tree species koa, ʻōhiʻa, kōlea (*Myrsine lessertiana*), kāwaʻu (*Ilex anomala*), and shrub species ʻākala (*Rubus hawaiensis*), naʻe naʻe (*Dubautia plantaginea*), and hoi kuahiwi (*Smilax melastomifolia*) (Figure 25). Remnant individuals of the four native tree species mentioned (koa, ʻōhiʻa, kōlea, kāwaʻu) are distributed throughout the Nakula NAR site and are fairly common. Most of the outplantings of these four tree species were not used by the kiwikiu because those plants were still too young; although one individual (WILD1) was seen when he was first released in the oldest planting plots (from 2013) of pilo (*Coprosma* spp.), koa, and ʻōhiʻa. The native shrubs mentioned (ʻākala, naʻe naʻe, and hoi kuahiwi) are typically found within gulches and/or in restoration planting plots. All of these plant species are known as preferred food plants for kiwikiu in the wild elsewhere in their range (Mountainspring 1987, Stein 2007). Therefore, it is not surprising that they found or sought these plants out. There are few patches of ʻākala and hoi kuahiwi in Nakula NAR, yet WILD7 settled in a gulch that appeared to have a greater density of these patches.

Besides extracting larvae from woody tissue, several individuals were observed gleaning caterpillars from leaves. Although they mostly foraged in larger established trees and shrubs, several wild individuals were observed foraging in the terminal branches of young koa, presumably searching for insect larvae (e.g. caterpillars). These were some of the only planted trees in which the released kiwikiu foraged. We observed kiwikiu, both wild translocated and reared under managed care, successfully capture and consume insect larvae on several occasions. Although specific identification of the prey items could not be made, we observed the released kiwikiu capturing Lepidoptera and Coleoptera larvae (Figure 25).

The female, WILD11, foraged in a unique way among the released kiwikiu and in a manner not often observed in the windward range, though coincidentally a male was observed doing this exact behavior in Hanawī NAR in March 2020 after the release. This bird was found almost exclusively in the top canopy of ʻōhiʻa, prying open terminal leaf clusters (Figure 25). This reliance on ʻōhiʻa may explain why she settled in the area she did, as this area contained a greater density of ʻōhiʻa than in most parts of Nakula NAR. This foraging method resembles that of ʻakepa (*Loxops* spp.) whose diet consists largely of spiders

(Lepson and Freed 2020). Peck et al. (2015) found that spiders made up 1–2% of wild kiwikiu diets in Hanawī NAR but up to 6.5% of the diet of some females in TNC's Waikamoi Preserve. These authors also found spiders were (by far) the most abundant arthropod within ʻōhiʻa foliage and had a greater relative abundance in Nakula NAR compared to the windward sites, particularly in the fall. It is possible that this female was taking advantage of a seasonally abundant food resource, even if that resource is not generally among the most common kiwikiu prey items (e.g. caterpillars).

Many observers noted that the foraging manner of the kiwikiu from the conservation breeding facility was inefficient and ineffectual and unlike that of the wild birds. These birds were only observed in koa and ʻōhiʻa and, while they chewed on branches, observers noted fewer successful captures. We observed MP026 capture at least one large caterpillar, which he gleaned from the bark surface of a small koa near camp. In the few days MP022 and MP026 were observed after their release, both birds fed primarily from food trays (of mealworms) provided at camp or at their aviary. Foraging attempts by the birds from the conservation breeding facility were restricted to some branch splitting and bark peeling, particularly in small koa trees.

Vocalizations – In the windward range, kiwikiu exhibit three types of calls, "chip" notes, whistles, and songs (Mountainspring 1987). The atonal chip notes are generally considered to be contact notes that may have both intra- and interspecific purposes (Pratt 2005). The whistles are more often used for intraspecific communication, such as between mated pairs or parents and juveniles (MFBRP unpubl. data). Playback of these calls attracts kiwikiu more often than other call types during capture attempts. Songs are only exhibited by males and are likely used in mate attraction and territorial display, as in other passerines. The vocalizations exhibited by the released kiwikiu may offer a window into the psychological state of the birds and may indicate lack of breeding and/or territorial behaviors.

The wild kiwikiu released in Nakula NAR primarily vocalized by chip notes and occasional whistles, although the latter were comparatively rare. None of the males (from managed care or wild translocated) were observed singing after release. In general, the frequency of calls was great enough to allow observers to locate the birds before seeing them and to track individuals over short distances without the use of telemetry. Nakula NAR has a relatively open forest structure allowing for these calls to be easily heard. Additionally, since there were fewer Hawaiian honeycreepers in Nakula NAR, their vocalizations were distinct, allowing for definitive identification (e.g. Maui ʻalauahio exhibit a very similar chip note to that of kiwikiu but this species is not present in Nakula NAR). Hawaiian honeycreepers often mimic each other often making it difficult to identify kiwikiu aurally in their current range.

The wild kiwikiu were rarely observed near one another, but the birds vocalized even when they were alone. Vocalizing in this way may indicate some normalcy in their behavior after release because kiwikiu often become quiet when stressed or fearful (e.g. their response to pueo). None of the vocalizations indicated signs of territoriality or breeding.

Figure 25. Wild translocated kiwikiu foraging in Natural Area Reserve after release. The top left individual, WILD7, is shown with a large caterpillar in an ʻōhiʻa. The same individual is pictured in the bottom left, foraging in kawaʻu. The bird in the top right panel, WILD11, is shown foraging in terminal leaf clusters in ʻōhiʻa, a seeming favorite foraging method of this individual. The bottom right panel shows a male, WILD8, peeling bark in an ʻōhiʻa. Photos by Bret Mossman.

The kiwikiu from the conservation breeding facility exhibited more whistle-type calls than the wild birds, but they also frequently produced chip notes. MP022 (and sometimes MP026) produced odd palila-like whistles unlike any vocalizations heard in the wild. Kiwikiu, like many honeycreepers (Pratt 2005), are known to mimic other species' calls, particularly whistles (MFBRP unpubl. data). These kiwikiu almost certainly learned these vocalizations at the SDZG facilities where they were held in aviaries close to palila. In the wild, palila and kiwikiu do not coexist as they are found on different islands. These vocalizations made identification of these two individuals by ear easy. Another example of whistle mimicry came from WILD7 who would make whistles similar to the Warbling White-eye (*Zosterops japonicus*), a form of mimicry that has previously been observed on the windward slopes.

Interactions with other birds – Few interactions were observed between the released kiwikiu. The only interaction seen between wild kiwikiu was soon after their release from the aviaries, between two adult males, WILD7 and WILD8. During this interaction, the birds chipped back and forth across a narrow gulch but did not interact in other ways. Besides this interaction, most of the released birds were rarely in close proximity to each other, eventually settling in core areas far from other kiwikiu (Figure 21). The only time a male and female were released together (WILD7 and 11), they interacted briefly, chipping, and then both birds moved around independently. In the first few days after release, the female (WILD11) spent time in the same areas as WILD7 and (possibly) WILD5 before moving west, away from Nakula NAR and the other birds. WILD1's aviary was near where WILD7 had settled. When WILD1 was released, WILD7 was chipping nearby but we did not see any interactions between the two of them and WILD1 did not go towards WILD7.

We observed multiple interactions between the released kiwikiu and other bird species, particularly Hawai'i 'amakihi and Warbling White-eyes. On numerous occasions observers noted 'amakihi and white-eyes approaching kiwikiu as they foraged. In some of these interactions, the other birds became aggressive and chased the kiwikiu. Several male 'amakihi were seen chasing the female kiwikiu, WILD11, and a juvenile 'amakihi was observed following and begging from this female. In most of these interactions, the kiwikiu were much less interested in the other birds and would quickly fly away from the interaction, chipping profusely. While these interactions were unusual in their frequency and aggression, 'amakihi, white-eyes, and other Maui Hawaiian honeycreepers are often attracted to playback of kiwikiu calls in the windward range and some of these species have been seen chasing kiwikiu there as well. Additionally, birds from both the wild and from managed care were observed responding to pueo while in the aviaries and after release. Generally, their response was to freeze, sitting very still and quietly in a tree.

11. Survivorship/mortality

11.1. Survivorship

The overall survival time of the kiwikiu held and/or released in Nakula NAR was 20.4 ± 8.4 days (n = 13; excluding MP027 that was removed from the site alive). This does not include the one surviving male transferred back to MBCC. Included in this average are two wild translocated males and one male from the conservation breeding facility for which we did not determine final fate. We used the final observation dates of these individuals in the calculation of survivorship. In this way, the number of days each individual survived is a minimum value. Survivorship varied from nine (MP024) to at least 38 days (WILD1) in Nakula NAR, the latter of which was still alive as of the last monitoring attempt.

Survival time (i.e. the number of days birds survived in Nakula NAR), did not differ between managed care (n = 6) and wild (n = 7) kiwikiu (Type III Anova: F = 2.2, p = 0.164, df = 1). After release, however, the wild birds survived significantly longer than the managed care birds (Type III Anova: F = 10.7, p = 0.017, df = 1). Wild birds survived 16.24 ± 6 days (n = 5) on average after release while managed care birds survived 4 ± 3 days (n = 3). Collectively, the released kiwikiu survived for 11.75 ± 8 days on average in Nakula NAR.

11.2. Pre-release deaths

In preparing for this reintroduction, we took great care in creating a safe place for birds to acclimate prior to release. Our primary concern was preventing depredation in the aviaries. We controlled

mammalian predators for a year prior to the birds' arrival and constructed aviaries in such a way that it would have been very difficult for predators to access the birds. Fortunately, no birds were lost to predators in or out of the release aviaries (as far as we know). Sadly, the greatest threat to the kiwikiu in Nakula NAR came from avian malaria, not predators. As discussed in 3.4. Mosquito Mitigation in Nakula NAR, we were taken by surprise by the unprecedented increase in mosquito abundance in Nakula NAR in the fall of 2019. During the translocation, we had no idea mosquito numbers in Nakula NAR were experiencing a population explosion unlike what we had seen in previous fall months of the 2015/2016 assessment. Even as the first individual kiwikiu fell ill while being held in aviaries, we initially suspected other causes (e.g. aspergillosis).

Between 19 and 24 October, three kiwikiu that originated from the conservation breeding facility died in Nakula NAR or shortly after being transferred back to MBCC. On 18 October, eight days after being moved to Nakula NAR from MBCC, MP018, a 15-year old male, became lethargic and his food intake dropped. SDZG staff became increasingly concerned about this bird's health and he was transported from Nakula NAR to MBCC that evening. After returning to MBCC, MP018 received veterinary care that included administration of itraconazole and meloxicam. The veterinary exam also included a jugular blood draw, which resulted in extensive hemorrhage in the neck. This bird died at MBCC on 20 October. On the previous day, another male from the conservation breeding facility, MP024, showed similar symptoms of lethargy. This bird declined rapidly over the course of the day and died that afternoon while being transported to camp for veterinary care. Preliminary necropsy completed on 23 October of both MP018 and MP024 indicated avian malaria as the cause of death (although the neck trauma was a possible contributing factor for MP018). A third bird, MP009, became lethargic on 21 October. At this point SDZG attempted to administer itraconazole to this bird (in mealworms) as a proactive measure, but she did not eat the mealworms. Her health declined and she was brought back to camp and given subcutaneous fluids and Itraconazole. On 23 October, both MP009 and MP027 (the male from the conservation breeding facility that was not going to be released and was removed from Nakula NAR to prevent infection) were transferred back to MBCC for veterinary care; MP009 died overnight (early on the 24th).

When we received the preliminary necropsy results for these birds, indicating avian malaria was a factor in their death, we decided to increase the frequency of mosquito larvicide treatments (see 3.4. Mosquito Mitigation in Nakula NAR). At this time, it was not clear if these birds could have contracted the disease in Nakula NAR as these birds developed symptoms more rapidly than is typical and died more rapidly than has been reported for other honeycreepers. Following the third death, we became more concerned for the safety of all of the kiwikiu in Nakula NAR. However, all three individuals that died were among the smallest (MP024) or the oldest (MP009 and MP018) individuals. We hoped that these losses would be restricted to individuals with compromised immune systems and that healthier individuals might be spared the same fate. Following the deaths of three managed care birds as of 24 October, none of the other birds from the conservation breeding facility showed malaria symptoms, leading to optimism among the team.

Nevertheless, two wild birds developed symptoms 12–13 days after arriving in Nakula NAR, similar timing to the deceased birds from MBCC. On 28 October, after the transmitter replacement (see 8.2. Transmitter attachment in Nakula NAR), WILD10 appeared initially strong but notably stressed. After opening the aviary doors for her release and coaxing her out, she eventually fluttered to the ground outside the aviary. We quickly placed her back in the aviary to allow her time to calm down overnight as

it had become late afternoon. The following day she did not eat and remained largely sedentary. We became more concerned and eventually transferred her to a holding cage at camp for the evening of the 29th.

In the morning of 29 October, the subadult male, WILD9, showed some signs of lethargy during the application of a transmitter (see 8.2. Transmitter attachment in Nakula NAR). At this time, we were concerned that this bird was having an unusually strong stress response to the transmitter attachment. This was the first time any of the birds had shown such a negative response to having a transmitter attached. This bird's continued decline led us to remove his transmitter and transfer him to a holding cage at camp. Both WILD9 and WILD10 received subcutaneous fluids at camp, to which WILD10 initially responded well. WILD10 was observed feeding and bathing after the fluids were administered. Unfortunately, both WILD9 and WILD10 died in holding overnight of 29/30 and 30/31 October, respectively.

The deaths of the two wild birds following similar symptoms to those of the deceased birds from the conservation breeding facility indicated that the threat of avian malaria was not restricted to the managed care birds. However, just as in the deceased birds from MBCC, the two wild birds that died were also young and among the smallest translocated birds. We also discussed the possibility that holding birds in the release aviaries may have put them at greater risk of infection. If the birds had been allowed to roost higher in the trees where breezes and lower temperatures may occur overnight, they might have been less likely to be bit by mosquitoes. Further, we strategically placed the aviaries near gulches containing good habitat, but these gulches also likely served as mosquito breeding habitat. The deaths of the three managed care birds encouraged us to release the other birds more rapidly than initially planned in part based on this aviary hypothesis. By the time the two wild birds died, we only had one wild bird (WILD1) left to be released. After some discussion, we decided to ultimately release him (coincidentally, he ended up being the longest living kiwikiu in Nakula NAR).

In all, the birds that died prior to release survived less than two weeks in Nakula NAR. In these cases, the birds showed some signs of lethargy, decreased appetite, and usually died the following day. These birds survived an average of 1.4 days (0–3 days) after the first symptoms were noted. The older managed care male, MP018, survived longer than the others after receiving veterinary care at MBCC and may have survived longer had the neck hemorrhage not occurred. Other birds succumbed to the disease in as few as 10 hours after the first signs were noted. In laboratory studies of other honeycreepers, symptoms of acute malaria infection were first seen a minimum of seven days after infection and peak parasitemia occurred approximately 12 days after infection (Atkinson et al. 1995, Atkinson et al. 2000, 2001b). In these studies, death occurred between 13 and 31 days after infection (Atkinson et al. 2000, 2001b). This timeline would indicate that the kiwikiu that died prior to release contracted the parasite in as few as 1–2 nights after arriving in Nakula NAR. These individuals also died more rapidly than most previously studied honeycreepers, surviving only 12 days on average. Since *Culex* mosquitoes are strictly nocturnal, these birds were exposed as few as eight nights prior to death. The fact that at least five individuals died so quickly after they were first potentially exposed to mosquitoes in Nakula NAR likely speaks to the high densities of mosquitoes later determined to be present during the fall of 2019 in Nakula NAR.

11.3. Post-release deaths

On 17 October, we translocated the wild birds to Nakula NAR. The first deaths of the birds from the conservation breeding facility occurred in the following days and indications that avian malaria was

becoming a major problem were not apparent for another week after the translocation. The team decided to speed up the planned release period due to the possibility that holding the birds in the release aviaries put them at greater infection risk and issues with the birds removing and/or damaging the transmitters. We released all remaining eight seemingly healthy birds on 27–30 October.

After the birds were released, we began tracking and monitoring them and all birds appeared to be adjusting well in the first few days. (See 10.1. Tracking Kiwikiu in Nakula NAR for a description of the birds' movements after release). We lost track of one managed care male and one wild male after release due to transmitter failures. Besides these two, we were able to track all other six individuals daily (even MP022 without a transmitter). As more time passed, we became more hopeful that the released individuals might avoid the fate of those that died of malaria prior to release. As of 1 November, all released wild and managed care birds had been in Nakula NAR for 15 or 22 days, respectively, beyond the point that the deceased individuals developed symptoms.

On 2 November, we discovered MP022 dead underneath his release aviary. We had not observed any malaria symptoms (e.g. lethargy) prior to his death; however, he was not seen on 1 November. Given this bird's reliance on supplemental food, we hypothesized at the time that his death could have been the result of poor nutrition. Necropsy results later determined malaria to be the cause of death (as in all other birds) and there were no indications of malnutrition beyond pathologies consistent with malaria. A few days later on 4 November, MP026 was recaptured after being discovered huddled under the camp deck. He was transported to MBCC the following morning where he died shortly after arrival. This bird had been recaptured on 31 October so we could replace a faulty transmitter and the bird was judged to be in good health. Over the next three days, he was observed regularly feeding from the camp deck tray. The first signs of declining health were noted on the morning of 4 November, when he was seen sitting under the deck. At this time, he was energetic enough to fly away when people approached. By that evening, he appeared very lethargic and was captured for veterinary care and transportation back to MBCC where he died on 5 November. Both MP022 and MP026, survived in Nakula NAR for 23 and 26 days, respectively, but less than one week after release.

Over the next nine days, 6–15 November, each of the remaining released birds (all wild translocated) died or vanished. On the afternoon of 5 November, WILD8 was observed scarcely moving, feebly biting at branches, and losing his balance. We recovered this bird's body the following afternoon and the tower data indicates he died that same day. From his release on 27 October to 4 November, this bird was observed nearly every day actively foraging and moving around his core area. As such, this bird died ≥ 3 hours after the first sign that anything may be wrong. On 11 November, MFBRP staff discovered the body of WILD5. This bird had been observed the previous day and his behavior gave no indication that his health was failing. The tower data indicates that this bird showed some activity up through the morning of the 11th. Both of these birds were large, healthy male kiwikiu and seemingly adapting well to their new environment. We did at times observe periods of resting in trees, but we did not know whether this was unusual for wild kiwikiu.

As of 11 November, the remaining birds included one transmittered female, one male that had recently removed his transmitter, and a male without a functional transmitter that had only been observed once since release. The male that removed his transmitter, WILD7, was regularly found in a specific gulch every day since the first few days following release. His transmitter as found on 8 November, but he was observed without the aid of the transmitter for a few days following this find. However, after 12

November no further observations were made despite daily attempts to locate him in his core area and the surrounding region. The consistency in which he had been found prior to this leads us to believe this bird likely died or left the site on 13 November or shortly after. Without the aid of a transmitter, we were not able to locate his body or otherwise determine his fate, but this timeline is consistent among the individuals confirmed to have died. It is also notable that this bird disappeared within the same timeline of some palila that returned to their capture site (Banko et al. 2014). Thus, it is possible this bird emigrated from Nakula NAR.

We continued to track WILD11 who had moved approximately 1.5 km west of Nakula NAR onto Hawaiian Home Lands. This bird was very active, moving among ʻōhiʻa canopies rapidly and constantly feeding. Following a familiar tragic pattern, however, observers found her noticeably less active (e.g. sitting still for long periods) on 15 November and her body was recovered the following day. Like the others, the first sign of illness was noticed within 24 hours of her death. As in WILD5 and WILD8, the tower data helped narrow the time of death, indicating she died early on the morning of 16 November. At the time, we believed WILD11 had been the last kiwikiu alive in Kahikinui. However, observers found WILD1 on 24 November in the same area he was last observed 18 days prior. The final fate of this individual was not determined, and no further attempts were made to locate this bird after this date. The survival of this bird (> 38 days), well after the timing of the death or disappearance of the other birds (≤ 29 days), instills some hope that this individual may still survive. This length of time is also beyond the average length of time (although not beyond the maximum) palila emigrated from their translocation site (Banko et al. 2014).

In general, the kiwikiu died very rapidly after being moved to Nakula NAR, ranging 9–29 days among those known to have died. We do not know if the variation seen in survival time was due to variation in individual immune response or variation in when birds were infected. However, the kiwikiu deaths all occurred within the length of time when they could have conceivably been infected or reinfected on the first night in the aviaries, based on rates in other honeycreepers. A laboratory study on ʻiʻiwi (*Drepanis coccinea*) found survival time ranged from 19–37 days (average 28.9 ± 5.5 days, *n* = 7) in birds that died from acute malaria after being infected by a single mosquito (Atkinson et al. 1995). In studies on Hawaiʻi ʻamakihi, experimentally infected birds died 12–31 days after infection (Atkinson et al. 2000: average = 21.3 days, *n* = 9; Atkinson et al. 2013: average = 20–21 days). Experimentally infected Maui ʻalauahio, the closest kiwikiu relative tested in this manner, died between 13 and 26 days after infection (*n* = 3) (Atkinson et al. 2001b). The most rapid death from acute malaria recorded in a honeycreeper was nine days in an experimentally infected Hawaiʻi ʻamakihi (Atkinson et al. 2001a).

If all of the birds were infected or reinfected the first night (see 11.6. Further malaria analysis), the overall survival time for the translocated kiwikiu (20.4 ± 8.4 days) was consistent with that of other honeycreepers infected in laboratory studies. However, excluding the individuals for which we were not able to determine fate (WILD1, WILD7, and MP023) average survival time was 18.2 ± 7.1 days, among the most rapid rate of any honeycreeper. Further, 50% (5/10) of the deaths occurred within 14 days after arriving in Nakula NAR and included some of the most rapid individual mortality events recorded for any honeycreeper from acute malaria (9 and 10 days) (Atkinson et al. 2001b). Post-infection survival time in the translocated kiwikiu also represents a maximum value because these birds may not have been infected or reinfected on the first night.

11.4. Necropsy results

In total, we recovered the bodies of ten kiwikiu held and/or released in Nakula NAR. After we recovered a body, if possible, we weighed them and recorded body condition, e.g. fat score, keel prominence, and rigor mortis. The bodies were held at camp until they could be flown out of the field, transferred to MBCC, and shipped to San Diego, CA. SDZG staff refrigerated the bodies overnight at MBCC and then shipped them to San Diego Zoo for necropsy. Necropsies were performed two to seven days postmortem (Table 11).

The bodies were held at camp in foam coolers and kept cool with first-aid cool packs. Although this system likely did not keep the bodies at optimum temperatures to prevent autolysis, the goal was to reduce necrosis until the body could be properly refrigerated. Three birds died at MBCC after being transferred from Nakula NAR while still living and their bodies were properly refrigerated immediately upon death. The bodies of the other birds were held at camp in Nakula NAR for one to four nights (Table 11). Most birds were noted as being autolyzed to some degree during necropsy and those held at camp the longest, not surprisingly, were the most autolyzed. The degree of necrosis likely depended on how long the bodies laid in the field before recovery, how well the crew was able to keep the bodies cool at camp, and daily temperatures during this period. Based on previous observations of the birds, all bodies recovered in the field were found within 20 hours of death. Most deaths occurred overnight, and the bodies were recovered the following morning or early afternoon.

Table 11. Necropsy reports for the recovered deceased kiwikiu held and/or released in Nakula Natural Area Reserve. Cause of death wording is taken from the official necropsy reports from SDZG.

Bird ID	Death date	Nights held at camp (postmortem)	Necropsy date	Autolysis	Body condition	Fat	Pectoral muscle	*Plasmodium* positive	Notes	Cause of death
MP009	10/24/19	0	10/26/19	slight	poor	none	moderate atrophy	yes		acute hemoparasitic infection
MP018	10/20/19	0	10/22/19	moderate	fair	moderate	even with keel	yes		hemoparasitic infection; extensive neck hemorrhage
MP022	11/2/19	3	11/7/19	severe	poor	not noted	moderately below keel	yes		severe hemoparasitism
MP024	10/19/19	2	10/23/19	moderate	fair	small	reduced	yes		overwhelming *Plasmodium* infection
MP026	11/5/19	0	11/7/19	moderate	fair	none	not noted	yes	dehydration	overwhelming *Plasmodium* infection
WILD5	11/11/19	2	11/15/19	moderate	poor	none	decreased	yes		severe intra-erythrocytic infection
WILD8	11/5/19	2	11/11/19	moderate	poor-fair	scant	moderately below keel	yes	spirurid nematode in proventriculus	severe hemoparasitism
WILD9	10/30/19	1	11/2/19	moderate	poor-fair	scant	slightly below keel	yes	confirmed male	severe hemoparasitic infection
WILD10	10/31/19	0	11/2/19	slight	poor	small	moderately below keel	yes	intestinal trematode parasitism; active folliculogenesis	severe hemoparasitic infection
WILD11	11/16/19	4	11/22/19	severe	poor	none	moderately below keel	yes		avian malaria

All necropsies reported damage to multiple organ systems (e.g. lungs, liver, spleen, kidneys) consistent with acute malaria infection and protozoans were found in the blood and several internal organs. Further molecular analysis (polymerase chain reaction [PCR]) confirmed the presence of *Plasmodium relictum* in at least one tissue type in all cases. Atkinson et al. (1995) described what is often considered typical gross pathology of acute malaria infection in honeycreepers. These authors described enlarged and darkened livers, spleens, and kidneys in infected ʻiʻiwi. Similar pathologies were noted in infected Hawaiʻi ʻamakihi (Atkinson et al. 2000). The necropsies performed on the kiwikiu frequently noted enlarged spleens and livers. Among the most frequent gross pathologies noted was pigmentation and damage to at least one lung. They also noted the presence of *Plasmodium* schizonts in the lung tissue of several individuals. Atkinson et al. (1995) did not note damage to the lungs (or other internal organs) beyond minimal pigmentation. This suggests a slightly different pathology in kiwikiu compared to other honeycreepers potentially influencing the speed of mortality. If respiration was compromised, kiwikiu may have succumbed faster than from anemia alone. Damage to the lungs was likely further indication of the scale of the infection, affecting numerous organ systems simultaneously.

The recovered birds (n = 10) weighed 10.9 ± 6.8% less than their capture/holding weight. Because most birds gained weight while in the aviaries in Nakula NAR, the loss was greater between their final antemortem (e.g. transmitter attachment) and postmortem weights, averaging 15.4 ± 8.3% loss. These individuals weighed between 0.9 and 6.4 g less upon their death than their final antemortem weight. This weight loss may indicate that the deceased birds were symptomatic longer than was evident from field observations or tower data. The number of organ systems affected further indicates that the infection was advancing long before behavioral changes were noted in the field. While in holding in Hanawī NAR, the birds lost 1.7 g (8%) on average overnight while they were not feeding, only to regain that weight throughout the next day. So, any bird that died overnight may be expected to weigh up to 8–10% less than the previous afternoon even if they were healthy. However, the three released wild birds that were recovered weighed up to 20% (average 17%) less than their capture weights. Most of the birds from the conservation breeding facility and the wild birds that died prior to release were less than 10% of their pre-exposure weight after death. As such, the individuals that were not supported by supplemental food showed the greatest weight loss. This may indicate that the weight loss associated with disease was moderated by a readily supplied food source.

Most necropsies noted some degree of pectoral muscle atrophy and little evident fat stores. These birds were likely losing weight and becoming anemic for several days prior to the first signs of lethargy noted by observers. We do not know when each kiwikiu was infected but the disease appears to have progressed incredibly fast in these birds. In laboratory studies of other honeycreepers, deceased birds typically lost 13% of their pre-infection body weight (Atkinson et al. 1995, 2000). The 50% of kiwikiu mortalities that occurred ≤ 14 days after translocation to Nakula NAR, weighed only 8.3 ± 5.2% lower than their starting weight and showed only slight to moderate pectoral muscle atrophy. The individuals that survived longer were more consistent with the laboratory studies, dying after > 24 days on average and weighing 13.5 ± 7.8% less than their pre-translocation weight. However, we found no correlation in the number of days a bird survived in Nakula NAR (in aviaries and after release) and the percent body weight lost (t = 0.44, p = 0.204, df = 8).

A few of the wild birds were noted as having other parasites or diseases besides malaria upon postmortem analysis. Parasites were found in the digestive tracts of two birds but neither was associated with inflammation and the extent of these infections was not clear. In both cases, this was

considered minor and not a contributor to their deaths. Upon recovery of WILD11's body, observers noted a wound and swelling of the right distal tarsometatarsal joint (Figure 26). This was not noted in the necropsy but is a common symptom of avian pox (*Poxvirus avium*) in honeycreepers. Avian pox, another mosquito-borne disease, was rare among all birds banded in Nakula NAR in 2015 and 2016, 0.7% (Warren et al. 2019a). However, prevalence was higher among birds that also tested positive for malaria. This disease often leads to loss of digits and is not generally considered a direct cause of death in honeycreepers but may reduce agility, which may interfere with foraging or other activities (e.g. avoiding predators).

Figure 26. The legs of WILD11 postmortem showing a wound and swelling of the right distant tarsometatarsal joint consistent with avian pox infection.

Recommendations for the future

In planning to release kiwikiu into Nakula NAR, there were extensive discussions and preparations made to reduce threats to the birds before and after release. Avian malaria was one of several concerns (e.g. mammalian predators, habitat) but we underestimated the scale of the threat malaria posed. Had we known, we might have been able to reduce exposure to the disease and mortality. However, had we understood malaria would present such a risk we would not have proceeded with the translocation.

The aviaries in Nakula NAR were not designed to be mosquito-proof and did not have mosquito netting. In retrospect, mosquito netting might have greatly reduced the threat of birds becoming infected or reinfected before release. The placement of the aviaries near gulches and the fact that the birds were held so low to the ground might also have artificially increased their risk of becoming infected before release. In the end, none of the birds lived long enough after release to say that they were not infected in the aviaries. The deceased wild individuals died between 10 and 18 days after release, well within the 9–37 day period reported for malaria mortality in honeycreepers (Atkinson et al. 1995, 2000, 2001a, 2001b). However, if kiwikiu can succumb to the disease in as few as nine days, this could suggest that some birds may have been infected or reinfected after release, but we will never know. In the end, if we

had thought that the threat of malaria infection was great enough to warrant mosquito netting or changes to the placement of the aviaries, we would not have proceeded with the project at all.

We also did not have a good plan for treating birds in the field if they showed symptoms. Malaria medications (e.g. quinine) were not brought into the field until too late and emergency veterinary care at camp was restricted to providing subcutaneous fluids and/or Itraconazole (for treating aspergillosis). The field setting is also not ideal for providing intensive care and prescribed, detailed treatment of sick birds. Once MP018 died, Itraconazole was brought into the field to be used for preventative care, but malaria medications were only brought in briefly and after it was too late. No veterinarians were present at the release site, although at least one SDZG staff was present in the field at any one time and were trained to administer these medications but varied in experience and skill. A bird from the conservation breeding facility, MP027, was held in Nakula NAR for 13 days before being returned to MBCC. After his return, this bird developed some of the same symptoms seen in other birds and was held in a controlled environment, intensively monitoring, and treated with anti-malarial medications. This infection was confirmed by subsequent PCR analysis. MP027 has made a full recovery and is being continually monitored. This case indicates that, with proper intensive care and medication in a controlled environment, kiwikiu can recover from malaria infection. Whether the full extent of this intensive care could be adequately administered in the field is unknown. The treatment applied to MP027, for example, required multiple oral treatments per day. Even if birds were treated with anti-malarial medications, it is unclear if they could survive in the wild afterwards especially given the threat of additional infections and recrudescence after the effective period of the medication ends. There is some evidence that honeycreepers can survive reinfection and may develop some level of immunity (see 11.6. Further malaria analysis). Not having the medication on hand in Nakula NAR was an oversight as was not assessing all the birds for avian malaria prior to release, however, qPCR testing would have greatly increased the holding times for individuals. If all wild translocated birds were assessed for malaria prior to being moved to Nakula NAR, they could have been treated beforehand. Nevertheless, if we had known this, we might not have gone through with the reintroduction.

In addition to planning for treatment of sick individuals within the field, there should also be a plan of how and where to bring individuals if something is putting the translocated individuals at risk (e.g. disease, poor nutrition, depredation). For this release, this was not planned-out in detail beforehand. For some of the birds from the conservation breeding center, they were allowed back into that facility if recaptured; however, it was suggested that the wild birds go back to Hanawī NAR. But if we had moved birds back to Hanawī NAR, we would likely have moved sick individuals that may have died there anyways. Additional monitoring after returning birds to the capture site may be advisable. Some expressed concern that the stress of recapture might be detrimental to their health, but we were able to recapture MP026, hold him overnight, and successfully transport him to the conservation breeding facility. Re-capturing individuals would likely have been challenging, but in most cases we were able to determine regular “routes” (aided by transmitters), which could guide net placement. In the future, it would be advisable to ensure that a facility (e.g. MBCC) is available to care for translocated birds post-release and a decision-making tree of when individuals (and/or all translocated birds) should be re-captured, brought to this facility, and scenarios for release into the wild.

11.5. Post-release disease assessment

The deaths of both translocated and kiwikiu from the conservation breeding facility in Nakula NAR from avian malaria raised several questions. One possibility was that we incorrectly judged the disease risk in Nakula NAR to be acceptable in 2015–2016. Alternatively, mosquito densities may have increased between 2016 and 2019. A third possibility is that the release occurred during an unexpected, temporary peak in mosquito numbers within the site. To investigate if mosquito densities and/or infectivity of mosquitoes changed, we repeated the same sampling design used in 2015–2016 to trap adult mosquitoes in 2019–2020, utilizing the same traps and trap placement. We placed the traps in the five 2015/2016 locations as well as additional trap locations in the area, including near aviaries. Mosquitoes were trapped during three week-long sessions in November 2019, May 2020, and July 2020. The results from the mosquito trapping in November showed a pronounced spike in mosquito capture rates, low rates in May, and average rates in July compared to 2015/2016 (Table 12).

