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Recovery of the endangered Maui Parrotbill (Kiwikiu, *Pseudonestor xanthophrys*)

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Acknowledgements specific to each self-standing chapter are located at the end of each chapter.

Author's Declaration

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Chapter 2 Determining productivity of the Maui Parrotbill, an endangered Hawaiian honeycreeper:

The data collected for this chapter was a collaborative effort by the seasonal staff at Maui Forest Bird Recovery Project. Analysis was completed by HL Mounce with assistance of SR Wheeler, CL Rutt and J Milikowsky for the nest survival data. Co-authors all commented on drafts of the manuscript written by HL Mounce before it was submitted for publication. This chapter has been published,

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Chapter 3 Management implications derived from long term re-sight data: annual survival of the Maui Parrotbill (*Pseudonestor xanthophrys*):

This chapter used an 18 year encounter dataset from mark-resight data maintained by Maui Forest Bird Recovery Project. HL Mounce contributed field re-sights from 2006-2011. All other re-sights were provided by seasonal staff at Maui Forest Bird Recovery Project: TK Pratt, JC Simon, PE Baker, and SL Pimm. Analyses were undertaken by HL Mounce with input from EH Paxton. All co-authors reviewed and provided guidance and comments on the manuscript written by HL Mounce before submission for publication. This chapter has been published,

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Chapter 4 Spatial genetic architecture of the critically-endangered Maui Parrotbill (*Pseudonestor xanthophrys*): management considerations for reintroduction strategies:

Blood samples were collected by HL Mounce 2006-2012 and the contemporary samples prior to 2006 were provided by Maui Forest Bird Recovery Project. Museum toe-pad samples were provided by the museums listed in the acknowledgements. The microsatellite library was developed by Genetic Identification Services, Inc. (California, USA). Guidance and training in laboratory techniques were provided by C Raisin and S Kundu. Microsatellite optimization was assisted by H Wickenden at University of Kent. STRUCTURE and GENELAND analyses were assisted by S Simpson at University of Kent. KJ Iknayan, CC Warren, and RL Hammond assisted with GIS mapping. All other lab work, analyses, and writing were undertaken by HL Mounce. AC Frantz provided R code for the simulation to model proportion of alleles that could theoretically be transferred with differing numbers of founder individuals. All co-authors provided comments and edits on a draft manuscript prior to submission for publication. This chapter has been published,

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Chapter 5 Extinction risk and reintroduction options for the endangered Maui Parrotbill (Kiwikiu, *Pseudonestor xanthophrys*):

The data used in these models is a culmination of the chapters 2-4. Introduction to PVA methods and the use of their applications was initiated by KJ Swinnerton. CP McGowan and EH Paxton provided guidance on analyses and, in combination with the other co-authors, provided comments and edits. The R code used to evaluate translocation options was developed by CP McGowan and optimized by HL Mounce and CC Warren. This chapter has been submitted for publication and is currently under review.

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Abstract

Species recovery programs are tasked with reversing the declines of threatened and endangered speices and mitigating the threats to their populations. These goals must be accomplished in the face of a human dominated global landscape where habitat destruction and alteration is still increasing at an alarming rate. Hawaii, as common on many islands, has one of the highest historical extinction rates in the world. Here I use the Maui Parrotbill (Kiwikiu; *Pseudonestor xanthophrys*) to explore population demographics, genetics, population viability, and recovery options for one of Hawaii's most critically endangered passerines (Maui Island endemic, pop. ~500). The accurate estimation of key demographic parameters is invaluable for making decisions about the management of endangered wildlife. Due to the challenges of data collection on a rare and cryptic species that inhabits remote terrain, such estimates are often difficult to obtain and reliable basic demographic data was not before available for parrotbills. First I look at parrotbill productivity estimates through both nest success and annual reproductive success measures. Secondly, I look at annual survival based on an 18 year encounter history. These studies both suggest population limitations may be coming from fecundity, and juvenile and female survival. Maui Parrotbill once inhabited a variety of forest types throughout Maui Nui but are now restricted to a single strip of wet forest 40-50 km^2 in size. I quantified the levels of contemporary genetic diversity and structure in wild and captive Kiwikiu populations, and compared these genetic patterns to those observed within historical nuclear diversity derived from 100-year old museum samples enabling the design of a conservation translocation strategy that is tailored to the patterns of genetic structure across the species' range. Lastly, I combine these data into a comprehensive population viability model to assess the risks to this population and evaluate the impacts of recovery options to the overall viability trajectory of a species. In planning for a reintroduction of parrotbills to areas of their former range, this model provides managers with demographic benchmarks that the new population will need to meet in order for the reintroduction to be successful.

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Chapter 1 Introduction

1.1 REINTRODUCTION AS A TOOL FOR SPECIES CONSERVATION

In order to recover endangered species, their populations need to be returned to areas of their former range. More often than not, these species have been extirpated from former areas of their range. The release of such organisms back into historical habitats is known as a *reintroduction* (IUCN 1987). As this conservation tool is a varied and complex method of recovery, there are a variety of terms that must be defined to cover these actions. When moving threatened and endangered species where conservation is the main objective, these reintroductions are considered also *conservation translocations* (Hodder and Bullock 1997). For simplicity from here on, we will use the terms *reintroduction* to refer to both *reintroductions* and *conservation translocations*. These actions are not new to species recovery efforts. Reintroductions have been widely used throughout conservation programs worldwide and the number of such efforts is growing exponentially each year (Seddon *et al*. 2012).

There are many specific reasons for using reintroduction as a conservation tool in a given species, but put in its simplest form, restoring a species to an area where it has been extirpated will increase the total number of individuals for that given species over time and reduce extinction risk. Still, reintroductions should be approached with caution as despite notable successes, there is also a high rate of failure (Griffith *et al*. 1989; Wolf *et al*. 1996). This thesis introduces a system and an endangered species that could benefit from a well-planned and implemented reintroduction. Each section within is aimed at synthesizing data to be used to design such a reintroduction and aims to provide managers with the best information to facilitate responsible decision-making and an increased chance of success.

1.2 ECOLOGICAL AND GENETIC CONSIDERATIONS FOR REINTRODUCTIONS

Reintroductions are now widely applied as a conservation tool yet despite their extensive use, the ecological and genetic implications of using reintroductions as a tool for species recovery are still poorly understood (Robichaux *et al*. 1997; Groombridge *et al*. 2012). Furthermore, reintroductions are still often unsuccessful. The increasing use of reintroductions is not unexpected due to the accelerating rate of global ecological change (habitat loss and fragmentation, biological invasions and climate change) and the corresponding pressure on biodiversity. The International Union for Conservation of Nature (IUCN) Reintroduction Specialist Group recently updated their guidelines for reintroductions as a response to these increases in usage (IUCN/SSC 2013). The original guidelines were developed out of necessity in the face of rising numbers of global reintroductions taking place and many of these reintroductions observed to be failing (IUCN 1998). Reasons for these failures have been attributed to poor quality habitat at release sites (Moorhouse *et al*. 2009; White *et al*. 2012), too few individuals being released (Wolf *et al*. 1998; Fischer and Lindenmayer 2000), captive sourced individuals being less suitable than wild individuals (Jule *et al*. 2008; Aaltonen *et al*. 2009), depredation risks (Moorhouse *et al*. 2009; White *et al*. 2012), and failure to remedy the original causes for the species' decline (Fischer and Lindenmayer 2000). Unfortunately, the lack of post-release monitoring after many reintroductions leaves the causes and timing of many failures still unknown (Seddon *et al*. 2007).

While some of these ecological consequences may be difficult to predict, we can attempt to account for the genetic considerations pre-release. Genetic factors play an important role in assessing a species extinction risk, and thus genetic management too should be an important consideration for designing a successful reintroduction program (Groombridge *et al*. 2012). We can assume that reintroduced populations that are founded from small numbers of individuals may suffer the same associated genetic problems as other small or bottlenecked populations. These processes such as loss of genetic variation, inbreeding and inbreeding depression have all been widely accepted as increasing the risk of extinction in small populations and would therefore be expected to operate in the same manner on a reintroduced population (Keller and Waller 2002; Frankham 2005).

Although we have knowledge on the expected genetic mechanisms at play, we cannot always predict how these genetic processes will interact with the ecological processes at a release site. In other words, how a certain level of inbreeding affects the trajectory and/or fitness of a population may be predictable in the species' current range; but, once they are faced with lower quality habitat and/or increased depredation risk and/or naïve birds sourced from captivity, these effects become much more difficult to predict. Every reintroduction is unique and the incorporation of high quality data on genetic and ecological factors must be considered together in order to formulate a well-designed reintroduction.

Even with the best available data, the ideal reintroduction scenarios for a given species are not often possible. There are frequently various threats that managers are unable to mitigate in selected release sites. For a species to be successful in the long-term, a reintroduced population needs to be able to survive these threats while also retaining adequate levels of genetic diversity to adapt to future environmental change (Reed and Frankham 2003; Keller *et al*. 2012). When attempting to restore small and declining populations, models often predict idealized numbers of individuals adequate to maintain this genetic diversity that are as large as the total global population itself. In these cases, we may have to manage the genetics of the reintroduced populations in order to maximize the retention of genetic diversity but know that we are unlikely to achieve the ideal population sizes. Most species conservation programs do not have the luxury of time and are often faced with rapidly declining populations. These programs will most often be faced with the need to make a decision to reintroduce a population with a lower genetic potential. However, this option may be preferable to taking no action at all.

Many of these actions have been taken on islands, which is not unexpected given that islands hold a large percentage of our global avifauna diversity (Stattersfield *et al.* 1998). These actions also are trying to counteract some of the most rapidly declining avian populations. Some estimating that more than half of island bird species may be functionally extinct by the turn of the century (Şekercioğlu *et al.* 2004). Many of these islands have already suffered massive extinctions (ex. New Zealand, Craig *et al.* 2000; Mauritius, Cheke and Hume 2008), and reintroductions have become a common management practice for recovering the remaining island species (Jones and Merton 2012). Due to the inherent nature of island populations, these species do not have the luxury of large numbers of individuals that continental conservation programs might have and often rely on small numbers of founding individuals. Although historically the success of reintroductions has been positively correlated with the numbers of animals released (Griffith *et al.* 1989; Wolf *et al.* 1996. Wolf *et al.* 1998), there are many examples of successful and well-known island reintroductions around the world that started from low numbers of individuals. Some examples of these include but are not limited to:

- The Echo Parakeet (*Psittacula echo*) in Mauritius which recovered from ~10 individuals to more than 550 (Jones and Merton 2012).
- The South Island Saddleback (*Philesturnus carunculatus*) in New Zealand which recovered from ~36 individuals to more than 2000 (Masuda and Jamieson 2013).
- The Black Robin (*Petroica travers*) in New Zealand which recovered from ~5 individuals to ~200 (Butler and Merton 1992).
- The Laysan Duck (*Anas laysanensis*) in Hawaii which recovered from 42 founders on a new island to a breeding population of more than 500 (Reynolds *et al.* 2013).

- The Mauritius Kestrel (*Falco punctatus*) in Mauritius which recovered from ~4 individuals to more than 500 (Jones and Merton 2012).
- The Nihoa Millerbird (*Acrocephalus familiaris kingi*) in Hawaii which has recovered from 50 founders on a new island to a breeding population of more than 160 (Dalton *et al.* 2014)

1.3 ADAPTIVE RADIATIONS OF BIRDS AND CONSERVATION OF ISLAND ENDEMICS

Adaptive radiation is defined as “the evolution of ecological diversity within a rapidly multiplying lineage” (Schulter 2000). While naturalists have documented adaptive radiations of birds on islands across the globe, nowhere else in the world has there ever been such a complex diversity of avian species that have evolved in such a small place, in such a short amount of time as in the Hawaiian Islands. The Hawaiian Islands are large and extremely isolated. Thus, the evolutionary processes that occurred on them created a diverse flora and fauna and a multitude of new species from relatively very few founders.

The Hawaiian Honeycreepers comprise more than 50 species and the present count is continuing to grow as new information is contributed from the recent fossil record (James and Olson 2005; Pratt 2005; James and Olson 2006). The classic example of adaptive radiation popularly used in literature is that of the Galapagos finches, a group of only 14 species (Figure 1.3.1). Hawaiian Honeycreepers are the only diverse radiation of birds in the Hawaiian Islands, a fact that may account for the high number of species. While there are a few lineages of crows, monarchs, and thrushes across the Hawaiian Islands, they evolved to produce comparatively few species (Pratt 2009). Therefore, the honeycreepers were exhibiting such an array of different morphological and physical traits as to exploit the majority of different environments in the islands available for avian species.

At the heart of the evolutionary mechanism of adaptive radiation is the process of competition (Schulter 2000). The pressure of intense intraspecific competition leads individuals to exploit novel food resources, precipitating specialization in foraging strategies and life history changes (Pratt 2009). While this specialization may have allowed high densities of forest birds to coexist in relatively small areas/islands, these narrow feeding niches also may set up Hawaiian Honeycreepers to be highly vulnerable to environmental change.

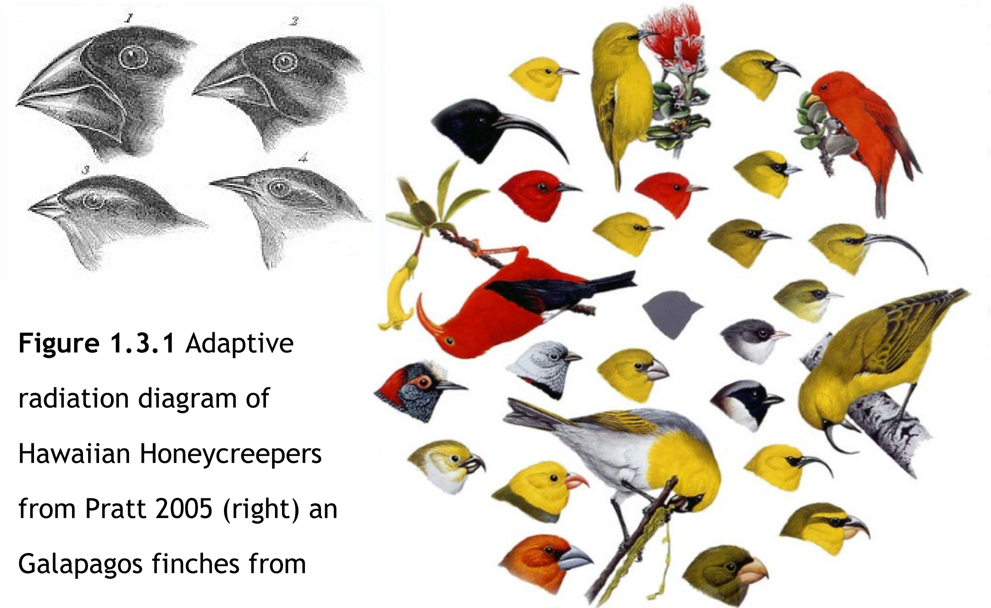

Figure 1.3.1 Adaptive radiation diagram of Hawaiian Honeycreepers from Pratt 2005 (right) an Galapagos finches from Darwin 1959 (left).

1.4 HAWAII AS THE 'EXINCTION CAPITAL' OF THE WORLD

The main Hawaiian Islands sit about 4000 km from the nearest continent and about 3200 km from any other high-island groups of any size (Howarth *et al.* 1988). The Hawaiian Islands, the most isolated archipelago in the world, often are referred to as the extinction capital of the world, having lost more bird species than anywhere else on earth. Similar as in other oceanic islands, the arrival of humans in Hawaii, and the non-native animals, diseases, and plants brought with them, had devastating effects on native flora and fauna which continue today.

The Hawaiian ecosystems were profoundly transformed first by the Polynesians approximately a millennium ago and then again by Westerners in the past two centuries. As little as 10% of the original avifauna of Hawaii persists today (Pimm *et al.* 1995), and almost all of the species that remain have undergone drastic population declines and range contractions (Banko and Banko 2009). Small ranges and minimal numbers of individuals, as exist in these island birds, predispose such species to extinction, and thus they are highly vulnerable to anthropogenic changes in their ecosystems (Hughes 2004; Sodhi *et al.* 2004). Each of these human arrivals brought a range of threats to the environment.

These threats included island-wide habitat destruction and degradation. Forest size, structure, and compositions were all altered after Polynesian contact by the introduction of rats (*Rattus exulans*), fire and agriculture (Pratt and Jacobi 2009). Nearly all bird species are impacted by massive deforestation (Davies *et al.* 2000; Zanette *et al.* 2000). These forests were further degraded by the European introduction of ungulates (hoofed-mammals). In places where these animals did not destroy the forest entirely, they removed the understory vegetation and disrupted the overall plant communities (Pratt and Jacobi 2009). Compounding these habitat alterations was the introduction of a variety of non-native predators and competitors (Lindsey *et al.* 2009). Following the introduction of mammals to the Hawaiian Islands, almost all flightless species disappeared (Olson and James 1991) and those avian species that survived nested as high in the trees as possible (Woodworth and Pratt 2009).