Table 12. Mosquito capture data from Nakula Natural Area Reserve prior to and after the kiwikiu release. Shown are capture rate (mosquitoes / trap nights), the number of mosquitoes tested for Plasmodium relictum using qPCR, and the percent of individuals that tested positive for P. relictum. Capture rates shown are for CO_2 traps only because oviposition traps were not used after 2016. CO_2 traps were baited with dry ice through November 2019 and tanks of CO_2 thereafter. Mosquitoes captured after November 2019 have yet to be tested for P. relictum.

Year	Month	Capture rate	# tested	*Plasmodium* +
Pre-release				
2015	May	0.11	2	0%
2015	May	0.17	3	0%
2015	July	0.93	13	0%
2015	November	1.62	21	0%
2015	December	0	1	0%
2016	March	1.86	26	0%
2016	April	6.39	115	6.1%
2016	July	0.47	7	0%
2016	August	1.26	24	8.3%
2016	November	0.25	6	16.7%
2016	December	0.24	4	0%
Post-release				
2019	November	7.07	181	3.9%
2020	May	0.06	-	-
2020	July	0.81	-	-

The alarmingly high number of adult mosquitoes captured in Nakula NAR in November 2019 was greater than had been detected in the past. During this session we captured 7.1 mosquitoes/trap night (mq/TN; 7.8 mq/TN with just the 2015/2016 trap locations), an exceptionally high number especially for the elevation. The overall capture rate across all capture sessions in 2015/2016 was 1.22 mq/TN and the previous high capture rate within a single trapping session was 6.4 mq/TN in April 2016 (Table 12). Warren et al. (2019a) considered the rate in April 2016 an outlier in 2015–2016, given that this rate was on the order of 3–60 × greater than the capture rate of any other session among those two years. Without that single April session, the overall capture rate for the 2015–2016 trapping efforts was 0.65 mq/TN. Similarly, the November 2019 capture rate was anomalous compared to the May and July 2020 trapping sessions, which were more comparable to the average rates found in 2015/2016 (≤ 1 mq/TN).

It is unclear if mosquito densities overall changed in Nakula NAR between 2016 and 2019 or if the November 2019 capture rate represented a temporary high-water mark as seemed to be the case in April 2016. The fact that these population explosions happened in different seasons between 2016 and 2019 (and not detected in 2015) is another intriguing facet of the disease story in Nakula NAR. A peak in mosquito numbers in the fall has been seen elsewhere in Hawai‘i (LaPointe 2000, Gaudioso-Levita et al.

2015). It seems likely, given these results, that the conditions leading to a population explosion can arise in various seasons in Nakula NAR. Regardless, a temporary population explosion in mosquitoes can potentially still have a devastating impact on the bird population demonstrated by the speed at which the kiwikiu fell ill. It may matter less what the overall or average mosquito densities are at a site and more what kinds of fluctuations in mosquito densities are possible or expected at a given site.

Hawai‘i has experienced increasing temperatures and decreased annual rainfall in the last few decades (Steven et al. 2017). These changes in weather patterns are known to effect mosquito and disease prevalence (Ahumada et al. 2009, LaPointe et al. 2012). MFBRP installed an Onset ® weather station in Nakula NAR in May 2013 and have monitored temperature and precipitation since. Despite state-wide patterns, there has not been a discernable change in the Nakula NAR climate since 2013 based on the temperature and precipitation collected on site. In general, the warmest months in Nakula NAR from 2013 to 2019 were July–October, peaking in August, while rainfall had two peaks, one in the spring (February–April) and another in October (Figure 27). Thus, October typically represented one of the warmest and wettest months in Nakula NAR. Although we have not seen an overall trend in climatic conditions since 2013 in Nakula NAR, 2019 was the second wettest year recorded (total precipitation). However, 2019 was also the second coldest year recorded (average monthly temperature). Most notably, the summer months in 2019 (June–September) received above-average rainfall. This may have translated into a greater-than-typical amount of larval mosquito habitat in Nakula NAR in 2019. McClure et al. (2018) found high cumulative summer rainfall and higher temperatures led to an increase in *C. quinquefasciatus* abundance. The temperatures in Nakula NAR in August and September 2019 were slightly above average, corresponding with increased precipitation.

The degree to which a given month sees both above-average rainfall and relatively high temperatures likely explains the spikes in mosquito numbers in Nakula NAR to a large degree. Unfortunately, the precipitation sensor on the weather station malfunctioned in 2015 resulting in a loss of those data through April 2016. As a result, we do not have precipitation data during the peak mosquito capture time in 2016. However, the fall of 2016 was exceptionally dry, including the driest October recorded in Nakula NAR. This may help explain why there was not a peak in mosquito numbers during the fall of 2016. The spring of 2020 appears to have been wetter than average (although we had some data loss in the precipitation sensor in April) but this appears to not have translated into a spike in mosquito numbers. This may in part be due to the lower than average monthly temperatures in January–March. Other factors (e.g. uphill winds) may also play a significant role in creating the conditions in which the Nakula NAR mosquito population undergoes an explosion.

In many ways, it seems obvious that a warm and wet month, like October, could be good for mosquitoes. Ahumada et al. (2004) presented a model that predicted higher densities of mosquitoes in the fall based on the frequency of precipitation and temperature at elevations similar to Nakula NAR. But the weather patterns that lead to increased mosquito abundance appear to have been absent during the fall months in 2015–2016. This may have led to false conclusions about mosquito densities, and thus disease risk, in Nakula NAR during the fall. The high cumulative rainfall in the summer of 2019, during the warmest months of the year, may have created ideal conditions to create especially high mosquito densities in Nakula NAR in the fall. It may well have been the case that the fall of 2019 was an especially poor choice for the translocation as far as reducing disease transmission risk. However, if we had conducted the translocation during a low-mosquito season, we might have eventually seen the same high mortality in the fall when mosquito numbers increased again.

The mosquitoes captured in Nakula NAR in November 2019 were tested for the presence of *Plasmodium* using quantitative PCR (qPCR) analyses. Prior to the analysis, we dissected each mosquito into two sections, abdomen and thorax. This was done to estimate the proportion of captured mosquitoes that were infectious versus those that contained only oocytes in the hindgut. Of 181 mosquitoes (out of 191 captured) tested from November 2019, 3.9% tested positive for *Plasmodium* and infectivity was 2.2% (contained *Plasmodium* in their thorax). In the 2015/2016 study, we tested only whole body samples and do not have information on infectivity. However, the overall positive rate in 2016 (5.5%) was greater than the 2019 rate (Warren et al. 2019a). The positive rate during the April 2016 spike in mosquito numbers was even higher at 6.1%. Based on the samples tested to date, it seems that the frequency of *Plasmodium* in mosquitoes at this site has not increased since the 2015/2016 study.

As part of a separate project with the USGS, blood samples were collected from seven common bird species in Nakula NAR during the spring of 2019 and tested for the presence of *Plasmodium* using qPCR. Of the 75 samples tested, 29.3% were positive for the presence of *Plasmodium,* including from six of the seven species tested. Positive rate varied among the species ranging from 0% in Japanese Bush-Warbler (*Horornis diphone*, n = 2) to 100% in House Finches (*Haemorhous mexicanus*, n = 3). The overall positive rate from the 2015/2016 study, including all of these same species, was lower at 10.4% (Warren et al. 2019a). The 2019 analysis detected positive samples in several non-native bird species in which no positive samples were found in 2015/2016 (House Finch, Warbling White-eye, and Scaly-breasted Munia [*Lonchura punctulata*]). In some cases, Warren et al. (2019a) may have failed to detect positive samples because they only tested five samples from these species. However, the 2019 samples also included some very low sample sizes including those from House Finches (n = 3, 100% infected) and Scaly-breasted Munia (n = 5, 40% infected). Perhaps the biggest difference was in the native species, with a lower rate among Hawai‘i ‘amakihi in 2019 (4.8%) compared to 2015/2016 (8.2%) and a much higher rate among ‘apapane (2015/2016 = 15.8%, 2019 = 50%). It is difficult to conclusively determine if the overall prevalence of malaria within the Nakula NAR bird population has increased. An increase seems to have taken place within ‘apapane but it seems odd that rates among ‘amakihi would go down while ‘apapane would increase this much, especially given the robust sample tested sizes from these species (n > 15).

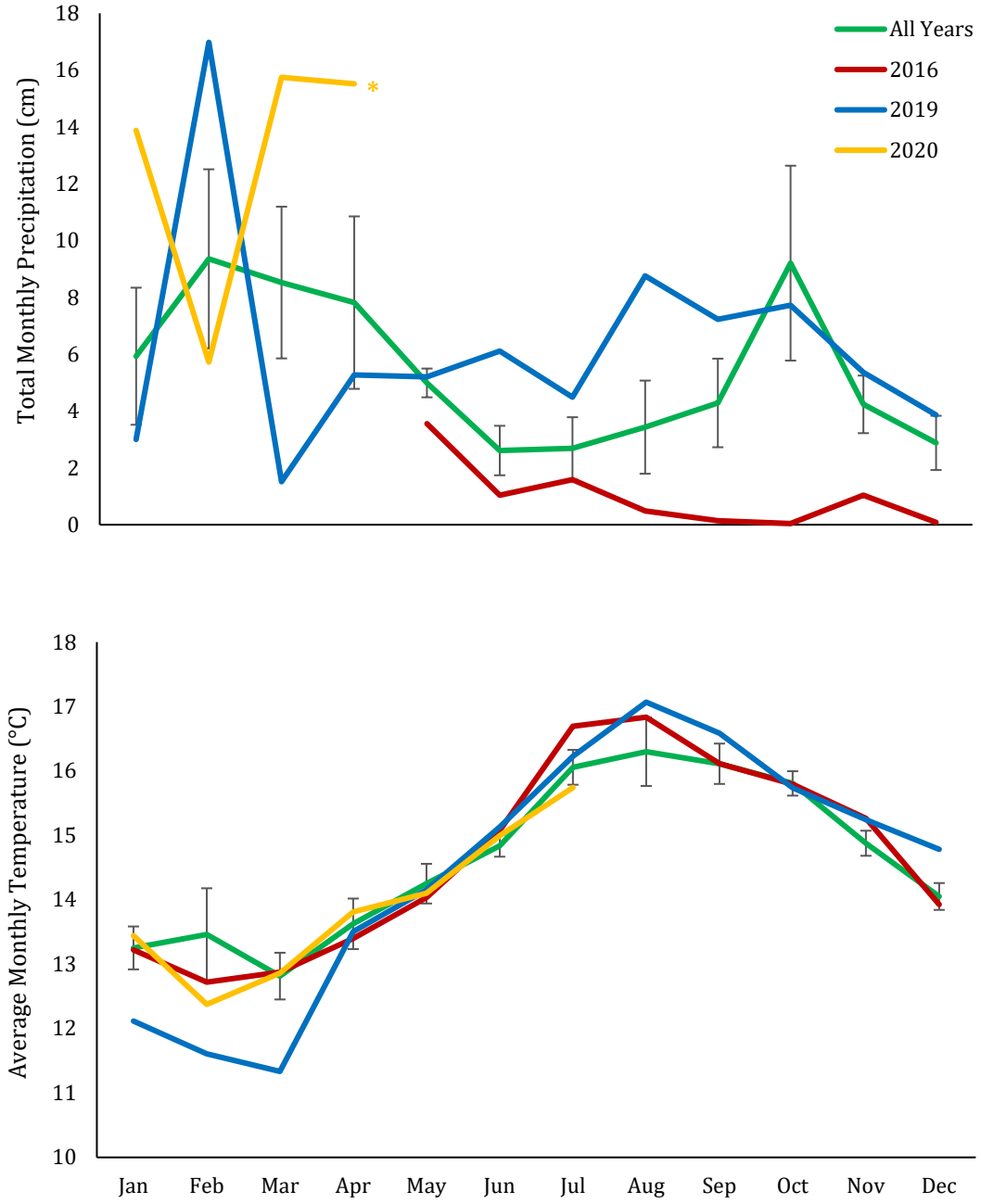


*Figure 27. Climate data from Nakula Natural Area Reserve from 2013–2020 showing total monthly precipitation (top) and average monthly temperatures. Shown are the average values for each month and monthly values for 2016, 2019, and 2020. The precipitation sensor malfunctioned in 2015–April 2016 and again in April 2020. * — The April 2020 precipitation value is based on eleven days only, prior to the sensor failure.*

Due to the deaths of the kiwikiu in Nakula NAR and the alarmingly high number of mosquitoes that we found there in November, we installed five mosquito traps in Hanawī NAR (where kiwikiu currently exist) at a similar elevation as the Nakula NAR release site (1627 m asl). During one week in December 2019 trip, we captured 14 mosquitoes in four nights, 0.7 mq/TN, a greater rate than previous sampling in December 2015/2016 in Nakula NAR (Table 12). However, in January 2020 we repeated this trapping effort at the same site and captured no additional mosquitoes. Two additional weeks of mosquito trapping at different sites within Hanawī NAR in June 2020, one at 1634 m and another at 2132 m asl, failed to capture any additional mosquitoes. Of the mosquitoes captured in Hanawī NAR at 1627 m asl in December 2019, one sample (7.7%) was positive for *Plasmodium,* although the parasite was only present in the abdomen of this individual. There is typically variation among sites, seasons, and years that makes comparisons challenging, but these data definitively show that mosquitoes are present, including those carrying the malaria parasite, in Hanawī NAR where kiwikiu occur.

11.6. Further malaria analysis

Prior to translocating the kiwikiu from Hanawī to Nakula NAR, we collected blood samples from all male birds held in the bird room, including the five translocated males and three males released back into Hanawī NAR. Blood samples were not collected from the females because they were captured at the end of the trip and we wanted to reduce their stress given the other procedures being conducted (i.e. veterinary check and banding). These samples were sent to the Jeff Foster and Katy Parise at the University of Northern Arizona for qPCR analysis. This lab previously analyzed blood samples of several Maui endemics collected between 2008-2016, including birds from the Frisbee Meadows study site in Hanawī NAR (MFBRP unpubl. data), but none of these samples tested positive for *Plasmodium relictum.* Given these prior results and the high elevation at which the birds were captured, we expected all of the pre-translocation samples to test negative as well.

Contrary to our assumptions, all eight samples came back positive for *P. relictum,* indicating that all of these birds had been exposed to the malaria parasite prior to their translocation. These represent the first positive-malaria cases documented in wild kiwikiu. This result has immense implications for both the fate of the translocated birds and the wild population as a whole. As such, all samples were re-analyzed and the second analysis confirmed the original finding. The second analysis was also conducted alongside 14 other samples from other species that had previously tested negative for *P. relictum.* All of the previous negative samples again tested negative for *P. relictum.* In all, the eight samples were tested twice and five assays were run in total for each individual. In these samples, *P. relictum* DNA amplified in at least one out of the five runs and up to 4/5 runs.

The conclusion from the qPCR analysis was that all eight individuals were “low-level” positive for avian malaria. During each analysis, all assays were run multiple times to ensure low-level positives were captured. The fact that *P. relictum* DNA failed to amplify in all runs is typical of low-level parasitemia (K. Parise pers comm). Parise and Foster consider a cycle threshold value (Ct) of amplified *P. relictum* DNA of 40 as an indication of a “positive” sample. The lower the Ct value below 40, the greater the parasitemia (parasite load) (e.g. Ct of 20 would indicate high parasitemia). The Ct values in the analysis of the wild kiwikiu ranged from 37.2 to 39.7. This indicates that all individuals tested had low parasitemia but were positive for *P. relictum* regardless. In the analysis of disease prevalence in Nakula NAR prior to the translocation, this same lab found low-level positives in ‘apapane, Hawai‘i ‘amakihi, and

Red-billed Leiothrix captured in Nakula NAR. As such, the results from the kiwikiu samples are consistent with other birds determined to be positive for malaria in the past.

Knowing that these individuals were exposed to malaria prior to being translocated to Nakula NAR raises several important questions. It is clear from the necropsies that all of these birds died of malaria. However, where they contracted the parasites that led to the infection that killed them remains unknown. Was mortality due to recrudescence of a chronic infection from parasites acquired in Hanawī NAR? Or were the birds subsequently reinfected in Nakula NAR leading to a new acute infection? The stress of the translocation on the birds may have played a role in the immune response in either case. Unfortunately, we cannot definitely answer these questions.

Clearly, the fact that these birds tested positive for *P. relictum* prior to their translocation to Nakula NAR indicates that they were infected in Hanawī NAR. Whether this infection was detected in the early stages of an acute infection or was a chronic infection remains unknown. We cannot rule out the possibility that these birds were infected in Hanawī NAR just prior to their capture. Survival time of the known malaria-induced deaths was 24.6 ± 6.5 days (18–32 days) starting from capture date in Hanawī NAR. This is a minimum value as it is likely these individuals were infected prior to the day of capture. This value is well within the time reported for malaria mortality in other honeycreepers. Although we cannot rule out this scenario, it may be more likely that these birds were infected in the more distant past and the positive qPCR results indicated a previous, likely chronic, infection. The fact that all eight of the birds tested positive supports this idea given the low likelihood that all birds would have been infected just prior to capture. We have no way to determine if these birds would have survived this infection in Hanawī NAR if they hadn't been translocated to Nakula NAR. It would be very difficult to find the three males that were released back into Hanawī NAR and impossible to be certain of their absence should we fail to find them. MFBRP resighted one banded male without color bands in December that was very likely one of these three released individuals. If true, this individual survived longer than the translocated individuals. This bird could not be positively identified as he was not color-banded but the chances of resighting another previously banded individual that had removed all of their color bands is extremely unlikely. Another released male (that did have color bands) captured during the October trip was resighted in June 2020. This male was caught later in the trip and was released at the net and unfortunately was not tested for malaria. In addition to this June resight, we visited three locations where kiwikiu were captured in October (see Figure 10) and kiwikiu were still detected in those locations included a juvenile in the same spot. There is no way to know whether these are the same birds from October, but it is likely that many of these were the same individuals detected in October. Additional blood samples from all species captured were collected in June and may shed more light on the current overall prevalence of avian malaria in Hanawī NAR.

One plausible scenario in regard to the outcome of the translocated Hanawī NAR birds in Nakula NAR is that they were suffering from a low-level chronic infection and that the stress of the translocation led to a recrudescence of the malaria infection and then mortality. The necropsies found parasites in multiple organ systems indicating a severe systemic infection that had moved out of the blood stream into other tissues. This could be the result of increased replication of the malaria parasite following some change in the immune system of the birds, such as in response to environmental stress. A second scenario is that the birds were reinfected (possibly multiple times) in Nakula NAR which led to an acute infection and death. The birds from the conservation breeding facility were unlikely to have been previously infected because aviaries are enclosed with mosquito-proof netting, although no blood samples of these birds

were taken prior to moving them to Nakula NAR. Based on the assumption that the birds from the conservation breeding facility were not infected, survival time for these individuals was similar to that of the wild birds. The fact that the birds from the conservation breeding facility were most likely infected in Nakula NAR raises the likelihood that the wild translocated birds were reinfected in Nakula NAR.

The fact that infectivity among mosquitoes was 2.2%, combined with how rapidly some individuals were infected, may suggest that the kiwikiu in Nakula NAR were bitten multiple times over a short time period. Recent evidence suggests *Plasmodium* may be able to manipulate the behavior of their mosquito hosts to increase transmission. For instance, mosquitoes have been shown to be drawn to the scent of previously infected birds (Díaz-Fernández et al. 2020). This may provide one explanation as to why some of the wild birds appeared to be reinfected so rapidly in Nakula NAR. But this does not explain the rapid transmission to the birds from the conservation breeding facility.

There is some evidence that previous malaria infection can lead to a certain level of immunity in honeycreepers. Atkinson et al. (2001b) re-challenged Hawai‘i ‘amakihi that had been previously infected. In this study, seven previously infected individuals that recovered from an initial infection were re-challenged with additional infective bites > 60 days after the initial infection. Of these, only one previously infected bird showed increased parasitemia after the re-challenge and subsequently died. This indicated that the previous infection imparted some immunity and prevented future symptoms in these birds. Although this was the case in this ‘amakihi study, we do not know if previously infected kiwikiu have a greater potential for survival when reinfected.

We also cannot discount the impact that stress may have on the immunological response of these birds to the threat of recrudescence or re-infection of malaria. Perhaps these birds would have been able to withstand another infection if not also stressed by the translocation or already battling an infection. It might also have been the case that the immunity imposed by the previous infection was limited to the strain of *P. relictum* endemic to the windward slope of Haleakalā. Very little is known about the genetic variation in *P. relictum* in Hawai‘i and we do not know if there is indeed differentiation in *P. relictum* populations between the windward and leeward slopes. Atkinson et al. (2001b) did not see any increase in parasitemia among Hawai‘i Island ‘amakihi re-challenged with *P. relictum* derived from sources on Kaua‘i Island. They concluded that either little differentiation existed in *P. relictum* between the islands or the antibodies the birds developed to the Hawai‘i Island strain were sufficient to protect against the Kaua‘i strain. There is also some evidence that the species make-up of the avian community has a role in the spread of avian malaria (McClure et al. 2020). Densities of native and non-native birds vary between Hanawī and Nakula NAR and the windward slope species composition is changing (Judge et al. 2019). However, the largest factor that McClure et al. (2020) found to positively influence malaria infection prevalence was the density of native birds; overall native bird density was higher on the windward slopes. More research is needed on the complicated relationships between species composition, host competence, mosquitoes, and disease transmission and strains.

Regardless of the outcome of the translocation, the positive pre-translocation samples may represent a ray of hope for kiwikiu. If these were in fact chronically infected birds, this may mean the wild kiwikiu population has some tolerance to avian malaria and that a low-level infection is not a death sentence. This could indicate that the species has more time than we thought. Of course, this is tempered by the fact that the translocated individuals experienced high mortality despite the previous infection.

Additionally, recent evidence shows that the kiwikiu range is rapidly contracting uphill and the best explanation for this is expansion of the mosquito range (Judge et al. 2019).

12. Conclusions

Clearly, the outcome of the 2019 kiwikiu reintroduction attempt was not what we anticipated or desired. It is impossible to remain completely objective about the outcome; to pretend otherwise would be disingenuous. This was, in many ways, the culmination of over a decade of work by innumerable people and organizations to save the kiwikiu. The manner and scale of the kiwikiu deaths in Nakula NAR and the ramifications for future conservation leave us with a sense of loss and grief. However, we as a conservation community are no less determined to save the kiwikiu from extinction. The outcome of the 2019 reintroduction attempt has increased our knowledge of the threats to kiwikiu and our resolve in meeting these challenges.

Prior to this project, we knew very little about how avian malaria directly affects kiwikiu. We suspected that the species was intolerant of the disease given their elevation range and laboratory studies with other honeycreepers. But we knew nothing of the pathology of malaria in kiwikiu nor the scale of the threat malaria poses to the species. ‘I‘iwi have long been considered among the most susceptible species based on a laboratory study in which 90% of individuals died after exposure to a single infected mosquito (Atkinson et al. 1995). In Nakula NAR, at least 10 out of 14 kiwikiu died, and all confirmed mortalities were from malaria, indicating that kiwikiu are at least as susceptible to the disease as are ‘i‘iwi. We also now know avian malaria can infect and kill kiwikiu in as few as nine days, meaning even temporary incursions of mosquitoes into upper elevations could quickly overwhelm a kiwikiu population. Further investigation spurred by the reintroduction attempt has led to the discovery that mosquito populations exist at much higher elevations within the kiwikiu range than previously thought (see 11.5. Post-release disease assessment). The current kiwikiu range is shrinking rapidly and if current climate models prove accurate, there may be little or no disease-free habitat for kiwikiu in five to ten years. The fact that translocation candidates captured in Hanawī NAR tested positive for malaria and infected mosquitoes were captured in Hanawī NAR suggests this may have already occurred. Without the kiwikiu reintroduction attempt, we might not have discovered this so soon.

Among the many unknowns prior to this project was whether the current habitat on leeward Haleakalā could support kiwikiu. The species’ success in this habitat would hinge on their adaptability and plasticity in habitat preference. While the released kiwikiu did not survive long enough for us to say the species can persist in the region throughout the year, their behavior was highly encouraging. All translocated birds showed signs of establishing home ranges and foraged successfully without support from supplemental food. We can be confident that future translocated birds would do the same and, without the limitation of disease, the species would likely do well in the Kahikinui region. The more time that passes will allow for additional habitat recovery that will improve the chances of a successful reintroduction.

Despite the outcome, we gained invaluable insight into the behavior of kiwikiu and the disease ecology in this species through the 2019 translocation attempt. We were able to successfully care for kiwikiu in temporary facilities and structures, transport them via helicopter, attach transmitters and track individuals, and successfully translocate them to a new area. Moving forward, this report and the valuable knowledge that we gained in this attempt will be vital as we recover the species in other ways.

Furthermore, we have a much clearer sense of the scale of the threat malaria poses, both in magnitude and time. Without being able to establish the species in new disease-free habitat and knowing the disease is present in the wild population means that the focus of conservation efforts for kiwikiu should be targeted on the control of avian malaria. Nothing else poses a greater threat to the species and failure to control this disease would mean failure to save kiwikiu from extinction. Fortunately, new tools are becoming available and supporting the development and deployment of these tools is a primary focus for those working to conserve kiwikiu and other honeycreepers.

Since the translocation, the Maui Forest Bird Working Group has re-evaluated recovery options for this species and outlined five key next steps. These actions will need to be swift and concerted in order to save this species. These steps include:

1. Bring birds into captivity with the intent to maintain and/or produce release-quality birds and release birds as soon as a suitable release site(s) can be identified and managed.

    Managed care propagation of kiwikiu has had only limited success without significant and robust breeding success over many years. This species has a challenging life history that requires an adaptive management plan to encourage pair banding and copulation and to avoid juveniles imprinting on humans, so as to produce individuals that can survive in the wild. New partners willing and able to take on the challenges presented with this species are needed. Capture and captive holding of kiwikiu can accomplish two main tasks: 1) keeping enough individuals alive and safe from disease to prevent the extinction of the species, and 2) providing additional individuals for eventual release if rearing techniques can be refined and improved. We may have a very short window of time before a large segment of the current population is swamped by a higher prevalence of mosquitoes and disease, thus losing those individuals and any conservation options for that part of the population.

2. Ensure landscape-scale vector control remains a high-priority for both agencies and is expedited for implementation.

    As the recent translocation efforts for kiwikiu made clear, avian malaria spread by nonnative mosquitoes represents a significant threat to the persistence of the species. The changing climate may allow for expansion of both the mosquito vectors and the malaria parasite. Larval control treatment is not a viable strategy. Mosquito dispersal and the relationship of abundance to breeding habitat is unknown. Landscape-scale control of mosquitoes has been a key management concern and significant resources have been put toward the effort already. The current strategy being pursued is an incompatible insect technique using different strains of a locally-sourced bacteria, *Wolbachia*, to eradicate mosquitoes over large areas. Another potential technique includes the use of *Wolbachia* not to eradicate mosquitoes, but to prevent them from serving as vectors for malaria. This is currently being researched simultaneously, although it will have a longer regulatory path. Finally, additional techniques using more advanced methods (e.g. gene drive and genetic modification technology) may also be useful in the future, but many of these are still in the early stages of development. The focus for now is on development and deployment of the *Wolbachia* cytoplasmic incompatibility technique for mosquito suppression.

3. Develop strategies and evaluate the feasibility of short- and long-term predator control tools to protect the wild population, including broader landscape-level methods for each predator species.

   Predator control strategies are well-developed for local control of rats and other predators and have been implemented successfully in multiple areas of the existing kiwikiu range (although there are no active predator control sites within the kiwikiu range currently). On-the-ground predator control is both labor intensive and requires some adverse impacts to the forests. However, the level of threat that depredation poses and the effectiveness of this action to produce population-level benefits is unknown. Nevertheless, given the life history of kiwikiu, saving even a few birds may be disproportionally important, given the small numbers of surviving individuals.

4. Continue and increase habitat management and restoration in the current and historical kiwikiu range to ensure habitat is available into the changing future.

   Continuing to maintain current habitat in TNC's Waikamoi Preserve, Hanawī NAR, and Haleakalā National Park is imperative to preserving the kiwikiu. Among the most pressing needs in these areas are the removal of any ungulates that have reinvaded fenced units (e.g. Kīpahulu), controlling habitat-altering invasive weeds, and restoration of denuded areas within fenced units. Future habitat can also be improved and expanded through restoration efforts. Nakula NAR and other properties on leeward Haleakalā may still provide important range expansion opportunities if disease can be managed. Continuing efforts to remove ungulates and planting native tree and shrubs will provide a future release site on the south slope of Haleakalā. Given the relative low elevation of the Nakula NAR release site, the steep slope, and the limited opportunities for expansion of the forest upslope, priority for forest bird-focused habitat restoration needs to be expanded beyond this one site, shifting to the west slope of Haleakalā as well. Finalization of the purchase of Kamehamenui by the State of Hawai'i and implementation of reforestation activities on this property would provide habitat for forest birds within 5–10 years and provide connectivity between the windward populations in Waikamoi and populations in Kula Forest Reserve and restored forests on leeward Haleakalā. Restoration back to native dominated forests of existing State lands, including Kula Forest Reserve, would also have positive impacts for forest birds.

5. Assess potential translocation sites on the island of Hawai'i for habitat suitability and impacts to or from other species.

   Hawai'i Island currently contains the most expansive high elevation forests and could provide needed refugia to kiwikiu. Since the kiwikiu extinction is predicted to be possible within a shorter time frame than that of implementation of the *Wolbachia*-incompatibility method on Maui, and the subsequent disease suppression, establishing the species in additional high-elevation forest may provide additional opportunities to save individual kiwikiu. The expansive forests above 6000 feet in elevation and the more gradual slopes on Hawai'i Island may provide

areas where kiwikiu can persist beyond what is possible on Maui. However, it is unclear whether disease rates in this area will remain low into the future.

We remain hopeful that this species can be saved and may soon be thriving on both the windward and leeward slopes of Haleakalā.

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14. Appendices

Kiwikiu Reintroduction Plan

August 2019

The Maui Forest Bird Working Group

American Bird Conservancy, Haleakalā National Park,

Leeward Haleakalā Watershed Restoration Partnership,

Maui Forest Bird Recovery Project, National Park Service Inventory and Monitoring,

Pacific Bird Conservation, Pacific Cooperative Studies Unit, San Diego Zoo Global,

State of Hawai'i Division of Land and Natural Resources – Forestry and Wildlife,

State of Hawai'i Division of Land and Natural Resources – Native Ecosystems Protection and

Management, The Nature Conservancy of Hawai'i, US Fish and Wildlife Service,

US Geological Survey – Biological Resources Division

Kiwikiu (Maui Parrotbill) Reintroduction Plan

Table of Contents

Table of Figures

Table of Table

Executive Summary

Kiwikiu (Maui Parrotbill; *Pseudonestor xanthophrys*) are among the rarest and most endangered Hawaiian passerine. Recent population assessments estimate total abundance of less than 312 individuals that occupy approximately 30 km^2. The species in its current range continues to be under threat from invasive mammalian predators and non-native disease. Kiwikiu are specialized insectivores that occupy large home ranges within intact native forest. Likely as a result of this specialization, pairs produce one young each year and offspring stay with the parents for an extended period, up to 18 months. Given low natural productivity, the species relies on adult survivorship and adults live upwards of 16 years in the wild; particularly long for a small songbird. Nest failures are often attributed to predators and heavy rain events, a common feature of the wet, windward forests that they currently occupy. For these reasons the US Fish & Wildlife Service has recommended establishing a second population on the leeward (or southwestern) slope of Haleakalā to increase total population size and protect the species from severe weather events or other catastrophic loss in their small current range. Nakula Natural Area Reserve (NAR) in the Kahikinui region of Maui was selected as the site of the first experimental releases of Kiwikiu to begin establishing a second population. The forest on the leeward (south-facing) slopes of Haleakalā, where Nakula is located, generally exists in a deteriorated state as a result of a century of browsing and grazing damage from non-native ungulates. However, large, intact forest sections remain and the vast majority of this habitat is now either fenced and protected, or will be shortly. Following fencing and eradication of ungulates, the forest in this area has begun to recover through natural regeneration and conservation restoration efforts. This forest is naturally dominated by koa (*Acacia koa*) and ōhiʻa (*Metrosideros polymorpha*) and was likely always a more open habitat than the forest that Kiwikiu currently occupy. Kiwikiu are now restricted to wet forest on the windward slopes where the canopy is almost exclusively ōhiʻa. When originally described to the scientific community, the species was thought to prefer koa as a foraging substrate. It is possible that Kiwikiu, once established, will do well in a habitat that sees fewer heavy rain events and where koa is a dominant tree.

Herein we propose to begin the process of establishing the species in Kahikinui and lay out the procedures for the first year of Kiwikiu releases in Nakula NAR. We propose to release all of the suitable captive individuals (8) if these individuals pass a pre-release exam, from the San Diego Zoo Global's facilities as well as translocate an additional 12 individuals from the current range. The Nature Conservancy's (TNC) Waikamoi Preserve is the preferred site to capture and translocate wild individuals due to its habitat similarities with Nakula NAR, Kiwikiu population genetics data,

and logistical considerations. This site lies at the western edge of the species' range and contains a genetically distinct population of Kiwikiu. All captive individuals originate from the eastern genetic sub-population. Thus, combining birds from both sub-populations will maximize genetic diversity in the new population. TNC Waikamoi Preserve is also one of the few places in the current Kiwikiu range that contains some koa and thus, translocated birds may be more familiar with this type of forest. Although most of the Maui Forest Bird Working Group preferred TNC Waikamoi Preserve for the first year, permission was not granted by TNC. Therefore, Hanawi NAR will be serving as the first year's source site with Waikamoi Preserve being the possible second year's source site. We propose a soft release in which birds will be housed in temporary field aviaries and provisioned with food within and outside the aviaries. Released birds will be carefully monitored through the use of radio transmitters and color-band resighting to evaluate foraging and breeding behaviors as well as monitor movements within and outside of the release site. This is the first step of a multi-year effort to implement actions explicitly identified in the USFWS species recovery plan to re-establish a population on southern Haleakalā. Following the first year of experimental releases, the results will be evaluated to determine if, and in what ways, additional releases should be conducted. The short term goal of this project is to create a disjunct population of Kiwikiu that survives multiple years. The ultimate goal is to establish a self-sustaining population of Kiwikiu in Kahikinui.

1. Background

1.1 Natural History

Population abundance and historical range

As is the case for nearly all extant native Hawaiian bird species, Kiwikiu or Maui Parrotbill (*Pseudonestor xanthophrys*) have undergone a significant reduction in range and population size since human contact with the Hawaiian Islands. Although apparently never among the most common of Hawaiian passerine species, subfossil evidence shows that Kiwikiu formerly occupied a large proportion of the islands of Maui and Molokaʻi (James *et al.* 1987, James and Olson 1991, Simon *et al.* 1997). Some of these subfossils have been found down to 200 m in elevation in the Kahikinui region (H. James, *pers. comm.*, in Mountainspring 1987) and the fossil sites on Molokaʻi are coastal dunes (James and Olsen 1991a). This historic range covered multiple forested habitat types from high elevation wet forests to lowland dry forests. No modern observations were made of the species outside of its current range, but Maui was historically under-sampled by early naturalists (Munro 1944). All historic specimens come from a single area of forest at Ukulele near present day The Nature Conservancy's (TNC) Waikamoi Preserve. Observations made in the late 19th century indicated a preference for koa (*Acacia koa*) trees as a foraging substrate in the limited areas that Kiwikiu were historically observed (Perkins 1903). Thus, although not present in large numbers throughout the current range, koa trees may have played an important role in the historical distribution of Kiwikiu.

The current range of the species may be in large part an artifact of the extent of the last remaining large tracts of high elevation native forest instead of a result of forest preference. Wide-scale deforestation for agriculture and livestock grazing has reduced the amount of forest cover on the island of Maui to a fraction of prehistoric levels. The subsequent addition of invasive plant and animal species further eroded the extent of native forest and reduced forest quality throughout the island. Furthermore, introduced avian diseases restrict Kiwikiu to forests above 1400 m in elevation, where disease prevalence is comparatively low. As such, the current range of the species is constrained by the combination of the distribution of high quality native forest and disease prevalence. These factors have resulted in a species range of approximately 30 km^2 on Maui; the species was extirpated on Molokaʻi. The current range of the species is located on the windward slopes of Haleakalā Volcano from TNC Waikamoi Preserve in the west to the Manawainui Planeze in Haleakalā National Park (NP) in the east (Figure 1).

The locally rare, low-density nature of the current Kiwikiu population has confounded efforts to accurately estimate population size since the initial population assessments were attempted by the Hawai'i Forest Bird Surveys (HFBS) in the early 1980s. Unlike many sympatric species, Kiwikiu behaviors and vocalizations are rather inconspicuous, adding to the difficulty in detecting the species using auditory-based surveys. As a result, precision of population estimates have historically been low and estimates have tended to include large confidence intervals. Range-wide estimates of population size that have been conducted in recent decades include Scott *et al.* (1986) which estimated the total population size at 502 ± 230 (95% CI) individuals. More recently, Camp *et al.* (2009) estimated the population at 590 ± 208 individuals as of 2001. Intensive point count surveys specifically targeting the species conducted between 2006 and 2011 throughout the Kiwikiu range, but excluding the area within Haleakalā NP, found similar densities to the 2001 range-wide estimates and from 209–674 birds (point estimate = 421) in 2011 (Brinck *et al.* 2012). Judge *et al.* (2013) surveyed the Haleakalā section the following year, and although there were only eight detections, the estimated population was 495 ± 261 birds within the NP. Although estimates have varied, the large confidence intervals associated with these estimates prohibit conclusions about long-term trends in abundance of the species (Camp *et al.* 2009, Brinck *et al.* 2011).

Maui Forest Bird Recovery Project (MFBRP) and The National Park Service Inventory and Monitoring Program coordinated the most recent range-wide surveys in 2017. Out of the 27 transects surveyed, 11 transects were surveyed within the Kiwikiu range and these legacy transects have information from previous surveys dating back to the original surveys in 1980. Using survey data from the last 15 years the survey coordinators also delineated a more accurate current Kiwikiu range, eliminating several areas where the species was not known to exist or had not been documented for decades despite good coverage from surveys. The recent range-wide study estimated Kiwikiu abundance at between 44 – 312 (95% CI; mean 157) individuals (Figure 2). While alarming, abundance estimates for the species have historically shown significant variability and the low precision of each estimate should not be ignored. However, realistically the overall abundance is fewer than 312, and the population may be declining, though the variance in the estimates precludes finding a significant statistical trend (Judge *et al. In prep*).

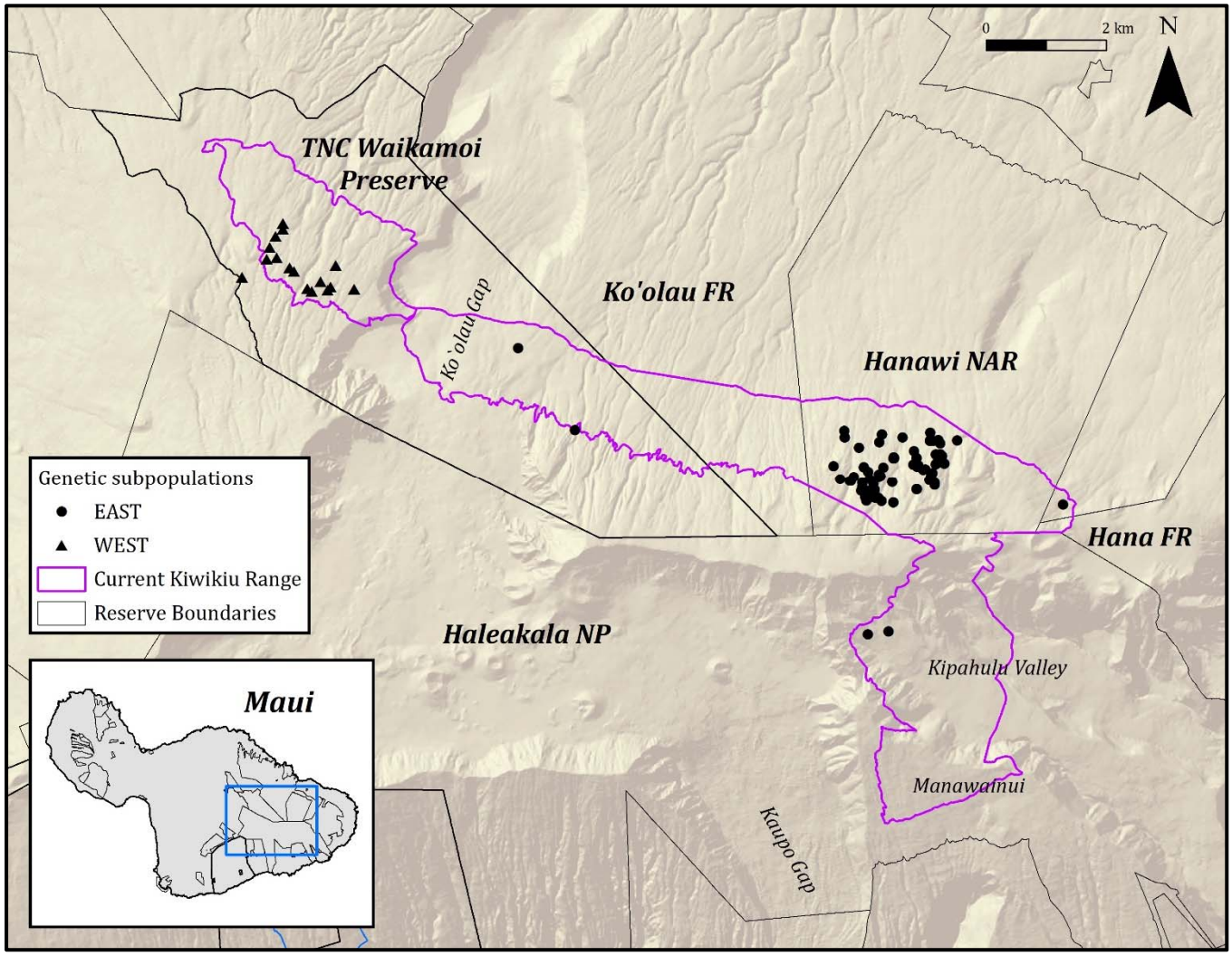


Figure 1. Current Kiwikiu (Maui Parrotbill; *Pseudonestor xanthophrys*) species range (Total area = 29.92 km^2) and land management areas. Also shown are the genetic sampling locations, including showing collection sites of initial captive individuals (east). Subpopulations, east and west, are based on analysis of genetic population structure by Mounce *et al.* (2015).

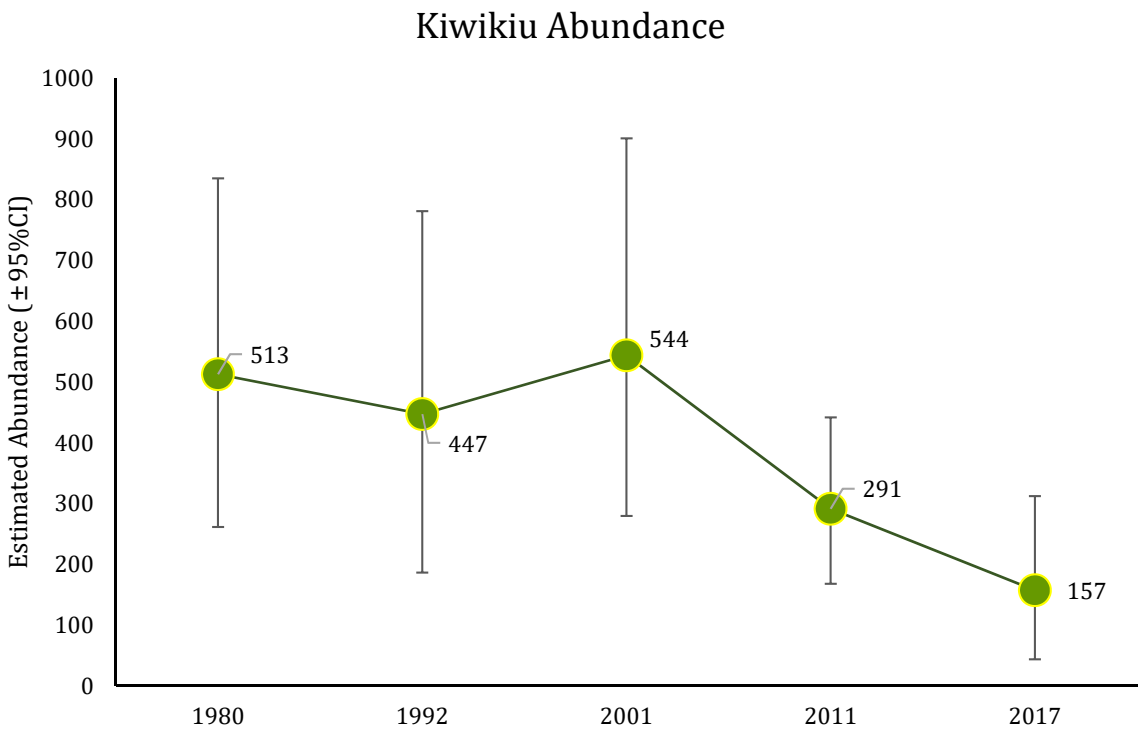


Figure 2. Estimated Kiwikiu abundance from 1980 to 2017 from the Hawai'i Forest Bird Surveys (HFBS). Estimates are presented (± 95% CI) from the five years where the entire species range was surveyed based on the current Kiwikiu range (29.92 km^2).

Threats and limiting factors

The long-term persistence of Kiwikiu is threatened by a wide variety of factors. Like many other native forest bird species, Kiwikiu are primarily threatened by non-native organisms, loss and/or alteration of habitat, and climate change. However, several specific traits put Kiwikiu at greater risk of extinction than other species. Kiwikiu have rarely been observed outside of pristine native forest and the only nests ever discovered have been in ʻōhiʻa (*Metrosideros polymorpha*), the dominant tree species within its current range. This apparent intolerance of non-native vegetation restricts the species to only those areas with contiguous native forest, now nominally windward Haleakalā. With the exception of the endangered ʻĀkohekohe (*Palmeria dolei*), all other extant sympatric Hawaiian finches on Maui tolerate non-native and/or mixed native-non-native forests to a certain extent (Motyka 2016). In these areas, these species often utilize ecologically similar non-native plant species to those used in fully native plant communities (e.g., ʻApapane [*Himatione sanguinea*] and ʻIʻiwi [*Drepanis coccinea*] forage on non-native *Acacia* spp. as well as the native koa). However, this kind of resource replacement behavior has not been observed for Kiwikiu. The species also

relies on many understory and subcanopy plant species for forage substrates (i.e., hosts for insect larvae prey) (Mountainspring 1987, Stein 2007). Typically, the understory and subcanopy layers of native Hawaiian forests shows the greatest damage following invasion of feral pigs and other ungulates thereby reducing or eliminating critical components of the forest for Kiwikiu (Pratt and Jacobi 2009). Seed predation of understory plants by rodents may also further reduce forest quality for Kiwikiu.

Several demographic and behavioral traits also put the species at risk. Similar to other native species, Kiwikiu are at risk of predation by invasive small Indian mongooses (*Herpestes palustris*), feral cats (*Felis silvestris catus*), and rats (*Rattus spp.*). The species' habit of foraging in the understory or shrub layer of the forest, closer to the forest floor, may increase this risk. Kiwikiu are also a long-lived species that typically lay single-egg clutches and have a long juvenile dependency period (Simon *et al.* 1997). Such low natural recruitment means that replacement of an individual due to loss from predation or disease is more difficult and the loss to the whole population is perhaps greater than in a more fecund species. Kiwikiu are vulnerable to predation of females on the nest by mammalian predators and this may explain the lower estimated female annual survivorship compared to males (Mounce *et al.* 2014).

Despite the fact that native forest exists at low elevation in places, Kiwikiu are only found above 1400 m. This represents evidence of the species' apparent lack of resistance to introduced avian diseases, principally avian malaria (*Plasmodium relictum*). The primary vector for avian malaria in Hawai'i, the southern house mosquito (*Culex quinquefasciatus*), is unable to persist and breed in high densities at high elevations due to unfavorable environmental conditions, i.e., low temperatures (Warner 1968, van Riper *et al.* 1986, Atkinson and LaPointe 2009). The *Plasmodium* parasite itself also has environmental tolerance levels, e.g., temperature, beyond which they cannot develop inside the mosquito host (LaPointe 2000). Generally, areas below 1200 m in elevation have been shown to have high transmission rates of avian malaria and often cited as the so-called "mosquito line" (Atkinson and Samuel 2010). In reality, temperature affects both vector densities and transmission rates of *Plasmodium* and cooler high-elevations rarely exclude the disease entirely (Atkinson *et al.* 2005, Samuel *et al.* 2015). Thus, upper elevations may protect species, like Kiwikiu, by reducing the risk of infection to the point that the bird species can persist rather than eliminating the risk entirely. The fact that Kiwikiu and 'Ākohekohe only persist at higher elevations, bottoming out closer to 1400 m may indicate that these species are particularly sensitive to the

disease. Any Kiwikiu that moves down in elevation into areas that support high densities of mosquitoes and promote greater development of the *Plasmodium* parasite inside vectors are at significant risk of contracting the fatal disease. The elevational distribution of avian malaria may change throughout the year when wetter, warmer conditions during certain seasons allow mosquitoes and parasites to proliferate at higher elevations only to decline at other times when conditions are less ideal. The area (e.g., elevation) where disease prevalence reaches unacceptable rates for a given bird species may be better modelled as a fluid "zone" rather than a rigid "line". As temperatures rise due to global climate change, the disease zone is also predicted to rise in elevation, thereby reducing the amount of suitable habitat for Kiwikiu even further. In addition, Kiwikiu nests have been shown to fail most commonly following severe weather events (Simon *et al.* 2000, Becker *et al.* 2010). As global temperatures rise, the frequency and intensity of major weather events may increase in Hawai'i as is predicted elsewhere (Emanuel 2005, Knutson *et al.* 2010). Such disturbances could have catastrophic effects on the single extant population of Kiwikiu either through reduced reproductive success (e.g., nest failure) or habitat loss.

Ecology

Productivity

Pairs of Kiwikiu typically remain together throughout the year and may attempt to breed whenever favorable conditions present themselves (Simon *et al.* 2000). Active nests have been found in all months except September (MFBRP unpublished data). However, the majority of pairs successfully breed between January and June each year (Mounce *et al.* 2013). Females lay single-egg clutches and males provision females on the nest (Simon *et al.* 2000). Offspring may be provisioned with food by the parents for up to 18 months after fledging. This extended parental investment may explain why only one offspring is typically produced per year; although two fledglings have been observed with pairs on very rare occasions (Baker and Baker 1997, Simon *et al.* 2000, MFBRP unpublished data).

The low-density and cryptic nature of the species has largely prohibited precise estimation of productivity from nests alone (Mounce *et al.* 2013). However, because Kiwikiu typically lay only single-egg clutches and re-nest only after failure, observing a pair with a single offspring is enough to indicate that a pair was successful during a given breeding season. Annual reproductive success can then be estimated using the proportion of successful pairs each year. Using this method, annual reproductive success was estimated to be 46% in Hanawi Natural Area Reserve (NAR) from 2008-

2011 (Mounce *et al.* 2013) and 40% in TNC Waikamoi Preserve from 2011-2014 (MFBRP unpublished data).

Habitat use (diet, foraging behavior, and home range size)

Kiwikiu are insectivorous and forage in a unique way among Hawaiian finches. They use their large parrot-like bill to extract insect larvae from wood and fruits. The Kiwikiu bill is laterally compressed and capable of reaching larvae in narrow openings and cavities. The large bill and strong jaw muscles of the Kiwikiu are used to remove strips of bark and other woody tissue in search of larvae inside. For small stems, Kiwikiu hook their culmen over the branch and cut out strips of woody material with their sharp mandible to expose larvae inside. Insects may also be gleaned from plant surfaces. Plant species with soft wood and/or hollow pith seem to be particularly favored, e.g., ākala (*Rubus hawaiiensis*). Larvae are also often extracted from the fleshy fruits of several understory species, primarily kanawao (*Broussasia arguta*), kōlea (*Myrsine spp.*), pilo (*Coprosma spp.*), and ʻōhelo (*Vaccinium spp.*). Kiwikiu are often observed carefully prodding or squeezing these fruits, presumably feeling for parasitic larvae inside. When a suitable fruit is discovered, the fruit is often cut in half, the larva is removed, and the fruit is discarded. The presence of Kiwikiu in an area can often be determined by bite or testing marks on ripe kanawao berries. Kiwikiu may also eat some small fruits, e.g., pilo, although little fruit consumption is observed in the wild. Some nectar may also be taken either by tongue probing exposed flowers, e.g., ʻōhiʻa, or biting closed flowers, e.g., ʻōhelo. Diet studies show Lepidoptera larvae make up the majority of the Kiwikiu diet as well as Coleoptera larvae to a lesser extent (Peck *et al.* 2015).

Home range size for individual Kiwikiu is estimated to be ~ 9 ha on average (9.29 ± 1.29 ha or 9.63 ha ± 1.51 ha [± SE] depending on the technique used) and no difference was found between sexes (Warren *et al.* 2015). These estimates were based on resighting data of color-banded individuals in Hanawi NAR and TNC Waikamoi Preserve from 2007-2014. Home ranges were found to be > 50% larger in Waikamoi than Hanawi. A fair amount of variation in home range size was also observed during this time period with home ranges varying between ~ 1 ha and 31 ha in size. Some of this variation can be attributed to the number of times each individual was resighted in a given year, e.g., the smallest home ranges may have been under-sampled individuals. The largest home ranges may be attributed to birds that shifted home ranges during the breeding season. Average pair home range size, i.e., the combined male, female and shared home range, was estimated to be 13.9 ± 10.5 ha or 17.8 ± 12.3 ha depending on estimation technique. Due to the fact that Kiwikiu remain paired throughout the year, an estimate of pair home range size may be a more appropriate metric for

space and habitat use than an estimate of home range for an individual. Pair home range size could also be more relevant in determining the number of breeding pairs the reintroduction area (i.e., Nakula) can support, and estimating carrying capacity of the region. The dataset currently available did not allow for estimation of variation in home range size based on age classes. However, subadult birds likely occupy a larger area than adults with established home ranges (Warren *et al.* 2015). The establishment of a home range and initial pairing has been observed in second-year birds although little information exists on movement patterns prior to this period (MFBRP unpublished data).

Dispersal, movement, survival

Juvenile dispersal remains one of the major unanswered questions regarding demographics of Kiwikiu. Hatch-year birds are difficult to capture and only a small number (15 out of 232 banded Kiwikiu) have ever been banded and only two of these have been resighted beyond their natal year (dispersing a maximum of 2.5 km). However, unbanded second-year birds are encountered regularly during productivity surveys in Hanawi NAR and TNC Waikamoi Preserve. When known pairs are not observed in a given year, i.e., a home range becomes vacant, the area is usually quickly recolonized either by second-year or adult birds by the next breeding season (MFBRP unpublished data). The origins of the new individuals in these cases are often not known. These individuals could be moving into the area from an unknown distance outside the study area or may have been "floating" in the area waiting for a space suitable for home range/territory establishment to become available. Downhill dispersal of juveniles is a potential source of mortality if individuals move into areas where they are under greater threat from avian malaria. Anecdotally, MFBRP banded one second-year bird in Hanawi NAR and subsequently resighted this individual a few months later constructing a nest about 1.5 km away from the capture location. Other second-year birds banded in Hanawi NAR and TNC Waikamoi Preserve have also established home ranges near their banding location. This represents our best current information on second-year bird dispersal and home range establishment. Adult dispersal has been documented in a number of cases within a study site wherein adults shift home range areas within or among years (MFBRP unpublished data). In a few cases pairs shifted home ranges ≥ 1 km following nest failure. Therefore, "missing" known pairs between study years may be the result of adult dispersal rather than mortality as is often assumed in other studies.

Due to the species' cryptic nature, the resight data from productivity surveys conducted by MFBRP typically have not resulted in enough observations per individual to estimate within-year movement patterns. However, behavioral observations suggest that variation in home range size likely occurs throughout the year. As a pair is not tied to a nest site during non-breeding times, a pair's collective home range may be largest outside of the breeding season (typically the fall and winter months). As breeding activities begin, males may defend a territory, a subset of their home range presumably surrounding the nest site. During this time, some males can be found singing from specific perches on a semi-regular basis as he makes his rounds defending the territory (Simon *et al.* 2000, Baker and Baker 1997, MFBRP unpublished data). During the incubation and nestling stages both parents are more reliably found in the area immediately surrounding the nest than at other times (Becker *et al.* 2010). In this period, males often provision females on or near the nest on a regular basis throughout the day, e.g., once per hour. He then may follow a somewhat predictable path to and from the nest. Females may only forage in a small area immediately surrounding the nest site. This period may mark the time when pair home ranges are smallest. After fledging, the hatch-year is provisioned regularly by one or both parents for several months. During this time, known pairs and associated hatch-years are often found well outside the area where they had been found previously (MFBRP unpublished data). Presumably, during this time territory defense has been energetically replaced by offspring care and birds wander wherever resources allow. Non-breeding individuals may also follow regular routes particularly when timing of resources is important, e.g., ripening of kanawao berries. These routes may then vary throughout the year with phenology of various plant species.

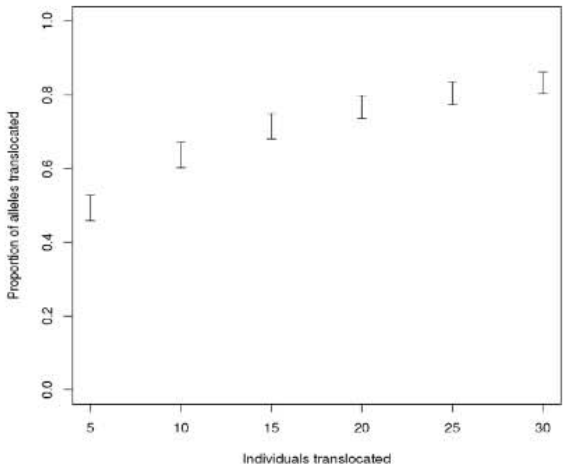
Kiwikiu are long-lived passerines capable of living ≥ 16 years in the wild (Mounce *et al.* 2012). Annual survivorship rates in Hanawi NAR was estimated to be high and vary by sex with 0.82 for males and 0.72 for females (overall adult survivorship = 0.78) (Mounce *et al.* 2014). This difference may be attributed to the risks and costs associated with nesting, e.g., predation by non-native mammals. Juvenile annual survivorship was estimated at 0.17 but a small sample size of juveniles (n = 10) combined with limited survey coverage across the breadth of the potential dispersal range limits confidence in this estimate (Mounce *et al.* 2014). Thus, juvenile survivorship remains a relatively unknown demographic variable.

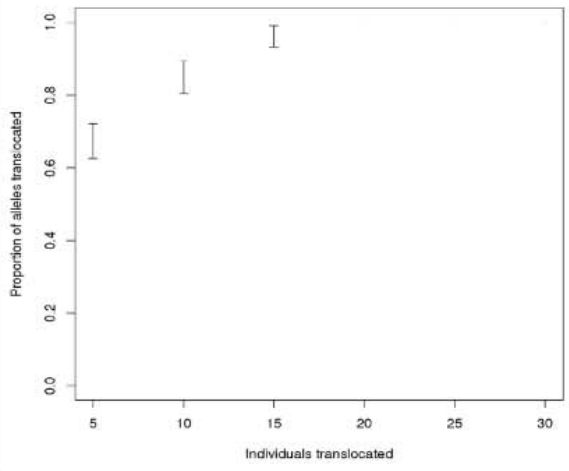
Genetics

Concerns about low genetic diversity arise with any organism that has undergone significant range and population size reduction. Knowledge of the current genetic diversity and structure is important when designing a reintroduction strategy to maximize the potential for long term success of the new population. Genetic analysis comparing historic and contemporary samples showed a 96% reduction in genetic effective population size and current genetic diversity to be low (global F_{ST} = 0.056) (Mounce *et al.* 2015). This is not unexpected for a species that has reduced dispersal opportunities due to habitat limitations and disease distribution. Increasing overall genetic diversity likely requires a larger population and/or increased metapopulation structure. Reintroducing the species to leeward Haleakalā may accomplish both goals provided the released group contains the maximum amount of genetic diversity that can be feasibly captured (see below).

Although small, the current ~ 30 km^2 range of Kiwikiu runs ≥ 20 km around the northwestern rim of Haleakalā volcano. Within this span several large topographic features exist that have the potential to limit gene flow and influence the genetic structure of the species as a whole. Analysis of contemporary samples indicates that genetic structure is influenced by the Koʻolau Gap showing that individuals in TNC Waikamoi Preserve west of the Gap, represent a genetically distinct subpopulation from those to the east (Mounce *et al.* 2015; Figure 1). The eastern subpopulation showed higher levels of overall genetic diversity and allele privatization than the western population. Mounce *et al.* (2015) estimated that a random capture of 25 individuals from the east would ensure the inclusion of 80% of the genetic diversity (Figure 3). Ten individuals would capture the equivalent genetic diversity from the west. A random selection of 30 individuals from across the species' entire range would capture 80% of the total contemporary genetic diversity, 60 individuals would capture 90% and 105 individuals would have to be selected to capture 100% of the genetic diversity. In order to capture the maximum amount of diversity from the current population, the reintroduction group should be comprised of individuals from both the east and west genetic subpopulations. Captive individuals are all descended from the eastern population and should be considered as part of this group when considering their impact on the overall genetic diversity of the release group. Comparatively few individuals from Haleakalā NP were included in this analysis but the relative distance and the presence of several significant topographic features suggest the possibility of further genetic structure particularly in individuals from the southwestern edge of the species' range in the Manawainui Planeze.

East Population			West Population			Wild Population		
No. individuals translocated	Mean % of alleles captured	SD	No. individuals translocated	Mean % of alleles captured	SD	No. individuals translocated	Mean % of alleles captured	SD
5	0.493	0.034	5	0.674	0.048	5	0.467	0.037
10	0.638	0.035	10	0.850	0.045	25	0.773	0.032
15	0.716	0.035	15	0.962	0.029	45	0.866	0.027
20	0.767	0.032	20	1.000	0.000	70	0.930	0.023
25	0.804	0.031	25	1.000	0.000	90	0.968	0.017
30	0.834	0.029	30	1.000	0.000	120	1.000	0.000





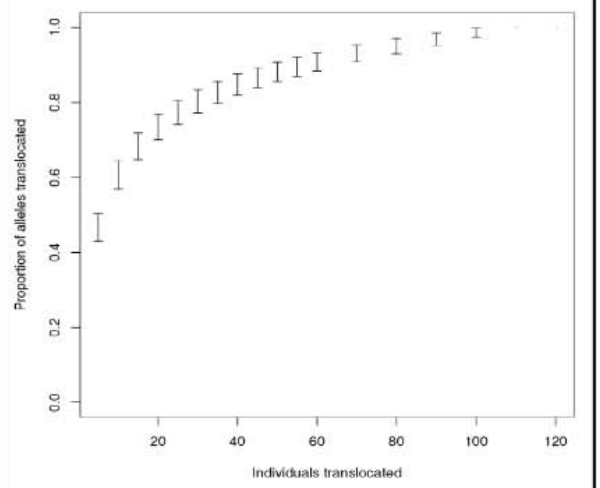


Figure 3. Probability values for capturing different percentages of the total genetic diversity available in the east, west and total wild Kiwikiu (Maui Parrotbill; *Pseudonestor xanthophrys*) population modelled while using different numbers of individuals in translocation efforts.

1.2 Conservation Breeding

Historical overview of conservation breeding flock

Conservation breeding efforts for Kiwikiu was initiated in 1997 and the genetic founders of the current flock were collected from the Hanawi NAR part of the eastern subpopulation in 1999, 2001, and 2005 (Figure 1). The current conservation breeding population was derived from six genetic founders (Figure 4). The mean lifespan of the deceased captive birds, both founders and descendants, is 6.86 years (5.43 SD, range = 0.26-16.36, n = 12), excluding three birds that died as nestlings (SDZG unpublished data). All conservation breeding efforts have been conducted by the San Diego Zoo Global (SDZG) staff at facilities on Maui and Hawai'i Islands.

Current overview of conservation breeding flock

As of January 2019, the conservation breeding flock consisted of seven males and two females (Table 1). Current breeding potential in captivity is limited by the relatively small number and old age of the females. The two females currently in captivity are non-reproductive (Table 1). In December 2015, a female was brought in from TNC Waikamoi Preserve, to provide an additional breeding pair. Unfortunately, this individual died in October 2016 and, as she was the only captive bird from the western genetic subpopulation and did not breed in captivity, the captive population now only contains birds with genes from the eastern subpopulation. At present there are eight captive birds (Table 1) that are suitable for release, although this may change if any birds do not pass the pre-release exam (see Section 2.5 for more details).

Future potential for conservation breeding

Reproduction in captivity has been relatively unsuccessful and inconsistent since the first full Kiwikiu breeding season in 2000, in large part due to the small number of birds (and thus breeding pairs) in captivity during this period (Figure 4). Only three Kiwikiu breeding pairs have produced more than two offspring in total over the history of the captive breeding program, and two of the three breeding pairs consisted of the same female (SDZG unpublished data). Various techniques such as increasing protein in the diet during the breeding season, adding carotenoids to the diet for more natural plumage coloration, and providing insects using new distribution methods have been implemented, but conclusions have been difficult to determine due to the small number of breeding pairs in captivity. Furthermore, the high intelligence and unique life history characteristics (e.g., one

egg per clutch and long dependency periods) of Kiwikiu may also contribute to the relatively low reproductive success within the conservation breeding program

Table 1. Current Kiwikiu from breeding facilities and ages as of November 2019.

Studbook	Sex	Founder/ Descendant	Hatch Date	Age	Reproductive history
MP009	Female	Founder	6/12/2001	18 yrs	Never laid an egg in captivity.
MP015	Female	Descendant	3/5/2005	14 yrs	Unreleasable, becomes egg bound
MP017	Male	Founder	1/1/2005*	14 yrs	-
MP018	Male	Founder	1/1/2005*	14 yrs	-
MP022	Male	Descendant	3/2/2012	7 yrs	-
MP023	Male	Descendant	3/2/2012	7 yrs	-
MP024	Male	Descendant	4/2/2012	7 yrs	-
MP026	Male	Descendant	4/15/2013	6 yrs	-
MP027	Male	Descendant	3/23/2014	5 yrs	-

*Estimated hatch date. Adult bird collected from the wild.
Note: in order to be released, each bird will need to pass a physical exam conducted by a SDZG veterinarian prior to release.