Possibly the most devastating introduction was that of foreign disease. Avian malaria (*Plasmodium relictum*) is thought to have arrived to the islands around 1920 and the widespread susceptibility of native forest birds was devastating with only a few lowland populations developing resistance (Woodworth *et al.* 2005; Woodworth and Pratt 2009). Avian malaria and its vector, mosquitoes (*Culex quinquefasciatus)*, are limited by cool temperatures and thus have restricted native birds to high elevation refuges. Elsewhere these introductions of disease, predation, habitat degradation and competition have reduced bird populations (Savidge 1987; Mack *et al.* 2000), but the culminating effects these had on small bodies of land and high levels of endemism in Hawaii were devastating.

1.5 CONSERVATION OF AVIAN SPECIES

Birds offer a unique opportunity to track global changes in biodiversity. This is because birds occur in nearly every habitat on Earth, and they are often one of the most visible forms of wildlife within a given area. As a result of this visibility, humans are often very familiar with the avian species around them. Despite our species awareness and often affinity for such species, human activities are undoubtedly to blame for the global biodiversity crisis that we are currently facing (Issac *et al.* 2007; Jones and Merton 2012). Dubbed the “sixth extinction” (Barnosky *et al.* 2011), this is the only global mass extinction that has been triggered by human activities (Leakey and Lewin 1996). One in eight of the world’s bird species is globally threatened (BirdLife International 2014a), and with predicted increases in extinction rates, estimates forecast that we could lose up to 20% of all remaining vertebrate species within the next century (Baillie *et al.* 2010; Sinervo *et al.* 2010). Islands, being particularly vulnerable, may suffer even higher losses than these predictions.

While island species deserve immediate conservation attention, there are not only numerous biological challenges facing their recovery but social and political barriers as well. The reductions in numbers of island species have in many cases isolated the birds from people. This lack of awareness and identity of such species has become a key social factor that affects support for avian conservation (Leonard 2009). Furthermore, the isolation of islands does not facilitate the appreciation and understanding of rare and endangered island fauna to a larger global audience (Pratt *et al.* 2009b).

Conserving avian biodiversity is an overwhelming task and it can be easy to become overly pessimistic in thinking about the future of endangered and imperiled species throughout the world. We are in a state of increasing human populations and the associated increasing human resource demands make this even more challenging. Avian conservation programs should focus on removing and lessening the adverse effects on these species in the immediate future. By minimizing these measurable threats, recovery programs can reverse the trajectory of decline in these species one at a time. As discussed earlier, this approach has successfully brought numerous species back from the brink of extinction (see examples in previous section 1.2).

Furthermore, there are many reasons for guarded optimism when considering the conservation of Hawaiian species in particular. Despite the many that have been lost, the surviving Hawaiian Honeycreeper populations are still large enough to recover and there are significant areas of protected native habitat remaining for these species (Pratt *et al*. 2009b). There has also been a marked increase in public awareness since the first Hawaiian Forest Bird Surveys (1976-1983) brought international attention to the plight of Hawaiian birds (Scott *et al*. 1986) and with this, more support for conservation efforts (Dayer *et al*. 2006). However, these facts can only provide guarded optimism because there are still major obstacles to overcome as these species battle to survive in the face of ongoing alien introductions, climate change, and growing costs of implementing recovery actions (an estimated $4.6 million per species per year; Leonard 2009).

1.6 ONGOING EXTICTIONS

Although there are multitudes of global efforts in avian conservation and the preservation of individual species, many still think of extinctions as things that happened in the past. School children are often taught to associate extinction with the Jurassic period but this ecological phenomenon is still a very current and ongoing process, especially on islands. While extinction rates have varied through time, the past 400 years have experienced a vertebrate extinction rate of 20-200 times that of "natural" or "background" extinction rates throughout history (Groombridge and Jenkins 2002) and as much as 100 times greater over the past 100 years (Mace *et al*. 2009). As discussed above and illustrated with the incidents of mass extinctions in the Hawaiian Islands, the process of extinction has turned from a natural process to a human-induced one.

An exact estimate of lost species is difficult to calculate as many Hawaiian species have not been seen in many years are still listed as endangered with the United States Fish and Wildlife Service. Three such forest bird species exist on Maui. The Maui Akepa (*Loxops ochraceus*), endangered, was last heard (but not seen) in 1995 (Reynolds and Snetsinger 2001), the Nukupuu (*Hemignathus lucidus*), endangered, has not been seen since 1996 (Reynolds and Snetsinger 2001), and the last known Poouli (*Melamprosops phaeosoma*) died in captivity at the end of 2004 (BirdLife International 2014b). The recent population declines and extinctions that have occurred in Hawaii in less than a century are numerous (Table 1.6.1).

Of the avian species that are left in the Hawaiian Islands, only 11 are common enough to suggest their future may be secure. Although habitat still exists for other Maui species which have not been seen in many years, extensive work being done on the conservation of other Maui avifauna has failed to yield any more recent sightings of these species (MFBRP unpublished data).

Table 1.6.1 Recent (1968-1983) and Current (2014) Status of Endangered and Recently Extinct Hawaiian forest birds (Banko and Banko 2009, Gorreson *et al*. 2009, VanderWerf 2013).

Species	**Hawaiian Island**	**Recent Population 1968-1983**	**Current Population**	**Last Seen**	**USFWS Listing**
Alala (*Corvus hawaiiensis*)	Hawaii	Rare	Extinct in the wild	2002	Endangered
Ooaa (*Moho braccatus*)	Kauai	Rare	Extinct	1987?	Endangered
Bishop's Oo (*Moho bishopi*)	Maui	Rare?	Extinct	1981?	
Millerbird (*Acrocephalus familiaris*)	Nihoa	Abundant	~380	.	Endangered
Kamao (*Myadestes woahensis*)	Kauai	Rare	Extinct	1985	Endangered
Olomao (*Myadestes lanaiensis*)	Molokai	Rare	Extinct	1980	Endangered
Puaiohi (*Myadestes palmeri*)	Kauai	Rare	~500	.	Endangered
Laysan Finch (*Telespiza cantans*)	Laysan	Abundant	> 10,000	.	Endangered
Nihoa Finch (*Telespiza ultima*)	Nihoa	Abundant	~3,000	.	Endangered
Ou (*Psittirostrata psittacea*)	Kauai	Rare	Extinct	1989	Endangered
Ou (*Psittirostrata psittacea*)	Hawaii	Rare	Extinct	1987	Endangered
Palila (*Loxioides bailleui*)	Hawaii	Rare	~1,260	.	Endangered
Maui Parrotbill (*Pseudonestor xanthophrys*)	Maui	Rare	~500	.	Endangered
Greater Akialoa (*Hemignathus obscurus*)	Kauai	Extinct	Extinct	1969	Endangered
Nukupuu *(Hemignatus lucidus)*	Maui	Rare	Extinct	1996	Endangered
Nukupuu *(Hemignatus lucidus)*	Kauai	Rare	Extinct	1990s?	Endangered
Akiapolaau (*Hemignathus munroi*)	Hawaii	Rare	~ 1,900	.	Endangered
Akikiki (*Oreomystis bairdi*)	Kauai	Rare	~1,300	.	Endangered
Hawaii creeper (*Oreomystis mana*)	Hawaii	Rare	~14,000	.	Endangered
Oahu Alauahio (*Paroreomyza maculata*)	Oahu	Rare	Extinct	1978	Endangered
Kakawahie (*Paroreomyza flammea*)	Molokai	Extinct	Extinct	1963	Endangered
Akekee (*Loxops caeruleirostris*)	Kauai	Rare	~3,100	.	Endangered

Maui Akepa (*Loxops ochraceus*)	Maui	Rare	Extinct	1980	Endangered
Hawaii Akepa (*Loxops coccineus*)	Hawaii	Rare	~12,000	.	Endangered
Akohekohe (*Palmeria dolei*)	Maui	Rare	~6,700	.	Endangered
Poouli (*Melamprosops phaeosoma*)	Maui	Rare	Extinct	2004	Endangered

1.7 MAUI AND ITS ENDEMIC AVIFAUNA

Maui is the second largest island in the Hawaiian archipelago (1,883 km^2) and is estimated to be around 800,000 years old (Howarth *et al.* 1988) (Figure 1.7.1). This volcanic island was formed from two volcanoes that overlapped one another. The last, erupting around 1790, is considered a dormant volcano but not extinct. The mountain of Haleakala forms the eastern half of the island. This summit rises 10,023 feet above sea level and has a peak exposed to both wet windward trade winds and drier leeward air (Giambelluca *et al.* 2013; Figure 1.7.2). This allows one mountain to host a wide variety of different ecosystems from dry shrub lands to wet rainforests in a relatively small area.

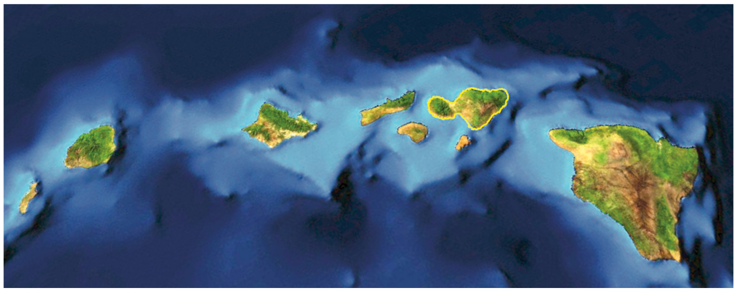

Figure 1.7.1. The Hawaiian archipelago with Maui Island outlined in yellow.

Today Maui is primarily a tourist destination. Visitors come seeking a lush tropical paradise and assume that the lowland vegetation that they are seeing is native to Hawaii. This belief is far from the truth, and much of the native vegetation on Maui has been destroyed or highly degraded. Likely due to its inaccessibility, the windward forests of Haleakala house some of the most pristine wet forests left in Hawaii. Contrastingly, the leeward forests have all but disappeared since the initial arrival of Polynesians. It is estimated that less than 10% of the original forests of leeward Haleakala remain today.

In the Hawaiian archipelago, no island has had more extinctions than Maui. Of those that remain, Maui has three forest bird species that are found across multiple islands, the Apapane (*Himatione sanguinea*), the Hawaii Amakihi (*Chlorodrepanis virens*), and the Iiwi (*Drepanis coccinea*). It also has three extant endemic forest bird species. The Maui Alauahio (*Paroreomyza montana*) is the most abundant with a population estimate of at least 55,000 (Brinck *et al*. 2012). The critically endangered Akohekohe, or Crested Honeycreeper (*Palmeria dolei*), is the next most abundant with a population estimate of 3,753 (± 373) (IUCN 2011; Scott *et al*. 1986). Lastly, the critically endangered Maui Parrotbill, or Kiwikiu, (*Pseudonestor xanthophrys)* is the rarest of endemic Maui forest birds. Maui Parrotbills have a population estimate of 502 (272-732 95% CI) individuals (Scott *et al*. 1986) across their current range of approximately 50 km^2 (or 421 (209-674 95% CI) individuals within a single 40 km^2 area (Brinck *et al*. 2012)) on windward Haleakala.

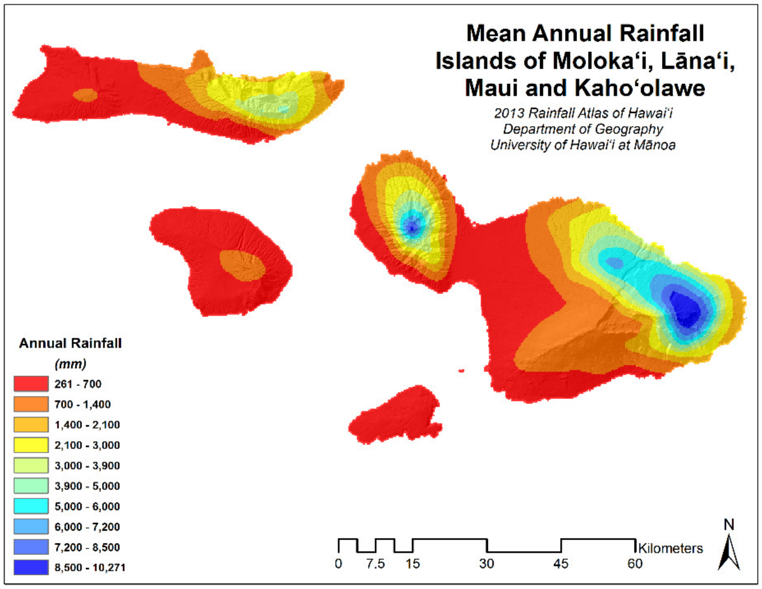

Figure 1.7.2 Mean annual rainfall for Maui Nui highlighting the different ecosystems formed on the Island of Maui (Giambelluca *et al*. 2013).

1.8 THE MAUI PARROTBILL- A STUDY SYSTEM AND STRATEGY FOR SPECIES RECOVERY

It is incredible to think that on an island as small as Maui, a species could go undetected for long periods of time. Indeed the Maui Parrotbill disappeared after its initial descriptions by Rothschild in 1893-1900 and was thought to be extinct until it was rediscovered in 1950 (Richards and Baldwin 1953). Because of this extreme rarity, Maui Parrotbill had no surviving Hawaiian name as Hawaiian was not a written language, and it had been lost over time. In 2010, the Hawaiian Lexicon Committee gave Maui Parrotbills a new Hawaiian name, Kiwikiu. Kiwikiu is named for its curved sickle-shaped bill, its whistle, and the weather on Haleakala, the mountain on which it now survives.

The natural history of this species was still largely unknown until the first active nest was discovered 1993 (Van Gelder 1993; Lockwood *et al*. 1994). Further ecological studies did not begin until the 2000s after the formation of Maui Forest Bird Recovery Project, an organization focused on the development and implementation of techniques that recover Maui's endangered birds and restoration of their habitats through research, development, and application of conservation techniques.

Maui Parrotbills are part of the subfamily Drepanidinae, of Fringillidae (finch family), with the other Hawaiian Honeycreepers. They were previously considered part of the “Hawaiian finches” like the Palila (*Loxioides bailleui*) and Laysan Finch (*Telespiza cantans*) (Berger 1981), but more recent phylogenetic studies have revealed the Akiapolaau (*Hemignathus munroi*) on the Island of Hawaii as the parrotbill’s closest extant relative (Lerner *et al*. 2011).

Maui Parrotbills are is one of the largest extant honeycreepers (length 14 cm, males 25 g, females 20 g) with a relatively large, parrot-like bill. It uses this bill to search for concealed invertebrates as well as rip into branches, bark, and stems, and to bite open fruit. Parrotbills are also one of the most sexually dimorphic of the honeycreepers with the males being approximately 20% larger than the females (Simon *et al.* 1997) (Figure 1.8.1).

Figure 1.8.1 Adult male Maui Parrotbill (right) and an adult female (left).

Parrotbills have very unique breeding behaviors. Males and females remain in established pairs year-round and only tend to re-pair after the death of a mate (MFBRP unpublished data). Pairs maintain home ranges of 9-11 ha throughout their range (Warren and Mounce 2014). Most nesting occurs January-July, but nests have been found in all months of the year except September, and pairs defend their territories year-round (MFBRP unpublished data). Pairs often will nest up to three times until they are successful, laying a single egg, although there have been several observations made of pairs caring for two offspring (Simon *et al.* 1997, MFBRP unpublished data). Once the chick fledges, it has a juvenile dependence period of up to 18 months (Simon *et al.* 1997, MFBRP unpublished data). To balance this low reproductive potential, parrotbills have a long life. The oldest known parrotbill is a minimum of 16 years old (Mounce *et al.* 2012).

Parrotbills occur in mesic and wet native montane forests. These remnant Maui forests are dominated by ohia (*Metrosideros polymorpha*), olapa (*Cheirodendron trigynum*), kolea (*Myrsine lessertiana*), and kawau (*Ilex anomala*). More important for their feeding ecology than this overstory, is the diverse understory of native plants including akala (*Rubus hawaiensis*), ohelo

(*Vaccinium calycinum*), alani (*Melicope* spp.), pilo (*Coprosma* spp.), and kanawao (*Broussaisia arguta*) (Simon *et al.* 1997, Stein 2007). Parrotbills forage mainly on the woody portions of native shrubs and trees using their powerful bills to excavate bark and wood for insects and other arthropods. Feeding primarily on the larvae and pupae of beetles and moths (as much as 90% coming from Lepidoptera larvae (Peck *et al.* 2015), they also forage on soft fruits to extract invertebrates (Perkins 1903, Mountainspring 1987, Simon *et al.* 1997, Stein 2007). Parrotbills are range restricted to high elevation forests above the “malaria line” on east Maui. This area gives the species only 50 km^2 of available habitat (USFWS 2006). This is a massive constriction of their historical habitat as they were once found on the island of Molokai as well as in low elevation forests (Gorreson *et al.* 2009).

The most comprehensive population survey for parrotbills was done in 1980 as part of Hawaii Forest Bird Surveys, run by the State of Hawaii. The population estimate at that time was 502 ± 116 individuals (Scott *et al.* 1986). While some subsequent surveys have shown densities in certain portions of their range as similar to 1980 (Simon *et al.* 2002), none have been able to conclusively show that the population is stable across their range (Gorreson *et al.* 2009). An intense population survey within 36.9 km^2 of parrotbill habitat in 2011 estimated 421 individuals (209-674 95% CI), but excluded some additional areas of known parrotbill habitat (Brinck *et al.* 2012). Regardless of exact numbers, recent distribution surveys have suggested that this habitat area is “full” (MFBRP unpublished data). Creating a second population of Maui Parrotbills has been identified as key to their long-term survival (USFWS 2006). Furthermore, USFWS has identified areas across Maui Nui that they consider “critical habitat” for the recovery of the species. In addition to the wet forest where the species is currently found, this includes montane mesic forest around Haleakala (USFWS 2012) (Figure 1.8.2).

In addition to the wild population, a captive population of Maui Parrotbills was initiated in 1997. A total of seven founding individuals were collected from the wild in 1997, 1999, 2001, and 2005. Two of the seven founders have never produced offspring, and thus the captive population is composed of five genetic founders. As of August 2015, the captive flock consisted seven males and five females (Table 1.8.1). Current breeding potential in captivity appears to be limited by the relatively small number of breeding females. Four of the five females currently in captivity appear to be non-reproductive due to obvious physical ailments (e.g. blindness) or based on their poor reproductive history. Although the remaining female (MP011) has been productive by producing six offspring throughout her 11 year life, she has laid a relatively large number eggs over this period and may be starting to show signs of senecence. During the 2015 breeding season, none of MP015's eggs successfully hatched for the first time since the 2011 breeding season.

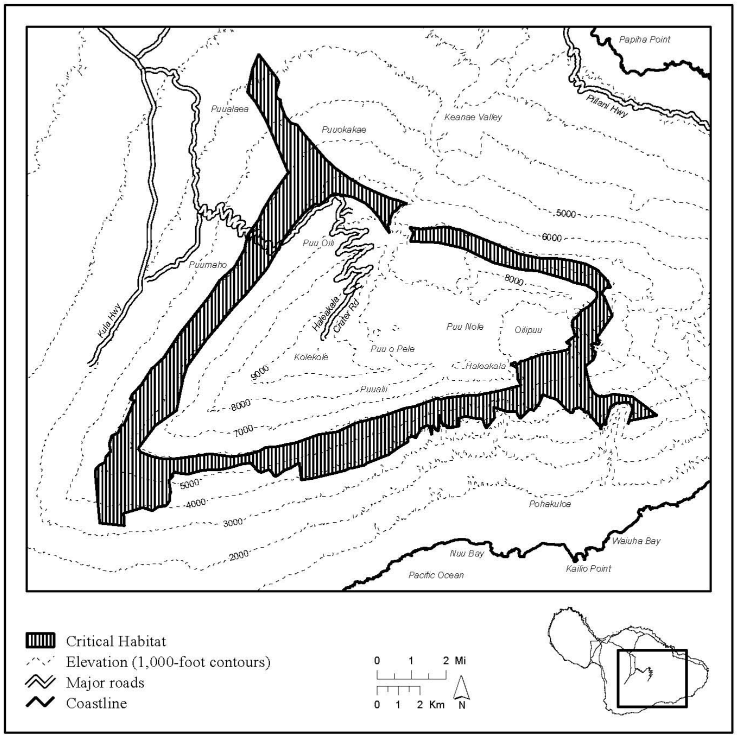

Figure 1.8.2. US Fish and Wildlife Service critical habitat for *Pseudonestor xanthophrys* on the Island of Maui within montane mesic forest (USFWS 2012).

Table 1.8.1. Current and historical inventory of Kiwikiu in captivity.

Studbook	Sex	Founder/ Descendant	Hatch Date	Status	Reproductive status
MP003	Female	Founder	4/30/1999	Alive	Blind
MP009	Female	Founder	6/12/2001	Alive	Never laid an egg
MP011	Female	Descendant	5/17/2004	Alive	Productive female
MP012	Female	Descendant	6/21/2004	Alive	Never laid fertile egg
MP015	Female	Descendant	3/5/2005	Alive	No descendants
MP017	Male	Founder	1/1/2005*	Alive	-
MP018	Male	Founder	1/1/2005*	Alive	-
MP022	Male	Descendant	3/2/2012	Alive	-
MP023	Male	Descendant	3/2/2012	Alive	-
MP024	Male	Descendant	4/2/2012	Alive	-
MP026	Male	Descendant	4/15/2013	Alive	-
MP027	Male	Descendant	3/23/2014	Alive	-
MP001	Male	Founder	1/27/1997	Dead	-
MP002	Female	Founder	3/21/1999	Dead	-
MP004	Female	Descendant	7/21/2000	Dead	-
MP005	Male	Descendant	9/18/2000	Dead	-
MP006	Female	Descendant	5/2/2001	Dead	-
MP007	Female	Descendant	6/17/2001	Dead	-
MP008	Female	Descendant	7/19/2001	Dead	-
MP010	Male	Founder	5/1/2001*	Dead	-
MP013	Unknown	Descendant	8/16/2004	Dead	-
MP014	Unknown	Descendant	9/13/2004	Dead	-
MP016	Female	Descendant	9/12/2005	Dead	-
MP019	Unknown	Descendant	7/4/2007	Dead	-
MP020	Male	Descendant	7/9/2008	Dead	-
MP021	Female	Descendant	3/21/2009	Dead	-
MP025	Female	Descendant	12/18/2012	Dead	-

*Estimated hatch date. Adult bird collected from the wild.

1.9 OVERALL AIM OF THE THESIS

Many species around the world are disappearing at a much faster rate than recovery efforts can be designed, including Hawaiian honeycreepers. There are more species becoming endangered every year and fewer resources to address the threats against them. The resources needed to tackle the biodiversity crisis in Hawaii are large, and Hawaii's isolation from the mainland United States limits support for species conservation as compared to continental species (Leonard 2008). With 17 species of Hawaiian birds of fewer than 1000 individuals remaining, conservation efforts need to be well planned and have a high rate of success.

As conservation managers, we need to be able to identify the genetic and ecological constraints in each species and be able to design studies to evaluate these constraints before reintroduction or other recovery efforts are implemented. However, many species do not have the luxury of time before conservation efforts must be undertaken. I undertook this research focused on Maui Parrotbill in order to inform conservation managers of the next best steps in planning a reintroduction for this species. This research made use of all past ecological data that had been gathered on this species in order to synthesize everything that is known about Maui Parrotbills within this thesis.

This includes:

- An evaluation of demographics for Maui Parrotbills, a rare and cryptic species for which large sample sizes are impracticable and conclusions must be carefully drawn from all quantitative and anecdotal data available
- An assessment of the current genetic profile of Maui Parrotbills
- The use of available knowledge on Maui Parrotbills to inform conservation managers as to the benefits and risks to conservation actions and no action for this species

THESIS OUTLINE

My thesis starts with examining nest success and annual reproductive success for Maui Parrotbill, **Chapter 2**. While productivity is at the heart of the demographics driving the trajectory of any population, this information was mainly unknown for this species due to the difficult field conditions for collecting it and the small sample sizes that we were able to achieve per given person efforts. Although nest success is a commonly used metric of productivity, I compared the more labour intensive annual reproductive success methods in order to legitimize the information we ascertained from the nest success alone and present both of these analyses.

In **Chapter 3** I examine the second largest factor driving population trajectories, survival. Parameters such as survival rates are an important component to understanding population ecology and informing management decisions but can be quite difficult to determine for rare species. This chapter makes use of a long-term dataset of mark-re-sight information for Maui Parrotbill. This 18-year encounter history allowed a comprehensive analysis that accounted for annual variations in survival and detection probabilities through time.

In **Chapter 4** I examine the genetic profile of the current Maui Parrotbill population. I used nuclear and mitochondrial DNA to quantify the levels of contemporary genetic diversity and structure in wild and captive parrotbill populations. I further compared these genetic patterns to those observed within historical nuclear diversity derived from 100-year old museum samples. While the contemporary population structure lends valuable information on how to manage this wild population, how to manage the captive populations, and how to plan reintroduction efforts for the species, the historical data yeilds a better perspective on the overall effects of the different threats that have harmed this species through time.

In **Chapter 5** I use all the information derived from the previous chapters to build a comprehensive population viability analysis for Maui Parrotbill. This analysis examined population growth rates and probabilities of extinction within the current population. Using a sensitivity analysis, I identified key parameters limiting the probability of persistence in the next 25 years. With few management options available to implement in their current range, I then explored various options for establishment of an additional new population and the cost/benefit of such for the population as a whole.

At the conclusion of my thesis in **Chapter 6**, I provide a synopsis of the key findings throughout these studies and use these to provide guidance on the best recovery strategiy for this endangered species.

Chapter 2 Determining productivity of the Maui Parrotbill, an endangered Hawaiian honeycreeper

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Key words: annual reproductive success, demography, nest success, population monitoring

ABSTRACT

Maui Parrotbills (*Pseudonestor xanthophrys*), critically endangered Hawaiian honeycreepers endemic to the island of Maui, are restricted to a single population of ~500 individuals located in remote, mountainous terrain. January to June 2006-2011, we located nests and fledglings in the Hanawi Natural Area Reserve (NAR) in east Maui, Hawaii, to document nest success and annual reproductive success. Nest success is a commonly used measure of productivity and is a central component of many demographic studies. Annual reproductive success is less frequently documented because greater effort is required to monitor the reproductive success of breeding pairs through time. However, for species whose nests are difficult to locate or access, such as Maui Parrotbills, the presence or absence of fledged young may provide a more accurate measure of breeding success than monitoring nests. During our study, we located and determined the outcome of 30 nests to document nest success, and monitored 106 territories for the presence or absence of fledglings to calculate annual reproductive success. Nest success probability was 19% (N = 30) and seasonal nest success was 46%. During our monitoring efforts, 49 of 106 breeding pairs produced a single fledged young. Because parrotbills typically have single egg clutches and only re-nest after nests fail, the presence or absence of a fledgling is an indication of a pair's overall reproductive success for a breeding season. The number of fledglings per pair produced an annual reproductive success estimate of 46%, confirming our initial productivity estimate from nests. Thus, our results indicate that the two methods, determining annual reproductive success by monitoring fledglings and calculating nest success, provide similar estimates of annual productivity for Maui Parrotbills. Based on our estimates, the parrotbill population appears to be demographically stable. However, our productivity estimate was based only on the population at Hanawi, an area representing just 3% of the total range of parrotbills. Thus, our results may not accurately reflect the status of parrotbills over their entire range.

2.1 INTRODUCTION

Maui Parrotbills (*Pseudonestor xanthophrys*) are a federally endangered and red-listed critically endangered species of Hawaiian honeycreeper (U.S. Fish and Wildlife Service 1967, IUCN 2011). The species is restricted to a single population occupying an area of ~50 km^2 on the northeastern slopes of Haleakala, Maui, Hawaii (Scott *et al.* 1986; U.S. Fish and Wildlife Service 2006). Historically, Maui Parrotbills (hereafter referred to as parrotbills) were distributed across the islands of Maui and Molokai (James and Olson 1991), where they may have preferred native koa (*Acacia koa*) forests (Perkins 1903). Clearing of lowland forests and introduction of alien diseases (i.e., avian malaria and pox) drastically reduced the range of parrotbills, and they are now restricted to high-elevation (1200 - 2350 m) wet montane forests, where cool temperatures limit disease vectors (i.e., mosquitoes) and consequently the spread of avian malaria (Scott *et al.* 1986; Mountainspring 1987; Simon *et al.* 1997). Population estimates of parrotbills based on data collected in the 1980s suggested a stable population of 502 ± 230 (95% CI) individuals (Scott *et al.* 1986). More recent surveys, however, have been inadequate to produce an accurate population estimate. Whereas range-wide surveys through 2001 yielded densities similar to those in the 1980s, the trend assessment was inconclusive regarding the stability of the population (Gorreson *et al.* 2009; Camp *et al.* 2009).

Parrotbills are insectivorous honeycreepers that defend year-round territories (Pratt *et al.* 2001) and frequently occur in family groups, in which young remain with parents for five to eighteen months after fledging (Simon *et al.* 1997; MFBRP unpublished data). Parrotbills breed from November to June, with most breeding between February and June. Males and females form long-term monogamous pair bonds, typically foraging together year round. Females typically lay single-egg clutches and only re-nest after nest failure, which often occurs during periods of heavy rain (Lockwood *et al.* 1994; Simon *et al.* 1997). Due to their rarity and tendency to nest high in the forest canopy, information about parrotbill reproductive success is limited and no recruitment data are available.

In the absence of a conclusive population estimate, population modeling may be crucial in guiding management efforts for this species. For example, population viability analyses (PVAs) provide managers with information about extinction risk that is useful in developing management strategies for endangered species (Boyce 1992; Akçakaya and Atwood 1997; Brook *et al.* 2000). However, all population models rely on accurate demographic data. Unfortunately, the quality of such data is often poorest for endangered species – species that are most commonly in greatest need of accurate PVAs to inform their conservation management (Beissinger and Westphal 1998).

One key demographic component of all population models is productivity, and nest success is a commonly used metric for estimating this variable (Woodworth *et al.* 2001; Renner and McCaffery 2008; Hartman and Oring 2009; Nappi and Drapeau 2009). The Mayfield estimator or more recently developed methods implemented in Program MARK (White and Burnham 1999) and SAS/STAT® software have been used to standardize data from nests found (Mayfield 1961, 1975; Rotella *et al.* 2004), but information about the success of individual nests does not always reflect reproductive output at the population level (Murray 2000; Jones *et al.* 2005), especially when it is not possible to monitor all nesting attempts (Thompson *et al.* 2001). This problem can be particularly acute for cryptic species that are difficult to locate and monitor. Therefore, alternative methods for estimating productivity are necessary for some species. One potential alternative method is the intensive monitoring of breeding pairs and calculation of annual reproductive success (ARS) based on the number of fledged young per pair. Given the difficulty of monitoring individual birds through an entire breeding season, few investigators have quantified productivity using this method (Porneluzi and Faaborg 1999; Jones *et al*. 2005, Vanderwerf 2009; Rogers 2011).

The choice of reproductive measure and the resulting fecundity estimates that different estimators produce can have far-reaching effects when determining population viability. Furthermore, models of population dynamics have been shown to be sensitive to small changes in such estimates (Powell *et al*. 1999; Woodworth 1999). Consequently, we estimated the productivity of breeding parrotbills using both nest success and annual reproductive success, and compared estimates to evaluate their relative performance in the Hanawi Natural Area Reserve.

2.2 METHODS

Study Area

The Hanawi Natural Area Reserve (NAR) covers 3036 ha on the windward slopes of Haleakala Volcano. Within the reserve, 800 ha above 1600 m in elevation are fenced and ungulate free; this is the core area used by the current parrotbill population (U.S. Fish and Wildlife Service 2006). We used two study areas in the Reserve, Frisbee Meadows (FSB) and Poouli Camp (HR3). The FSB study area (77 ha) is between 1600 and 2200 m asl, and the HR3 study area (56 ha) between 1550 and 1950 m asl (Figure 2.1). Non-native rodents are controlled on 35 ha of the HR3 site (Malcolm *et al*. 2008; Figure1). The area is characterized by steep, rugged terrain and supports a thick montane, wet forest dominated by ohia (*Metrosideros polymorpha)* and olapa (*Cheirodendron trigynum*; Jacobi 1989). The forest has an intact native understory and sub-canopy that provides high-quality foraging habitat for parrotbills.

Parrotbills were captured prior to and throughout the duration of our study using passive mist-netting and targeted mist-netting using playback. Of 212 adult (ASY) birds monitored over the 4-yr period, 130 were marked with a unique color band combination. Unmarked birds could be accounted for when paired with a banded individual during a single breeding season, but could not be identified between years.

Territories were defined by the presence of singing males, males counter-singing with neighboring males, and regular presence of foraging adults; little overlap was observed between adjacent territories. Birds were assumed to be paired if they were observed foraging and travelling together, occupied the same territory, and demonstrated typical breeding behaviors such as mutual preening, mutual feeding, and nest building. To prevent possible double-counting, pairs where both adults were unbanded were only classified as discrete pairs when their territories bordered those of marked individuals.

We searched for nests and fledglings along trails at each study site. Trails were 50 to 100 m apart in a network web that covered the entire study area and were systematically searched at least once per week from 07:00 to 17:30, each observer covering ~2 km per day. We conducted searches along 32.5 km of trails in the two study areas. Once an adult was detected, observers stayed for several hours to identify the individual and note behavioral activity. Three to six observers searched each site daily, except during severe weather. In addition to regular trail coverage, all territories in each study area were visited weekly to locate adults. We located fledglings either using their incessant begging calls (Simon *et al*. 1997) or by following parents to offspring.

Nest success

From January to June 2006-2011, nests were located by observing adults carrying nesting material and the location of courtship displays, copulations, and pair feedings, all of which usually occurred near nest sites. Because of individual variation in the timing of breeding and the length of the breeding season, we could not determine if nests we monitored were first, second, or third nesting attempts for the year. Nests were usually monitored daily for 3 to 6 hrs using spotting scopes or binoculars from a distance of ~30 m until chicks fledged or nest failure was confirmed. Because nest contents were usually not visible, parental behavior at nests was used to determine nesting stage (e.g., constructing, incubating, brooding, or fledged; see Becker *et al*. 2010). Only nests where an egg was presumed to have been laid, based on observation of apparent incubation, brooding, or food delivery, were included in our analyses. Nests were classified as successful if fledglings were observed, with young considered to have fledged when they left nest trees.