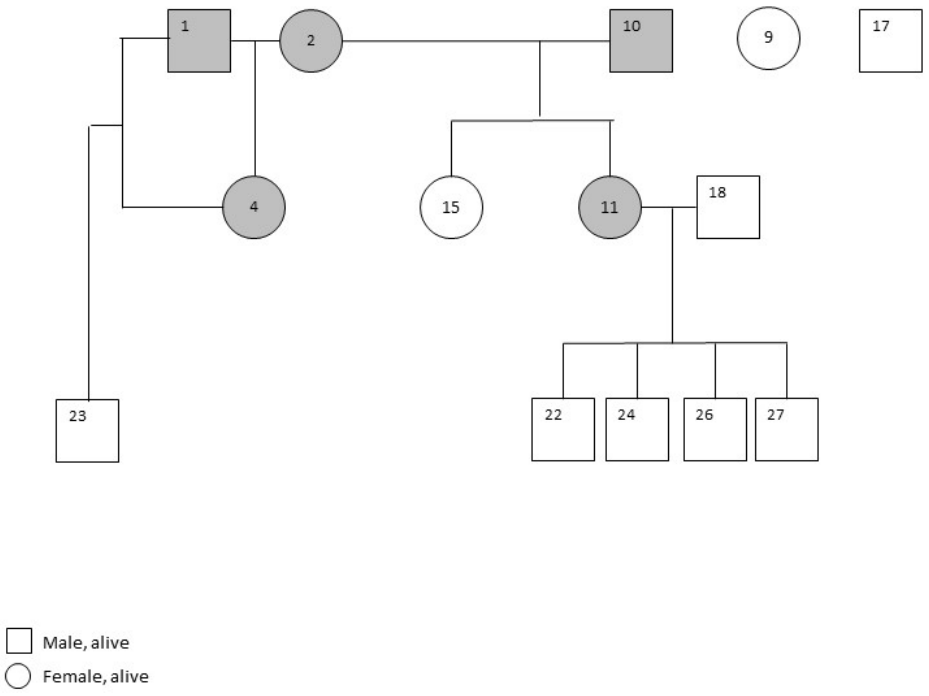


Figure 4. Pedigree of current Kiwikiu conservation breeding population as of June 2018, including alive birds (white shapes), as well as dead birds (gray shapes) that have descendants within the current population. Studbook numbers are indicated within each shape.

1.3 Study Sites

Kiwikiu current range

The current range of Kiwikiu is approximately 30 km^2 from observations made during the HFBS. This range falls exclusively within Haleakalā NP, Hanawi NAR, Koʻolau and Hāna Forest Reserves, and TNC Waikamoi Preserve (Figure 1).

Haleakalā National Park

At 134.62 km^2 Haleakalā National Park (NP) is the single largest unit of conservation land on Maui. The majority of the park is enclosed by ungulate management fencing and is managed for ungulates. The Kīpahulu Valley Biological Reserve in the eastern portion of the park is

characterized mainly by native wet forest containing a wide variety of rare and threatened native flora and fauna. Manawainui Planeze to the south of the Kīpahulu Valley is situated in a transition zone of high precipitation and comparatively dry leeward slopes. The vegetation community in this area reflects this transition zone showing characteristics of the ʻōhiʻa-dominated wet forest and the mixed koa-ʻōhiʻa community of mesic forests. Manawainui marks the southern edge of the Kiwikiu range. In all, roughly 8.5 km^2 of the total 30 km^2 Kiwikiu range falls within Haleakalā NP.

History of Kiwikiu conservation in Haleakalā National Park

The upper Kīpahulu Valley has a long history of important historical observations of Kiwikiu. Following the initial collections and anecdotal observations in the late 19th century, the Kiwikiu went unreported until it was rediscovered on the windward slope in what is now Hanawi NAR (see below) by L. Richards in 1950 (Richards and Baldwin 1950). Following those observations, the species again went unreported until 1967 when it was observed in Kīpahulu by W. Banko (1968). No demographic data have been collected in Kīpahulu. However, the Manawainui area was investigated as a potential reintroduction site (Stein 2007). Although the species was not present in high densities in this area, this study highlighted the importance of specific plants, such as ʻōlapa (*Cheirodendron trigynum*) and ʻalani (*Melicope spp.*), associated with Kiwikiu occupancy. Additionally, several HFBS transects traverse the park, including Kīpahulu, Manawainui, and the Upper Hāna Rainforest and many of these transects (as well as additional transects) were recently surveyed in 2012 and 2017 and documented the presence of Kiwikiu mostly along the upper elevation portions of the transects (Judge *et al.* 2013, Judge *et al. In prep*).

<u>Hanawi Natural Area Reserve</u>

The 30.35 km^2 Hanawi NAR was created in 1989 to protect critical watersheds and a number of threatened plants and animals. Prior to the creation of the NAR this area was part of the larger Koʻolau Forest Reserve. The highest elevations (> 2000 m) within the reserve contain subalpine native shrubland and bogs and the remainder of the reserve (600-2000 m) is characterized by wet, primarily native forest. The upper elevation forest (> 1600 m) is contained within an ungulate-proof fence protecting some of the highest quality native forest remaining on Maui. The reserve also contains one of the highest concentrations of rare and endangered native forest birds in the state. Hanawi is the site of many of the last sightings of the most critically endangered and possibly extinct bird species on Maui including Poʻouli (*Melamprosops phaesoma*), Maui Nukupuʻu

(*Hemignathus affinis*), and Maui ʻĀkepa (*Loxops ochraceus*). Hanawi is situated in the center of the Kiwikiu range and is thought to contain the highest density of the species (Brink *et al.* 2012).

History of Kiwikiu conservation in Hanawi NAR

The need for significant conservation actions for the critically endangered Poʻouli and other rapidly disappearing native birds in the upper Hāna district of Maui became apparent by the 1970s (Banko 1971, Scott and Sincock 1977). Following important management actions like ungulate exclusion and removal, research efforts on the status and causes of declines in native forest birds increased during the 1980s and 1990s. During this time, key data on the behavior and ecology of Kiwikiu were collected in Hanawi (Berlin *et al.* 1981, Carothers *et al.* 1983, Mountainspring 1987, Lockwood *et al.* 1994, Simon *et al.* 2000). Maui Forest Bird Recovery Project (MFBRP) was formed in 1997 to research the causes of the declines in bird populations and Hanawi was initially the primary field site for the Project due to the presence of the last remaining individuals of the most endangered species. Following the last sightings of the Poʻouli in 2004 and the lack of sightings of Maui Nukupuʻu or Maui ʻĀkepa during this time, MFBRP switched its primary research focus to Kiwikiu. From 2006-2011 MFBRP intensively studied the Kiwikiu population in Hanawi and estimated productivity in two study areas within the reserve, Frisbee Meadows and Home Range 3. The datasets collected during this period have been used to estimate a number of important demographic and behavioral variables, such as annual reproductive rates, survivorship, and home range size, vital for conservation efforts including planning the reintroduction of the species to leeward Haleakalā. Rodent removal efforts in Hanawi from 1996-2004 demonstrated that reduction in rodent densities is possible (Malcolm *et al.* 2008); however, beneficial effects of rodent reduction on demographics of native birds at this site were not definitively shown (Sparklin *et al.* 2010).

Koʻolau and Hāna Forest Reserves

The 125.7 km^2 Koʻolau Forest Reserve (FR) is a very large management unit that wraps around Hanawi NAR (formerly part of the FR) and covers the area of the Kiwikiu range between TNC Waikamoi Preserve and Hanawi NAR. Koʻolau FR and the 53.11 km^2 Hāna FR cover the small portion of the Kiwikiu range between Hanawi NAR and Haleakalā NP. Relatively little research has been done on Kiwikiu in the areas of these reserves where the species is thought to persist beyond the HFBS transects that go through portions of the reserves. It is thought, however, that densities of Kiwikiu in these narrow portions of their range may be lower than those found in other parts of

their current range based on relatively few observations during HFB surveys. However, the entire area of these reserves where Kiwikiu are found is fenced and protected.

The Nature Conservancy's Waikamoi Preserve

Waikamoi Preserve is a 36.2 km^2 land parcel owned by Haleakalā Ranch and East Maui Irrigation and managed by The Nature Conservancy. Nearly the entire preserve is contained within ungulate-proof fencing and is ungulate free. The preserve contains a mixture of native and non-native forest. The area between 1500–1800 m in particular is dominated by high quality native forest. While most of the native forest is ʻōhiʻa-dominated wet forest, the preserve contains some areas of mesic koa-ʻōhiʻa forest. Waikamoi marks the western extreme of the current Kiwikiu range.

History of Kiwikiu conservation in TNC Waikamoi Preserve

The area that would become TNC Waikamoi Preserve was historically an area popular among many of the early European naturalists and collectors due to access from the Ukulele dairy. All Kiwikiu specimens were procured in the vicinity of the future preserve (Banko 1986). The first active Kiwikiu nest was discovered in the preserve in 1993 (Van Gelder 1993) and some of the first nesting behaviors were observed at subsequently discovered nests in the preserve (Lockwood *et al.* 1994). Though some banding was conducted there previously, MFBRP significantly expanded research efforts into TNC Waikamoi Preserve in 2011. This was primarily to answer questions about variation in demographics and genetic structure of the species throughout its range. Productivity surveys were conducted by MFBRP during 2011–2014. Despite being on the edge of the species' range, productivity surveys indicate comparable annual reproductive rates but slightly lower densities of Kiwikiu compared to Hanawi NAR (MFBRP unpublished data).

Reintroduction Site

Nakula Natural Area Reserve

Nakula NAR was created in 2011 in an effort to protect some of the last remaining koa-ʻōhiʻa mesic forests on Maui. The 6.7 km^2 reserve sits within the center of the Kahikinui region on leeward Haleakalā. After over a century of browsing and grazing damage by feral ungulates the entire preserve is now fenced and ungulates have been removed from the majority of Nakula. The forest within the preserve varies from pockets of mature native forest to savanna and non-native grassland. The reserve is divided into three units, Wailaulau, West Pahihi, and Mauka, based on

fencing and restoration timelines (Figure 5). The Wailaulau unit was selected as the future Kiwikiu release site for the reintroduction program due to the remaining native habitat and high potential for restoration. This unit ranges in elevation from 1100-1900 m and contains the largest area of remaining mature native forest within the reserve. Remnant pockets of mature native forest containing native understory species are found mostly in steep gulches where they were afforded some protection from ungulates. Other areas, particularly higher in elevation, have lost all standing trees and mostly only grasses remain with some native shrubs. The remaining area is characterized by a mosaic of non-native grasslands and savanna containing mature koa, ʻōhiʻa, and ʻaʻaliʻi trees, and little understory.

Ungulate Removal

In 2007, the State of Hawaiʻi Division of Forestry and Wildlife (DOFAW) erected an ungulate-proof fence along the western and southern boundaries of what would later become Nakula NAR. With the addition of another internal fence section in 2012, the 170 ha (1.7 km^2) Wailaulau unit of the reserve was the first section to be enclosed by fences and ungulates were removed within the same year. The West Pahihi unit to the east of Wailaulau was fenced in 2015 and restoration efforts are underway in this area. This unit, also incorporating a section of the adjacent Kahikinui FR, experienced heavy grazing and browsing damage and now contains mostly remnant forest with few mature stands of trees. Ungulates were removed from the West Pahihi unit by 2017. The Mauka unit contains the remainder of the reserve above the other two units and contains mostly subalpine shrubland and talus. This area is open to the larger adjacent Kahikinui FR and both areas are now ungulate-free as of 2018.

Habitat Restoration

Restoration efforts began shortly after the Wailaulau unit was fenced and ungulates were eradicated. In 2012, MFBRP initiated experimental restoration trials within this unit to investigate the most efficient and effective techniques for restoring forest in this area. This experiment was completed in January 2016 (Warren *et al.* 2019). The trials showed that natural regeneration within the first few years following ungulate removal was limited to a few species, but that regeneration could be stimulated by disruption of non-native grasses, exposing topsoil. Outplanting success was high for most species and growth rates were enhanced in some species by herbicide application prior to planting. Large-scale outplantings were established by MFBRP and the DOFAW Native Ecosystem Protection and Management (NEPM) program starting in 2012. The NEPM program

began large-scale planting in the West Pahihi unit in 2014. Together NEPM and MFBRP planted > 160,000 native seedlings in Nakula from 2012-2018. MFBRP also established a camp in the center of the Wailaulau unit in 2012 to be used by those conducting restoration and reintroduction work. Due to the protected status of the area, the Plant Extinction Prevention Program has also outplanted several rare and endangered plant species in the Wailaulau unit.

Predator trapping and removal

The presence of mongooses, feral cats, and rats has been documented within Nakula NAR and all likely occupy much of the Kahikinui region. A predator abundance study was conducted by MFBRP in the Wailaulau unit of Nakula in 2014-2015. Densities of rats were comparatively low in Nakula compared to densities seen in Hanawi NAR (MFBRP unpublished data). Black (*Rattus rattus*) and Polynesian rats (*Rattus exulans*) were captured. Three mongooses were also captured but no cats were trapped during this study (although one was seen). This may indicate a low density of cats in the reserve, although the traps utilized may have been inadequate to assess cat numbers. Alternate trapping methods are now being devised. High numbers of mice (*Mus musculus*) were observed during this study in Nakula. While not likely direct predators of forest birds, mice could have a significant impact on regeneration of native flora and invertebrates. As has been seen at the nearby Auwahi Restoration Project, rat densities are expected to increase as forest cover increases (Medeiros *et al.* unpublished). Control methods will be implemented during the Kiwikiu release process. Rodent removal efforts in Hanawi indicated that the reduction of overall rodent density for the larger Nakula area would require great effort and the density reduction is likely to be temporary. However, new trapping technology may increase efficacy of long-term reductions in rodent densities.

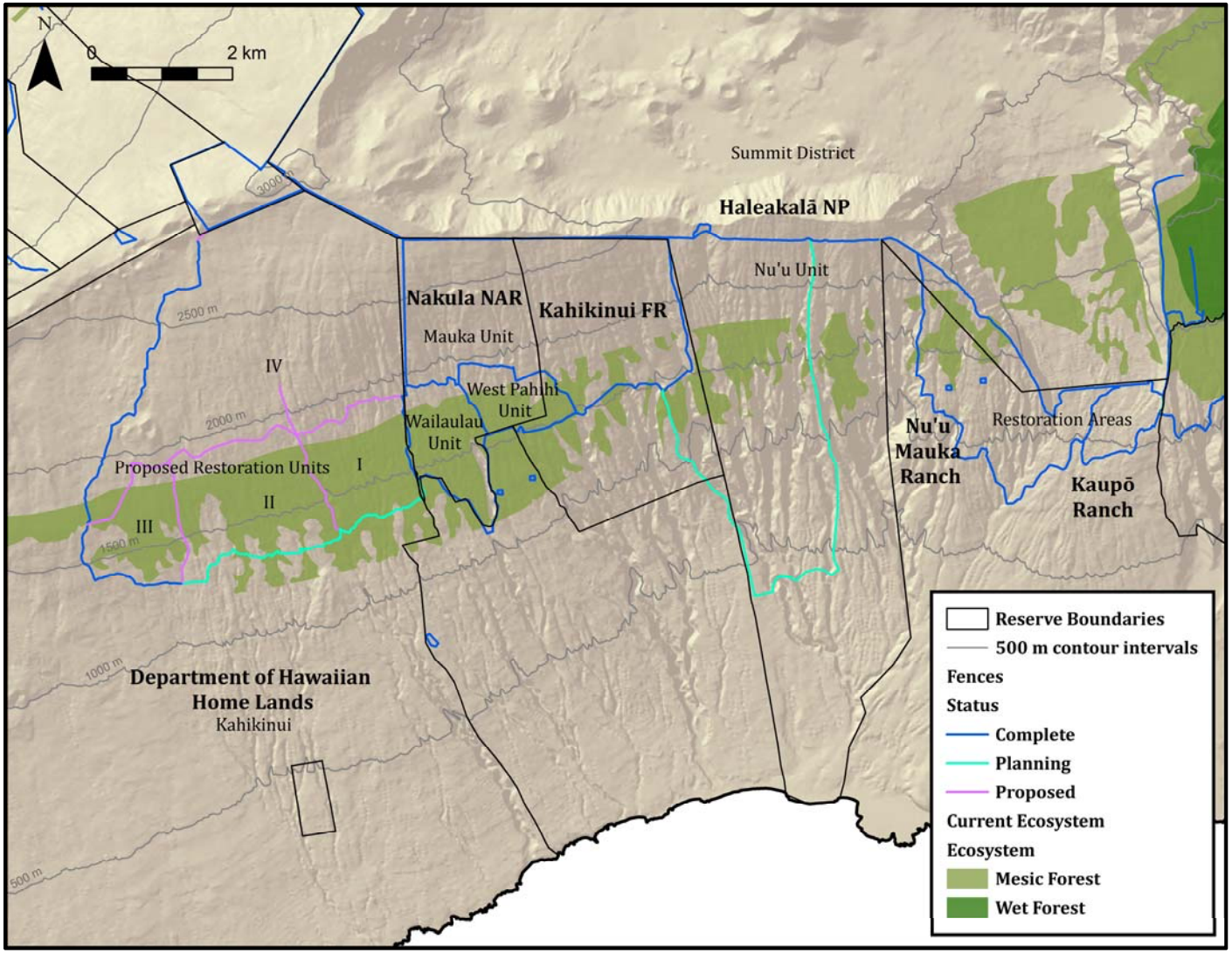


Figure 5. Kiwikiu (Maui Parrotbill; *Pseudonestor xanthophrys*) reintroduction site land management areas showing completed and planned fencing as of February 2018. The "mosquito line" is approximately 1400 m, and is commonly used to describe the area below which have high rates of avian malaria transmission.

Department of Hawaiian Home Lands

Contiguous with Nakula NAR to the west is a 96.67 km^2 parcel of land managed by the Department of Hawaiian Home Lands (DHHL). Leeward Haleakalā Watershed Restoration Partnership (LHWRP) is working with the resident community to fence and remove ungulates from an 18.7 km^2 section of this land containing remnant native forest adjacent to the Wailaulau unit (Figure 5). This section will likely be divided into four management units and collectively will protect the largest remaining section of the existing leeward koa-ʻōhiʻa forest. Although feral ungulates are still present, this area still contains pockets of mature native forest similar to those in the Wailaulau unit of Nakula. Ungulate control efforts in this area were initiated in 2018 and are on-going. The final fence enclosing the greater area is likely to be completed in 2019, followed by the internal units in the future and ungulate removal throughout the area.

Haleakalā National Park – Nuʻu Parcel

Adjacent to Kahikinui FR to the east is a large 17.4 km^2 parcel recently added to Haleakalā NP and the National Park Service is finalizing fencing to enclose a large ~ 7.6 km^2 section of land incorporating a portion of the FR. The fence should be completed in 2019 and ungulate removal will commence immediately after. Like Kahikinui FR and Nuʻu Mauka Ranch, much of this area has seen significant loss of top soil resulting in large erosion scars. Little native forest remains except for small stands of ʻōhiʻa.

Haleakalā Ranch

Haleakalā Ranch leases a ~ 1.25 km^2 parcel of state-owned land just south of Nakula NAR. This area is divided by the lower half of the Wailaulau unit of Nakula and extends from approximately 1100 to 1550 m. This parcel is actively used by Haleakalā Ranch to graze cattle and sheep. Feral ungulates are also present. LHWRP established two experimental koa outplanting units in this area in 2009. The protection of the last forest patches in this parcel is contingent on the cessation of grazing activities, removal of feral ungulates, and fence installation. However, some remnant forest remains particularly in steep gulches, and there is the possibility Kiwikiu will disperse onto this ranchland.

Kahikinui Forest Reserve

Kahikinui FR is divided into two disjunct units; a 2.87 km^2 section contiguous with Kula FR and, up until 2011, a 15.64 km^2 section to the east. Nakula NAR was withdrawn from the eastern unit of Kahikinui FR in 2011 and the FR and NAR are now contiguous along their western and eastern boundary, respectively. The current eastern unit of Kahikinui FR is 8.94 km^2 in size and contains

some areas of remnant forest, but is largely grassland, talus, and subalpine shrubland. The area above 1500 m is enclosed by an ungulate proof fence and ungulates have been removed from the fenced area. Restoration of the forest has begun in this section as well, and several thousand seedlings were planted in 2014-2018. This section, combined with the Nuʻu unit of Haleakalā NP, despite currently containing little remaining forest, has the great potential to increase the overall amount of leeward mesic forest.

Nuʻu Mauka and Kaupō Ranches

Two private ranches, Nuʻu Mauka and Kaupō, have committed to forest restoration on portions of their lands. These restoration efforts are being conducted by LHWRP in two fenced units, collectively protecting 4.96 km^2. These units contain large areas of bare rock and soil as well as some patches of ʻōhiʻa and koa. Much of these areas, however, are below 1200 m in elevation. However, these sections may be critical to connecting the future Kahikinui Kiwikiu population to the windward population across the Kaupō Gap.

2. Reintroduction

2.1 Objectives

The creation of an additional population of Kiwikiu is a critical management action that is necessary to improve the long-term population viability of the species and is a high-priority action listed in the species' recovery plan, and for US Fish and Wildlife Service (USFWS), DOFAW, and MFBRP. The USFWS (2006) recovery strategy stated that "Reestablishment in southern or western areas of Haleakalā is needed to promote natural demographic and evolutionary processes", and the Kahikinui region was identified as the leading location. This action will be a conservation translocation and is classified as a reintroduction by the International Union for Conservation of Nature (IUCN) standards because it is releasing Kiwikiu into an area of its indigenous range from which it has disappeared (IUCN/SSC 2013). This plan is focused on the actions needed for reintroduction to succeed over the short-term and to begin the process of achieving long-term success for the population in Nakula. The initial Kiwikiu responses and results from the initial reintroduction are unknown. Planning how to achieve the long-term objective and determining if any modifications or improvements are needed can only occur after assessing the first introductions. The current habitat protection and species management activities must continue, but they are insufficient to prevent the Kiwikiu's continued decline. Reintroduction is a serious and drastic management activity, but as justified by the USFWS recovery plan (2006) and updated and detailed in this plan, it is necessary to protect this species. Delaying or choosing not to begin the

reintroduction will likely result in a continued population decline (Figure 2). Based on apparent population trajectories, there will not be a better time to begin the actions to create an additional population and avoid the possible extinction of Kiwikiu.

Short-term objective

The short-term goal of this reintroduction is to create a disjunct population of Kiwikiu, separate from the main source population, which survives through multiple years. This plan details the steps necessary to accomplish this objective, and start the leeward Haleakalā population on the trajectory to achieve the long-term objective.

Long-term objective

The long-term objective of the overall reintroduction effort is for the newly established population of Kiwikiu to be self-sustaining, successfully breeding, and to achieve sufficient size to provide significant protection from extinction in case the source population is threatened or extirpated. All of the actions described here work towards accomplishing this objective, but achieving this goal will require substantial resources, committed over a long period, so a detailed strategy is beyond the scope of this plan's recommendations. One of the keys in confidently assessing population establishment is determining the long-term status and fates of the released birds (Seddon 1999). The management and monitoring actions should be adaptively extended into the future to collect these data (IUCN/SSC 2013). A second critical component is conducting additional reintroductions, building upon lessons learned and knowledge gained from the first one. A single reintroduction is insufficient to build a stable and genetically-healthy population, and subsequent efforts will move Kiwikiu from other portions of their current range.

2.2 Site Selection

Selection of a suitable reintroduction site was based on a number of factors, including historical distribution of Kiwikiu, the need to promote natural demographic and evolutionary processes, establishment of a disjunct population to reduce extinction risk, and to increase the ecological breadth of the species to help buffer against climatic fluctuations. Based on these factors, the Revised Recovery Plan for Hawaiian Forest Birds delineated 470.27 km^2 as recovery habitat for the species on East Maui (315.24 km^2), West Maui (90.58 km^2), and Molokaʻi (64.45 km^2) (USFWS 2006). This recovery plan identifies reintroduction to leeward Haleakalā as one of the high priority actions for Kiwikiu.

The USFWS 2006 recovery plan prioritizes evaluating, selecting, and preparing sites for releases and/or translocation of endangered birds to ensure long-term persistence of reintroduced populations, including potentially suitable habitat outside the species' known historic range. The goal is to select and restore habitat that fulfills the year-round requirements for the species to ensure that birds remain in the managed habitat (e.g., sufficient seasonal food resources, nesting and roosting sites). Site selection and subsequent management should include the evaluation of the species' natural history requirements, vegetative analysis, physical qualities (area), elevation, elevational gradient, topography, soil characteristics, prevailing weather patterns, corridor potential, proximity to other conspecific populations, biological limiting factors (e.g., diseases, mosquitoes, predators, food availability, feral ungulates, alien competitors), anthropogenic threats, historical habitat modification and cultural practices of pre-contact Hawaiians, and current level of management and landowner cooperation and integration (habitat conservations plans, safe harbor agreements, etc.). Methods also should consider prevalence of threats identified, and the species' likely response to novel habitat and threats. If areas available for releases do not provide all requirements during some periods of the year but logistical or other concerns necessitate release in these areas, then technologies must be available to support released birds during periods when essential niche characteristics are temporarily absent. Species and areas currently in need of habitat evaluation and selection for releases of endangered birds include:

Leeward Haleakalā, West Maui, and Molokaʻi for Maui forest birds

Kiwikiu currently occupy roughly 20% of the identified recovery habitat on East Maui on the northern and eastern slopes of Haleakalā. It is hoped that fencing and ungulate removal below the current range on these aspects of the mountain will allow regeneration of a complex subcanopy and reduce mosquito densities to allow expansion of the population into these areas and increase densities in currently occupied habitat.

The recovery habitat on West Maui and Molokaʻi is predominately fenced and ungulate-free currently, but much of the habitat lies below the 1200 m elevation where mosquitoes become more plentiful and *Plasmodium* is able to complete its life-cycle. While the long-term goal for the recovery of the Kiwikiu may be dependent on establishing a second viable population in one or both of these areas, more work is needed to assess the current mosquito abundance and disease prevalence in

the areas and potentially develop methods to reduce or eliminate this limiting factor before reintroductions can begin.

A study of disease prevalence in Nakula NAR and TNC Waikamoi Preserve conducted by MFBRP found higher prevalence of *Culex quinquefasciatus* mosquitoes in Nakula than Waikamoi at similar elevations (Warren et al. *In Prep*). However, this study also indicated comparable rates of *Plasmodium* infections in the bird populations in Nakula compared to other sites at similar elevations on Hawai'i Island, including areas containing 'Akiapōla'au (*Hemignathus wilsoni*), the closest living relative to Kiwikiu (Atkinson *et al.* 2005, Samuel *et al.* 2015). Despite comparable infection levels, mitigating any disease risk is preferable. Larval mosquito habitat (particularly for *C. quinquefasciatus*) is largely in stagnant pools in drainages. These locations may be amenable to specific control measures and possible mosquito reductions. Given that these pools are readily accessible, there is a high potential for the reduction in the mosquito populations in Nakula NAR using currently available techniques. Since July 2019, VectoMax, a permitted insecticide to control mosquito larvae, has been applied in gulches prior to the Kiwikiu release. This will continue to be done monthly as long as necessary.

While the presence of *Culex* in Nakula was unexpected given the elevation and drier climate, the rates of avian malaria in the native bird population do not appear higher than comparable sites and the conditions in Nakula may increase our ability to control mosquitoes in this habitat. While controlling mosquito vectors are likely how avian malaria can be contained, it is also important to remember that presence of the vector does not mean that the disease is also present. The *Plasmodium* parasite has more restrictive environmental tolerances that its mosquito host (LaPointe 2000). Kiwikiu occupy all of the extant native forest on the windward slopes of Haleakalā above 1400 m and yet the species appears to be in decline or, at the very least, exists at precariously low population levels (Camp *et al.* 2009, Judge *In Prep*). The presence of avian malaria has been documented in upper elevation habitats within the current Kiwikiu range. However, rates of infection in the current range are not known and cannot be compared to those in Kahikinui. Although not strictly disease-free, Kahikinui is among the only unoccupied high-elevation habitat that can support Kiwikiu. In the long-term, we may be able to manage mosquitoes better in this habitat compared to windward forests.

Non-native mammalian predators represent a significant threat to Kiwikiu in their current range and may be one of the largest contributing factors to their apparent decline; perhaps second only to avian malaria. MFBRP has conducted predator control programs in Hanawi NAR and TNC Waikamoi Preserve to reduce the pressure these mammalian predators exert on native forest birds. It has been demonstrated that rat densities can be reduced in these areas, but the effort required is extremely high (Sugihara 1997, Malcolm *et al.* 2008). These efforts highlight how difficult it is to control these predators in the current Kiwikiu range. New trapping technologies may aid in this, such as Good Nature™ A24 and A18 traps, but these efforts are currently not feasible across the entire range. Another benefit to the more open mesic forest is that rat densities tend to be lower in areas with less forest cover (Medeiros *et al.* unpublished). Current predator densities appear to be lower in Nakula NAR than TNC Waikamoi Preserve (MFBRP unpublished data). While the partners' long-term goal is to increase forest cover in Kahikinui, and thus potentially cause an increase in rat densities, it is possible that predator densities will naturally be lower in this more open habitat. Even after understory density increases in the leeward forests, the height of the lower canopy will likely lead to less connectivity between the understory and canopy. In theory, less connectivity may mean fewer routes for rats and other predators to reach Kiwikiu nests in the canopy.

The western and southern slopes of Haleakalā offer the most immediate opportunity to create a disjunct second population and expand the range and population size of Kiwikiu in the near term. The original mesic forests on these slopes were destroyed or severely degraded by ranching, fires, and feral ungulates over the past few centuries, which likely caused the local extirpation of Kiwikiu. As previously mentioned, some forest exists on state-owned land within the Kahikinui FR and Nakula NAR and on land administered by the DHHL. Restoration and enhancement of this area has begun by the State of Hawai'i, MFBRP, LHWRP and partner agencies and when restored, these areas will provide a mesic koa-'ōhi'a forest, which was once a major component of the Kiwikiu range.

Initial restoration has focused within a 170 ha area of the Nakula NAR (Wailaulau unit), which contains some of the most intact portions of this forest, especially in gulches inaccessible to ungulates. Peck *et al.* (2015) found the, "total arthropod biomass and caterpillar biomass at Nakula was as great or greater than that observed at Hanawi and Waikamoi", however their results were limited to the scale of the individual branch or tree – the vegetation density and quality still need to be compared across these sites, but overall woody plant density is almost certainly lower in Nakula. This area is sufficiently physically separated from the current population and creating an additional

population would improve the conservation status of Kiwikiu by reducing the risk of extinction from demographic or environmental stochasticity. It would also serve as the founding population for an eventual connection to the current population through National Park-owned lands to the east. Finally, the reintroduction site is under state control and work can begin immediately when planning is complete.

2.3 Guidelines for starting Kiwikiu reintroduction

Habitat restoration, regeneration, recovery

In 2007, NAR staff completed a fence enclosing much of Nakula NAR and Kahikinui FR in an ungulate-proof fence. Following the boundary fence, DOFAW completed an internal fence in 2012 creating the later-named Wailaulau Unit, with the final stream pass-throughs and ungulates removed in 2013. In spring 2013, MFBRP began field trials to test different restoration methods in the Wailaulau unit (MFBRP 2013). The field trials showed that outplanting seedlings was necessary to restore the habitat and promote a diverse and functional forest throughout the site; neither seed scatter nor unaided natural regeneration were effective techniques. Most species had high (>90%) 24-month survival rates, with the notable exception of ākala and māmaki (Mounce *et al.* 2015). Trials also indicated that natural regeneration of certain species could be enhanced through removal of the non-native grass mat. This treatment effectively promotes natural regeneration, although the remnant seed bank seems to be species-depauperate, with koa and ʻaʻaliʻi being the two most common species. This treatment can be effective as the initial step at landscape restoration, if the effort is followed with outplanting of understory plants. MFBRP and NEPM have built upon these results, and are restoring habitat in the Wailaulau unit to support Kiwikiu and increase the native forest within the reserve. The current outplanting strategy is focused on corridors connecting the remaining forest in the gulches (Figure 6), and along the erosion scars to prevent further soil loss.

In addition to the Wailaulau unit of Nakula NAR, at the regional scale there is forest protection and natural regeneration occurring in the larger leeward Haleakalā region (see Section 1.3 – Reintroduction Site). NEPM is actively restoring other parts of Nakula NAR, particularly the West Pahihi unit (Figure 5) and LHWRP is restoring ranchland and will soon begin outplanting within the DHHL lands. These external restoration efforts are crucial because Kiwikiu are unlikely to remain within the Wailaulau borders. Having a larger restored area with a range of forest types and ages increases the likelihood of the translocated birds remaining in the area. Given the high likelihood

that Kiwikiu will use habitat in areas of the adjacent DHHL and Haleakalā Ranch properties, communications are being facilitated by LHWRP and access issues must be resolved before the translocations begin.

As part of the habitat assessment, aerial imagery is being used to track the forest recovery and the effects of the forest restoration on the overall canopy cover. This will allow the project to control for the original, remnant forest and track the canopy cover that has been produced through the outplanting efforts. At the start of the restoration effort in 2011, canopy cover in the Wailaulau unit of Nakula NAR was roughly 16.5% of the area (Mounce *et al.* 2015). As of 2018, > 40 ha have been planted in the Wailaulau unit, and if all these trees survive and mature, there could be up to 40% canopy cover within the unit not including that produced by natural regeneration. While some of the remaining 170 ha cannot support native forest (e.g., exposed rock or cliff faces, ~ 58 ha), there are still approximately 44 ha of grasslands that could be restored. Many areas contain canopy trees but the understory no longer remains. In these areas outplanting of subcanopy and understory species will likely be needed to restore full ecological function.