Previously active nests where no activity was documented for ≥3 h were classified as failures. Over a 3-h time period, adults typically visit nests two to three times (Becker *et al*. 2010). All failed nests were checked at least once more 1-3 days after failure was documented. Causes and timing (nest stage) of failures could not be determined for most nests because nests were located high (~ 11 m) in the canopy. When possible, we used mirrors or climbed nest trees to view nest contents.

Parrotbill nest success was calculated using PROC GENMOD (SAS Institute 2008) to fit a logistic-exposure model (Shaffer 2004). This generalized linear model with a modified link function uses the appropriate likelihood estimator for interval data, avoiding assumptions about when failure occurs and allowing variable intervals between observations. We pooled nest data across all years to increase our sample size because there was no apparent annual variation (Kershner *et al.* 2001). For nests found under construction, the first day of incubation was determined by female behavior (i.e., when first observed incubating). Because only single-egg clutches have been documented (U.S. Fish and Wildlife Service 2006), we assumed incubation began immediately after an egg was laid. Because we were unable to determine the contents of most nests, we did not differentiate between egg and nestling survival.

Annual reproductive success

From January to June 2008 - 2011, we systematically monitored the territories of 106 pairs of parrotbills for the presence of fledglings to calculate annual reproductive success. Because parrotbills typically have single-egg clutches and only re-nest after nest failure, the presence or absence of a fledgling is an indication of a pair's reproductive success for a breeding season (Simon *et al.* 1997). Therefore, annual reproductive success was estimated by dividing the number of pairs with offspring by the total number of pairs observed during a breeding season.

Population growth model

To determine the overall effect of each estimate of productivity (nest success and annual reproductive success), we calculated the finite rate of population growth (λ) using the formula:

$$\lambda = P_A + P_J \beta(0.5),$$

with P_A = adult survival, P_J = juvenile survival, and β = average productivity per pair. Values of $\lambda > 1$ indicate a population increase and values of $\lambda < 1$ indicate decline. Adult and juvenile survival estimates derived from the same study population were drawn from Vetter *et al.* (2012).

Due to re-nesting, our nest success estimate did not reflect seasonal productivity (Streby and Anderson 2011). Parrotbills have been observed to make up to three nesting attempts per season after nest failures (MFBRP unpublished data). We adjusted our ß value for nest success with the following equation to have comparable seasonal productivity estimates based on each method:

Seasonal nest success = Observed nest success + (Observed nest success *(1- Observed nest success)) + (Observed nest success *((1- Observed nest success)*(1- Observed nest success)))

2.3 RESULTS

Nest success

During six breeding seasons (2006-2011), we located 30 Maui Parrotbill nests (24 at HR3 and six at FSB). Eight nests either did not progress past the nest-building stage or nest outcome could not be determined; these nests were not included in our analyses. All nests were located in ohia trees, most in outer canopy branches 5.2 to 18.2 m above ground (mean = 10.9 m). Fifteen of 22 nests failed (68.2%). The logistic-exposure method resulted in a nest success probability of 0.185 ± 0.056 and a daily nest survival probability of 0.953 ± 0.007. One egg that did not hatch after 31 days of incubation was presumed to be infertile. Seven of the 15 failures occurred during the first 10 days of the nestling period, and one chick was predated by a Pueo (*Asio flammeus sandwichensis*; Mounce 2008). The cause of failure of the other 14 nests could not be determined. Seasonal nest success, adjusted for re-nesting, was 46% (*N* = 22 nests).

Annual reproductive success

During four breeding seasons (2008-2011), we monitored 43 pairs at HR3 and 63 pairs at FSB. Annual reproductive success estimates were 51% and 43% for HR3 and FSB, respectively, resulting in an overall estimate of 46% (Table 2.1). For all four years combined, we found no difference in productivity between the two study sites ($x^2 = 6.5$, $k = 3$, $P = 0.10$).

Population growth model

According to Vetter *et al.* (2012), adult survival in our population of parrotbills was estimated at 0.84 ± 0.04 and juvenile survival at 0.76 ± 0.09. Based on both our seasonal nest success estimate and our annual reproductive success estimate, our model predicts a stable population ($\lambda = 1.02 \pm 0.07$).

2.4 DISCUSSION

Demographic modeling relies on accurate estimates of reproductive success. Therefore, using the reproductive monitoring method that provides the most accurate productivity data is critical. We suspected that our estimates of annual reproductive success based on observations of family groups would be the superior method because we calculated annual reproductive success using a larger subset of the population than for nest success, and because this method more accurately reflected season-long productivity. However, our results suggest that using either method (seasonal nest success or annual reproductive success) to estimate annual productivity is adequate for studies of this species. Both productivity calculations are indeed confirmations of one another, both resulting in a 46% annual productivity estimate.

Our productivity estimate suggests a stable or potentially increasing population. This is supported by results from population monitoring using point transect distance-sampling throughout the species' range that were unable to detect any recent changes in population size (Gorreson *et al.* 2009; Camp *et al.* 2009). However, these transect surveys are only repeated every 5 yrs and, because parrotbills are long-lived birds (up to 16 yrs), any changes in population could take several years to detect. Thus, our productivity values may be more valuable to managers than range-wide survey data.

For species like parrotbills that occur at low densities (as few as 10 birds/km^2) and have difficult-to-locate nests, determining productivity by documenting the number of young fledged per pair confirmed the validity of our seasonal nest success estimates despite low sample sizes. However, these two methods may not yield similar results for all species. Although monitoring nests is critical for identifying factors that might limit productivity (i.e., weather or predation; Jones *et al.* 2005), nest success has been shown to provide inaccurate estimates of productivity in other passerines (Murray 2000; Underwood and Roth 2002; Grzybowski and Pease 2005). In a review of methods for estimating productivity, Anders and Marshall (2005) noted that quantifying the season-long productivity of individuals in a population provides the most accurate estimate of population productivity. When obtaining such data is not logistically practical, productivity can still be estimated more accurately by incorporating other variables into population models (Anders and Marshall 2005). For example, for species where nests are difficult to locate or access, like those of Maui Parrotbills in our study, surveying territories for the presence of fledglings can be less time-consuming than locating and monitoring nests because fledglings often beg loudly and adults give alarm calls or chips (contact calls) when potential predators approach (Anders and Marshall 2005).

Although our data suggest some variation in annual reproductive success of parrotbills between our two study areas, we found no significant spatial and temporal differences, even though predator control was conducted at over 62% of the HR3 site during our study, but not conducted at FSB. Populations of non-native mammalian predators, including rats (*Rattus* spp.) and mongooses (*Herpestes javanicus*), were controlled using bait stations loaded with rodenticide, snap traps, and mongoose body traps (Malcolm *et al*. 2008). These non-native mammals are considered major threats to Hawaiian birds and, although rats have not been documented as predators of parrotbill nests, they have been documented predating Akohekohe nests at heights similar to those of parrotbill nests (Scott *et al*. 1986; Simon *et al*. 2001). Predator control efforts may need to be expanded to cover at least 100% of each pairs' home range, with perhaps an additional buffer of control around each home range, if any resulting difference in nest success is to be realized from these management efforts.

Based on our annual productivity estimate, the parrotbill population appears to be demographically stable at Hanawi, close to the core of their range. However, our productivity estimate was based only on the population at Hanawi, an area that represents just 3% of the total range of parrotbills. Thus, our results may not accurately reflect the status of parrotbills over their entire range. Survey efforts using point transect distance-sampling throughout their range indicate that parrotbill densities may be lower outside of Hanawi (Maui Forest Bird Recovery Project unpublished data), but there has been no detailed demographic monitoring in other areas of the species' range. Expanding our productivity estimate techniques to the outer edges of the species' range will enhance the utility of population modeling studies and will help managers to develop a more sophisticated assessment of population-wide levels of productivity.

ACKNOWLEDGMENTS

Maui Forest Bird Recovery Project operates under the sponsorship of Pacific Cooperative Studies Unit and Research Corporation of the University of Hawaii. State of Hawaii Division of Forestry and Wildlife, and Pacific Island Office of the U.S. Fish and Wildlife Service provided funding, oversight, scientific input, access into the Hanawi Natural Area Reserve and the required state and federal permits. We would like to thank the following partners for logistical support and/or access to their properties: Haleakala National Park, The Nature Conservancy Hawaii, Tri-Isle Research, Conservation & Development, Pacific Helicopters, and Windward Aviation. S. Wheeler, C. Rutt, and J. Milikowsky assisted with data analyses. Finally, we thank all of the seasonal biologists who contributed to the data set used in this study.

2.5 TABLES AND FIGURES

Table 2.1. Maui Parrotbill annual reproductive success based on number of pairs observed with fledglings at two study sites (FSB and HR3) in Hanawi NAR 2008-2011.

Site	Year	Number of pairs observed	Number of pairs with juveniles	Percent success
FSB	2008	11	3	27.3%
	2009	15	8	53.3%
	2010	18	6	33.3%
	2011	19	10	52.6%
HR3	2008	10	4	40.0%
	2009	8	6	75.0%
	2010	9	5	55.6%
	2011	16	7	43.8%
FSB totals		63	27	42.9%
HR3 totals		43	22	51.2%
Totals		106	49	46.2%

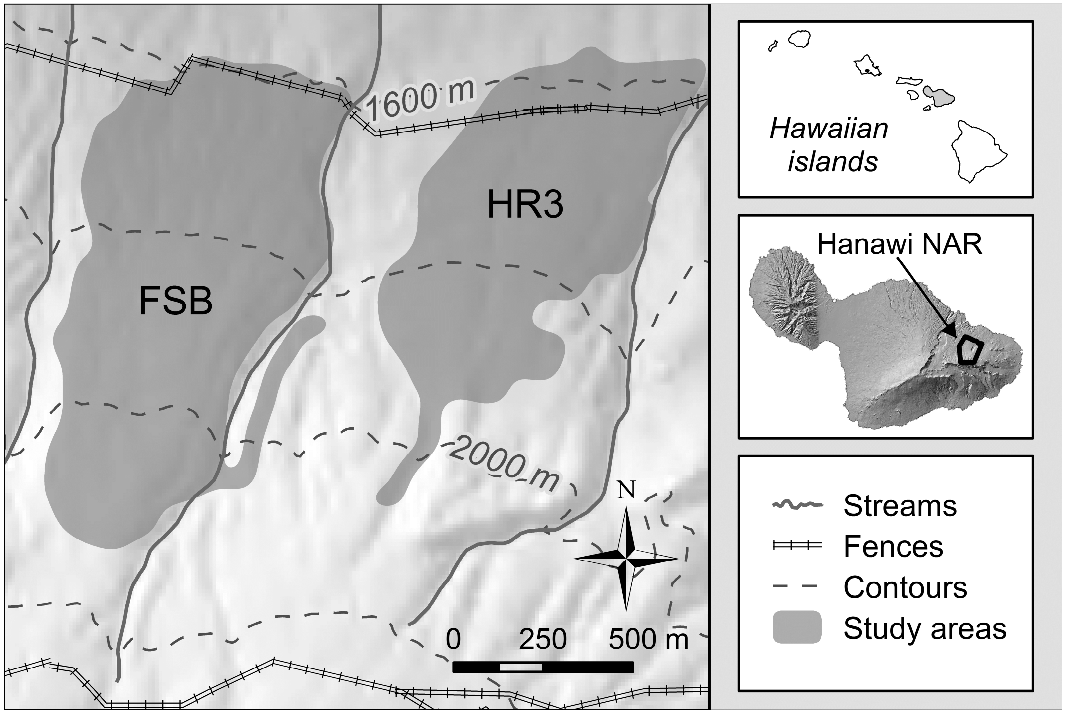
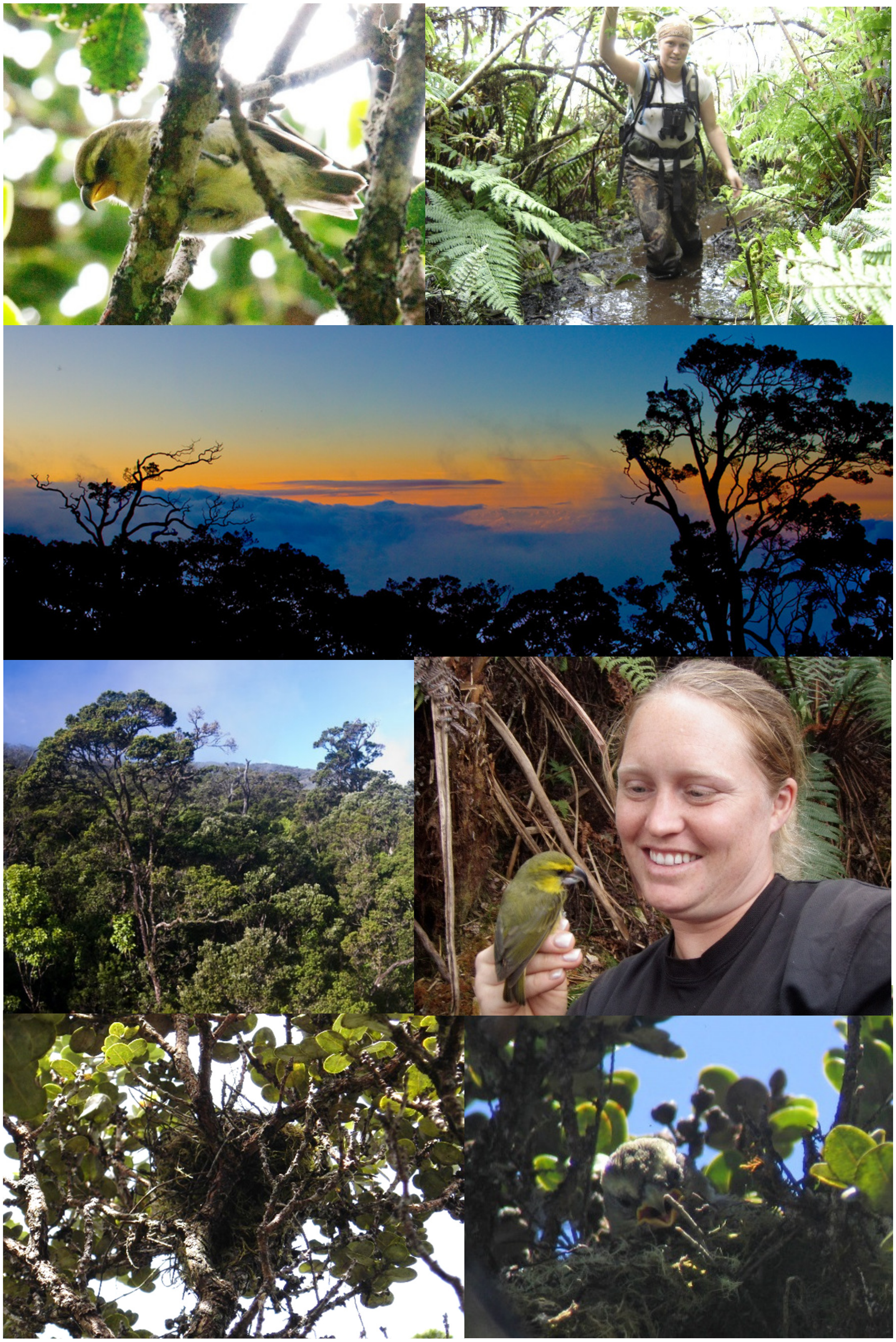


Figure. 2.1. Study sites where the productivity of Maui Parrotbills was examined in our study. Both Frisbee Meadows (FSB, 77 ha) and Poouli Camp (HR3, 56 ha) are located in the Hanawi Natural Area Reserve, Island of Maui, Hawaii.



Chapter 3 Management implications derived from long term re-sight data: annual survival of the Maui Parrotbill (*Pseudonestor xanthophrys*)

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Keywords: apparent survival, endangered species, demography, Maui Parrotbill, population monitoring

ABSTRACT

The accurate estimation of key demographic parameters is invaluable for making decisions about the management of endangered wildlife but such estimates are often difficult to obtain. Parameters such as species-specific apparent survival rates are an important component to understanding population ecology and informing management decisions. Maui Parrotbills (*Pseudonestor xanthophrys*) are 'Critically Endangered' Hawaiian honeycreepers endemic to the Island of Maui. We used an 18 year encounter history dataset comprising 146 marked individuals to estimate apparent survival between sexes and age classes (juvenile, adult). A difference in survival rates between sexes was strongly supported; 0.72 ± 0.04 for adult females and 0.82 ± 0.03 for adult males. This difference may be a reflection of either reproductive costs or additional risks of incubation and brooding, such as depredation. We also found support for age-biased survival, but limited information for juveniles did not provide a well-supported model fit for our data (juvenile survival = 0.17 ± 0.15; adults = 0.78 ± 0.02). However, apparent adult survival was similar to that of other Hawaiian passerines (mean 0.78 ± 0.03, n = 16). These results suggest that efforts to prevent the extinction of this species may benefit from future management strategies focused on increasing female survival such as predator reduction.