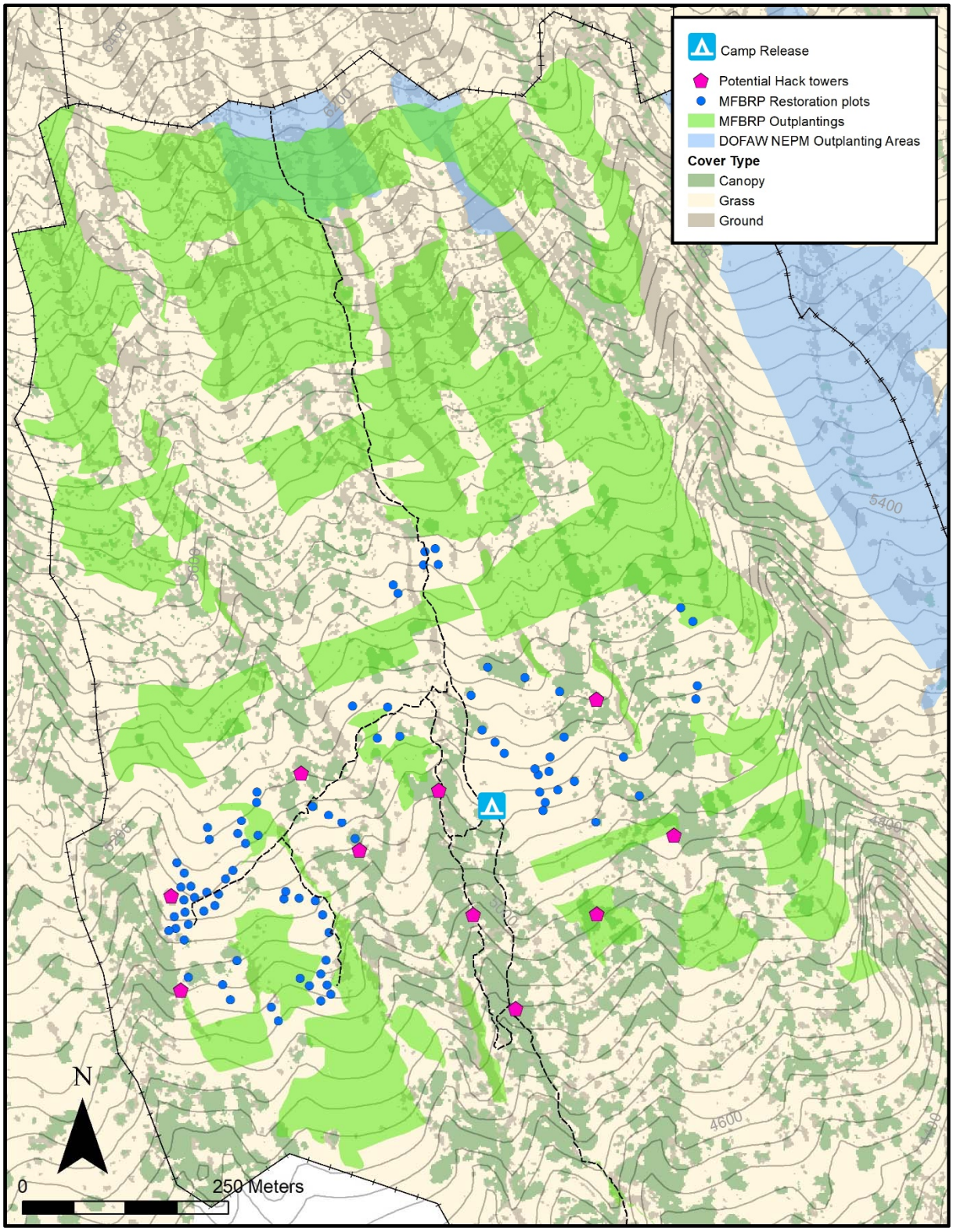


Figure 6. Nakula NAR reintroduction site in the Wailaulau unit for Kiwikiu (Maui Parrotbill; *Pseudonestor xanthophrys*) with base camp for reintroduction operations, forest restoration areas as of 2018, and proposed release aviary sites for reintroduced individuals/pairs.

2.4 Seasonal Timing

The initial translocation will take place in October/November, 2019. This time period would minimize the disruption of the source population. During this period, both adults and second-year (SY) birds could be captured. This time period is typically outside the peak in Kiwikiu nesting activity, so there would be a reduced chance of removing birds with dependent nestlings. Before and after a bird is captured, the adult birds' natural behavior will be observed to determine if there are dependent fledglings or juveniles. Any begging juveniles in the immediate vicinity of a captured adult will be assumed to be dependent and the adult will be returned to the wild unless it can be quickly determined that this juvenile is not the offspring of the captured adult (i.e., two adults are travelling with the juvenile). If we cannot capture the desired 12 birds from the wild, due to poor weather or lower-than-anticipated capture rates, we may have additional capture trips to attempt to capture the desired number of Kiwikiu, but we will halt efforts by the end of January to avoid disrupting the source population.

2.5 Cohort Composition

The goal is for the first translocation cohort to be roughly equal parts wild and captive birds. At present there are eight captive birds (Table 1) that are suitable for release, although this may change if one or more birds do not pass the pre-release exam. This plan assumes that all eight birds are releasable. As such, we will attempt to capture and translocate 12 wild Kiwikiu. This will make an initial release cohort goal of 20 birds.

We will attempt to capture breeding pairs from the wild. If we are unable to capture and translocate pairs, we will move single, ideally unpaired birds (e.g., second-year) to the site and pair them in separate, but adjacent, aviary cells to allow them to become familiar and possibly pair up and mate at the new site. The actual number and sex ratio have to be flexible to account for the difficulty in capturing birds. As discussed further below, we will hold the wild birds in temporary field aviaries at the capture site for up to ten (10) days. If we have not captured sufficient birds (n = 12) at that time, we will continue to mist-net in the source population, while a portion of the translocation team moves the captured birds to Wailaulau.

The precise sex ratio of the released birds will depend on what individuals can be captured. Wild males are usually easier to capture than females and capturing the precise number of males and females to equal a 50:50 sex ratio may be challenging. Even if we do release an equal sex ratio of

birds, there is no guarantee that birds released from paired aviaries will form a pair and we expect some mate switching to occur. The captive individuals to be released are mostly male (7/8). To maintain a balanced sex ratio including all eight captive birds, we would need to capture six (6) wild females to be paired with the six (6) captive males (one would presumably be paired with the captive female). The remaining six (6) wild individuals to capture would then ideally be three (3) males and three (3) females making the overall translocation goal nine (9) females and three (3) males; a heavy take on females from the source population. Based on the ages of the captive males, only 4-5 individuals are likely to become part of the breeding population. As such, we may be able to achieve an *effective* sex ratio close to 50:50 even if we capture < 9 wild females. The combination of all these factors means a release cohort of more males than females is very likely and acceptable given the composition of the captive flock. An exact 50:50 sex ratio is not necessary in the first year of releases, given that additional releases will be needed to establish a population. A more realistic goal is to capture and translocate 6-8 females and 4-6 males. Combined with the captive birds, the first release group would then be 7-9 females and 11-13 males.

Ideally, we would capture and translocate second-year Kiwikiu (birds in the second year of life; one year old). Birds of this age are likely in the early stages of establishing a home-range, looking for a mate, and establishing a pair. Second-year females may attempt to breed, but most birds of this age do not have the opportunity. Thus, removal from the source population will have less impact on the overall population than breeding adults. In addition, translocating monogamous and territorial passerines of this age may result in higher post-release survival and settlement (Masuda and Jamieson 2012). We also know very little about juvenile (including second-year) movements. It is possible that if an individual does not find a suitable home range area by the time they are ready to breed, they may emigrate from the site including into lower elevation areas where they may be at greater risk of contracting avian malaria. However, while MFBRP has captured and banded a fair number of second-year Kiwikiu in TNC Waikamoi Preserve and Hanawi NAR, they typically make up a very small proportion of captured individuals in a given year. Thus, we are unlikely to have the opportunity to capture 12 second-year birds and some portion of the translocated cohort will have to be older adults.

Hatch-year Kiwikiu will not be translocated. This will avoid removing a bird that is still dependent or being supplemented from its parents. A bird aged one- to approximately 6 months (post-fledging) can be readily aged as a hatch-year bird (Figure 7). Birds younger than one month are known as fledglings and are easily discernible from older hatch-years. However, as birds approach a year in age, they appear physically the same as second-year birds. As such it may not be possible

to avoid taking a bird that is between 12 and 18 months in age (the oldest an individual was observed being fed by parents). Only birds definitively defined by plumage as a second-year (SY; which may include birds ≥ 12-months) or after second-year (ASY; birds at least in their third year of life) will be selected for translocation. See the Kiwikiu sexing and aging guide in section 2.8 of this plan as well as Figure 7 below.

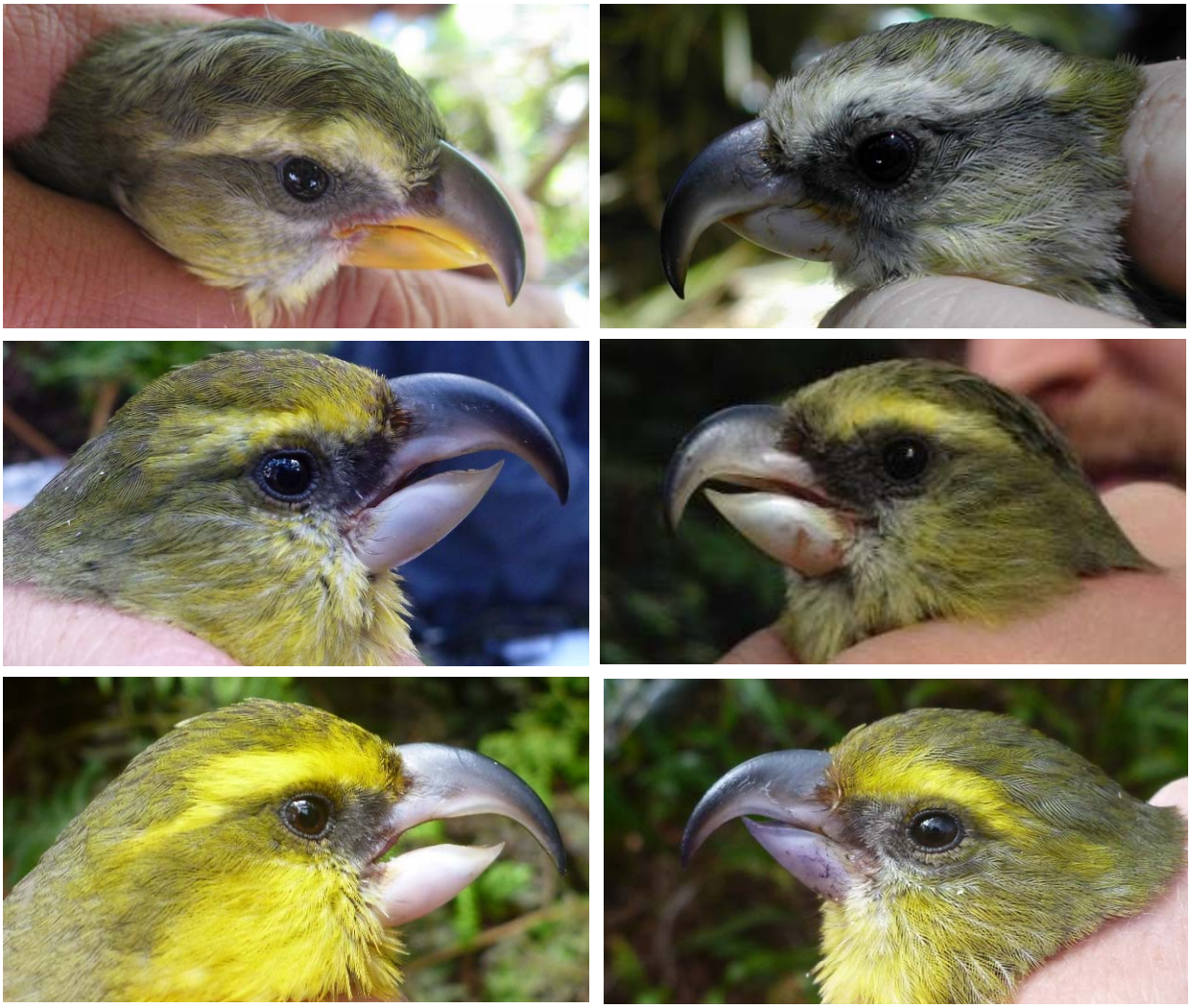


Figure 7. Photos of banded Kiwikiu demonstrating the plumage differences in the head and face among hatch-year (HY), second-year (SY), and after second-year (ASY). Males (or presumed males in the case of HY) are in the left column and females are in the right. The top row shows two HYs, the middle SYs, and the bottom shows ASYs.

Quite often in both TNC Waikamoi Preserve and Hanawi NAR field sites, MFBRP observed cases where "empty" home range areas become occupied by new individuals. Usually this occurs when a known, banded pair is observed in one year but they are not found the following year. But, a new, often unbanded, pair is observed in the same area as where the known pair had been the previous year. Sometimes these "empty" home ranges are filled by SY birds, but quite often the "new" birds are ASY adults. Usually the "new" birds attempt to breed in the new home range the first year they are observed. These cases do not include times when a neighbor simply expands into the "empty" space but rather times when the known neighboring individuals remain and new birds of unknown origin appear in the gap. This likely indicates that even when an individual or pair dies or emigrates from the site, there are enough additional birds "floating" nearby to fill any available space. Similarly, MFBRP has observed many cases where one half of a known pair (male or female) disappears from one year to the next and the remaining bird nearly always is then seen with a new mate in the new year. One male in TNC Waikamoi Preserve was observed with three different females in three successive years and produced a chick with two of them. For Waikamoi Preserve (2011-2015) and Hanawi NAR (2006-2011) mate identity was known for 13 and 32 individuals, respectively, in more than one year. For these banded birds, we know the identity of their mate in more than one year, and 46% and 34.4%, respectively, switched mates between years (MFBRP unpublished data). There are several cases when a bird switched mates more than once. Fifty-five percent (55%) of newly formed pairs in Hanawi resulted in a hatch-year in their first year as a "new" pair. This challenges the narrative that this is a long-term monogamous species as we observed more readily a decade or so ago. While Kiwikiu seem to naturally be a long-term socially monogamous species, increased adult mortality may be reducing the average length of time pairs are together.

It would be extremely difficult, if not impossible, to determine the impact of the removal of 12 individuals on the source population as a whole. At both MFBRP field sites in TNC Waikamoi Preserve and Hanawi NAR, natural turn-over ("missing" known birds and arrival of "new" birds) was common. Many pairs were consistently found year after year, while others were observed in one year and then never again. Some of this can be attributed to shifting of home ranges outside the study site, while others are certainly due to mortality. Each year however, regardless of which individuals were present, few areas in each study site did not contain a Kiwikiu home range. This turn-over has led many to hypothesize that the species is "saturated" in its current range and that many individuals in the population are non-breeding "floaters" waiting for a home range to become

available. If this is indeed the case, the removal of a pair or individual could provide the opportunity for "floater" individuals to move in and occupy the removed birds' home ranges. In this scenario, removal of an individual or pair would not represent a net loss in the breeding population for a given site. The removal of a non-breeding "floater" would similarly have a negligible impact on the breeding population.

Capturing already paired individuals could prove important to establishing the species in Kahikinui. However, the chances of capturing six (6) established pairs is very low. Importantly, these six (6) "pairs" are unlikely to all be known breeding pairs. MFBRP has successfully captured a male and female travelling together at the same time on a number of occasions. Often these turn out to be breeding pairs following further observations. However, this is not always the case and several times when MFBRP captured a male and female in the same net, they were later determined to be paired with other individuals. In 2014, MFBRP captured three Kiwikiu, two males and one female, in one net in the span of just a few hours. After further observations it was determined that these belonged to three separate pairs. Thus, even if the capture teams are lucky enough to net a male and female together, there is no guarantee that they are a mated pair. The high frequency of mate switching (presumably after the death of a mate) in both TNC Waikamoi Preserve and Hanawi NAR study sites (as discussed above), indicates that even if a pair is split up, there is a high likelihood that both individuals will be able to find a new mate at the capture and the release sites.

Additionally, annual reproductive success (the proportion of pairs that successfully produce a chick each year) at both TNC Waikamoi Preserve and Hanawi NAR averages < 50% each year. Pairs may re-nest at least three times in a given year but only in the case of nest failure. On average, approximately half of the birds we remove from the source population would not have produced a chick that year. This additionally lessens the impact on the overall productivity of Kiwikiu at a capture site. Limiting captures at a site to a single year may mean that the source population has one bad year of productivity for the breeding adults in the population and may actually provide opportunity for young or "floater" individuals to take their place in the breeding population.

2.6 Cohort Source

There are currently eight captive Kiwikiu in the SDZG facilities that may be suitable for reintroduction (Table 1). SDZG supports releasing all these birds and effectively ending the Kiwikiu breeding program.

Due to the logistics of transporting and housing both field staff and captured Kiwikiu, only one capture site will be used per release year. Hanawi NAR will be used as the source of the wild birds for the first release year. The preferred source of the wild birds for the second release year is TNC Waikamoi Preserve. Other source locations could be areas within Haleakalā National Park. In order to capture genetic diversity from the current population, both birds from the western and eastern populations will need to be translocated (Mounce *et al.* 2015)(Figure 1, Figure 3).

2.7 Permitting and Compliance

DOFAW staff and staff employed under PCSU contract (including MFBRP) are listed as subpermittees on DOFAW's bird banding permit for bird banding activities, including capturing, banding, and affixing color bands and radio transmitters. DOFAW's bird banding permit is currently being renewed on an annual basis, and expires April 30 2019. We will renew the permit before the expiration date. SDZG holds a federal recovery permit for captive individuals. DOFAW staff and staff employed under PCSU contract are covered by DOFAW's Section 6 cooperative agreement with USFWS for bird banding and recovery activities.

Competitive State Wildlife Grant (C-SWG) activities covered by DOFAW's cooperative agreement on listed species include:

- capture of wild Kiwikiu and holding for less than 45 days
- banding of wild Kiwikiu, attaching radio transmitters and color bands
- release of wild Kiwikiu into an area within their historical range

A Section 7 compliance form is being prepared. All activities will fall under NEPA categorical exclusions.

2.8 Logistics

Organizational Responsibilities

MFBRP

- Predator control
- Establish protocols for sourcing birds for translocation
- Coordinate reintroduction plan objectives among partners
- Organize community outreach meetings (in conjunction with DOFAW public relations and LHWRP.)

- Apply for funding and grants
- Capture and translocation of wild Kiwikiu from source populations to Wailaulau
- Post-release monitoring
- Continued habitat restoration

SDZG

- Design and construct release aviaries at Wailaulau
- Transport captive birds from the Maui Bird Conservation Center to Wailaulau
- Care of captive and wild birds in release aviaries at Wailaulau
- Conduct necropsies on dead birds

ABC

- Apply for funding and grants
- Advise reintroduction plan
- Field operations support at capture and release sites

Pacific Bird Conservation

- Care of captured wild birds in field aviaries at capture site
- Advise and aid in construction of field aviaries and transfer cages

DOFAW-Wildlife

- Apply for funding and grants
- Outreach & Public communications
- Organizational support
- Advise and approve reintroduction plan

DOFAW-NARS

- Continued habitat restoration
- Apply for funding and grants
- Organizational support
- Maintain fences
- Invasive species control

DOFAW-Forestry

- Continued habitat restoration
- Fire prevention and suppression
- Ungulate control
- Field operations support at capture and release sites
- Funding and logistical support
- Habitat restoration
- Forest health surveys and monitoring
- Public communications

USGS-BRD

- Advise/assist in radio transmitter attachment and maintenance
- Assist with radio tracking tower equipment and installation

USFWS

- Apply for funding and grants
- Advise and approve reintroduction plan

Proposed Predator Control

Birds are especially susceptible to predators when awaiting release in aviaries and immediately following release as they become acclimatized to the site. To reduce or eliminate the threat of predators, a predator grid of 215 stations and additional traps deployed near the release aviaries (395 traps total) were installed in Nakula NAR during June-August 2018 and activated in October, 2018 (only DOC250s and body-grips were set, A24s will be set March 2019) (Figure 9). The predator control grid will then be active for a full year prior to the first translocation. The predator grid will consist of four trap varieties, GoodNature™ A24, Belisle-brand body grip, DOC250, and Victor® snap traps. An A24 will be placed at each grid station (215 total traps). There will be 40 stations of body grip traps, 20 ground-style stations which allow for two traps per/box (40 traps) and 20 elevated-style stations (20 traps) (60 Belisle-brand traps total). DOC250 traps will be placed at 40 stations (40 traps). Five Victor® snap traps (50 total traps) will be deployed in the immediate vicinity of each release aviary. Unless they are deployed to target a specific cat (see below), one leg hold or other live cage trap will be deployed at each release aviary. Live traps will be monitored daily and will only be active while daily monitoring is possible.

Placement of traps within the grid will be based on spacing from another trap of the same type and spacing from the release aviaries. We will maintain a distance of 50 m between A24-only stations, 50-100 m between DOC250 stations and body grip stations. With few exceptions, we will also maintain a minimum distance of 50 m between DOC250/body grip stations and release aviaries to avoid attracting predators to the aviaries. There will be no minimum spacing from release aviaries for Victor® snap traps. Additional traps will be used to target cats detected on game cameras. "Floating" game cameras will be used to pinpoint an individual cat's (identified through unique markings) movement patterns. Additional pheromone-baited traps and/or leg-holds will be deployed along identified routes.

Traps will be monitored at different time intervals for the different trap types. The A24 traps will likely only need to be monitored every 3-6 months. Initially we will monitor A24-only stations on a 3-month cycle and the monitoring interval may be modified later based on capture rates. These stations will always be monitored a minimum of every 6 months as per the manufacturer recommendation. Stations containing body grip or DOC250 will be monitored monthly or bimonthly. These stations will be placed on select contours to reduce the number of trails that are accessed at this interval (see Proposed Trail Access below). The traps at the release aviaries (i.e., Victor® snaps) will be checked daily while birds are in the aviaries and as long as birds are utilizing the supplemental feeders. Once birds are no longer using the release aviary sites, these traps will be monitored on a monthly basis along with the body-grip and DOC250 stations or removed. When deployed, leg-hold or other live traps will be monitored daily. If daily access is not possible, these traps will not be used.

We will use a variety of bait types to attract animals to the body-grip and DOC250 traps. Each bait type will be used for a minimum of three months at a time to allow enough time to determine the efficacy of the bait. We will use four bait types initially that have been successful at other sites; sardines (canned in oil), dry cat food mixed with used fry oil, oily fish mixture, and fresh eggs. Additional baits, including pheromone baits, may be used in the "floating" traps to target cats.

Prior to trapping, MFBRP will estimate baseline densities of rats using tracking tunnels along transects within and outside of the trapping grid. We will be following the DOC tracking tunnel guide (Gillies and Williams 2013) for our tunnel monitoring procedures. Gillies and Williams (2013) suggests that tunnels are in place at least three weeks prior to the first survey period. The

tunnels were placed in the field in June, 2018. Baseline estimates were collected in August, September, and October, 2018 just prior to activating the predator grid. These pre-treatment baseline surveys will establish relative abundance of mammalian predators inside the predator reduction grid and outside. Although conducting only three baseline surveys at a similar time of year is not ideal, we feel it is important to make the grid active as early as possible given the timeline for releasing Kiwikiu. We conducted an *a priori* power analysis to estimate the number of tunnels that may be required to detect declines in rodent densities. This analysis indicated two things relevant to this design (Figure 8):1) If pre-treatment densities of rodents are exceptionally low (i.e., 0.1 rats/ha), we would need at least 35 tunnels (inside or outside the grid) to detect any trend. 2) We are unlikely to be able to accurately estimate a very small decline in rodent densities unless we put out a very large number of tunnels. Given the spacing recommended for these tunnels in Gillies and Williams (2013), it is not possible to have enough tunnels to have very high power in estimating a trend. If we relax the recommended spacing between tracking tunnel lines from 200-m to 100-m, we can easily monitor ≥ 40 tunnel stations inside and ≥ 40 stations outside the grid without getting too far outside the same habitat type (Figure 9). Eighty stations are a manageable number to monitor and this number will allow us to analyze the effects of habitat variability into our results. If the baseline survey indicates higher densities of predators than we think, > 0.3 rats/ha, we may consider adding additional tunnels to increase our power.

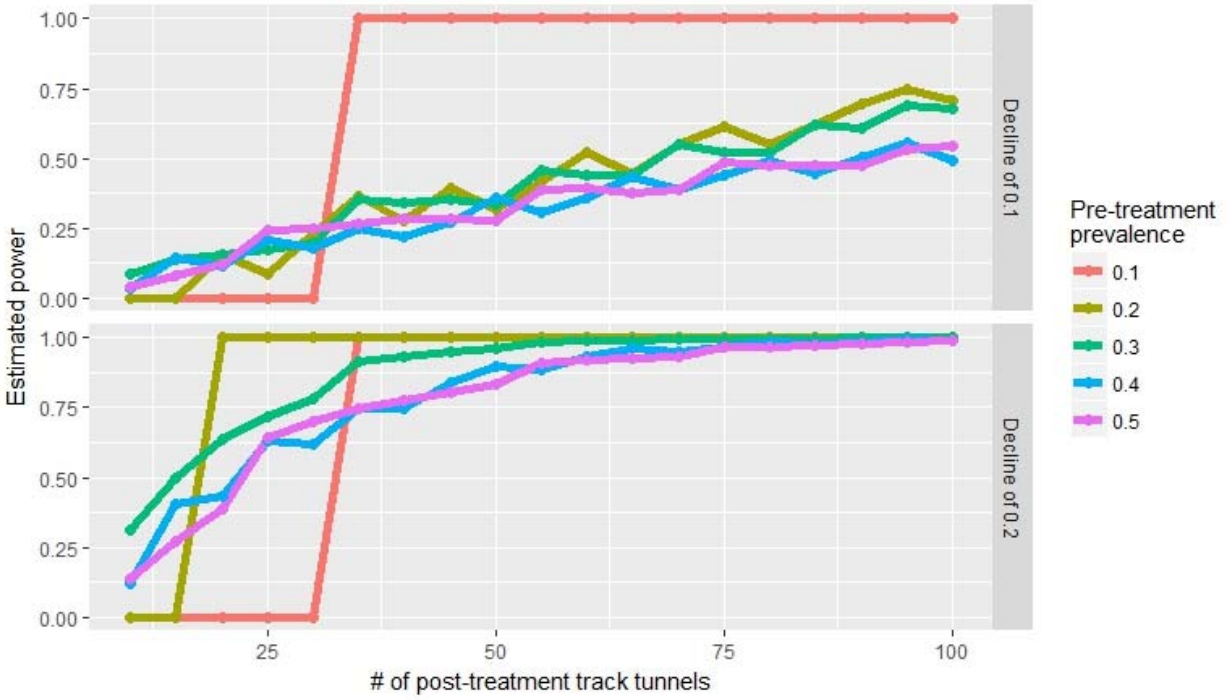


Figure 8. *A priori* Power analysis results showing the minimum number of tunnels required (inside or outside the grid) to be able to accurately detect a trend in predator density in the two treatments. This analysis indicates that a minimum 35 tunnels are required to detect a trend if pre-treatment densities of predators are 0.1/ha. This also indicates that we are unlikely to be able to detect a trend of 0.1 unless pre-treatment density is exceptionally low (i.e. 0.1) or unless we have a very large number of tunnels.

Once the trapping grid is active, we will monitor the tracking tunnels (inside and outside of the predator grid) on a quarterly basis (four times per year) to estimate rodent densities and evaluate trapping efficiency. Designated tracking tunnel stations will be deployed 50 m apart along four separate lines (following contours), 10 tunnels per line, inside and another four lines outside the predator removal grid. All "inside" tracking tunnel stations will be place > 50 m from the edge of the grid and "outside" stations will be placed ≥ 100 m from the edge of the grid. Tunnel stations inside the grid will be established between trap stations. There will be 40 tracking tunnels deployed inside the grid and 40 deployed outside the grid (Figure 9). Our goal is to reduce rat densities by ≥ 30% in the first year. Comparison of tracking tunnel data inside and outside the grid as well as pre- and post-trapping will allow us to evaluate if we are meeting our predator reduction goals.

Given large home range sizes, cat and mongoose densities will be difficult to estimate with any degree of accuracy within such a small area. For example, the recommended spacing between tunnel lines for mustelids (which is often used as synonymous for herpestrids in this context) is

1000 m and spacing between stations is 100 m. Even if we greatly relax the spacing recommendation between lines, we are unlikely to be able to monitor more than 10 stations resulting in very low power to detect a trend. As such, trapping efficiency for these larger predators will be evaluated through capture rates and game camera captures. A series of eight game camera stations (4 inside and 4 outside the grid) will be established along trails at fixed locations to estimate prevalence of cats and mongooses and possibly target specific individuals. Cameras will be placed a minimum of 350 m apart. A scent and physical lure will be placed in front of each camera. Scent and/or physical lures (e.g., tinsel) will be changed every month at the same time the data are downloaded from the cameras. The response variable will be the number of days (24-hour period) per month (30 days) during which a cat or mongoose is captured on camera. All identified individual cats (through unique markings visible in game camera photos) not captured in the grid will be targeted for removal via additional trapping methods deployed (e.g., leg-hold traps). Additional cameras will be used to establish regular foraging routes and facilitate targeted trapping efforts.

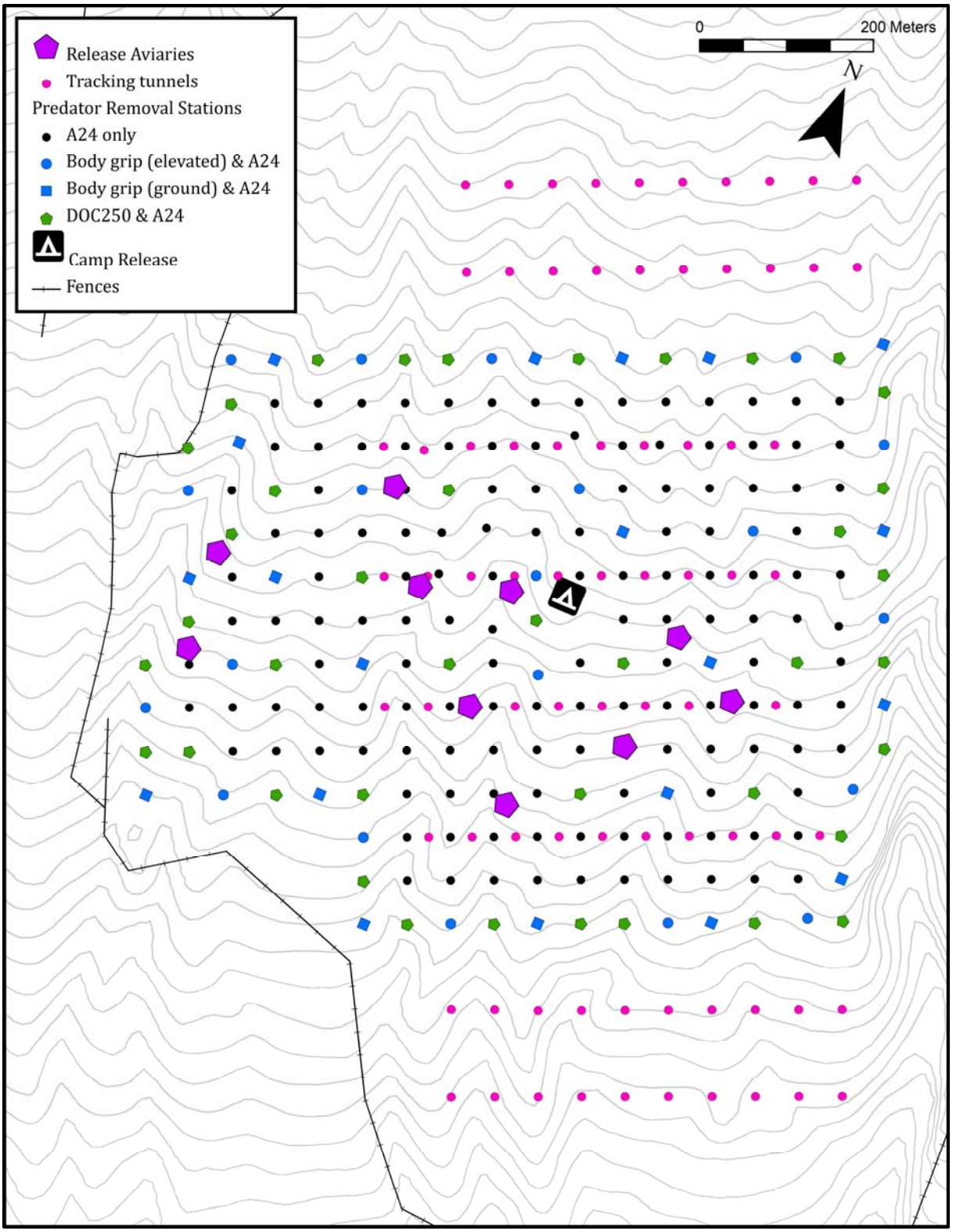


Figure 9. Wailaulau unit, Nakula NAR reintroduction site for Kiwikiu (Maui Parrotbill; *Pseudonestor xanthophrys*) with base camp for reintroduction operations, proposed release aviary sites for reintroduced individuals/pairs, and proposed predator reduction grid.

Proposed Trail Access

An additional goal of this project is to cause the least long-term negative impacts within the NAR, so walking will be used for all trail access; no ATV/UTV use will be needed. Four use-levels of trails have been determined based on activities and projected use rates (Figure 10); release aviary trails (high use during releases only), predator control trails (DOC250/body grip trap lines; high use for limited time), predator control trails (A24-only trap lines; low use), other trails (low use).

The highest-use trails will be the release aviary trails, but these trails will only be high-use during releases when daily access is necessary for a three- to four-week period. However, the release aviary trails take advantage of existing flagged trails and overlap some proposed high-use predator control trails. Based on the location of the release aviaries, most of the trails will be two directional (walked in both directions), although one loop is possible to access three of the towers. Sections of the highest-use trails will be regularly evaluated for damage or erosion, and a modified route taken before significant erosion is observed.

Some predator control trails will be high-use for a limited time. For the months prior to the first release, access to body grip stations within the predator removal grid will be needed monthly or bi-monthly. During and after releases, monthly access will be required until the predator detections are negligible. The body grip and DOC250 traps will be placed around the outside border of the grid and three row lines (contours) within the middle of the grid. Body grip and DOC250 traps will be checked along contour trails, and the connector trails snaking mauka-makai the mountain. To reduce impact on each trail, these contour trails can be used in a unidirectional manner in 3-4 loops when checking traps.