3.1 INTRODUCTION

Extinction risk is high for many Hawaiian bird species and this situation creates an urgent need for reliable assessment of their density and distribution. Maui Parrotbill (hereafter parrotbill) (*Pseudonestor xanthophrys*) is a critically endangered insectivorous forest bird endemic to the island of Maui (U.S. Fish and Wildlife Service 2006; IUCN 2012). Limited range, combined with small population size and low densities (U.S. Fish and Wildlife Service 2006) puts parrotbills at particular risk of extinction. Like most Hawaiian forest birds, parrotbills are currently limited to high elevation forests that are relatively free of mosquitoes and avian malaria (*Plasmodium sp.*) (Scott *et al.* 1986; Mountainspring 1987; Atkinson and LaPointe 2009), but these forests are otherwise likely suboptimal habitat (Simon *et al.* 1997; U.S. Fish and Wildlife Service 2006; Becker *et al.* 2010). In addition, widespread habitat loss, especially of koa (*Acacia koa*) forests, parrotbills' preferred foraging habitat (Perkins 1903), has contributed to their current limited distribution on the windward slopes of east Maui (U.S. Fish and Wildlife Service 2006). The depredation of nests, juveniles, and adults also may limit the parrotbill population and, while the cause and importance of depredation is unclear, rodents (*Rattus spp.*), feral cats (*Felis catus*), and the invasive small Indian mongoose (*Herpestes auropunctatus*) are present throughout the area where the current population persists (Sugihara 1997; Malcolm *et al.* 2008).

Based on the 1980 Hawaii Forest Bird Survey that used island wide point counts, the parrotbill population was estimated at 502 ± 116 (Scott *et al.* 1986). As a rare species with low detection rates, more recent surveys have been unable to confirm the stability of the parrotbill population (Gorreson *et al.* 2009). Indeed, Brinck *et al.* (2012) found that the repeated sampling frequencies and number of visits that would be necessary to increase the power of these surveys to detect trends in the parrotbill population would be particularly high and therefore logistically unfeasible. Due to the limitations of these survey efforts for estimating the population size of a rare species, demographic analysis is perhaps the only alternative means for providing a better insight into the population dynamics of the parrotbill. An understanding of population demography, coupled with an understanding of the factors limiting population growth, is essential for recovering populations of endangered species, designing effective conservation strategies and making informed management decisions (Anders and Marshall 2005). While studies of population dynamics depend heavily on mortality and recruitment rates (Lebreton *et al.* 1993), such information is often lacking for endangered species, but it is often these same species of conservation focus that would benefit the most from such studies (Beissinger and Westphal 1998).

Fundamental to population demography is an understanding of the variability in survival among individuals (Lack 1954; Eberhardt 1985). Accurate measurements of population-specific survival are essential for estimating reliable rates of population change, as many models of population dynamics are sensitive to small deviations in estimates of demographic measures (Noon and Sauer 1992; Porneluzi and Faaborg 1999; Woodworth 1999). Constant effort mist-netting and banding has historically been a common method used to estimate survival rates of passerines (DeSante and Burton 1994). This technique is limited by the fact that previously banded individuals are not always recaptured even though they may still be alive (Chase *et al.* 1997). Indeed, whether or not a banded bird is subsequently detected is a function of probabilities: survival, emigration, and detection. Re-sighting and re-capturing marked individuals has since improved this method for generating the most accurate estimations of survival in forest bird species (Sandercock *et al.* 2000; Gardali and Nur 2006; Johnson *et al.* 2006). Furthermore, understanding variation in age-specific and sex-specific survival can provide valuable insights to inform the ecology and conservation of a species (Sandercock *et al.* 2000; Martin 2002).

Considering the limitations of accurately estimating the population of rare species, accurate demographic data would provide managers with a yardstick to monitor the population trajectory of a species. However, the low densities and few individuals indicative of a rare and endangered species results in mark-recapture studies requiring large amounts of time and effort. In addition, parrotbills inhabit very rugged and remote terrain. Both these characteristics make collecting demographic data a challenge. Indeed, long-term demographic data for rare species inhabiting remote areas are uncommonly available for managers. Here we improve upon previous demographic estimates for this species (Vetter *et. al* 2012) by summarizing survival probability of parrotbills in the core of their population range using 18 years of encounter data, and we examine differences in age- and sex-specific survival probabilities.

3.2 METHODS

Study Area

We conducted this study within the Hanawi Natural Area Reserve (NAR) on the windward slope of Haleakala volcano, Maui, Hawaii (Figure 3.1). Our 180-hectare (ha) study site extended from 1600 to 2100 metres in elevation. This study area is located within an 800 ha portion of the reserve, managed by the State of Hawaii and has been fenced and free from invasive ungulates since 1997; it protects some of the most pristine native forest remaining in Hawaii. The area is mainly a montane wet forest characterized by rugged and steep terrain. Ohia (*Metrosideros polymorpha*) and olapa (*Cheirodendron trigynum*) are the dominant canopy species, although subalpine scrub and subalpine grassland occur at the highest elevations (Jacobi 1989). The study site supports the highest known density of parrotbills (Scott *et al*. 1986; Pratt *et al*. 2009a).

Mark-Recapture

Mark-recapture was a combination of recapture and re-sight efforts which varied across years, beginning at the higher elevations in 1994-1997 and resuming in 2006–2011 (Simon 1998; Simon *et al.* 2000; Berlin *et al.* 2001a; Berlin *et al.* 2001b; Pratt *et al.* 2001; Simon *et al.* 2001; Simon *et al.* 2002) and beginning at the lower elevations in 1998 and continuing through 2011. Most recapture and re-sight effort has occurred from January – June during the peak of the breeding season.

Banding occurred across two field sites connected by an extensive trail system (Figure 3.1). Individuals were initially captured in mist-nets and banded with a unique combination of a US Fish and Wildlife Service numbered band and three darvic plastic colored leg bands. To increase the capture rates above that of passive mist net efforts, playbacks were used in areas where unbanded individuals had been located. Once captured, parrotbills were aged and sexed using plumage and morphometric criteria (Berlin *et al.* 2001a). Both passive and targeted banding continued annually in different locations covering each study site. Re-sights were obtained by searching for banded individuals systematically across all trails, during each breeding season, as well as opportunistically in the same areas throughout the rest of the year. Subsequent re-sights were documented along with GPS locations.

Data Analyses

Based on capture, recapture, and re-sight histories from 1994–2011 for individually-marked parrotbills, we used Cormack-Jolly-Seber (CJS) models of live recaptures in program MARK, version 6.0, (White and Burnham 1999) to estimate apparent annual survival (φ) and encounter probability (ρ). While not explicitly designed for the combination of re-sight and recapture data, CJS is the most appropriate mark-recapture model for this type of data and has been widely used with similar data sets (Nur and Sydeman 1999; Sandercock *et al*. 2000; Vanderwerf 2009). We used an encounter period of one year given that (i) many individuals were only detected once a year and (ii) that subsequent encounters were often 10-12 months apart. Because of the rugged terrain, the fate of subsequently undetected individuals was unknown, thus the sampled population was defined as open, and survival estimates represent apparent survival. Using dates for the initial capture, and all subsequent recaptures and re-sights, we compiled an encounter history for each individual across the 18-year period.

In separate analyses, parrotbills were grouped by sex (male or female) and age class (juvenile or adult). Hatch-year (HY, juvenile) parrotbills cannot be conclusively sexed and were excluded from the sex-specific analysis. For each analysis, we started with the simplest model in which φ and ρ were both constant. Using standard model notation, this model is represented as φ(.)ρ(.) for each model set (Lebreton *et al*. 1992).

Each model was compared with Akaike's Information Criterion corrected for small sample size using the quasi-likelihood adjustment (QAICc), as calculated by Program MARK. The model with the lowest $QAIC_c$ value was considered to have the best fit (Burnham and Anderson 2002). To test that the arrangement of our data met expectations based on the assumptions underlying the model, we evaluated goodness-of-fit of our global (highest parameterized) model using the Program RELEASE GOF provided in Program MARK. We adjusted both analyses to the goodness-of-fit calculated value of $\hat{c}$ (variance inflation factor or lack of fit) from 1000 simulations before model selection. For both age and sex, we present the most parsimonious model and all models with QAICc weight in addition to the null (φ(.)ρ(.)), global (φ(g*t)ρ(g*t)), and fully time dependent (φ(t)ρ(t)) models.

3.3 RESULTS

Between 1994 and 2011, 146 individual parrotbills were banded in the study area (see Appendix A) and included in the age-specific analysis. Of these, 136 (64 females, 72 males) were included in our sex-specific analysis. Ten were HY birds and were excluded from the sex analysis. The number of individuals recaptured and re-sighted varied each year (see Appendix A for recapture histories), an average of 18.11 unique individuals were detected annually.

The best-fit age-specific and sex-specific models for parrotbills showed apparent survival that varied with age and sex but was constant across years and showed an encounter probability that varied with time (Table 3.2, Model 1; Table 3.3, Model 1; Figure 3.2). No other models were of a reasonable fit for either group. We found strong support for sex-specific differences in survival with males showing higher survival rates than females (males 0.82 ± 0.03; females 0.72 ± 0.04). We also found juveniles to show lower survival rates than adults (juveniles 0.17 ± 0.15; adults 0.78 ± 0.02) (Figure 3.3). While our sex-specific model had good fit to the CJS model selected (GOF Test 2 + Test 3 x^2=51.320, df=57, p-value=0.687, $\hat{c}$=0.900), our age-specific model did not (GOF Test 2 + Test 3 x^2=56.164, df=35, p-value=0.013, $\hat{c}$=1.605) due to insufficient data in the HY group to calculate independent x^2 results.

3.4 DISCUSSION

As with most survival studies of an open population, mortality and emigration cannot be separated and thus survival is likely to be underestimated (Cilimburg *et al.* 2002). This effect is especially true in rugged terrain which limits detectability. Even so, adult apparent survival was similar to that of other Hawaiian avifauna; Akohekoke (*Palmeria dolei*) show the highest annual survival of any Hawaiian passerine at 0.95 (Simon *et al.* 2001) but the average annual adult survival of 16 Hawaiian passerines averaged 0.78 ± 0.03 (Pratt *et al.* 2009a;

Woodworth and Pratt 2009). Conversely, juvenile apparent survival was lower than expected. Although the greater dispersal of young can contribute to differences in adult and juvenile survival (Greenwood and Harvey 1982), juvenile parrotbill survival was lower than that demonstrated for other Hawaiian birds. Woodworth and Pratt (2009) reported that the average annual juvenile survival of 13 other Hawaiian passerines was 0.32 ± 0.03. However, the juvenile parrotbill survival estimate was based on only 10 individuals, which contributes to the large standard error and only a moderate fit to our CJS model. While acknowledging that our estimate lacks precision, juvenile parrotbill survival is certainly lower than that of adults and it would not be surprising if juvenile survival of this species is particularly low. Unpublished data on territory occupancy in the Hanawi study site indicates that most available habitat is occupied. Young birds may be forced into poorer quality habitat, limiting their chances of survival. Although, we currently have little information on juvenile dispersal, and a larger sample size is necessary for a more precise survival estimate, it should be noted that the juvenile individuals included in Vetter *et al.* (2012) were never re-sighted in the subsequent years of this study. The lack of detection for any of these juvenile individuals in the years that followed, combined with high estimates of juvenile survival during years of low detection probability early on in this study, accounts for the large difference in juvenile survival estimates between the two datasets (Figure 3.4). Several re-sights of juvenile individuals during years of low overall detection probabilities artificially inflated the early juvenile survival estimates used in Vetter *et al.* (2012). We appreciate that both data sets are still sparse in their data on juvenile individuals but given that none of the juveniles marked during the years of high detection probabilities were seen again, we believe that this brought the average juvenile survival down to a more representative value.

Vetter *et al.* (2012) also did not detect strong differences in apparent survival between male and female parrotbil. As both analyses were conducted using similar methods, the increased detection probability in the survey years

2008 to 2011 in this study provided a more robust sample with which to demonstrate the sex-specific apparent survival. We found that parrotbills maintain strong pair bonds throughout the year and are often seen together. We found little bias for sex-specific encounter probabilities and re-sights were nearly evenly distributed between the sexes (females 410, males 465). Additionally, if we consider the $\Phi_{sex}.\rho_{sex}$ model (QAICc weight = 0.000), and assume that sex-specific encounter rates had a larger effect than the models suggested, males had a detection probability of 0.457 (± 0.043) and females 0.666 (± 0.060) (see Appendix A for more details). As with many species, the difference in apparent survival between males and females is more likely a result of higher reproductive costs for females, a higher rate of depredation, and/or higher emigration rates. While we can only speculate on the latter due to a lack of data, females do incur high energetic demands associated with egg production and incubation and have a higher risk of being depredated on the nest (Nur 1998; Ghalambor and Martin 2001; Fontaine and Martin 2006). Although female parrotbills alone incubate eggs and brood nestlings, the cost of reproduction for females may be similar to the cost to males of establishing and defending territories as well as provisioning females and offspring. Owens and Bennett (1994) found that provisioning chicks can have a higher direct mortality cost to adults than nest building and incubation. In parrotbills, higher female mortality is more likely the result of higher rates of depredation. Rodents (*Rattus spp.*) are predators of native island birds and have been documented depredating incubating and brooding females (Atkinson 1977; Moors *et al*. 1992; Robertson *et al*. 1994). In Hawaii, rats are responsible for the high female mortality in the Oahu Elepaio (*Chasiempis ibidis*) (Vanderwerf and Smith 2002) and may account for the sex-specific survival difference noted in the parrotbills.

Our highest selected models all incorporated a detection probability that varied considerably through time. Annual survey effort was influenced by the remoteness of our field sites and the rugged terrain. Access to the study area (by helicopter and on foot) was typically influenced by weather. Poor weather further influenced the probability of detecting individuals as re-sighting individuals in rain or mist was difficult. Despite uneven detection probabilities, the differences in survival estimates between this study and Vetter *et al.* (2012) illustrates the importance of long-term data sets for rare and cryptic species as well as those which may provide a scarcity of data within any period of years. We analyzed just the 2003-2011 datasets using the same methodologies to better understand the differences between these two studies. While the first portion of this data set used in Vetter *et al.* (2012) may have overestimated juvenile survival and was not able to resolve sex-based differences in apparent survival, the same is true of the latter half of the dataset when considered independently. Although these years had very high detection probabilities associated with them, and found similarly low juvenile survival, this subset of data had unresolved model rankings.

Our results suggest that conservation management focused on increasing female (and possibly juvenile) survival would likely benefit the recovery of the parrotbill population. Male survival would appear to be high for a small passerine, but although not necessarily so for a tropical species (see VanderWerf 2009). Landscape-scale rodent control would likely benefit the parrotbill and other native forest birds on Maui. Female survival of the Oahu Elepaio has been shown to increase following rodent control (Vanderwerf and Smith 2002). Other strategies could include intensive management such as supplemental feeding. Food supplementation has been a successful strategy in recovering endangered birds including the San Clemente Loggerhead Shrike (*Lanius ludovicianus mearnsi*) (Heath *et al.* 2008), Florida Scrub Jay (*Aphelocoma coerulescens*) (Schoech *et al.* 2008), Hihi (*Notiomystis cincta*) (Castro *et al.* 2003), and Kakapo (*Strigops habroptila*) (Clout *et al.* 2002) and has served as a short-term measure to support populations while longer-term habitat restoration occurs.

Finally, restoring high elevation forests that are buffered from extreme weather and that have a high abundance of koa trees may provide the greatest opportunity to increase the parrotbill population size (Simon *et al*. 1997; U.S. Fish and Wildlife Service 2006; Becker *et al*. 2010). More data may be able to resolve our juvenile survival estimates for this model. If juvenile survival is indeed low as a result of all suitable habitats being already occupied, the addition of new high quality habitat may be the only management strategy capable of increasing juvenile survival. Currently an 1100-ha area of mesic koa forest is being restored on leeward east Maui, possibly the single most significant conservation action taken for the parrotbill since the exclusion of feral ungulates from Hanawi in 1997. Experimental releases of parrotbills into this habitat are scheduled to occur in the next five years.

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3.5 TABLES AND FIGURES

Table 3.1. Apparent survival (φ) and encounter probability (ρ) models for Maui Parrotbills grouped by age (juvenile and adult). Subscripts indicate whether parameters differed among groups (e.g. φ_{age}) or time (φ_{t}) or were constant ($\varphi_{.}$). Overdispersion is corrected to 1.605 ($\hat{c}$) based on goodness of fit test on global model ($\varphi_{age*t}\rho_{age*t}$). $\Delta QAIC_{c}$ is the difference from the best (lowest AIC_{c}) model. AIC_{c} weight is the relative likelihood of each model.

#	Model	ΔQAICc	QAICc weight	No. Parameters	Deviance
1	$\Phi_{age}\rho_{t}$		0.8756	19	153.1149
2	$\Phi_{.}\rho_{t}$	4.6759	0.0845	18	160.1123
3	$\Phi_{age}\rho_{.}$	7.5464	0.0201	3	195.6714
4	$\Phi_{.}\rho_{age}$	8.8355	0.0156	3	196.9604
5	$\Phi_{age}\rho_{age}$	9.4988	0.0076	4	195.5618
6	$\Phi_{.}\rho_{.}$	12.5860	0.0016	2	202.7570
7	$\Phi_{t}\rho_{t}$	39.1413	0.0000	33	157.6439
8	$\Phi_{age*t}\rho_{age*t}$	47.3016	0.0000	48	149.6518

Table 3.2. Apparent survival (φ) and encounter probability (ρ) models for Maui Parrotbills grouped by sex (juvenile birds omitted). Subscripts indicate whether parameters differed among groups (e.g. φ_{sex}) or time (φ_{t}) or were constant ($\varphi_{.}$). Data underdispersed ($\hat{c}$ = 0.900) based on goodness of fit test on global model ($\varphi_{sex*t}\rho_{sex*\ t}$), $\hat{c}$ left at 1.00. $\Delta QAIC_c$ is the difference from the best (lowest AIC_c) model. AIC_c weight is the relative likelihood of each model.

#	Model	ΔQAICc	QAICc weight	No. Parameters	Deviance
1	$\Phi sex\rho t$		0.7923	19	349.9260
2	$\Phi.\rho t$	2.6782	0.2077	18	354.9384
3	$\Phi_{.}\rho_{.}$	55.8461	0.0000	2	442.9450
4	$\Phi t\rho t$	34.1433	0.0000	32	351.8188
5	$\Phi sex_{*t\rho sex*t}$	92.2258	0.0000	64	311.8119

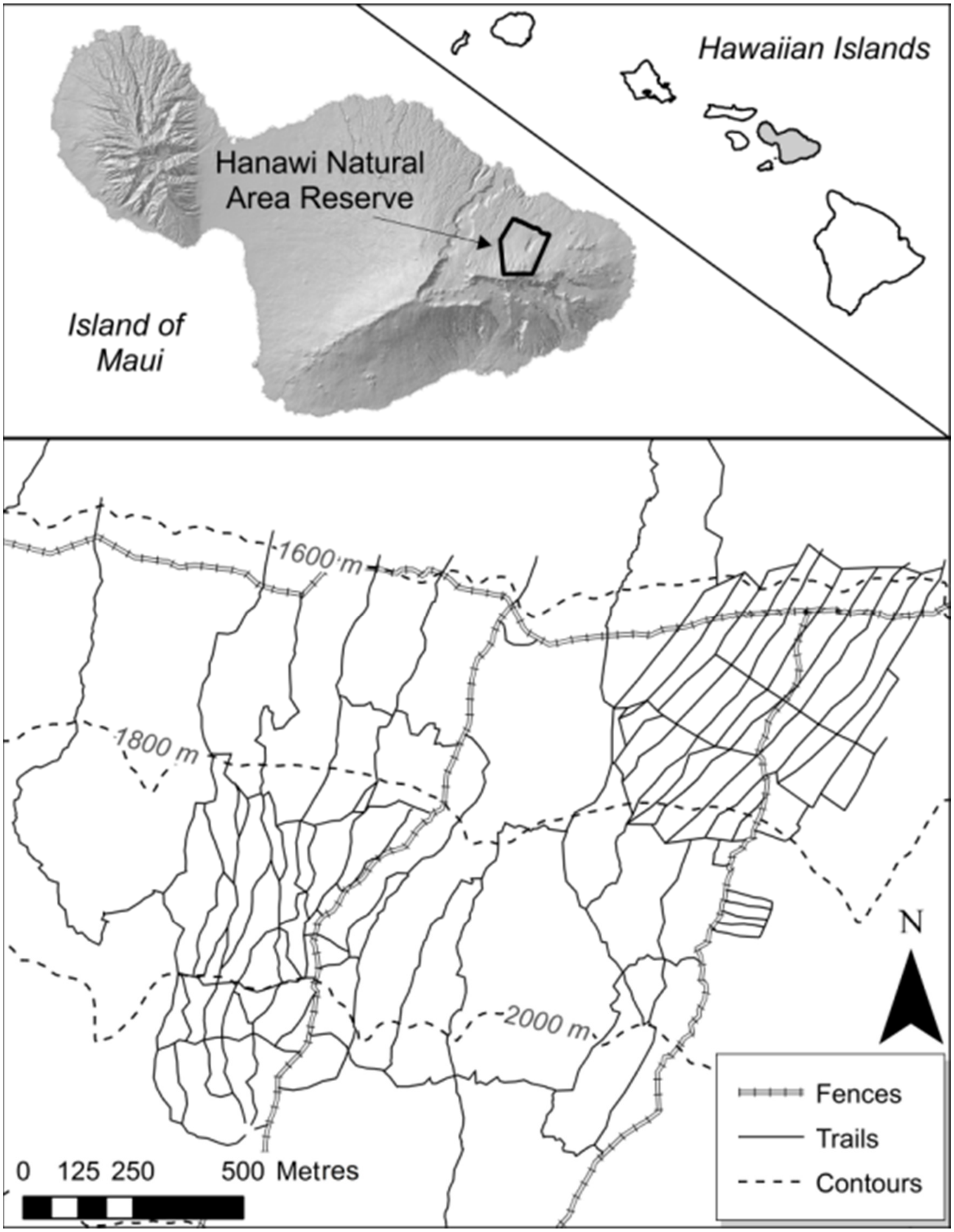

Figure 3.1. Study area within the Hanawi Natural Area Reserve, east Maui, Hawaii.

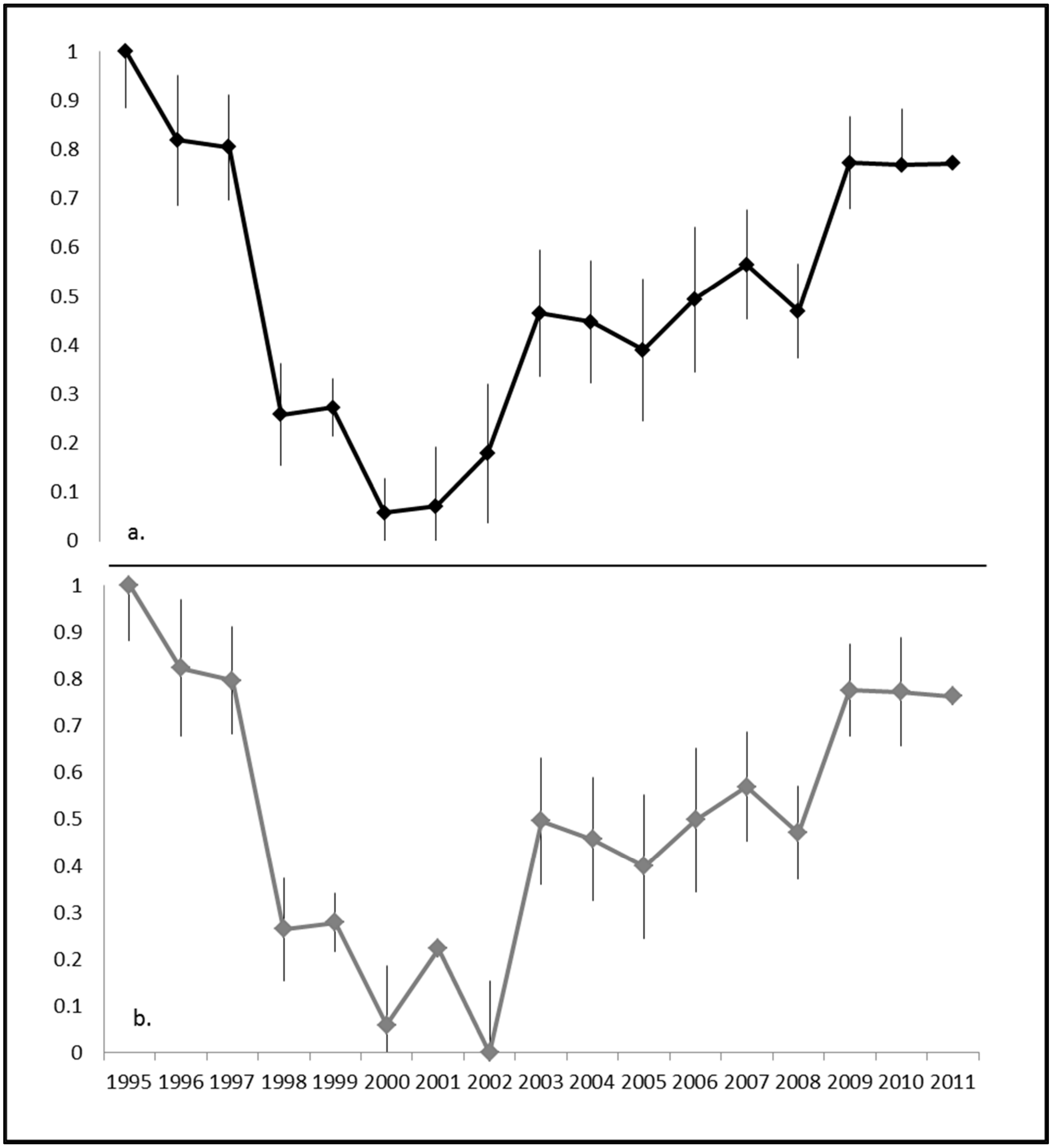

Figure 3.2. Detection probability variation over time in Maui Parrotbill survival analyses for age-specific analysis (a) and sex-specific analysis (b). Error bars indicate standard error for each year 1996-2011.

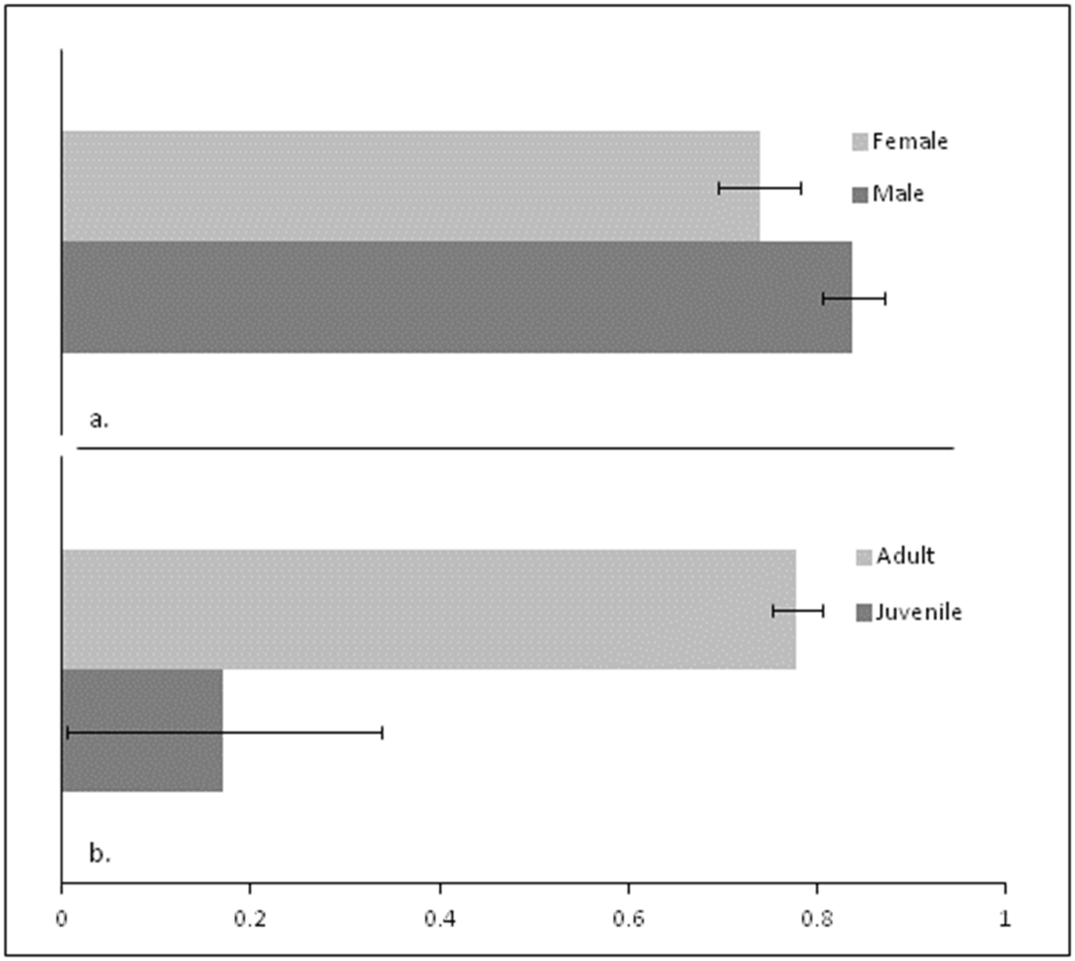

Figure 3.3. Apparent survival probability in Maui Parrotbill by sex (a) and age (b). Error bars indicate standard error for each individual group.

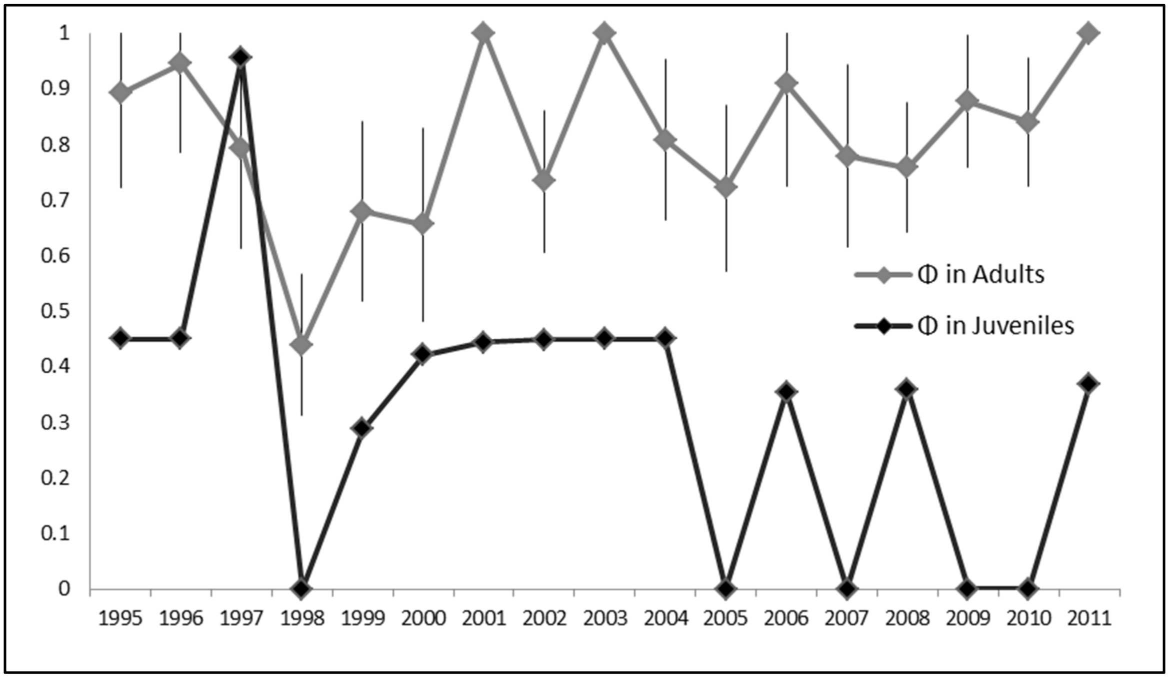

Figure 3.4. Apparent survival for juvenile Maui Parrotbill varied much more through time than did adult apparent survival illustrating the limitations of using a smaller data set may distort the results to suggest higher juvenile survival.

Chapter 4 Spatial genetic architecture of the critically-endangered Maui Parrotbill (*Pseudonestor xanthophrys*): management considerations for reintroduction strategies

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Keywords: ancestral DNA, control region, genetic structure, Maui Parrotbill, microsatellites, reintroduction

ABSTRACT

Conservation translocations are an important tool to circumvent extinctions on oceanic islands. A thorough understanding of all components of a species' biology, including genetic diversity and structure, can maximize their likelihood of success. The Maui Parrotbill (*Pseudonestor xanthophrys*) is an endangered Hawaiian honeycreeper endemic to the island of Maui. With a population of approximately 500 individuals restricted to 50 km^2 of habitat, this species is at high risk of extinction. Using nuclear and mitochdondrial DNA, this study quantified the levels of contemporary genetic diversity and structure in wild and captive parrotbill populations, and compared these genetic patterns to those observed within historical nuclear diversity derived from 100-year old museum samples. Substantial differences in the effective population sizes estimated between contemporary and historical parrotbill populations highlight the impact that introduced disease had on this species just before the turn of the century. Contemporary parrotbill diversity was low (global $F_{st} = 0.056$), and there has been a 96% reduction in genetic effective population size between contemporary and historical samples. This should not eliminate a conservation translocation (or reintroduction) as a viable recovery option. Measures of population differentiation (pairwise F_{st} and R_{st}) between different sections of the current population on either side of the Koolau Gap suggest that current genetic structure may be the result of this topographic barrier to gene flow. These data can enable the design of a conservation translocation strategy that is tailored to the patterns of genetic structure across the species' range.