Low-use predator grid trails will be used every three (3) to six (6) months to access the stations that only hold an A24 trap. The trails are likely to be unidirectional and can be checked in loops. Tracking tunnel trails will also be low-use and accessed quarterly. Two additional trails are currently flagged and have been used sporadically in the past few years to access planting sites and will either be decommissioned or incorporated into the high-use trails. Three HFBS transects established in 2015 extend mauka-makai throughout the Wailaulau Unit and are flagged and

stations are marked with PVC. These transect trails are rarely used but sections of these routes will be incorporated into the proposed high-use trails.

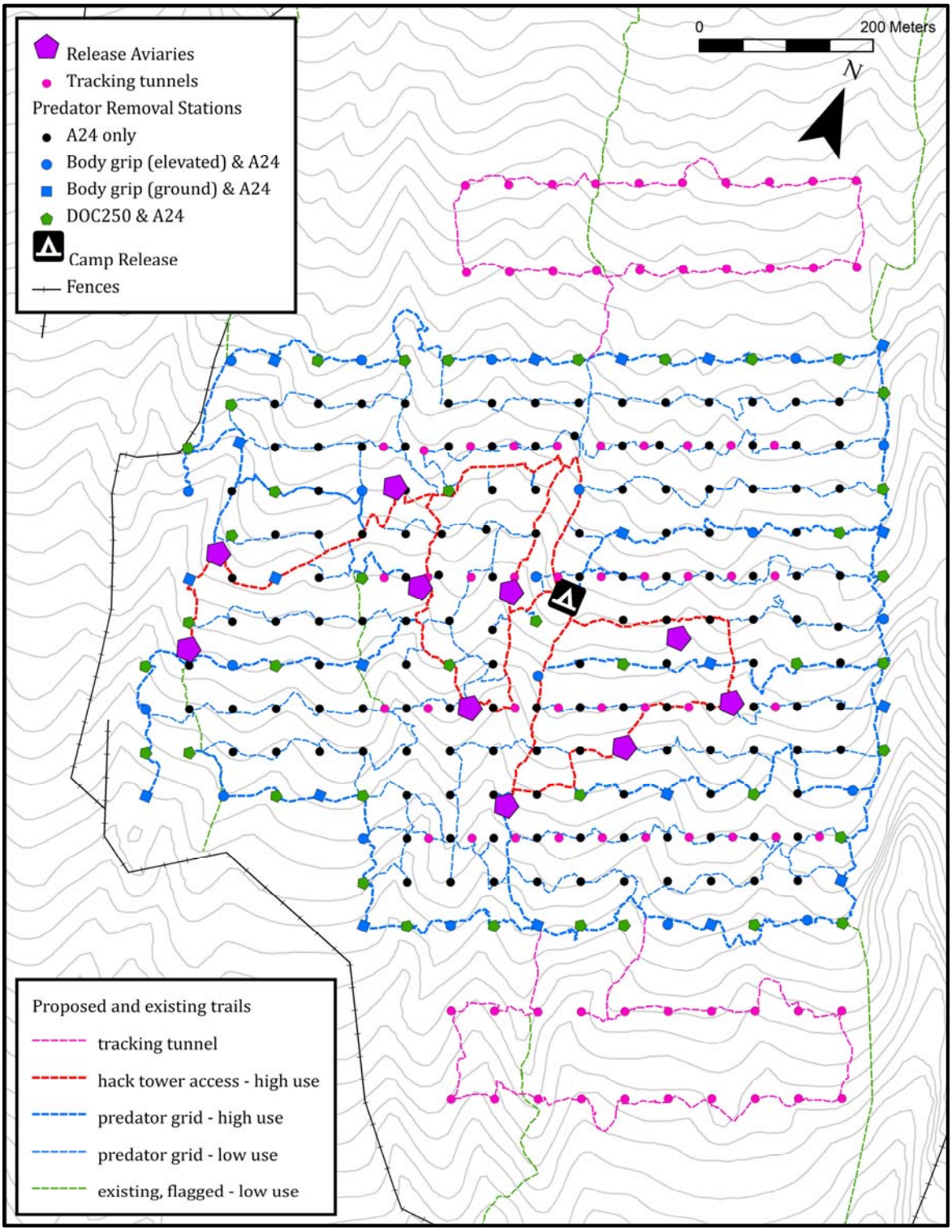


Figure 10. Wailaulau unit, Nakula NAR reintroduction site for Kiwikiu (Maui Parrotbill; *Pseudonestor xanthophrys*) with levels of use for proposed and existing trails.

Capture

MFBRP will lead and supervise the capture efforts. Multiple teams will operate at the field site to maximize the capture rate. Once captured, the birds will be moved to a set of central holding aviaries at the capture site. Capture attempts will begin October, 2019. No capture attempts will be made until all suitable captive Kiwikiu have been moved to the release site. Captures will be attempted only during acceptable weather conditions following MFBRP protocol; wind < 15 mph and rain < code of 2 (i.e., light rain capable of accumulating on nets). MFBRP protocol also allows continuous playback for up to one hour per net location and limits playback to 30 minutes if a Kiwikiu is observed in the area but not captured. If an individual is not captured on the first attempt, e.g., playback time limit exceeded when bird present, additional attempts will not be made until the following day.

Following capture, each Kiwikiu will be processed as it would under normal banding operations. This includes banding (USFWS steel band and unique color band combination), measurements (needed for sexing), photos, and blood sampling. The blood sample will be collected for all individuals captured, either during the banding operations in the field or prior to release. Only staff that are permitted to band Kiwikiu will lead capture teams and make final decisions regarding transferring a captured bird to a holding aviary. At present permitted banders on staff are Hanna Mounce, Christopher Warren, and Laura Berthold. Others may be present at the capture site and aid the banders in net set up, data recording, and secondary handling (e.g., no measurements, no blood sampling, may carry transfer cage, removing non-target species from nets). In the extremely unlikely case when a bird captured from the wild that is considered to be "unreleasable" (see key below), the bird will be transferred to the Maui Bird Conservation Center (MBCC) or Keauhou Bird Conservation Center (KBCC). Following transfer to a holding aviary, an individual may be released back into the wild based on the recommendations of the on-site veterinarian. The capture team may also decide to release a healthy bird back into the wild to achieve a more ideal sex ratio if additional birds are captured.

Below is a key to be used by the capture teams to determine the eligibility of a captured wild Kiwikiu to be translocated to Nakula. Also included are the key and chart used by field teams to age and sex individual Kiwikiu, necessary for determining eligibility for translocation.

Key to determining eligibility of a captured individual Kiwikiu for translocation.

1A. Hatch-year (HY) ..... **release**

1B. Second-year (SY) or After second-year (ASY) ..... **see 2**

2A. Sex determined as Male or Female ..... **see 3**

2C. Sex unknown ..... **return to 1, if still 1B, see 3**

3A. Injury noted ..... **see 4**

3B. No injuries ..... **see 7**

4A. Old injury, scar tissue visible, no open wounds ..... **see 5**

4B. Active injury, open wound, e.g., active pox lesions, broken leg(s), broken wing(s). (Does not include missing nails.) ..... **see 6**

5A. The injury affects range of motion or otherwise *significantly* influences foraging potential/efficiency of the bird ..... **see 6**

5B. The injury is not as described in 5A..... **see 7**

6A. The bird can be released safely back into the wild ..... **release**

6B. The bird is at significant risk of death if released into the wild ..... **transfer to MBCC**

7A. Vascularized brood patch present ..... **release**

7B. No vascularized brood patch present ..... **see 8**

8A. Begging juvenile observed in close proximity ..... **see 9**

8B. No juveniles in vicinity ..... **see 10**

9A. Can it be determined that the bird in hand is <u>*not*</u> the parent of the juvenile? Two additional adults in the area feeding the juvenile or being followed by the juvenile ..... **see 10**

9B. It cannot be determined if the bird in hand is <u>*not*</u> the parent ..... **release**

10A. Bird shows signs of undue or unusual stress, i.e., gaping, sustained raised crest, closing eyes..... **see 6**

10B. No unusual stress noted, e.g., bird is active, bright and open eyes, trying to bite ..... **see 11**

11A. After second-year …… **see 12**

11B. Second-year …… **transfer to holding aviary, see 13**

12A. Current translocation cohort sex ratio supports additional individuals of this sex (predetermined) (6-8 females and 4-6 males) ..... **transfer to holding aviary, see 13**

12B. Translocation cohort is maxed out for this sex …….. **verify with team, release**

13A. Observed feeding on provided food within 24 hours of being placed in holding aviary ... **see 14**

13B. Not observed feeding on provided food in 24 hours ..... **release following expert evaluation**

14A. Bird shows signs of unusual stress in the holding aviary, e.g., flying repeatedly into walls, gaping, feather plucking, lethargic, crouched or fluffed.... **release following expert evaluation**

14B. Not as above, or these behaviors cease after 2 hours …… **see 15**

15A. Passes final vet evaluation within holding aviary …… **eligible for translocation**

15B. Fails final vet evaluation …….. **follow vet recommendation which may be to: i) release; ii) hold and continue to observe; or iii) transfer to MBCC, prioritized respectively**

Key to Age and Sex Maui Parrotbill

1A. Dull grayish-olive body plumage, loosely fitting feathers, possibly underdeveloped bill, mandible pink in color, squeaks ..... **Fledgling**

1B. Plumage not as above ..... **see 2**

2A. Dull grayish-olive dorsal plumage and dingy white plumage on breast, abdomen, throat, cheeks and superciliaries with or without wing bars (pale yellow-white tips) on median and greater coverts ..... **Hatch-year (HY)**

2B. Plumage not as above but partial wing bars present or absent ..... **see 3**

3A. White or yellow wing bars are present, mandible *not* orange/pink, mottled yellow and white superciliaries, white in auriculars and throat ..... **Second-year (SY)**

3B. Plumage not as described, particular attention to color of auriculars and supercilium and presence of wing bars ..... **see 4**

4A. Yellow-olive or gray-olive plumage on nape, back, wing, and tail with solid yellow to bright yellow plumage on the breast, abdomen, throat, auriculars, and superciliaries ..... **After-second-year (ASY)**

4B. Plumage not as above, some mixture of traits including bright yellow on face and breast and possibly retained wing bars ..... **After-hatch-year (AHY)**

5A. Unflattened wing chord length (wing) less than 70.4 mm ..... **see 6**

5B. Wing greater than or equal to 70.4 mm ..... **Male**

6A. 2.386 (wing) – 168.212 < 0 ..... **Female**

6B. 2.386 (wing) – 168.212 ≥ 0..... **Male**

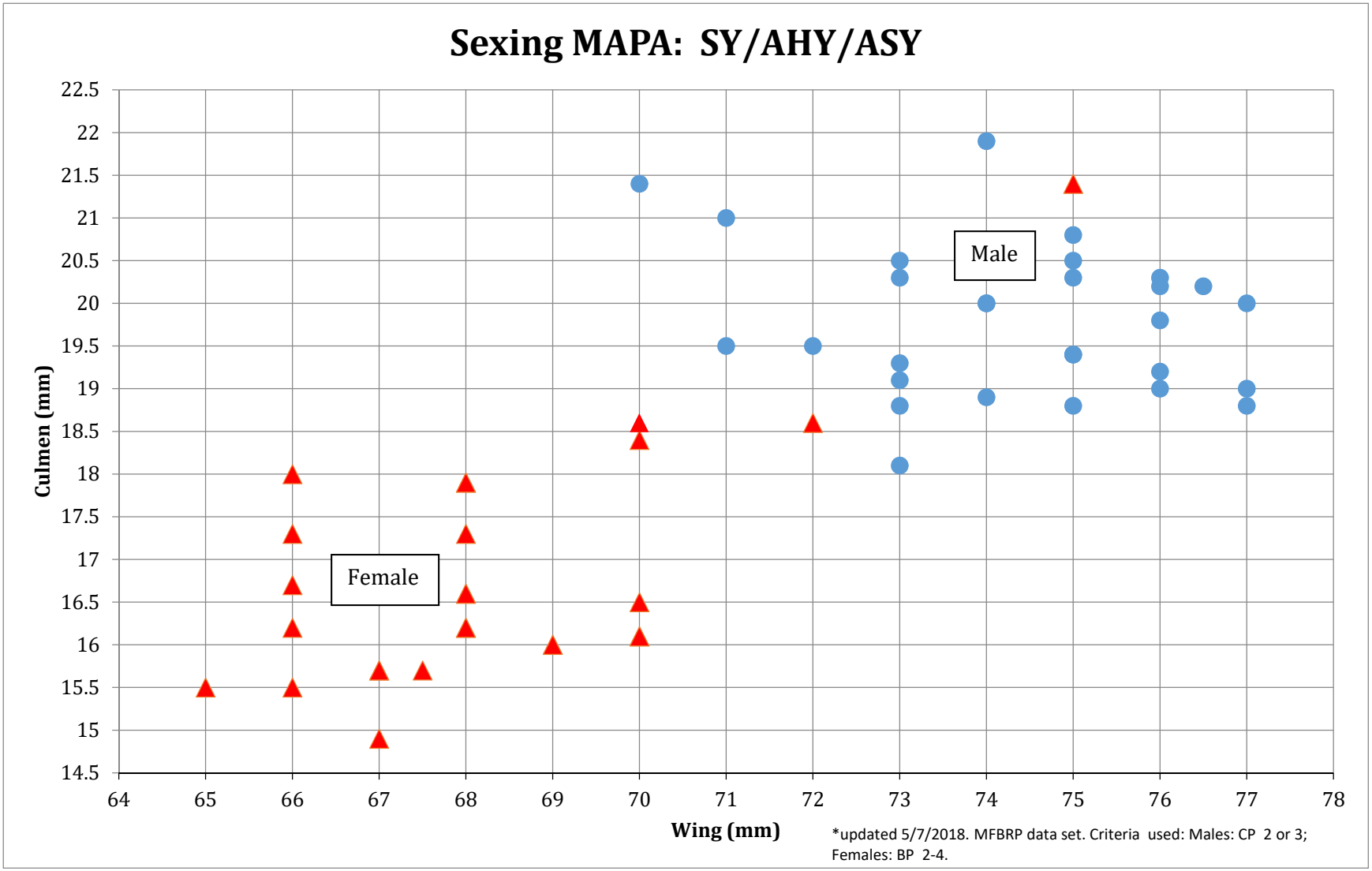


Figure 11. Sexing key for Kiwikiu (Maui Parrotbill; *Pseudonestor xanthophrys*). Regression chart of wing (unflattened wing chord) and culmen (exposed) lengths. Individuals shown are only those found to be in breeding condition, showing a brood patch (Bandit code of 2 - 4), indicating females, and a clocal protuberance (Bandit code of 2 or 3), indicating males.

Holding

Holding at Capture Source Location

When managing birds brought into captive conditions, we will do everything we can to minimize stress. Prolonged stress can and will have detrimental effects on the health of the bird being managed. There are three basic issues that will drive our management decisions:

1. Birds will be housed in cages that will optimize their conservation of energy and minimize the stress due to the extremes of weather.

    a. TNC Waikamoi Preserve and Hanawi NAR are can see fairly inhospitable field conditions with lots of rain and very cold nights with temperatures getting down to freezing. We will place birds into solid wall holding boxes. The boxes will provide the birds with an environment that is very stable and moderated. The bird boxes will be placed on a pipe shelf that is in a covered structure (tent). The tent will need to be set up so it can minimize the impacts of the wind and rain, while also allowing adequate ventilation during the hottest part of the day.

    b. Whenever a bird is held in a bag or holding box, their container will always be placed in the shade whenever possible to avoid overheating.

2. The birds must be able to consume some form of food to maintain its body weight and condition.

    a. The extremely cold temperatures this species endures requires that each bird be able to consume enough calories to compensate for the energy it expends to maintain proper health. Birds that are not consuming adequate calories will see fat reserves and body tissue consumed, resulting in reduced body condition.

    b. Birds will be provided gut-loaded mealworms and bee larvae that are likely to be readily consumed based on observations of Kiwikiu previously brought into captivity. A pelleted food mix will be available. Additional vitamin and mineral supplements developed for insectivorous birds will also be provided.

    c. Weights on birds will be taken in the early morning and also in the later afternoon. These data will be used to monitor and asses the birds' condition daily.

    d. Feeding activity will be documented. Notes will be taken on what items are consumed and diets modified accordingly.

e. Fecal output will be monitored. Feces is a good indicator of food consumption. A normal feces should have a large volume of feces (dark portion) with minimal urates (white portion). If the feces are composed of mainly urates that indicates inadequate nutrition and that the bird is now consuming body tissue and its condition is deteriorating. This will signal the need to provide additional and alternative food sources and to more closely monitor the bird's behavior and health.

3. The psychological state of the bird.

   a. Wild birds that are exposed to signs of danger are able to flee, while captive birds are not. Being in close proximity to a source of danger and not able to flee will cause stress and an increase in corticosterone levels. High levels of corticosterone for long periods of time can have negative impacts on the bird's health.

   b. Maintaining birds in solid boxes with minimal visual contact with the bird handlers and other birds will reduce their stress levels.

   c. Wherever the birds are held will need to be away from the staff facilities. All staff working around the birds will need to talk softly and not make any sudden loud noises that may startle the birds.

Transfer from field to base camp: Once the field team has finished processing a bird following capture and it has been determined that the bird is eligible for translocation (see above), it will be placed into a transport box and transported to the field camp (hiked) where the birds will be housed. The following information will be attached to each transport box: date of capture, time of capture, net, capture weight, band number, and band combination. The bird transport box should be handled carefully and kept steady at all times. When the bird arrives at the bird holding facility it will be taken inside the bird room and placed on the table until it can be transferred to holding cage.

Housing: Each bird will be placed in a single compartment holding cage at the capture site's central camp. The cage will measure approximately 23 cm (9") wide, 20 cm (8") high, and 40.5 cm (16") deep (Figures 12). The cage will be constructed out of 6 mm (¼") polyvinyl chloride (PVC) sheet material. The cage will be collapsible and able to be stored flat. The two side walls are solid, and will have a series of 2.5cm ventilation holes along the upper portion of each wall. The right side wall will have ventilation holes along the upper portions of the wall plus a trap door measuring 7.5 cm wide by 10 cm tall. This opening is covered with a sliding door. The door is placed on the upper back portion of the right side wall. The trap door is used to facilitate removing birds from the cage with

minimal stress. The back wall has an opening covered with mosquito-proof pet screen to encourage ventilation of the cage. The cage has two perches. The back perch is secured to the sides of the cage. The front perch is part of the remote weighing system - two support dowels that are attached to the horizontal perch extend up through the top of the cage and attach to a platform (Figures 12). There are two holes cut into the top of the cage that measure 9/16" and are spaced apart so the 3/16" support dowels to the front perch can easily pass through them. Once the cages are built they will be placed onto a pipe frame constructed from EZ Corners pipefittings. http://ezcorners.com/index.asp and 1" conduit pipe EMT. The rack will have two shelves and be able to hold 13 cages. The boxes will be labeled with a number to aide in observations and record keeping.

Figure 12. Kiwikiu (Maui Parrotbill; *Pseudonestor xanthophrys*) single compartment holding cages to be used at the capture site's central camp with remote weighing system and removable perches and floor.

*Set up***:** When the bird arrives at the bird room (tent), the holding cage will be prepped to house the bird. Paper will be placed on the cage floor tray. Food and water containers will also be placed in the cage. The information tag will be moved from the transport box and placed on the holding cage. The bird can then be transferred to the holding cage.

Diet: A diet will be developed by Peter Luscomb (Pacific Bird Conservation) and Bryce Masuda (SDZG). We will identify food items that are readily accepted by this species in captivity. It usually takes a while for wild-caught birds to take to a captive diet. We will have a variety of options available to ensure that we are able to provide the birds with a well balance diet.

One of the primary foods that we will be giving the Kiwikiu is gut-loaded mealworms. Mealworms will be placed into Repashy gut-loading formula (http://www.rainbowmealworms.net/repashy-superload/) and allowed to remain for up to 48 hours. Prior to feeding the mealworms to Kiwikiu, they will be sifted out of the mix and then distributed to feeding containers. The mealworms will be weighed to ensure feeding consistency and the ability to compare across birds and feeding bouts.

General Management: Birds will be managed starting at 6:00 am and ending at 6:00pm. The bird room staff will look at each bird and determine its basic status. Once all birds have been accounted for, then the weights for each bird will be collected using the remote weighing system. Once weights are complete, food prep will begin. A double set of feed and water bowls will be used to allow one set to be cleaned while the other is filled and offered to the birds. Feed and water bowls will be placed on a table and food items will be distributed among the bowls. When all food and water bowls have been prepped, then preparations for cleaning and feeding will begin. Paper for the cage floors will be precut. Papers will be numbered sequentially with a magic marker and stacked in order. When everything is ready then feeding and cleaning will begin.

To remove the service tray from the cage, the front door will be lifted up off of the service tray by no more than ½", and the tray slowly pulled out. We will be using 4" bowls that are ½" high for some of the food items. When the bowl hits the back of the front door, we will lift the door just enough so the tray and feed bowl are able to pass under the bottom of the door. We will always lower the door so there is about ¼" of space between the bottom of the door and the object on the service tray we are trying to remove. Two 3" D cups will be hung on the back plate of the service tray, when these hit the back of the front door, we will lift the door just enough so the last bowls can fit under the door and the lower the door as soon as possible.

When the service tray is removed from the cage, the paper will be placed onto a table. The food bowls will be placed on the paper. The cage's service tray will be cleaned as necessary, fresh paper placed on the service tray, and the food and water containers on the tray. The service tray will be placed on the bottom front edge of the cage and the tray will be inserted back into the cage.

When all birds have been fed, each bird will be directly observed again and its location, activity, and posture noted. Birds are creatures of habit, and differences or changes from each individual's baseline behavior are critical in early detection of problems. Anything out of the ordinary may indicate a concern, requiring further observation.

Once all behavioral observations are made, the food consumption and fecal output will be documented.

All unconsumed food and tray paper will be placed into a trash bag. The food and water bowls will be placed into a cleaning bucket and then taken to the field camp. All food prep and cleaning will be done away from the bird room so as to minimize disturbances.

Feeding will occur three times per day; 7:00am, 11:00am and 3:00pm. Cage cleaning will only occur during the first feeding. During the lunch and afternoon feeding the tray will be pulled out and food will be checked. Food will be added as needed.

Removing birds from holding cages: At any time a bird needs to be removed from the holding cage, the cage will be taken off of the shelf and placed onto the table. Two people will be needed to remove the bird from the cage. One person will stand next to the right side of the cage and place a small hand net over the trap door opening. This person will hold the net bag so it extends out from the cage providing the bird with an area that it can easily enter. Once the net is properly located and secure, the other person who is stationed at the front of the cage will pull up the sliding door on the trap door. They will then pull up the front service door just enough so they can fit their fingers under the door. This is usually enough to encourage the bird to enter the net. Once the bird has entered the net, the first person will grab the net and contain the bird.

Weight management: All birds will be weighed twice daily. The top plate to the weighing perch will be lifted and an Ohaus HH120 scale will be placed under it so the scale is sitting on the top of the cage with the top plate on top of the scale. The scale will be positioned so it is centered between the two opposing perch support dowels. The perch support dowels need to project out of the box and attach to the top plate are centered in the holes in the top of the cage and are not touching the sides. We will tare the scale so it reads 0. Once everything is ready, we will place one hand over the cage

and gently tap the back window. This will usually result in the bird hopping to the weighing perch. When the bird settles and the scale gets a reading, we will document the weight on the weight log and proceed to the next bird. All weights will be carefully monitored and the first morning weight will be our target weight to maintain and used to assess the bird's condition.

Monitoring status of birds: The birds will be evaluated using weight data, activity patterns, posture, body condition, food consumption, and fecal output.

The best time to monitor birds is after their first morning feeding. The birds should be hungry and spend much of their time acquiring food. Viewing can be made from the rear of the cage where there is a large screened wall. Depending on the type of structure we use for the Bird Holding area we may be able to set it up so the birds are placed in the structure so they can be viewed from the back of the holding structure. We may consider using a remote camera to monitor the birds between feedings.

Decision on status of bird: A bird will be determined to be suitable to be moved to, and released at Wailaulau based on the flow chart in the "Capture" section on page 52. This flow chart includes consultation with husbandry experts and veterinarians.

*Intensive care***:** There will be a veterinarian at the capture site to provide immediate care for any unforeseen medical issues and to evaluate whether a bird can be translocated or released. A veterinarian will be available to go to the reintroduction site if needed; otherwise San Diego Zoo staff are prepared to do minor treatment there in the field.

Holding at Release Site

SDZG will supervise the care and maintenance of the Kiwikiu in the field aviaries for staging in Nakula NAR, while the release cohort of 12 birds is captured. The reintroduction site will have ten paired release aviaries with dimensions of approximately 2.5 m long × 2.5 m wide × 2.25 m tall (8.2 ft long × 8.2 ft wide × 7.4 ft) tall, and made of PVC and wire mesh, with a wood platform (Figure 13). These dimensions have been determined based on observations of Kiwikiu behavior within aviaries used by SDZG's conservation breeding program. At each release aviary site, two paired aviaries will be placed side-by-side allowing two individual birds to see and hear each other, but not physically interact prior to release. There will be a removable visual barrier if it is deemed necessary for the birds not to be able to see each other due to aggressive behaviors. The barrier between the aviaries will also have doors that can allow physical interaction between individuals. A minimum of ¼ of

each aviary will be covered by a roof and shade cloth to allow birds to get out of the sun. Each aviary will be covered in mosquito netting.

Aviary prototypes have been built in Olinda and will be placed in the field in September, 2019. The wooden platforms have been built and are completed in Nakula NAR (Figure 13). The wooden platforms are 2.5 × 2.5 m, constructed with pressure-treated wood, and suspended on 6 cm diameter square steel posts.

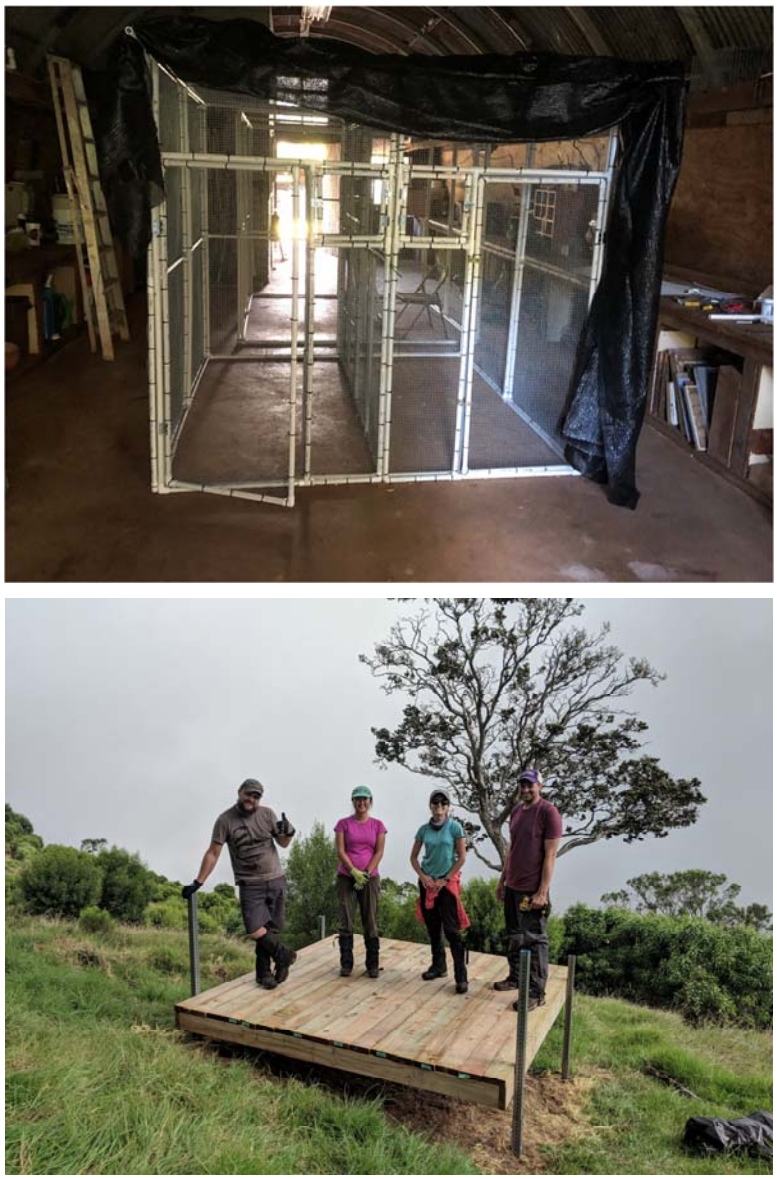


Figure 13. Release aviary prototype (Top) and release aviary platform in Nakula (Bottom).

Release aviary sites were selected based on close proximity to intact forest sections (e.g., gulches; ≤ 150 m from another aviary (so birds can hear other Kiwikiu), within 10-15 minutes hike from Camp Release, and in areas where construction of aviary will result in minimal disturbance to native plants (Figure 9). Aviaries will be constructed in a way that they can be easily broken down and removed following the final releases. Two supplemental feeders will be placed in each aviary (one for each bird).

Supplemental feeders will be placed within the aviaries and these feeders will remain accessible to the birds within each habitat area following the release (Figure 15). Captive birds will already be accustomed to the feeders. The food within each feeder will consist primarily of dry pellet food and/or live mealworms. Dried insects or other items may also be added to the supplemental food (pending ongoing nutritional analysis). The feeders will be similar to (or modified) commercially purchased feeders raised above ground on a metal pole within a PVC pole and/or aluminum flashing to repel rodents. Timing of food replenishment will be dictated by how long it takes for the feeders to be emptied. Each release aviary will be surrounded by five rodent traps to protect the birds from any potential predators while in the aviaries or near the feeders.

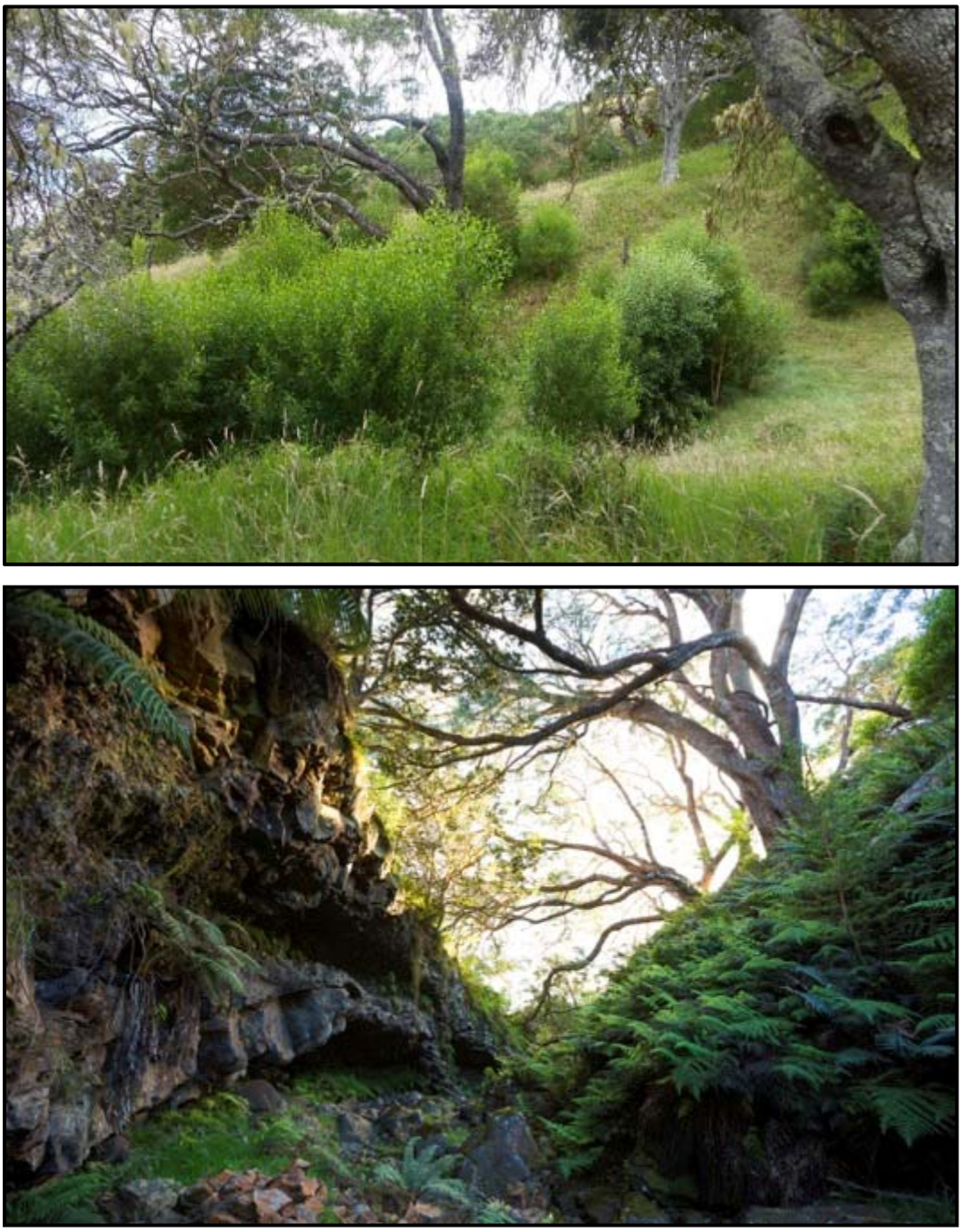


Figure 14. Release aviary Site D (Top) and a nearby gulch providing good habitat (Bottom).

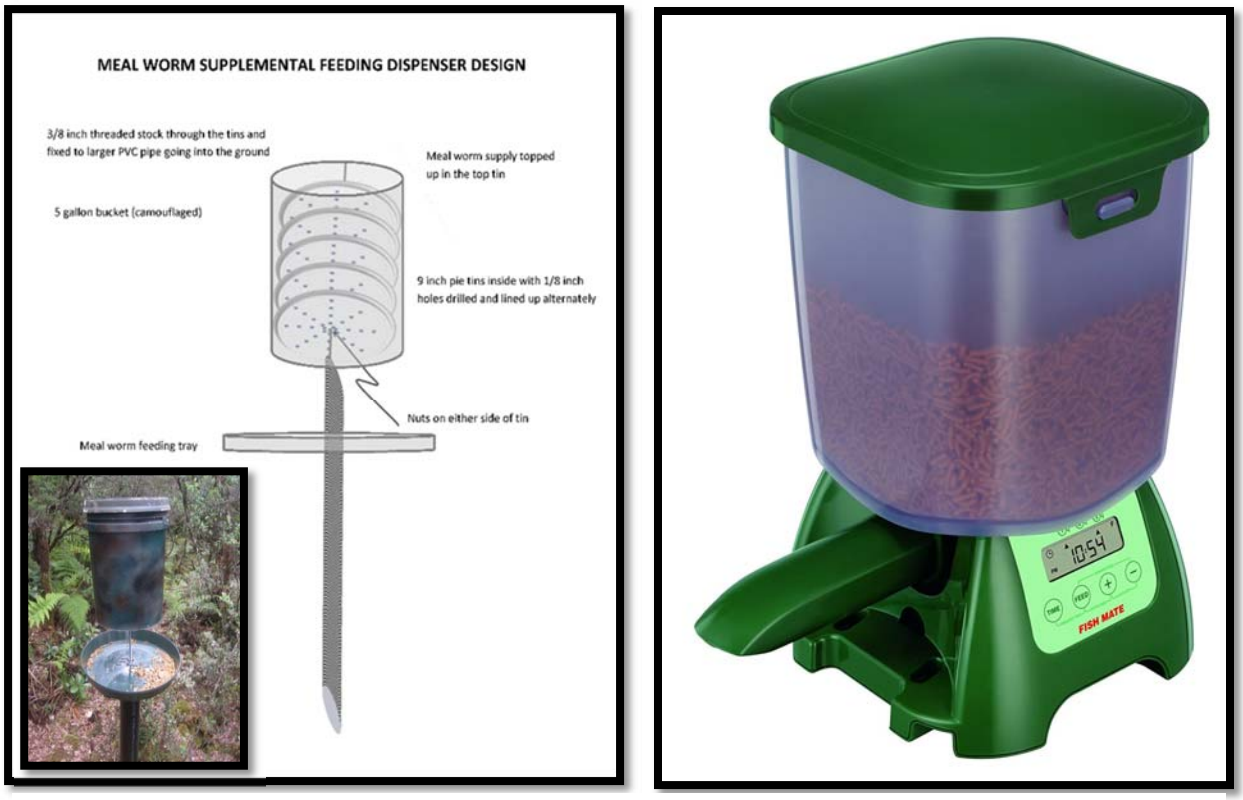


Figure 15. Prototype supplemental feeding dispenser designs to be placed inside each aviary cell and outside of the release aviary. The exact feeder used will be slightly different modification designed as a hybrid of the two pictured.

Transport

The captive-bred birds will be moved to the release site at the beginning of the reintroduction process, and before any capture attempts are made for the wild birds. Prior to being moved to the release site, all captive-bred birds will undergo an examination by a SDZG veterinarian. A helicopter will land at a landing zone near or above the Maui Bird Conservation Center to pick up the captive-bred birds and transport them to the field. These individuals will be held at the release site for approximately one week prior to when wild captured birds will be moved to the release site (see below). A portion of the release team will arrive at Camp Release in Nakula NAR, before these birds to open the camp, prepare release aviaries, and care for the birds once they arrive.

After being captured, the wild Kiwikiu will be hand-carried in transfer cages to a central field aviary near the capture site. Once the 12 bird cohort is assembled, the birds will be helicoptered to Camp Release on the next morning with safe flying conditions. Once the birds arrive at Wailaulau, the wild Kiwikiu will be immediately placed into release aviaries. If the full cohort of 12 birds is not captured within ten (10) days of the first capture, a smaller cohort may be moved to Wailaulau, followed by additional cohorts (no wild bird will be held in capture site field aviaries for > 10 days). Wild birds may be housed in paired aviaries (separate compartments) with captive birds, so that the captive bird can tutor the wild bird on how to use the supplemental feeders. Another priority will be to maintain pairs of wild-wild birds that are believed to be an existing pair based upon their capture behavior and history. There will likely be more wild birds than captive birds being released, so it is unlikely all wild birds will have captive tutor. This is not a great concern due to past SDZG observations of wild Kiwikiu acclimating to feeders relatively quickly.

Release

We will use a soft-release technique for all 20 Kiwikiu. The birds will be held in separate, but paired aviaries (with removable visual barriers), at the ten locations shown in Figure 9. As stated previously, these aviaries will be 2.5 m maximum in its longest dimension (approximately 2.5 m long × 2.5 m wide × 2 m tall), and constructed with PVC with wire mesh. The aviaries will not be permanent structures, and will have no long-term impact on the NAR landscape. The release aviaries will be removed following the final release.

The captive birds will be held for approximately one week prior to when the wild birds are transferred and placed into the release aviaries. This one-week duration will allow the captive birds

to acclimate to the release site prior to a wild bird being placed in an adjacent chamber. Once the wild birds are placed in the adjacent chamber, both the captive and wild birds will be held for approximately 1-4 weeks prior to release. This duration is the expected period of time it will take for wild birds to become comfortable eating out of the supplemental feeders and acclimate to both the conspecific and the release site. In total, captive birds will be held at the release site for approximately 2-5 weeks. Behavioral observations, including food consumption monitoring, will be conducted to inform factors that may affect post-release survival and settlement for future Kiwikiu releases. It is not known which specific behaviors, as well as the intensity and frequency of the behaviors, are associated with post-release survival and settlement for Kiwikiu.

Food will be provided to each bird while in the release aviaries and the same diet will continue to be provided after the birds are released into the wild. This diet will consist primarily of dry pelleted food, and may also include dried insects (pending ongoing nutritional analysis) and live prey items. Following the acclimation period, the aviary doors will be opened. Once Kiwikiu are outside of the aviary and away, the supplemental feeders will be moved outside of the aviary and the aviary doors closed. The aviaries can be used at a later time, if necessary, to recapture birds. Supplemental food will continue to be offered from within or in the vicinity of the release aviary for as long as the Kiwikiu are returning (minimum once a week service) and game cameras will be used to determine the frequency that the birds (Kiwikiu or other species) are feeding from the trays.

The release will be conducted incrementally in order to help anchor birds to the release site, and to ensure that post-release monitoring is manageable. On the first day of the release, one-two pairs being held in a centrally located release aviary will be released. Then, on the following days, one-two additional pairs from adjacent aviaries will be released. If there are no post-release difficulties, the releases will be conducted over a period of 10-14 days, since there will be ten release aviaries.

Each bird will have two to four bands (one to three colors, one USGS/USFWS steel band) that will be applied prior to movement into the release aviaries (at capture site for translocated birds and at MBCC for the captive birds). Weight of bands and transmitters adds up on these small birds so smaller individuals may have color bands eliminated to accommodate the transmitters and stay under 5% of their weight. Every bird will also have a radio transmitter to allow for monitoring the individual birds' behavior and movements. These transmitters will be attached approximately a week after the birds have been in the aviaries (longer for the captive birds). It will be critical to

follow the birds if they go onto the adjacent parcels or move across the many drainages within Nakula. Transmitters will also provide the ability to locate deceased birds to determine sources of mortality. The radio transmitter will be attached 3-5 days (or once they have returned to normal behavior) before the birds are released to monitor how the birds are interacting with the transmitter. Although not perfect surrogates for the wild birds, we attached dummy transmitters to captive birds at MBCC prior to the move to Wailaulau to allow for extended observations of how the birds may interact with the transmitters and harnesses. Of particular concern is if Kiwikiu bend the antennae, as Palila (*Loxioides bailleui*) have done, or are able to cut the harness with their sharp mandibles. Depending on the size of the transmitter (determined by the size of the bird), the transmitter batteries should last between 50 and 88 days after activation. If possible, the birds will be recaptured in the field before the transmitter battery dies, and the transmitter will be replaced. As a result of the dummy trials on Kiwikiu, we will be:

- Using a leg-loop harness made of elastic material
- Any birds that do not tolerate a harness (i.e. sit on the floor or won't feed) will have it removed within a 24-hour period and a glue on transmitter will be applied. We understand it is very likely that the birds will remove the glue-on transmitter but it is worth an attempt given that the alternative is to release birds without any monitoring devices or not to release them at all.
- The transmitter harnesses are going to be applied using the "pencil" measurement method. The tightness of a harness can be very subjective. While we have used the most experienced banders to feel these harness attachments out, the species may still not tolerate the same snugness that other birds do. Thus, inserting a pencil under the transmitter and tightening the harness to that and then removing the pencil will ensure a looser attachment. We understand that the compromise here is that birds may be more likely to slip out of transmitter in the field prematurely but this "pencil measurement" technique is recommended by the manufacturer as well as other experienced banders whom acknowledge and accept this trade off.
- A small layer of neoprene is going to be glued to the bottom side of the transmitters so that the plastic potting on the transmitter itself is not in direct contact with the birds' skin or feathers. This was suggested by the transmitter manufacturer as well as other experienced banders whom have worked with transmitters on other more problematic species.

During all of these activities, we want to minimize all disturbance to the birds in the aviaries. We will schedule the release and monitoring teams at the site in such a manner to limit helicopter activity around the aviaries as much as logistically possible. We have established landing zones 300 meters away, 500 meters away, and 800 meters away from the closest aviaries (approximately 1,000 ft in elevation above camp). We will be able to fly in supplies at the beginning of the translocation operations and then land people at these new alternate landing zones (less than an hour hike to the camp) depending on how we see the kiwikiu responding the helicopter noise/activity in the area.

As noted with helicopter activity above, there is additional concern about aviary stress in general. Kiwikiu have been held in small cages in the field for 48+ hours as well as transporter via helicopter out of the field without any ill effects (MFBRP and SDZG personal communications from 2007 capture efforts). Even so, the team observing the birds in the release aviaries on site will ensure that:

- Each bird's behavior in the aviaries has returned to normal following transmitter attachment before release.
- If birds show signs of stress, fluids will be administered in the field.
- Supplemental food resources will be of the highest nutritional value possible. This includes high protein and nutritious items similar to what the birds would consume in the wild such as, bird pellets that contain a comprehensive suite of nutrients, egg protein supplement mix, fly larvae, and mealworms., so will not be entirely natural or available in the wild.

Veterinary Care

All reasonable efforts will be made to have a veterinarian on site during captures. These individuals are not available locally and will be brought in from the mainland for the two weeks of field time. San Diego Zoo Global staff will be on site for releases and we have an on call veterinarian on Maui to use for emergencies but birds would need to be brought out of the field.

Death and Necropsy

This reintroduction is a significant management action that will have large benefits to the long-term conservation of Kiwikiu. However, translocations involve lots of capture, handling, and movement of the birds, and unfortunate events can happen and mortality can result. If this occurs, the bird's

carcass will be retrieved as soon as possible, and stored on ice (being careful for the carcass to not touch the ice directly, and only for the carcass to be kept cool by the ice) until it can be transferred to a qualified veterinarian for a necropsy. SDZG will conduct the necropsies for all the captive and wild birds. The project leads from all involved agencies will be notified of the necropsy results as soon as the results become available.

3. Post-Release Monitoring and Assessment

3.1 Protocols and data collection

Short term (50-88 days)

We will attempt to resight each bird every day (or at least every other day) while the transmitters are active. Although this will be very demanding on the monitoring personnel, because there is so much uncertainty in the birds' behaviors we must maximize our data collection while the transmitters are active. This intensive monitoring will allow us to detect an individual's absence and potential emigration or attempt to return to the source population. It will also allow us to determine the home ranges and pair status of all the birds. The home range data is especially critical because it is unclear how the translocated and released Kiwikiu will perceive the recovering forest, and the actual habitat suitability is critical for evaluating the carrying capacity of the Wailaulau unit and greater Nakula area. Similarly, it will provide important data on foraging and behavior to help assess the bird's health and ability of the habitat to support Kiwikiu and future translocations.

This intensive data will allow us to determine the survival of the birds and detect any mortality caused by the capture, transport, and release process. Intensive monitoring while the transmitters are active will assist in determining the timing of mortalities and timely retrieval of carcasses. This will increase the likelihood of determining causes of mortality and ultimately methods to mitigate these factors during subsequent translocations. We will also conduct focal behavioral observations of each bird during the resighting period. These short observations will allow us to determine foraging behaviors, habitat preferences, and possible social interactions. The length of these focal observations will depend on how long the bird remains within close distance to the observer. This information will be critical in assessing possible difficulties (e.g., insufficient food plants available) with the reintroduction. Beyond survival and foraging behaviors, every effort will be made to determine if any pairing, territorial, or breeding behaviors are being exhibited. In the marked wild

populations in Waikamoi and Hanawi, establishing pairing and breeding status often took multiple encounters with individuals over an extended period. The transmitters will greatly enhance our ability to determine the status of each bird during the life of the transmitter battery.

Extended term (89-365 days)

We will monitor all the movements during the first 50-88 days (depending on sex and size of bird due to battery life) while the radio transmitters are active. This will allow us to track any short-term movements. We will use our daily resights to determine each bird's home range and the habitat they are using.

Monitoring after the radio transmitters' batteries are no longer active is still important, but much more difficult. The Kiwikiu's territoriality should make it easier to locate birds that remain within the Wailaulau unit. However, some birds will presumably continue to slowly leave the release area, into the adjacent habitat or searching for their previous territory. Additionally, the remote nature of Wailaulau means that field teams cannot always be present. The most important data to collect is the survival and continued persistence of birds at the translocation site, as well as maintaining supplemental food resources if the birds are still observed using them. As stated above, this is an experimental release into an area with different habitat than either the Hanawi or Waikamoi sites. Evaluation of the success, or potential success, of the translocation depends on determining the survival and persistence of the Kiwikiu in this restored forest.

Monitoring the persistence, home range size, and foraging behavior over the first year will provide the data to evaluate whether subsequent translocations can occur. To reach our short- and long-term objectives, the Kiwikiu must persist at the site, which requires them finding sufficient food in the recovering forest. Estimating home range area is important because it determines how many Kiwikiu the area can support, i.e., carrying capacity. Given the differences in habitat between the current and reintroduced range, predicting carrying capacity in Nakula is very difficult. Through their intensive monitoring efforts, MFBRP found 24 pairs per km^2 in Waikamoi and 52 pairs per km^2 in Hanawi on average (0.23-0.52/ha) (MFBRP unpublished data) and pair home range size was estimated to be 14.5 ha (Warren *et al.* 2015). Based on the home range densities seen in Waikamoi and Hanawi we may expect that an area of equivalent habitat quality the size of Wailaulau (170 ha) could support between 40 and 88 pairs. Given the current state of the habitat in Nakula, we expect the current carrying capacity to be much lower than this. Based on non-overlapping home ranges,

Wailaulau may hold 11 pairs (170 ha/14.5 ha pair home range). However, pairs often overlap home ranges with neighboring pairs on the windward slope thereby increasing density (more overlap = higher pair density) and we expect similar behavior at the release site. Thus, comparing home range size and overlap in the released birds to those estimated in Waikamoi and Hanawi may give us an idea of the eventual densities we can expect (i.e., carrying capacity) and the number of birds to release over three years. The habitat is unlikely to be saturated in the first year, even if all 20 birds survive, but comparing the home range size to currently occupied habitat will also provide an index of the habitat quality.

Breeding

The long-term objective is for the established population to be self-sustaining, which requires local breeding and recruitment. During the first spring after the translocation (e.g., April-June 2020), there will be additional effort dedicated to find and monitor the remaining birds, and detect any nesting that occurs.

Longer Term (>1 yr)

MFBRP and the project partners will continue to resight and collect foraging and behavioral data on the translocated birds on their subsequent trips to the site. Continuous monitoring will be done until January 2020 and intermittent monitoring will be conducted at least until November 2020, one full year following the release. Annual point counts will be conducted in Nakula in April and May 2020. MFBRP will continue to visit Nakula monthly for predator control and monitoring though the fall. These data will be used to determine long-term persistence and ability of the Wailaulau area to support Kiwikiu and continue additional releases. If MFBRP and partners decide to move forward with additional releases, preparation will be made to capture wild birds from TNC Waikamoi Preserve in year 2 (captures to occur sometime between November 2020-January 2021). At this time, no releasable birds will be available from SDZG and cohort will be made up of all wild birds.

3.2 Alternatives and future actions

Guidelines for determining releases

In the first year, we will be released or translocating 20 or fewer birds from one wild source (Hanawi NAR) so we will not be capturing all the genetic diversity necessary. We know that multiple translocations will be necessary to create a healthy, self-sustaining population in Nakula.

However, one translocation from a single source population will meet the short term goals of the reintroduction and will be a more efficient use of resources. If the birds persist into the summer and appear to be doing well, then planning for a second translocation operating from a different source (e.g., Waikamoi) can begin. If the birds fail to persist, the first priority will be to determine why and how to correct this, and immediately work on resolving these reasons for emigration or mortality (if possible), before conducting subsequent and future translocations.

Any subsequent translocation will not occur until the second year, i.e., October 2020 – January 2021. If possible, factors that reduced the first year survival or persistence will be addressed and mitigated. However, it is important to keep in mind that 20 birds is a relatively small number to release, and that post-release mortality and dispersal is inevitable due to the stressful translocation and reintroduction process. Therefore, the plan is to release birds each year for at least three consecutive years, keeping in mind that the reintroduction protocols will change based on data collected during post-release monitoring and necropsy analysis. Due to the logistical difficulties in capture, transport, release, and monitoring, it is not feasible to attempt more than one release each year. Due to the release of nearly the entire captive flock, subsequent releases will be entirely wild translocated birds.

4. Resources Needed

4.1 People and Roles

Staff Needed

Installing Release Aviary Team (MFBRP/SDZG): Building release aviaries will involve up to 10 people at camp and within the area for up to 10 days per trip.

Capture team (MFBRP/ABC/DOFAW): Three teams of three people (9 total). These staff will work in the capture site, and then shift to Wailaulau. Some of these could be short-term experienced volunteers and/or partners that would not stay at Wailaulau for the entire monitoring rotation.

Holding Team (MFBRP/SDZG/Pacific Bird Conservation): Two teams of 2-3 (4-6 total). One team will be stationed at the capture site and the second team will be at Wailaulau. The holding team at the capture site could get assistance from SDZG staff, Pacific Bird Conservation, or the capture team. Once the wild birds are moved to Wailaulau, a second team would not be needed.

Release & Monitoring Team (MFBRP/ABC/DOFAW): 4-6 people. Initially, this team will only be two people to open Camp Release and prep it for birds, open and run the predator grid, and assist the Holding Team that is caring for the captive-bred birds in the aviaries. Once the birds are released, the team will need to increase to four to six people to monitor all the birds and collect the necessary behavioral data to assess the translocation. The number of staff needed for monitoring will depend on the difficulty in locating and observing all released birds. Constant staff presence will be required during the four to eight-week period of soft release, and then three to four months for initial monitoring. Team members will be rotated to maintain constant presence at the site for monitoring and maintenance of supplemental feeders. It is possible that only two people will be required for the latter part of the monitoring period. MFBRP has a field staff usually consisting of three to four people. We plan to hire three temporary field assistants in addition to our 1-2 interns.

Community volunteers and staff from partner organizations have been instrumental in the success that MFBRP has had conducting restoration work in Nakula NAR in the last five years. With the help of volunteers, MFBRP have been able to increase their effectiveness on the ground. MFBRP plans to continue using volunteers throughout the Kiwikiu reintroduction and for future restoration work. Volunteers during the release are likely to be longer-term, skilled persons given the specialized and sensitive nature of the work. Four to six volunteers will be involved with the aviary construction and continued predator control while one to two skilled volunteers will be recruited for monitoring.

Partners and collaborators

Maui Forest Bird Recovery Project – translocation from capture site to release site, release of cohort, post-release monitoring of cohort to collect behavioral data, predator control and rodent traps, future mosquito control.

Pacific Bird Conservation – advise design and construction of field aviaries and care of translocated birds in temporary field aviaries at capture and release sites.

San Diego Zoo Global – design and construct release aviaries at Wailaulau, transport captive birds from the Maui Bird Conservation Center to Wailaulau, care of captive and wild birds in release aviaries at Wailaulau, conduct necropsies on dead birds

American Bird Conservancy – continued funding and advise adaptive reintroduction plan informed by success and mortalities of released birds, support and assist with initial capture and monitoring.

DOFAW-NEPM – continued funding, and continued restoration in Wailaulau and West Pahihi Nakula NAR units. Access to NARs.

DOFAW-Wildlife – continued funding, and organizational support

DOFAW-Forestry – continued restoration in Kahikinui FR and access to Forest Reserve lands for monitoring if needed

USFWS – continued funding, possible personnel support

The Nature Conservancy – organizational support and access to TNC Waikamoi Preserve as a source for translocated Kiwikiu

Haleakalā National Park – access to Nuʻu unit during post-release monitoring and the Kīpahulu and Manawainui areas are possible future source of translocated Kiwikiu

LHWRP – coordinate access to Department of Hawaiian Home Lands with community leaders during post-release monitoring, continued restoration across the leeward slope

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Appendix I. Master timeline for Kiwikiu reintroduction

2018 Completed Tasks	NARS commission presentation. Secured funding. Finalized Reintroduction Plan. Conducted baseline tracking tunnel survey. Installed predator grid. Run predator traps (A24s are not set). Constructed prototype field aviary and feeder in Olinda. Finalized design and ordered materials. Constructed release platforms for aviaries in Nakula.
2019	Community Meeting/Communication with landowners. Construct telemetry towers when equipment becomes available, prior to October
January 2019	Finalize and order telemetry supplies.
2019 February	MFBWG conference call meeting
2019 March	Nakula predator control (all traps), tracking tunnels, planting Test harnesses and transmitters on captive-bred birds.
2019 April & May	Nakula point counts, banding, radio tracking HAAMs Filming/Production for Community Outreach.
2019 Summer	Nakula predator trapping, tracking tunnels, planting, possible mosquito control?
2019 September	Nakula predator trapping, planting, possible mosquito control? Install aviaries on the platforms in Nakula. Hanawi camp prep, trail work, scouting
2019 Oct	Nakula: predator trapping and quarterly tracking tunnels.

	Move captive-bred birds to Nakula release sites. Provide supplemental food in release aviaries. Begin capturing wild birds in Hanawi.
2019 November	Move Hanawi wild birds to Nakula release sites. Staggered releases of pairs. Supplemental feeding. Radio tracking Kiwikiu. Predator Control.
2019 Dec to 2020 Feb	Monitoring and tracking released birds. Provide supplemental food as long as birds rely on it. Continue predator trapping and quarterly tracking tunnels.
2020 March on	Continue resighting and behavioral observations of Kiwikiu. Point-counts in Nakula NAR (April-May). Continue predator trapping and quarterly tracking tunnels. Evaluation of release and planning for future translocations.

Appendix II. Equipment Needs for Capture Site

Equipment:

1 tent for Bird Holding area
13 collapsible holding cages
Brackets for the shelf, 1" EMT for shelf
2 folding field tables
55 3" D cups
26 4" plant saucers
2 hand nets for trapping birds out of holding cage
1 hand net for catching birds that escape into Bird Holding area.
2 plastic wash pans
2 Ohaus 120 scales
1 measuring spoon set
Pans for mealworms

Supplies

Mealworms
Bran meal
Repashy
Chlorox
Dawn dish soap
Scrub pads
Nekton I

Potential sources of equipment and supplies

http://www.ezcorners.com/index.asp
https://www.homedepot.com/p/1-in-EMT-Conduit-101568/100400409
https://www.campmor.com/c/coleman-outdoor-compact-table
https://www.backcountrygear.com/nrs-roll-a-table-blue-one-size.html?utm_keyword=NRS9R10490&utm_medium=cpc&utm_campaign=roi+plas+-+all+products
https://www.outdoorgearlab.com/reviews/camping-and-hiking/camping-table/alps-mountaineering-dining-table-regular
http://www.avian.nl/EN/delikat-insectivores-di.html
http://www.wombaroo.com.au/birds/finch/insectivore-rearing-mix
http://www.mazuri.com/mazuriinsectivorediet5MK8.aspx
https://www.haiths.com/prosecto-insectivorous-wbsf01004/
https://www.birdsupplynh.com/catalog/product_info.php?products_id=3271&osCsid=9ee52e164930e887715dac0f5f9d9f81

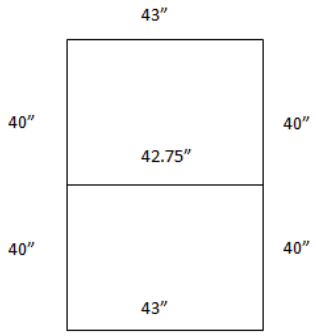
Kiwikiu Release Aviary Prototype

Overview

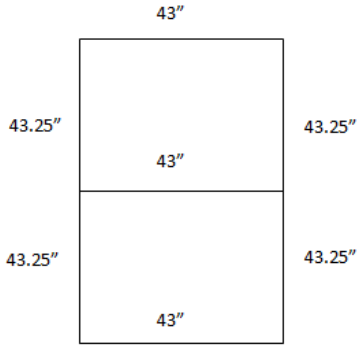
- ¾” PVC pipe aviary frame with panels that fit outside the frame, attached to a level wooden base (8’ across)
- Panels are just under 4’ wide, designed to accommodate 4ft wide wire paneling and fit on an 8’ base
- Two panels with doors and hatches, and an interior wall with two hatch doors on two aviaries
- Frame would be color coded for easy construction in the field
- Panels will be pre-constructed with wire attached and flown in together
- There will be a top roof panel that will cover that back 2’ of the aviary, there will also be a 2’ roofing panel on the top of the back wall. These two panels will provide a protected area from the weather.

Measurements

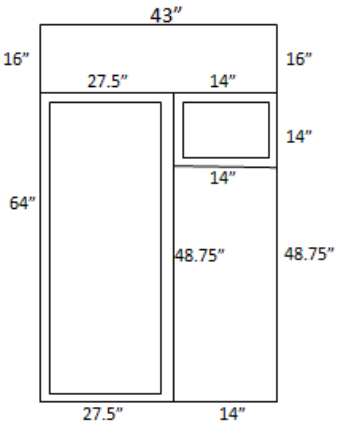
- Side Panel Dimensions
  - 6 per aviary



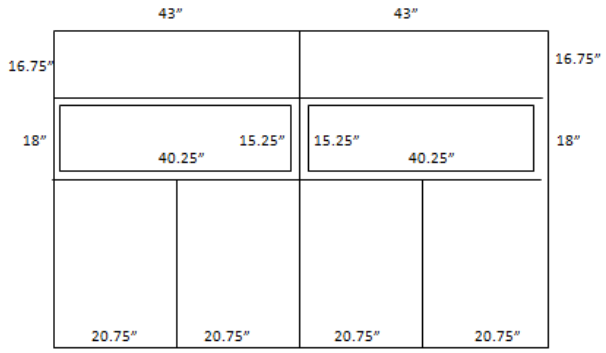
- Roof Panel Dimensions
  - 2 per aviary



- Door Panel Dimensions
    - 2 per aviary
        - Hatch Door: 11”x11”
        - Keeper Door: 61”x24.5”
        - No keeper area designed; could do shade cloth drape in front of the door and hatch door
        - Weather stripping would be installed around the edges of all doors
        - Doors will have two latches (top and bottom of the doors) to ensure it aligns properly

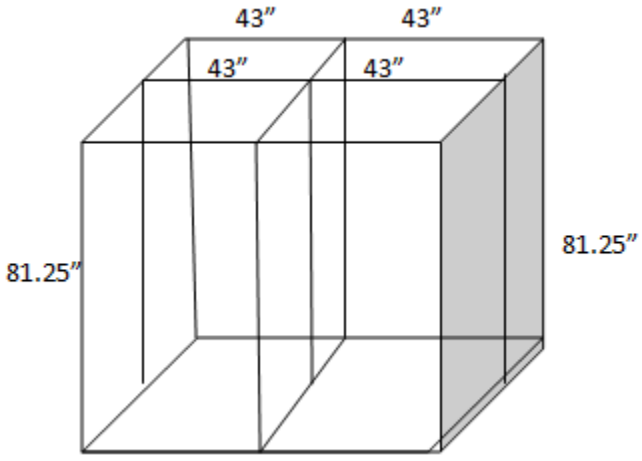


- Frame Divider Cross Section Dimensions
    - Built into the frame
    - Allow for Hatch door access between aviaries for breeding pairs (only two aviaries will have doors in between)
    - Shade cloth would be installed on the dividing panel to limit visibility between aviaries and reduce stress



- Wooden Platform Concept Picture
  - 8x8 plywood base

- Frame Dimensions



- Designed for the panels to align outside the frame and be attached with pipe clamps to the frame

Totals

Item	Amount **(1 Aviary)**	Amount **(9 Aviaries)**	Weight **(10 Aviaries)**	Price **(9 Aviaries)**	Link to Price Source
¾” PVC	463 ft	4167 ft	Approx. 900 lbs	20’ poles from Maui Irrigation	

				(26 poles needed per aviary, 234 in total)	
½" by ½" Hardware Cloth	Approx. 88ft	792 ft (8 rolls of 100ft)	65.1 lbs/100ft roll	$151.79/100ft roll Approx **$1,214.32** for 9 rolls	https://www.amazon.com/Garden-Zone-100ft-Hardware-Cloth/dp/B000OWDKQK/ref=sr_1_2?ie=UTF8&qid=1527280095&sr=8-2&keywords=1%2F2%22+hardware+cloth+4+foot
¾" PVC 90 degree slip elbows	64 (for aviary with hatch doors)	520	0.08lb/piece 41.6 lbs	$3.52/10-pack **$183.04 total**	https://www.homedepot.com/p/DURA-3-4-in-x-3-4-in-PVC-Sch-40-90-Degree-Slip-x-Slip-Elbows-10-Pack-CP406-007/203041000
¾" PVC Tee	42	378	.19lb/piece 79.8 lbs	$12.07/8-pack **$570.30**	https://www.homedepot.com/p/Formufit-3-4-in-Furniture-Grade-PVC-Tee-in-White-8-Pack-F034TEE-WH-8/205749498?MERCH=REC-_-SearchPLPHorizontal1_rr-_-NA-_-205749498-_-N
¾" PVC 4-way elbow	4	36	0.35lb/piece 14lbs	$17.51/8-pack **$78.79 total**	https://www.homedepot.com/p/Formufit-3-4-in-Furniture-Grade-PVC-4-Way-Tee-in-White-8-Pack-F0344WT-WH-8/205749450
¾" PVC cross	2	18	15.5ounces/ pack 3 lbs	$16.80/8-pack **$37.80 total**	https://www.amazon.com/FORMUFIT-F034CRX-WH-8-Cross-Fitting-Furniture/dp/B00MNIYSGO/ref=sr_1_1?s=hi&ie=UTF8&qid=1528226160&sr=1-1&keywords=3%2F4%22+pvc+cross+fitting
¾" PVC 3-way elbow	8	72	TBD	$14.94/8-pack **$134.46 total**	https://www.homedepot.com/p/Formufit-3-4-in-Furniture-Grade-PVC-3-Way-Elbow-in-White-8-Pack-F0343WE-WH-8/205749438
Weather stripping	8	72	TBD	$12.45/49in **$896.40 total**	https://www.amazon.com/Efficient-Stopper-Soundproofing-Weather-Stripping/dp/B079N7YVQ4/ref=sr_1_7?ie=UTF8&qid=1531716268&sr=8-7&keywords=long+door+sweep
Pipe Clamps (3")	54	486	TBD	$9.99/6 clamps **$810.00 total**	https://www.amazon.com/Stainless-Steel-Hose-Clamp-inch/dp/B076G1YG3N/ref=sr_1_18?ie=UTF8&qid=1528224853&sr=8-18&keywords=3%22+pipe+clamps
Shade cloth	1 roll per 3 aviaries	4	6.46lbs/roll 25.84 lbs total	$42.99/8'x25' **$171.96 total**	https://www.amazon.com/Fence4ever-Beige-Sunscreen-Shade-Fabric/dp/B015VOZDKG/ref=sr_1_11?s=lawn-garden&ie=UTF8&qid=1531717353&sr=1-11&keywords=shade+cloth+8%27
Hinge hardware	12 hinges (with hatch doors in between)	76	TBD	$5.50/2-pack **$209.00**	https://www.homedepot.com/p/Everbilt-2-1-2-in-x-2-1-2-in-Stainless-Steel-Narrow-Utility-Hinge-Non-Removable-Pin-2-Pack-20257/204727560?MERCH=REC-_-PIPHorizontal1_rr-_-204205210-_-

					204727560-_-N
Latch Hardware	10 latches (with hatch doors in between)	58	TBD	$4.15/each **$240.70 total**	https://www.homedepot.com/p/National-Hardware-3-in-Satin-Chrome-Slide-Bolt-V860-SLIDE-BOLT-SCHR/204380682
Bolts	1 box (100 total) of ½ in screws	10	TBD	Approx $6/box **$60 total**	Can buy in store
Zip ties	1 bag (100)	10	TBD	Bag of 1,000 **$26 total**	https://www.homedepot.com/p/National-Hardware-3-in-Satin-Chrome-Slide-Bolt-V860-SLIDE-BOLT-SCHR/204380682
Plywood	4 sheets of 4'x8' plywood	20 sheets	TBD	40 sheets of plywood	
2"x4" wood	TBD				
L-brackets	TBD				

Considerations

- PVC is very lightweight
- Can construct it at the facility, color code, and send broken down
- PVC easily warps which can throw off measurements so we will need to be cautious to inspect all pieces when buying 20' poles
- Connecting pieces must stay the same brand or can risk throwing off measurements

Construction

Total 20' poles for one aviary: **26**

Total 20' poles for additional 9 aviaries; **234**

Piece Sizes	**Number needed per aviary**	**Number needed for 9 additional aviaries**
43"	40	360
40"	24	216
14"	10	90
20.75"	8	72
43.25"	8	72
11"	8	72
81.25"	6	54
42.75"	6	54
44"	5	45
40.25" *Hatch door aviaries only	4	4
15.25" *Hatch door aviaries only	4	4
24.5"	4	36
16"	4	36
27.5"	4	36
48.75"	4	36
61"	4	36

18”	3	27
16.75”	3	27
64”	2	18

All aviaries were put together and gaps around the doors were covered with weather stripping prior to being sent into the field on 8/1/19. All aviaries were color coded so all parts would remain with the other parts that we had confirmed all fit together. 9/9/19 all of the food platforms were taken out and setup in the aviaries as well as shade cloth being added to the fronts of the aviaries. Perches were added on this trip and on the trip on 10/6/20. There were large gaps between the deck boards on some of the platforms. These were covered with flashing metal on the trip of 10/6/20 and all of the aviaries were checked and verified to be secure to hold birds.