4.1 INTRODUCTION

Reintroductions are a form of conservation translocation used to re-establish self-sustaining populations in areas from which species have been extirpated (Griffith *et al*. 1989; Armstrong *et al*. 2002; IUCN/SSC 2013). Such reintroductions have been particularly important on oceanic islands where exposure to extinction risk is higher than on continents (Steadman 2006; Jones and Merton 2012). Island species continue to be impacted by numerous anthropogenic threats; and, as threats are removed, reintroductions are often essential to re-establish species into areas of their former range (Castro *et al*. 1995; Armstrong *et al*. 2002; Cristinacce *et al*. 2009). To maximize success, reintroductions need to be carefully planned and have a solid understanding of the species, their threats, and habitat requirements. Historically low reintroduction success within species conservation (Griffith *et al*. 1989; Wolf *et al*. 1996; Fischer and Lindenmayer 2000;) has stimulated interest in integrating genetic information into the design of reintroductions to improve long-term viability of the resulting new populations (Seddon *et al*. 2007; Ewen *et al*. 2012).

Maximizing retention of genetic diversity to enhance long-term evolutionary potential is one way of achieving a successful reintroduction, an aim widely accepted as a fundamental component of population management for conservation (Frankel and Soule 1981; Frankham *et al*. 2010). There is a need to incorporate this more explicitly into the design of reintroduction programs (Groombridge *et al*. 2012; Keller *et al*. 2012). Loss of genetic diversity, increased levels of inbreeding, and accumulation of deleterious mutations in small populations are factors likely to have important consequences for the long-term persistence of reintroduced populations (Groombridge *et al*. 2012), and therefore it is important to ensure that a reintroduced population genetically represents the source population (Beck *et al*. 1994; Fischer and Lindenmayer 2000).

Before undertaking reintroductions, conservation managers should ideally know not only the genetic diversity of the source population, but also how that diversity is distributed spatially and temporally within a historical framework. Genetic structure arising from natural processes such as dispersal barriers or habitat gradients can then be distinguished from those that have arisen as a consequence of anthropogenic factors such as habitat loss and fragmentation. In addition to the benefits of applying this information at a practical level, an understanding of the ecological and historical context is also valuable for interpreting observed patterns of genetic diversity as knowledge of historical levels can often provide valuable perspective on contemporary genetic diversity (Groombridge *et al*. 2012).

The Maui Parrotbill (Kiwikiu; *Pseudonestor xanthophrys*) is an endangered insectivorous forest bird endemic to Maui, Hawaii, at high risk of extinction (U.S. Fish and Wildlife Service 2006; IUCN 2011; IUCN/SSC 2013). The population is estimated at 502 ± 116 (Scott *et al*. 1986). Due to the relatively low detection rates of parrotbills, more recent surveys have been unable to generate a more precise range-wide population estimate (Brinck *et al*. 2012) or to confirm the stability of the population (Gorreson *et al*. 2009).

The current range of parrotbills is small, 40-50 km^2 on the windward slopes of Haleakala volcano (Simon *et al*. 1997; Brinck *et al*. 2012) and is likely an artifact of past habitat loss, especially of the native koa (*Acacia koa*) forests, the possibly preferred foraging habitat of parrotbills (Perkins 1903), and current disease distribution (U.S. Fish and Wildlife Service 2006). Sub-fossils indicate a species' range that included the entire islands of Maui and Molokai (James *et al*. 1987; James and Olson 1991; Olson and James 1991; Simon *et al*. 1997; U.S. Fish and Wildlife Service 2006; Becker *et al*. 2010). Parrotbills are currently restricted to high elevation wet forests that are relatively free of avian malaria (*Plasmodium relictum*) and its primary vector, the non-native southern house mosquito (*Culex quinquefasciatus*)

(Scott *et al*. 1986; Mountainspring 1987; Atkinson and LaPointe 2009). Based on productivity and nest survival data (Mountainspring 1987; Mounce *et al*. 2013), these forests may be suboptimal habitats for this species.

The Koolau Gap, a 2 km-wide valley, cuts through a portion of the current parrotbill population range (Figure 4.1). The floor of Koolau Gap averages 600 m lower in elevation than the adjacent uplands. This feature is one of two such valleys that were formed as deep dissections in the landscape, which channeled lava into the ocean when the Haleakala volcano was formed prior to avian inhabitants (Morgan 1996). Since avian malaria and its mosquito vector are cold intolerant, and normally do not occur at elevations over 1500 m (Atkinson and LaPointe 2009), the Koolau Gap is thought to provide suitable habitat for these disease threats within an otherwise malaria-free forest. Although some Hawaiian forest birds have exhibited resistance to avian malaria (Foster *et al*. 2007), parrotbills have no known tolerance (Atkinson and LaPointe 2009); this is considered a primary factor limiting the habitat available for the parrotbill population (Scott *et al*. 1986; LaPointe *et al*. 2009). The Koolau Gap therefore presents a potential dispersal barrier within the current parrotbill population. Furthermore, climate change predictions suggest that high elevation rain forest habitats will become degraded (Loope and Giambelluca 1998; Giambelluca *et al*. 2008;) and unsuitable for parrotbills as warmer temperatures facilitate the persistence of avian malaria at higher elevations, such that up to 75% of current parrotbill habitat may become unsuitable in the future (Benning *et al*. 2002) (Figure 4.1).

As a consequence of these predictions, there is a need to establish a second population of parrotbills in other high-elevation disease-free habitats on Maui. Doing so would re-establish the species in its former range, and fulfill a critical recovery action as outlined in the species' recovery plan (U.S. Fish and Wildlife Service 2006). One potential location for reintroduction is the leeward side of Haleakala. Historically, these areas supported mesic and dry forests dominated by koa and state-owned lands have been prioritized for large-scale habitat restoration (Nakula NAR, Figure 4.1) specifically to provide habitat for parrotbills. Establishment of a reintroduced population will require translocation of individuals from the windward population and/or from the small captive breeding population in Hawaii (Mounce and Leonard 2012).

This paper describes the first population-level genetic study of parrotbills, quantifying levels of genetic diversity across both wild and captive populations using a suite of species-specific microsatellite markers and avian mitochondrial DNA (mtDNA) control region primers. In addition, we use microsatellite markers to quantify historical levels of genetic diversity in the parrotbill population by genotyping 100-year-old museum samples. We use these genetic data to: (*i*) characterize loss of historical genetic diversity and interpret it alongside the known and suspected ecological history of the species, (*ii*) utilize observed spatial patterns of genetic structure to suggest a reintroduction strategy optimized to maximize retention of genetic diversity, and (*iii*) estimate, based on contemporary levels of genetic diversity, how many individuals would be required for reintroduction in order to capture the majority of diversity.

4.2 METHODS

Samples

Contemporary genetic samples (*n*=129, comprising 118 wild individuals and 11 captive birds) were collected across the species' range between 1996 and 2011 and within two captive breeding facilities managed by San Diego Zoo Global (Figure 4.1). Wild birds were caught opportunistically throughout the year using mist nets and recorded playbacks. Blood samples were collected from the brachial vein using a 27 G needle and a 1.2 mm x 75 mm capillary tube. Blood was stored in a 'Queen's Lysis buffer' (0.01 M Tris, 0.01 M NaCl, 0.01 M EDTA, and 1% *n*-lauroylsarcosine, pH 7.5) (Seutin *et al.* 1991) solution at 4 °C. Feather samples consisting of 4-6 breast feathers were collected per individual and were also stored dry at 4 °C. Birds were banded with unique combinations of metal and plastic color-bands to facilitate ongoing demographic studies (Vetter *et al.* 2012; Mounce *et al.* 2013; 2014) as well as to prevent re-sampling. Geospatial location data was collected for each capture. Individuals were referred to as captive or wild. The latter were further grouped geographically as those captured east or west of the Koolau Gap (hereafter east and west; Figure 4.1). Historical samples (*n* = 34) were obtained by sampling toe-pad tissue of all known museum specimens (see Appendix B).

Laboratory Methods

DNA extraction

Genomic DNA was extracted from blood samples using an ammonium acetate precipitation method (Nicholls *et al.* 2000) and from feathers using DNeasy® Blood and Tissue Kit (Qiagen, West Sussex, UK) protocols for animal tissues. The DNA concentration was estimated using HyperLadder™ 100 base-pair (bp; Bioline, London, UK) on 1.0% agarose gels stained with either ethidium bromide (Fisher Scientific, Leicestershire, UK) or SYBR® Safe (Fisher Scientific, Leicestershire, UK).

The laboratory work on museum samples was carried out in a dedicated museum DNA laboratory where no contemporary avian DNA had been present. Furthermore, all work with museum samples/DNA was carried out in a UV-irradiated fume hood to further eliminate any potentially contaminating DNA before and after each laboratory session. Genomic DNA was extracted from museum samples in batches of three-five samples, with negative controls, using QIAamp DNA Micro kits (Qiagen, UK), following the manufacturer's protocol for isolation of genomic DNA from forensic case work samples, with an extended overnight step of incubation with Proteinase *K*.

mtDNA amplification

For contemporary samples, we used polymerase chain reaction (PCR) to amplify a 677 bp fragment of the mitochondrial (mtDNA) control-region using primers LCRL1 (5'-CGCTATGACCCTCCACGAA-3') and HCR1045 (5'-GAGACGACCTTATCCGCAAA-3') (Tarr 1995; Tarr and Fleischer 1995).
In each sample, the fragment was amplified in 50 µl reactions containing 1x *Taq* reaction buffer [160mM $(NH_4)SO_4$, 670 mM Tris-HCl], 1.5 mM $MgCl_2$, 200 µM each dNTP, 0.2 µM each primer, 2 units *Taq* DNA polymerase (Bioline, London, UK), and 40 ng template DNA. Amplification was performed under mineral oil using the following cycling conditions: 94 ^{o}C for 7 min; then 30 cycles of 94 ^{o}C for 30 sec, 52 ^{o}C for 30 sec and 72 ^{o}C for 1 min; followed by a final step of 10 min at 72 ^{o}C. All reaction products were checked using electrophoresis on agarose gels and those that had contamination in the negative controls or did not show strong amplifications were discarded and re-amplified. PCR products were sequenced using an ABI 377 DNA sequencer (Macrogen Genomics, South Korea, and Source BioScience, Nottingham, United Kingdom).

Characterization and amplification of microsatellite markers

Microsatellites were isolated from an enriched genomic library developed for parrotbill by Genetic Identification Services (Chatsworth, CA USA) following procedures in and Jones *et al.* (2002) and Cristinacce *et al.* (2009). Recombinant plasmids were produced by ligating restriction fragments (350-700 bp) from pooled genomic DNA from five individual wild parrotbills [two males (#1371-04952 and #1371-04937) and three females

(#1371-04951, #1371-04954 and #1371-04948)] into the *Hind* III (AAGCTT) cut site of the pUC19 plasmid. DNA sequencing was accomplished using Amersham's DYEnamic™ ET Terminator Cycle Sequencing Kit (Amersham Biosciences P/N US81050) with an M-13 forward primer (5'-AGGAAACAGCTATGACCATG -3'), followed by electrophoresis on an Applied BioSystems Model 377 DNA Sequencer. PCR primers were designed to the flanking regions of 24 microsatellites using DesignerPCR, version 1.03 (Research Genetics, Inc.). These primers were used to amplify five assumed unrelated parrotbill individuals to assess polymorphism. Sixteen loci (13 polymorphic and 3 monomorphic) were selected for further optimization.

Using the previously extracted and diluted genomic DNA for contemporary samples, a range of annealing temperatures (50-68 ^{o}C) were tested, and the temperature producing the cleanest PCR product, as observed on 1.0% agarose gels stained with ethidium bromide, was selected for subsequent PCRs. Each locus was amplified separately in 12.5 µl reactions containing 1x *Taq* reaction buffer [160mM $(NH_4)SO_4$, 670 mM Tris-HCl], 1.5 mM $MgCl_2$, 200 µM each dNTP, 0.2 µM each primer, 0.5 u *Taq*, and 10 ng template DNA. PCR amplification was performed under mineral oil using the following cycling conditions: 94 ^{o}C for 5 min; then 30 cycles of 94 ^{o}C for 30 s, 57 or 62 ^{o}C for 30 s and 72 ^{o}C for 1 min; followed by a final step of 10 min at 72 ^{o}C. All amplifications were checked on agarose gel and weak amplifications were discarded and run again. Twenty percent of the samples were also amplified a second time. Multiplexing was performed post PCR amplification. Fluorescently labelled DNA fragments were detected using an Applied Biosystems 3730 DNA Analyzer with GeneScan ROX-500 size standard (DBS Genomics, Durham, United Kingdom).

Historical samples were amplified for each locus separately in 4 µl PCR reactions containing 1 x Qiagen multiplex PCR master mix (Qiagen, UK), 1 µM of each primer and 2 µl of template DNA at unknown concentrations following

Kenta *et al.* (2008). PCR amplification was performed for each locus under mineral oil using the following cycling conditions: 95 °C for 15 min; then 46 cycles of 94 °C for 30 s, 60 °C for 90 s and 72 °C for 60 s; followed by a final period of 60 °C for 30 min. PCR products from museum samples were genotyped individually for each locus using the same ABI 3730 DNA Analyser as used for the contemporary samples. While there is a greater potential for allelic dropout when genotyping ancient and degraded DNA (Taberlet *et al.* 1996; Nielsen *et al.* 1999), re-amplifications were not possible for extracted museum samples due to the small quantities of DNA extracted from each tissue sample.

Data analysis

mtDNA

PCR products obtained from the first 10 DNA extractions (8.3% of the total samples) were sequenced in both the forward and the reverse directions. After end clipping and careful visual examination of the sequence reads, each was aligned. Alignments for each individual showed no discrepancies in base calls between the forward and reverse strands and subsequent samples were sequenced using the forward primer only. Chromatograms were edited using FINCHTV (Geospiza Inc.). Sequences were aligned in CLUSTAL X V2 (Larkin *et al.* 2007). Each polymorphism was visually scrutinized on the chromatograms. Samples with new or rare polymorphisms were sequenced twice, in both forward and reverse directions, by Macrogen Genomics Inc. and Source BioScience to confirm polymorphisms and haplotypes. Individuals were referred to as captive or wild. The latter were further grouped geographically as those captured east or west of the Koolau Gap (Figure 4.1). Standard DNA polymorphism and genetic differentiation measures were calculated using DNASP v4.00 (Rozas *et al.* 2003). All sequences have been deposited in European Nucleotide Archive (see Appendix D).

Microsatellite genotyping

Genotypes were scored using GENEMAPPER v3.7 (Applied Biosystems, Inc.). Twenty percent of the samples were scored a second time from subsequent PCR amplifications to check for allelic dropout. We calculated allelic and heterozygosity patterns using GENALEX 6 (Peakall and Smouse 2006) and tested for significant deviations from Hardy–Weinberg equilibrium (HWE) and for linkage disequilibrium at each locus for each sampling locality using GENEPOP v4.0.10 (Raymond and Rousset 1995). We corrected the linkage disequilibrium tests for multiple comparisons using a sequential Bonferroni correction (Rice 1989). We calculated allelic richness per locus for each sampling site using FSTAT 2.9 (Goudet 1995). Null allele frequencies were estimated using CERVUS (Marshall *et al.* 1998).

Patterns of genetic structure

For the entire contemporary population, we applied an analysis of molecular variance (AMOVA) using GENALEX 6 (Excoffier *et al.* 1992). AMOVA provides estimates of traditional *F*-statistics (Weir and Cockerham 1984), as well as their analogues (R_{st} and ϕ_{pt}) to examine subpopulation structuring and calculate pairwise F_{st} and R_{st} between the east, west, and captive populations. Given that a limitation of the AMOVA framework is that it requires *a priori* clustering of samples, microsatellite genotypes were grouped according to their geographic location (east, west and captive). As a source for comparison, we also generated similar population differentiation results using FSTAT 2.9 (Goudet 1995) incorporating an exact G-test (Goudet *et al.* 1996), and to account for sample size bias we calculated allelic richness and private allelic richness for each population using the rarefaction technique in HP-RARE 1.1 (Kalinowski 2005).

To further describe genetic structure of the wild population, a Bayesian clustering method that did not take spatial location designations into account using STRUCTURE v2.3.3 (Pritchard *et al.* 2000) was used. STRUCTURE implements a Bayesian approach to estimate the most likely number of population clusters (*K*) based on the genotypes of the individuals included in the analysis. We specified an admixture model, whereby a proportion of the genome of each individual is probabilistically assigned to each cluster according to allele frequency by minimising deviations from Hardy-Weinberg equilibrium.