On 8/7/19 Tess went into the field and started to install the aviaries on the platforms that MFBRP had set up prior.

Notes from the trip:

Aviary Notes from August trip to Nakula (TH).

Overall, not much to fix. All aviaries should be checked for gaps. ¼ inch is OK. Should not be able to fit thumb through. The two biggest fixes that need to be made are on HACK F and HACK G where we had trouble with the doors not fitting properly.

Across all aviaries: Gaps in platforms- Bryce/Jennifer may access and address in Oct before birds go out?
Grass overgrown around platform.
Difficult to step up to aviary for servicing.

Items for September trip in addition to aviary adjustments:
-Shade cloth attachment. These are all at MBCC and will need to be taken out. Attached to aviaries with zip ties.
-Install all Kiwikiu feeders (everything except auto food dispensers) and secure them to platforms.
-Hardware installment for ratchet strap anchor. Eye-hooks or something similar. 4 per aviary= 40.

Supplies needed: (most supplies still available in action packer from last trip)
MBCC step stool was left at camp. Extra PVC pieces were also left at camp.
Drills, back up battery, and charger. We can charge batteries at camp.
Drill bit set, star bit, Phillips bit. Bit needed to change out hasps/hinges if needed.
ZIP TIES. Should have enough left from last trip.
Screws. Long screws for Kiwikiu feeders. Self drilling hex screws.
Gloves (only one pair in action packer)
Wire cutters
Rubber mallet

Notes by Avairy

HACK E / Orange:
-Check gaps between frame and panels.
-May need to shift panels around.
- May need to add zip ties in some areas.

HACK F / Green:
*Left door, top hasp not fitting. Either need to shift frame around or re-do hasp.
-Grass overgrown around platform.
-Check for gaps between pvc and wire. Add more zip ties as needed.

HACK G / Pink:
*****Doors not fitting properly. When you shift the top over to the left by pushing it, it lines up better. May have to loosen pipe clamps and shift panels around. Some pipe clamps were left loose. Doors were zip tied shut.
*Gap in right corner of back left panel where rood meets the pipe.
-2 gaps in platform
-Grass overgrown in front of platform

HACK H / Silver:
-Need a step stool or plank ramp to access hatch for servicing. ~5 ½ ft for the left door and shorter for the right. Please access and measure this for best options.
-Gaps between wire and pvc all around
-More zip ties for middle panel.

HACK I / Bronze:
-Some gaps in platform

HACK J / Light Blue:
-Some gaps in platform

HACK D / Red:
-3 or 4 large gaps in platform. At least 5 that are ¼ inch.

HACK C / Cherry:
-Gap in frame, top left corner of left side.
-Some gaps in platform

HACK B / Dark Blue:
-Check for gaps around frame. There are some small ones but may be OK. Check around for entire aviary for wire bowing out. Add zip ties as needed.

HACK A / Purple:
-No notes. Check for gaps or other mistakes.

Pathology #: 64546 ID #: MPW05 Pseudonestor xanthophrys/Maui parrotbill

Sex: Male
Age: 2Y 5M 12D (estimated)
Bands: L White/Black:R Silver 1971/12181

Facility/Project: Kiwikiu reintroduction
Location: Nakula NAR release site
Hatch date: Unknown, estimated >1/Jun/2017
Death date: 11/11/2019
Weight when found: Not reported

Historical weights and date
Not reported

History and physical examination:

Kiwikiu MPW05 was captured in the wild by partners on 10/11/19 and moved into release aviary H at the Nakula Natural Area Reserve release site on 10/17/19. The bird adjusted well to the move. On 10/28/19 the bird was released into the wild with a vhf radio transmitter.

MPW05 was observed on 11/10/19 at 12:09pm in an area of the forest where it had been spending time previously. The bird was foraging actively in a koa tree, vocalizing (chipping) and occasionally flying to a different branch. The following day 11/11/19 at 12:05pm the carcass was found in tall grass in the same general location. There was no indication of rigor mortis. There were no visible injuries. The bird appeared to be thin and in poor condition.

Gross Findings:

1. Blood: Numerous hemoparasites consistent with avian malaria (cytologic diagnosis)
2. Lungs: Pale
3. Poor body condition

Final diagnoses:

1. Blood, multiple organs: Intraerythrocytic protozoan trophozoites and schizonts, large numbers, and hemozoin pigment, compatible with Plasmodium sp.
2. Liver: Hepatitis, severe, lymphohistiocytic with intraerythrocytic and intrahistiocytic protozoa and hemozoin pigment
3. Lungs: Diffuse hypoperfusion

Ancillary tests and results:

Molecular Diagnostics Report, lung cPCR result FINAL 2019/11/22:
Plasmodium relictum Positive
Haemoproteus Not detected
Leucocytozoon Not detected

Final comment:

The cause of death of this Maui parrot bill was severe protozoal infection in the blood caused by avian malaria. Infection with Plasmodium relictum was confirmed by molecular testing performed on a sample of lung. Inflammation in the liver, and accumulation of malaria pigment (hemozoin) within tissues was also significant. Hypoperfusion of the lung is suggestive of anemia secondary to hemolysis from the intra-erythrocytic Plasmodium organisms. This bird was in poor body condition, based on the absence of adipose stores.

PRIVILEGED INFORMATION, NOT FOR PUBLICATION

Pathology #: 64522 ID #: MPW08 Pseudonestor xanthophrys/Maui parrotbill

Sex: Male (determined by morphology)
Age: 2Y 5M 7D (estimated)
Bands: NB/AL:BK/GR

Facility/Project: Kiwikiu reintroduction
Location: Nakula NAR release site
Hatch date: Unknown, estimated >1/Jun/2017
Death date: 11/6/2019; 4:15pm
Weight when found: 22g

Historical weights and date
10/13/2019 25.7g
10/20/2019 27.6g

History and physical examination:

Kiwikiu MPW08 was captured in the wild by partners on 10/12/19 and moved into release aviary J at the Nakula Natural Area Reserve release site on 10/17/19. The bird adjusted well to the move. On 10/27/19 the bird was released into the wild with a vhf radio transmitter. MPW08 was behaving normally in the wild through 11/4/19. On 11/5/19, staff reported the bird was very inactive for long periods on this day. At one point the bird tried to bite a branch in front of him and appeared to nearly stumble. On 11/6/19 the transmitter signal was tracked to the bottom of a gulch where the body was found in the grass. The body seemed to be slightly stiff. The body appeared to be thin and weighed 22g (without the transmitter). Quite a few snails and slugs were on the body when it was found.

Gross Findings:

1. Blood: Numerous hemoparasites consistent with avian malaria (cytologic diagnosis)
2. Poor body condition

Final diagnoses:

1. Blood, multiple organs: Intraerythrocytic protozoan trophozoites and schizonts, large numbers, and hemozoin pigment, compatible with Plasmodium sp.
2. Liver: Hepatitis, severe, lymphohistiocytic with intraerythrocytic and intrahistiocytic protozoa and hemozoin pigment
3. Lungs: Hypoperfusion, severe, diffuse

Ancillary tests and results:

Molecular Diagnostics Report, lung cPCR result FINAL 2019/11/15:
Plasmodium relictum Positive
Haemoproteus Not detected
Leucocytozoon Not detected

Final comment:

The cause of death of this parrotbill was a severe hemoparasitism due to avian malaria. Plasmodium relictum was confirmed by molecular testing performed on a sample of lung. The was one of several recently relocated animals to die due to malaria infection. Hypoperfusion of the lung is compatible with anemia secondary to red blood cell loss from malaria infection. Autolysis hindered examination of the intestine. This bird was in poor body condition based on nearly absent adipose stores.

PRIVILEGED INFORMATION, NOT FOR PUBLICATION

Pathology #: 64479 ID #: MPW009 Pseudonestor xanthophrys/Maui parrotbill

Sex: Male (determined by morphology)
Age: 1Y 4M 31D (estimated)
Bands: GR/BL:NB/AL

Facility/Project: Kiwikiu reintroduction
Location: Nakula NAR release site
Hatch date: Unknown (appears to be second year age based on plumage), estimated >1/Jun/2018
Death date: 10/30/2019; 6:00am
Weight when found: 19.6g

Historical weights and date:

10/12/2019 20.7g
10/14/2019 20.5g
10/15/2019 20.2g
10/16/2019 19.9g
10/20/2019 21.5g
10/24/2019 21.8g
10/30/2019 19.6g

History and physical examination:

Kiwikiu MPW09 was captured in the wild by partners on 10/12/19 and moved into release aviary B at the Nakula Natural Area Reserve release site on 10/17/19. The bird adjusted well to the move. On 10/29/19 the bird seemed slightly more inactive than usual during observations very early in the morning. The bird was responsive to the keeper despite being more inactive than usual. The bird was caught and a radio transmitter was attached. The attachment went well. However, upon release back into the aviary the bird did not fly. Instead, the bird remained on the ground. As a result, the bird was recaptured and the transmitter was removed, and the bird re-released into the aviary. However, the bird did not move from the perch afterward, and was fluffed with its head tucked. Shortly thereafter, the bird was caught and subcutaneous fluids was administered. The bird was held in a carrier box. On 10/30/19 the bird was found dead first thing in the morning.

Gross Findings:

1. Numerous hemoparasites consistent with avian malaria (cytologic diagnosis)
2. Poor body condition

Final diagnoses:

1. Blood and vasculature (multiple tissues): Intraerythrocytic protozoan trophozoites, large numbers, with intrahistiocytic pigment, congestion, and occasional inflammation, morphology consistent with Plasmodium sp.

Ancillary tests and results:

Molecular Diagnostics Report, lung cPCR result FINAL 2019/11/08:
Plasmodium relictum Positive
Haemoproteus Positive
Leucocytozoon Not detected

Final comment:

Similar to other recently submitted parrotbill cases, the cause of death in this bird was due to severe avian malaria. As with the other cases, Plasmodium relictum was confirmed by molecular testing to be the causative agent. Haemoproteus was also identified molecularly in this and the other bird that was submitted at the same time as this one (Pathology # 64478); the role of this second hemoparasite in the death of the animals is unknown. Post mortem autolysis hindered evaluation of multiple tissues. The bird was in poor body condition.

PRIVILEGED INFORMATION, NOT FOR PUBLICATION

Pathology #: 64478 ID #: MPW010 Pseudonestor xanthophrys/Maui parrotbill

Sex: Female (determined by morphology)
Age: 1Y 5M 1D (estimated)
Bands: NB/BL:NB/AL

Facility/Project: Kiwikiu reintroduction
Location: Nakula NAR release site
Hatch date: Unknown, estimated >1/Jun/2018
Death date: 10/31/2019; 6:00am
Weight when found: 14.3g

Historical weights and date:
10/13/2019 16.6g
10/20/2019 17.6g
10/24/2019 18.4g
10/31/2019 14.3g

History and physical examination:

Kiwikiu MPW10 was captured in the wild by partners on 10/13/19 and moved into release aviary G at the Nakula Natural Area Reserve release site on 10/17/19. The bird adjusted well to the move. On 10/26/19, a radio transmitter was attached to the bird. On 10/28/19, the transmitter was found on the ground, presumably removed by the bird. Later the same day on 10/28/19, a new radio transmitter was attached to the bird. On 10/29/19, the bird appeared to be lethargic and was not observed to be eating. In the late afternoon, the bird was captured and held in a carrier box and kept warm. On 10/30/19 the bird was fluffed and inactive in the carrier box. The bird was provided with continued care and support through heat and being held in a quiet area throughout the day. On 10/31/19 the bird was found dead first thing in the morning.

Gross Findings:

1. Numerous hemoparasites consistent with avian malaria (cytologic diagnosis)
2. Fair body condition

Final diagnoses:

1. Blood and vasculature (multiple tissues): Intraerythrocytic protozoan trophozoites, large numbers, with intrahistiocytic pigment, congestion, and occasional inflammation and thrombosis, morphology consistent with Plasmodium sp.
2. Renal tubular necrosis, moderate to marked, with urate stasis
3. Intestinal trematode parasites, low numbers

Ancillary tests and results:

Molecular Diagnostics Report, lung cPCR result FINAL 2019/11/08:
Plasmodium relictum Positive
Haemoproteus Positive
Leucocytozoon Not detected

Final comment:

As with other parrotbills that have been submitted recently, the death of this bird is attributed to severe avian malaria. The infection was confirmed by molecular testing to be caused by Plasmodium relictum. Hemoproteus was also confirmed molecularly in this bird and the other bird that was submitted at the same time as this one (Pathology # 64479); the significance of this second hemoparasite in the death of the animals is unknown. Renal changes were consistent with dehydration. Unlike other examined cases, there was minor intestinal parasitism by trematodes in this bird, which was considered an incidental finding.

Pathology #: 64585 ID #: MPW011 Pseudonestor xanthophrys/Maui parrotbill

Sex: Female (determined by morphology)
Age: 2Y 5M 17D (estimated)
Bands: NB/YE:NB/AL

Facility/Project: Kiwikiu reintroduction
Location: Nakula NAR release site
Hatch date: Unknown, estimated >1/Jun/2017
Death date: 11/16/2019; 11:00am
Weight when found: 13.8g

Historical weights and date
10/14/2019 17.3g
10/16/2019 18.0g
10/26/2019 19.0g

History and physical examination:

Kiwikiu MPW11 was captured in the wild by partners on 10/14/19 and translocated and moved into release aviary C at the Nakula Natural Area Reserve release site on 10/17/19. The bird adjusted well to the move. On 10/28/19 the bird was released into the wild with a vhf radio transmitter. MPW11 was observed almost daily after release. The bird was active and foraging frequently on wild food items, spending its time mostly around 5800-6200 feet in elevation. The day before mortality on 11/15/19 at 3pm, the bird was frequently resting for 5-10 minute periods, which was unusual, because typically it was very active. The following day on 11/16/19, the bird was found dead at 11:00am. The body was stiff when found. There were no obvious signs of mortality. The bird was found around 5600 feet elevation. Due to the isolated field location, the bird was not transported out of the field until 11/20/19, shipped to San Diego on 11/21/19, for preliminary examination on 11/22/19. As a result, significant autolysis is likely.

Gross Findings:

1. Numerous hemoparasites consistent with avian malaria (cytologic diagnosis)
2. Poor body condition

Final diagnoses:

1. Blood and vasculature (multiple sites): Intraerythrocytic and intrahistiocytic protozoan trophozoites and schizonts, large numbers, with focal thrombosis and intrahistiocytic pigment, morphology consistent with Plasmodium sp.

Ancillary tests and results:

Molecular Diagnostics Report, lung cPCR result FINAL 2019/12/06:
Plasmodium relictum Positive
Haemoproteus Not detected
Leucocytozoon Not detected

Final comment:

As with the other nine Maui parrotbills that have died in the last month, the death of this young female was due to avian malaria. Similar to those cases, Plasmodium relictum was confirmed by molecular testing performed on a sample of lung. The bird was in poor body condition. Postmortem autolysis hindered histologic evaluation in most tissues.

Pathology #: 64446 ID #: MP009 Pseudonestor xanthophrys/Maui parrotbill

Sex: Female
Age: 18Y 4M13D
Bands: RD/AL:YE

Facility/Project: Kiwikiu reintroduction
Location: Nakula NAR release site
Hatch date: 06/12/2001
Death date: 10/24/2019; 1:48pm
Weight when found: 15.3g

Historical weights and date:

10/24/2019 15.3g
10/22/2019 17.2g
10/21/2019 17.9g
10/20/2019 18.1g
10/19/2019 19.0g
10/16/2019 18.8g

History and physical examination:

Kiwikiu MP009 was moved on 10/10/19 from MBCC to a release aviary at the Nakula Natural Area Reserve to acclimate to the site before release into the wild. The bird adjusted well to the move and following the move, was active, eager, begging, and quick to come to food. On 10/21/19, the bird seemed to be less active than normal and was started on itraconazole. On 10/22/19 the bird was slower to come to food and appeared lethargic. The bird was caught and taken to the field camp where itraconazole and subcutaneous fluids were administered. The bird was held in a carrier box and provided with heat and a quiet area. On 10/23/19, the bird was transported out of the field and back to MBCC, where it was given itraconazole, primaquine, meloxicam, and subcutaneous fluids. The bird continued to appear weak. On 10/24/19, the bird was found dead.

Gross Findings:

1. Numerous hemoparasites consistent with avian malaria (cytologic diagnosis)
2. Poor body condition

Final diagnoses:

1. Blood and vasculature (multiple tissues): Intraerythrocytic protozoan trophozoites and few schizonts, large numbers, with intrahistiocytic pigment and occasional inflammation, morphology consistent with Plasmodium sp.
2. Lung: Fungal pneumonia, regional, marked, morphology consistent with Aspergillus sp.

Ancillary tests and results:

Molecular Diagnostics Report, lung cPCR results FINAL 2019/11/04:
Plasmodium relictum Positive
Haemoproteus (cytB) Not detected
Leucocytozoon (cytB) Not detected

Final comment:

The most significant finding and major contributor to the death of this parrotbill was severe avian malaria, which is similar to other recently submitted parrotbills. Plasmodium relictum was confirmed by molecular testing from a sample of frozen lung. In addition, there was regional fungal pneumonia (aspergillosis), which was likely the result of debilitation. The bird was in poor body condition.

PRIVILEGED INFORMATION, NOT FOR PUBLICATION

Pathology #: 64420 ID #: MP018 Pseudonestor xanthophrys/Maui parrotbill

Sex: Male
Age: 14Y 9M 21D
Bands: R Blue (x2):L Yellow, Silver 1971-12167

Facility/Project: Kiwikiu reintroduction
Location: Clinic
Hatch date: Unknown
Death date: 10/20/2019; 5:45am
Weight when found: 24.5g

Historical weights and date:
05/23/2019 26.2g
10/08/2019 25.5g

History and physical examination:

On 10/10/19, MP018 was moved from MBCC to a release aviary at the Nakula Natural Area Reserve to acclimate to the site before release into the wild. The bird quickly adjusted well to the move and had good food consumption and exhibited normal behaviors. On 10/18/19, the bird suddenly exhibited concerning behaviors: open mouth breathing, not active, not vocalizing, not coming down to eat fresh mealworms, not moving or flying away when staff slowly approached. The bird was caught and taken to the field camp where subcutaneous fluids were administered. The weight was only slightly lower than the previous weight. Nevertheless, the bird was transported out of the field and back to MBCC a few hours later. Upon arrival at MBCC, the bird was examined by Dr. Matt Kinney and given oxygen, fluids, itraconazole, and meloxicam. The bird exhibited a rapid respiratory rate and open mouth. On 10/19/19, a blood sample was collected from the bird. The bird did not respond well and was placed in an AICU with oxygen. On 10/20/19, the bird was found dead.

Gross Findings:

1. Numerous hemoparasites consistent with avian malaria (cytologic diagnosis)
2. Fair body condition

Final diagnoses:

1. Blood and vasculature (multiple tissues): Intraerythrocytic protozoan trophozoites and schizonts, large numbers, with occasional inflammation and thrombosis, morphology consistent with Plasmodium sp.
2. Kidney: Tubular necrosis, moderate, with urate stasi

Ancillary tests and results:

Molecular Diagnostics Report, ante-mortem blood cPCR results FINAL 2019/10/23:
Plasmodium relictum Positive
Haemoproteus Not detected
Leucocytozoon Not detected

Final comment:

This is the first of several parrotbills that died due to severe avian malaria. Plasmodium relictum, a causative agent of avian malaria, was confirmed by molecular testing in a sample of blood taken at clinical examination before the bird's death. Kidney changes were consistent with dehydration. Post mortem autolysis limited the evaluation of multiple tissues.

Path #: 64500 ID #: MP022 Pseudonestor xanthophrys/Maui parrotbill

Sex: Male
Age: 7Y 8M 5D
Bands: GR/AL:YE/BK

Facility/Project: Kiwikiu reintroduction
Location: Nakula NAR release site
Hatch date: 03/02/2012
Death date: 11/02/2019; 4:00pm
Weight when found: Not Reported

Historical weights and date
10/08/2019 23.5g
10/16/2019 41.0g
10/21/2019 23.7g
10/24/2019 24.8g
11/04/2019 19.7g

History and physical examination:

Kiwikiu MP022 was moved on 10/10/19 from MBCC to a release aviary at the Nakula Natural Area Reserve to acclimate to the site before release into the wild. The bird adjusted well to the move and following the move exhibited normal behaviors. The bird was released into the wild on 10/29/19. This bird was released without a radio transmitter. This bird was observed periodically in the vicinity of the release aviary in the days following release, although the bird was not observed on the trail camera to visit the supplemental feeder at the release aviary. On 11/2/19, staff was in the vicinity of the release aviary and noticed MP022 sitting under the release aviary platform with its feathers fluffed. Staff called a co-worker and requested a carrier transfer box be brought to the release aviary right away. While waiting for the carrier transfer box to arrive, MP022 died. There were no obvious injuries on the body.

Gross Findings:

1. Advanced autolysis

Final diagnoses:

1. Blood: Severe hemoparasitism with numerous erythrocytic intracytoplasmic schizonts and trophozoites (Plasmodium relictum)
2. Blood: Scattered suspect erythrocytic and leukocytic shizonts (Leukocytozoon spp)
3. Kidneys: Mild, mutifocal urate stasis and tubular mineralization

Ancillary tests and results:

Molecular Diagnostics Report, lung cPCR results FINAL 2019/11/15:
Plasmodium relictum Positive
Haemoproteus Not detected
Leucocytozoon Positive

Molecular Diagnostics Report, lung cPCR results FINAL 2019/12/05:
Isospora Not detected

Final comment:

The cause of death is severe hemoparasitism from Plasmodium relictum (avian malaria), possibly complicated by a second hemoparasite, Leukocytozoon. This is the only bird in the recent group of Maui parrotbills that has had both hemoparasites. Unfortunately, the tissues had undergone advanced autolysis, hindering microscopic examination. However, of the tissues examined, there did not appear to be inflammatory lesions. Nevertheless, blood dyscrasias associated with parasitism are still considered the primary cause of mortality as with the other recent Maui parrotbills. Body condition was considered fair based on small fat stores.

Path #: 64429 ID #: MP024 Pseudonestor xanthophrys/Maui parrotbill

Sex: Male
Age: 7Y 6M17D
Bands: BK/AL:BK/RD

Facility/Project: Kiwikiu reintroduction
Location: Nakula NAR release site
Hatch date: 04/02/2012
Death date: 10/19/2019; 12:00pm
Weight when found: 18.2g

Historical weights and date
10/08/2019 19.3g
10/11/2019 20.6g
10/16/2019 20.2g
10/19/2019 18.2g

History and physical examination:

On 10/10/19, MP024 was moved from MBCC to a release aviary at the Nakula Natural Area Reserve to acclimate to the site before release into the wild. The bird quickly adjusted well to the move and had good food consumption and exhibited normal behaviors. On 10/19/19, the bird was found around 1015am sitting in a large feeder pan under cover with its head tucked. Staff responded immediately and captured the bird and took it back to camp. The bird was found dead upon arrival. There were no obvious signs of the cause of mortality. This bird was behaving and eating normally before the mortality. So the mortality was completely unexpected. Due to the remote field location, the carcass was in the field in a cooler with ice until 10/21/19. On 10/21/19 the carcass was transported out of the field and stored in a refrigerator until it was shipped for necropsy.

Gross Findings:

1. Numerous hemoparasites consistent with avian malaria (cytology diagnosis)
2. Fair body condition
3. Empty upper GI tract

Final diagnoses:

1. Blood: Severe hemoparasitism with myriad erythrocytic intracytoplasmic trophozoites and schizonts (consistent with Plasmodium spp)
2. Liver: Single cell necrosis and abundant hepatocelular pigment (iron and hemozoin)

Ancillary tests and results:

Molecular Diagnostics Report, lung cPCR results FINAL 2019/10/28:
Plasmodium relictum Positive
Haemoproteus Not detected
Leucocytozoon Not detected

Final comment:

The cause of death in this bird is an overwhelming Plasmodium infection (avian malaria). Innumerable parasites were seen in peripheral blood smears and vessels throughout microscopic sections. Molecular results confirm the infection to be from Plasmodium relictum. This is one of several recent mortalities in this species, all of which succumbed to malaria. Most of the tissues had undergone advanced autolysis, hindering optimal evaluation. Cell death in the liver was interpreted as secondary to anemia or directly from circulating hemoparasites. Urate stasis is compatible with dehydration. There did not appear to be recent feeding. Body condition was considered fair based on only small subcutaneous and intracavitary fat stores.

PRIVILEGED INFORMATION, NOT FOR PUBLICATION

Path #: 64499 ID #: MP026 Pseudonestor xanthophrys/Maui parrotbill

Sex: Male
Age: 6Y 6M 22D
Bands: BL/AL:GR/GR

Facility/Project: Kiwikiu reintroduction
Location: Nakula NAR release site
Hatch date: 04/16/2013
Death date: 11/05/2019; 2:30pm
Weight when found: Not Reported

Historical weights and date
- 10/08/2019 22.3g
- 10/12/2019 21.6g
- 10/19/2019 23.4g
- 10/24/2019 22.7g
- 10/31/2019 23.0g
- 11/05/2019 20.0g

History and physical examination:

Kiwikiu MP026 was moved on 10/10/19 from MBCC to a release aviary at the Nakula Natural Area Reserve to acclimate to the site before release into the wild. The bird adjusted well to the move and following the move exhibited normal behaviors. The bird was released into the wild on 10/29/19 with a radio transmitter. This bird stayed in close proximity to the field camp after release. This bird was recaptured on 10/31/19 to replace a suddenly non functioning radio transmitter. The bird was observed to be possibly sick on 11/4/19 and was recaptured by mist-net in the evening and held overnight at camp in a holding box. The following day on 11/5/19, the bird was transported out of the field and back to MBCC first thing in the morning where the bird received subcutaneous fluids, cholorquine, and primaquine. The bird died later that day.

Gross Findings:

1. Numerous hemoparasites consistent with avian malaria (cytology diagnosis)
2. Fair body condition
3. Empty upper GI tract

Final diagnoses:

1. Blood: Severe hemoparasitism with myriad erythrocytic intracytoplasmic trophozoites and schizonts (consistent with Plasmodium spp)
2. Liver: Moderate, extensive perivascular lymphohistiocytic hepatitis with abundant intralesional and sinusoidal hemozoin pigment (secondary to #1)

Ancillary tests and results:

Molecular Diagnostics Report, lung cPCR results FINAL 2019/11/15:
Plasmodium relictum Positive
Haemoproteus Not detected
Leucocytozoon Not detected

Final comment:

The cause of death in this bird is an overwhelming Plasmodium infection (avian malaria). Innumerable parasites were seen in peripheral blood smears and vessels throughout histologic sections, and associated inflammation was seen most extensively in the liver. Molecular results confirm the infection to be from Plasmodium relictum. This is one of several recent mortalities in this species, all of which were determined to have died due to malaria. There was evidence of dehydration in the kidneys, and body condition was fair based on small fat stores.

PRIVILEGED INFORMATION, NOT FOR PUBLICATION

Field Packing List for the Translocation

Sling load gear

- Nets
- Swivels, lines
- Emergency crash kit box and helmet

Sleeping gear for Frisbee Meadows Field Camp

- Pillows
- Pillow cases
- Sleeping bags
- Cots
- Thermarests
- Tents

Camp Gear for Frisbee Meadows Field Camp

- Propane
- Lighters
- Toilet Paper
- Paper towels
- Dish towels and rags
- Sponge and dish soap
- Duct tape
- Hand soap
- Hand sanitizer
- Bleach
- Alcohol
- Zip ties
- Sharpies and pencils
- Lantern Mantles
- Gallon and quart Ziplock bags
- Blue, pink, and orange flagging
- InReach Devices, Chargers for InReach
- Charger for regular radios
- Chargers for MP3 players and speakers
- Digital radio and charger for digital radio
- Lua Buckets
- Bags of shavings
- Trash bags
- 24 AA
- 10 AAA batteries
- Inventory Sheet

- Maps
- Detection maps, coloring pencils, and sharpener
- Generator (backup to solar that is Frisbee) and fuel
- Power strip and extension cord for generator
- SOP Binder
- Extra camp chairs
- First Aid Supplies (there are some at camp but these items needed to be resupplied)
    - Benadryl, aspirin, ibuprofen
    - iodine
    - antibiotic ointment

Banding Gear:

- 40 banding poles
- 10-6m nets, 10-9m nets, 3- 12 m nets
- Three bags of clothespins (3-4/net)
- Six bags of bird bags
- Tieback for banding poles (80 pieces)
- Three Banding Binders with datasheets and color combos
- Three Bandings kits (make sure have MAPA tools and bands) (add duct tape)
    - Banding plyers, banding spreaders, metal bands, color bands, soldering iron, leg gauge, calipers, Pesola scale, stop ruler, tail ruler, capillary tubes, vial of alcohol, vial of bleach, scissors, seam ripper, tweezers, pencils, sharpies, hand sanitizer, hand warmers, Quik stop, anti-biotic ointment
- Fecal (containers with alcohol, labels, parafilm, and tape) and blood sample kits (needles, vial with queen lysis buffer, Q-tip, cotton balls) for three banders
- Extra banding kit stuff for camp (batteries, labels, parafilm, sharpies, tape, etc)
- Playback speakers, cords (for some speakers), AA/AAA batteries, MP3 players (with playback on them), Boostaroos in three dry bags
- Machetes

Equipment for the Bird Holding Room

- Extra lights
- 3 Tables
- Bucket for soaking dishes
- Dish pan
- Dish soap/Bleach
- Dust pan and broom
- Garbage bags
- Garbage bucket
- Paper towels
- Mealworms
- Apples/Potatoes for mealworms

- Bran Meal
- Containers for mealworms
- Dry mix (1/3 part Mazuri softbill pellets, 1/3 part dried fly larvae and 1/3 part Quicko egg food)
- Mesh strainer sifter
- Plastic food trays
- Repashy Superload gut loading food for mealworms
- 3" D cup
- measuring spoons
- Plastic 4" food saucers
- Dowels
- Extra lights
- Shelving Unit
- Paper for paper liner
- Ant spray
- Cutting mat
- Head lamp
- Holding cages
- Paper cutter
- Paper template
- Paper template, paper cutter and cutting mat
- Short and long handle Hand nets
- Snap traps
- Transfer boxes
- Vaseline to be placed on shelf legs for ants
- Rubbermaid Containers for bird carriers (with towels and cardboard)
- Tote Boxes
- Camera
- Color dots
- Computer
- Duct tape
- Index cards
- Log sheets,
- Ohaus scales
- Pens and pencils
- Record keeping sheets
- Sharpie

<u>Veterinary Supplies</u>

- 3ml syringes - #15
- TB syringes (0.5ml) - #15
- 27G needle - #10

- 25G needle - #10
- Sterile darcon cotton tipped applicator - #10
- Fecal collection cup (#1 large) and ~ #20 small with lids
- CTA (non-sterile) - #10
- Fine pointed magic marker
- Sodium Chloride bottles (wash)
- Medications
    - Meloxicam injectable – diluted to 1 mg/ml - #1 vial
    - Itraconazole 10mg/mL – 30ml - #1 bottle
    - 0.45%NaCL + 2.5 dextrose – unopened – 1 bag
    - Chloroquine and Primaquine
    - Heparin mircovials
    - Small bottle of isopropyl
    - Chlorohexidine scrub with gauze
    - Quickstop styptic powder
    - Tissue adhesive – 1 bottle
    - QuickAid hemostatic bandage – large piece left – some used
    - SSD cream
    - Triple abx ophthalmic ointment
    - Eyewash - #1 bottle
    - Bandage material – 2x2 gauze, CTA, cotton balls, white tape 1', waterproof tape 1", vetwrap 1", and cast padding 1"
- Handwarmers (Hothands)
- Sharps container
- Gloves – nitrile
- Scissors (various types)
- Stethoscope

<u>Items for the Translocation Site</u>

- Dry bag for supplies
- Bird food
- Water for the birds
- Hand net
- Leatherman
- Zipties
- Binoculars
- Scale and food pan
- Bird feeder
- Supplies for cleaning the feeder
- First aid kit
- Subcutaneous fluids
- Radio
- GPS

- Work phone and mount
- Camouflage ghillie suit
- Cold pack and ziplock bags for carcass
- Handwarmers (Hothands)
- Medications (from Vet List above)
    - Meloxicam injectable – diluted to 1 mg/ml - #1 vial
    - Itraconazole 10mg/mL – 30ml - #1 bottle
    - 0.45%NaCL + 2.5 dextrose – unopened – 1 bag
    - Chloroquine and Primaquine
    - Heparin mircovials
    - Small bottle of isopropyl
    - Chlorohexidine scrub with gauze
    - Quickstop styptic powder
    - Tissue adhesive – 1 bottle
    - QuickAid hemostatic bandage – large piece left – some used
    - SSD cream
    - Triple abx ophthalmic ointment
    - Eyewash - #1 bottle
    - Bandage material – 2x2 gauze, CTA, cotton balls, white tape 1', waterproof tape 1", vetwrap 1", and cast padding 1"