The data were grouped geographically (see Figure 4.1) but genotypes from all wild individuals were pooled into a single dataset and analyzed for signals of genetic structure. STRUCTURE allows the input of predefined populations to enable easier comparison with the allele frequency based structure, but it does not use this information prior to analyses. This approach allowed a comparison between signals of structure in the ecologically-inferred east and west populations and any signal of structure evident from allele frequencies alone. Geographic grouping was delineated using the natural topography of east Maui. Since the overall range for parrotbills is small (40-50 km^2), and other than the Koolau Gap, there are no geographic barriers to the existing population, we did not believe that high *K* values would be biologically feasible in this population. Therefore, we considered a maximum value for K of 1-5. We completed 20 runs of 1000000 iterations with thinning of 100 after an initial burn-in of 100000 as per revised recommendations in Gilbert *et al.* 2012. The assignment values, log likelihood scores, likelihood rate of change, and Δ K (Evanno *et al.*2005), were examined using STRUCTURE HARVESTER (Earl and vonHoldt 2012) to infer the optimal number of clusters.

GENELAND v3.3.0, a spatial Bayesian clustering program, also was used to assess population structure in the wild population (Guillot *et al.* 2005).

Unlike STRUCTURE, GENELAND assumes that genotypes are spatially correlated. This assumption is based on a hidden partition model that estimates the number of populations (based on genetic criteria) within the study area, assigns individuals to their populations of origin, and potentially detects immigrants. Inferences from spatial Bayesian clustering programs can be reasonably robust when the number of available polymorphic loci in a study is limited (Chen *et al.* 2007). A mixed analysis was first implemented to identify the number of clusters in the data considering a maximum of K = 1-5, with five repetitions. The results of these mixture analyses were then used to conduct the admixture analysis (Corander and Marttinen 2006).

Tests for patterns of isolation by distance for the wild population were performed using ISOLDE program within GENEPOP v4.0.10 (Rousset 1997; Rousset 2000). This program regresses estimates of $F_{st}/(1-F_{st})$ to the natural log of the geographic distance between populations and compares the distance with a simple Mantel test (Rousset 1997). Geographic distances between each were calculated as linear distance between UTM (Universal Transverse Mercator geographic coordinate system) locations for each captured individual.

Estimation of temporal change in effective population size

To estimate changes in effective populations size (N_e) between contemporary and historical populations, we used TMVP (Beaumont 2003) and NEESTIMATOR (Ovenden *et al.* 2007). Using a Markov chain Monte Carlo (MCMC) approach in a Bayesian framework, TMVP samples independent genealogical histories using importance sampling. The program makes explicit use of collection dates for all individuals and combines historical and contemporary data to obtain a posterior distribution of N_e at the time of the oldest sample and at the most recent sample (N_A and N_0 respectively; Beaumont 2003). We specified a mean generation time of three years based on known reproductive biology, and field observations of parrotbills, and after confirming the robustness of the TMVP program by running several sensitivity analyses at generation times of 1-4. We ran 20000 MCMC updates (an initial 10% were discarded as burn-in) with 10 updates between estimate outputs and used a rectangular prior of 0-1000 for both N_A and N_0. NEESTIMATOR uses a linkage disequilibrium approach and provides an estimate of N_e between two generations (N_A and N_0; Ovenden *et al.* 2007).

Estimating sufficient individuals for reintroduction

To assess the proportion of genetic diversity that could be captured within a given number of individuals, we performed a simulation in R (Team 2010). This calculated the range in proportion of alleles that could theoretically be transferred to a new population with differing numbers of founder individuals. The model first randomly selected numbers of founders in increments of five for up to 30 birds from the east and west populations and ran 1000 replicates. The number of founders was relatively small due to the low availability of potential founding individuals in either population. Second to look at what might be genetically desirable albeit biologically improbable, the model was repeated for numbers of founder individuals between 5-120 birds for 1000 replicates for each number of individuals. These estimated individuals to be translocated for a probability of capturing 100% of the genetic diversity in the founders of the reintroduced population.

4.3 RESULTS

mtDNA

Sequences from 85 individuals (56 east, 18 west, and 11 captive) identified three haplotypes (A, B, and C). It was decided that it was unlikely that the remaining 44 parrotbill samples would identify additional haplotypes. Each haplotype was defined by a single bp change. Haplotype B had a G/A replacement and haplotype C had a C/T replacement. While haplotypes A and B were found across the species range and captivity, haplotype C was restricted to one adult female captured in the east. Haplotype diversity (H_d) was 0.382 overall, nucleotide diversity (π) was 0.001 and haplotype frequencies varied (Table 4.1). Restricting the analysis to the wild individuals resulted in a slightly lower H_d (0.365), while nucleotide diversity remained the same. There was no significant difference in the mtDNA genetic differentiation between the wild and captive samples (x^2 = 1.792, df = 2, p = 0.408) or between the wild east and west samples (x^2 = 0.810, df = 2, p = 0.667; Table 4.1).

Characterization and amplification of microsatellite markers

We found 15 polymorphic loci. Based on sequence homology, all loci could be assigned a chromosome location on the Zebra Finch (*Taeniopygia guttata*) genome and mapped using MapChart 2.2 (Voorrips 2002) to identify Z-linked or other closely aligned loci based on chromosome locations (see Appendix B). Two loci were found to be Z-linked (sex-linked), therefore only males were characterized at these loci as females were always homozygous. One locus could not be scored cleanly due to spurious bands, one locus deviated from Hardy-Weinberg in all populations, and one locus was found in linkage disequilibrium with two other loci. These three loci were excluded, leaving 12 loci for final analyses (Table 4.2). Sequences and primer information for these 12 loci have been deposited in European Nucleotide Archive (See Appendix D). A total of 86% of loci across contemporary samples were amplified and genotyped successfully. No evidence suggestive of allelic dropout was apparent in re-amplification runs. A total of 56% of loci across historical samples were amplified and genotyped successfully. Five historical samples yielded no accessible DNA which may be a result of past storage conditions and preservation treatment of specimens (Lindahl 1993; Wandeler *et al*. 2003).

Genotyping analyses

Mean allelic richness for the contemporary population was 4.05 (1.67-6.04). The differences between observed, expected, and unbiased expected heterozygosity patterns were similar across each of the three populations (Table 4.3). The east population had the highest level of heterozygosity and a significantly higher number of private alleles per loci (Table 4.3). Rarefaction analyses found similar patterns of allelic richness and private allelic richness with analyses across only 10, 20, and 30 genes (see Appendix B). Global F_{st} for the entire dataset was low (F_{st} = 0.06). There were significant differentiations in pairwise F_{st} and R_{st} between east and west (F_{st} = 0.05; R_{st} = 0.06) and between west and captive populations (F_{st} = 0.10; R_{st} = 0.16) but not between east and captive birds. The mean numbers of alleles per locus, effective alleles per locus, and private alleles per locus were all higher in the historical samples compared to the contemporary population (Table 4.3).

Population patterns of genetic diversity

STRUCTURE and GENELAND analyses resolved a clear pattern of population structure in the wild. Applying the Evanno *et al.* (2005) correction to the STRUCTURE output yielded a signal of ΔK= 4 (Figure 4.2). Individuals were mapped according to the probability of assignment to each cluster from GENELAND (K = 2 and 4; Figure 4.3). No significant evidence for isolation by distance was detected within the genotype dataset.

Estimates of historical and contemporary N_e

The temporal change in effective population size (N_e) from TMVP analyses is given in Figure 4.4. The density of points is proportional to the probability density of population size at the time of the oldest sample and the most recent sample. An off diagonal distribution therefore indicates a change in N_e. The resulting output for the contemporary population provides strong evidence for a severe decline in N_e across the past 110 years. The modal mean for the contemporary population in 2011 is 37 (95% HPD limits 22-71) compared to a modal mean of 991 in 1892 (95% HPD limits 575->1000). NEESTIMATOR calculated a contemporary N_e estimate of 52.5 (45.7-61 95% CI).

Estimating sufficient individuals for reintroduction

Random capture of 25 individuals from the east would ensure the inclusion of 80% of the genetic diversity. 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 (Figure 4.5).

4.4 DISCUSSION

Our results reveal a 96% reduction in genetic N_e over the past 110 years, as well as a signature of spatial structuring of the current parrotbill population that is underpinned by topographical features and reflected in a skewed distribution of private alleles. Together, these data provide a valuable new perspective on the conservation management of this species and an important framework for the design of a reintroduction strategy to maximise future retention of genetic diversity.

Contemporary levels of genetic diversity

Contemporary mitochondrial DNA haplotype (H_d) and nucleotide (π) diversity was low compared to other more common Hawaiian honeycreeper species (Hawaii Akepa $H_d = 0.980$, $\pi = 0.006$ Reding *et al.* 2010, Hawaii Amakihi $H_d = 0.882$, $\pi = 0.008$ Foster *et al.* 2007). Nuclear DNA revealed similar levels of observed and expected heterozygosity (0.574 and 0.534, respectively), which were within the expected range for a population with a history of small population size (Frankham *et al.* 2002). Island species often exhibit low levels of genetic diversity (Frankham 1997; 1998), commonly interpreted as being a consequence of their having a history of long-term isolation and restricted population size.

Historical loss of genetic diversity

While it is impossible to assume that any historical museum samples are representative of a panmictic population, knowledge of historical patterns of genetic diversity can provide insight into a population's demographic trajectory and can reveal indications of genetic impoverishment. Nuclear DNA markers showed temporal losses in genetic diversity across all diversity measures. Mean number of alleles per locus and proportion of polymorphic loci were both reduced by 12%, mean number of effective alleles per locus was reduced by 30%, mean number of private alleles was reduced by 10% amongst the east and by 100% amongst the west population, whilst expected heterozygosity was reduced by 19%. These reductions are not unsubstantial and suggest that the current parrotbill population has experienced severe reduction in size over the last century. The species has also experienced a reduction in N_e of approximately 96%, from an ancestral N_e of 991 to a contemporary N_e of 37. The estimate of current census population size (N) for parrotbills in the area of their range from which our samples were taken is 421 (209-674 95% CI) individuals (Brinck *et al.* 2012). This estimate of N aligns closely with our genetic estimates of contemporary N_e, assuming a N_e /N ratio of 10% for wildlife populations (Frankham *et al.* 2002).

The finding of an historical reduction in N_e of at least an order of magnitude across the past 110 years enables some interpretation of what ecological or environmental mechanisms may have been responsible for this decline. While Hawaiian forest birds have suffered a plethora of threats that have reduced their populations over time (Banko and Banko 2009), it is perhaps most likely that the bottleneck required to precipitate the observed reduction in N_e since 1894 occurred relatively quickly, imposing a reduction in population size within a short period of time and to a level that subsequently induced the substantial loss of genetic diversity (Frankham *et al.* 2010). Such an interpretation and the timing of this event could be explained by the arrival of avian malaria on the Hawaiian Islands (Atkinson and LaPointe 2009). Mosquitoes were introduced to Maui in 1826 (Dine 1904; Hardy 1960), providing a mechanism for spreading disease (avian malaria, *Plasmodium sp.),* which is believed to have arrived to the islands sometime in the early twentieth century and effected non-native passerines soon thereafter (Atkinson and LaPointe 2009). While Hawaii extinctions have been attributed to post Polynesian (James and Olson 1991; Olson and James 1982; James *et al.* 1987) and post European (Banko and Banko 2009) contact, the arrival of mosquitoes and avian malaria was potentially the most serious threat to the parrotbill population and the resulting population contraction.

Patterns of contemporary genetic differentiation and structure

F_{st} and R_{st} values showed significant deviations on either side of the Koolau Gap, a division supported by both STRUCTURE and GENELAND analyses. This suggests that the Koolau Gap is acting as a topographic barrier that is limiting gene flow between these two sections of the current population.

Further subdivision within the east and measures of genetic diversity calculated per population lend support to this result. These higher levels of overall diversity and allele privatization in the east cannot be explained by sample size alone. While sampled disproportionally, overall population estimates for these areas (the global population comprises ~¾ on the east and ~¼ on the west) show that our sample sizes represented ~20% of the west and ~22% of the east population.

Optimizing reintroduction strategy for genetic diversity

The low detection rate during field surveys has made it difficult to confirm the apparent stability of this species' population over the past 30 years (Gorreson *et al*. 2009). This fact, coupled with the low N_e and the presence of a rare mtDNA haplotype, emphasizes the need to move forward the recovery program for this species. There are a number of different recovery options; below, we discuss how integration of genetic data into each of them can maximize the retention of genetic diversity within the restored population and potentially enhance the success of reintroduction efforts.

(i) The first recovery option would be to raise the quality of the current habitat to increase population density and in turn increase the global population and reduce the continued loss of genetic diversity. Rat (*Rattus spp.*) densities are very high across much of the current population's habitat (Sugihara 1997; Malcolm *et al*. 2008) and intense rodent reduction efforts might increase survival and/or productivity by reducing depredation risk. However, recent demographic work suggests that although low fecundity and productivity may complicate this species' recovery, it is not the main factor limiting the population (Mounce *et al*. 2013).

(ii) The second recovery option would be to establish a second population through the release of captive bred individuals. The significant

deviation in F_{st} and R_{st} values for the west and captive populations raises concerns as to whether the captive population should play a significant role in future reintroduction efforts. If managers choose to rely heavily on the captive population for reintroduction, the overall genetic diversity of the captive flock would need to be increased to reflect the overall diversity in the wild. We would suggest that this include a combination of new genes from the east to incorporate the private alleles not represented in captivity, as well as genes from the west. Due to the low reproductive potential of this species and the minimal breeding success that they have had in captivity thus far, we do not recommend this strategy as the time line for developing a genetically diverse captive population would greatly delay the recovery efforts.

(iii) Lastly, we feel the recovery option that has the greatest chance of success and maximizes the potential for retention of genetic diversity, would be to establish a second population through translocating wild individuals from both the east and the west to leeward east Maui. There is the potential of capturing varying levels of genetic diversity in the reintroduction design and the presence of low levels of genetic diversity observed highlights the importance of capturing as much diversity as possible amongst the founding individuals (Groombridge *et al.* 2012) in order to retain maximum evolutionary potential to adapt to environmental change.

The patterns of population structure are designed to allow managers to weight the genetic considerations in moving different numbers of individuals from different areas of the population. Furthermore, the estimation of sufficient individuals for reintroduction informs managers of the numbers necessary in structuring a reintroduction through translocations. With a small and endangered population like Maui Parrotbill, the numbers of individuals needed to capture 100% of the available genetic diversity are probably unrealistic as they represent up to one quarter of the global population.

Instead, managers will need to determine what an acceptable amount of genetic diversity is and how to best design efforts to attain that goal as no one single strategy to cope with the genetic trade-offs in reintroduction designs has been universally accepted (Groombridge *et al.* 2012). We feel that the reintroduction design should attempt to capture all available genetic diversity in the wild while considering that the global population size itself may limit the number of individuals that can be taken from any one area. This population is unlikely to suffer a reduced level of fitness from outbreeding depression and the loss of local adaptations (Frankham *et al.* 2010) given that parrotbills have been extirpated from the koa-dominated mesic forest similar to that of the reintroduction sites available (U.S. Fish and Wildlife Service 2006), and it is unlikely that adaptations to the drier forest still exist in the current genetic diversity of the parrotbill population.

Although these data reveal the dramatic affect avian malaria has had on the parrotbill population, there are currently no management techniques available to control its spread in Hawaiian honeycreepers. Fortunately, the mosquitoes and *Plasmodium* do not thrive at higher elevation habitats, and thus the current wild population is relatively secure from their effects at these high elevation refugia. This not only explains the current distribution of parrotbills across Haleakala above ~1500 m in elevation, but also enforces future management efforts to be restricted to these high-elevations areas. Reintroductions and recovery actions will be limited to currently “malaria free” zones on Haleakala. Currently 1100 ha of mesic koa forest is being restored on leeward east Maui and is the best suitable location for the reintroduction. Knowledge of population genetics can inform the choices managers will have to make, but genetics is just one of a variety of ecological and other factors needed in order to best design these reintroductions whether through conservation translocations of wild individuals, the release of captive-bred birds, or a combination of both. While the best reintroduction design will most likely incorporate components from several of the options outlined, the reintroduction of wild-sourced individuals selected from across the different genetically structured parrotbill populations provides the greatest potential for success going forward.

ACKNOWLEDGEMENTS

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4.5 TABLES AND FIGURES

Table 4.1. Sample size (*n*), number of haplotypes (Hp), haplotype (gene) diversity (Hd), nucleotide diversity (π) and the frequencies (*f*) of haplotypes A, B and C for Maui Parrotbill (*Pseudonestor xanthophrys*) mtDNA analyses.

	n	**Hp**	**Hd**	**± SD**	**π**	***f* (A)**	***f* (B)**	***f* (C)**
East	56	3	0.350	± 0.067	0.001	0.786	0.196	0.018
West	18	2	0.425	± 0.099	0.001	0.722	0.278	0.000
Captive	11	2	0.509	± 0.010	0.001	0.636	0.364	0.000
Total	85	3	0.382	± 0.050	0.001	0.753	0.235	0.012

Table 4.2. Characteristization of Maui Parrtobill (*Pseudonestor xanthophrys*) microsatellite loci. Annealing temperature (T_a), allelic diversity (*A*), observed heterozygosity (H_o), expected heterozygosity (H_e), Hardy-Weinberg equilibrium test *P* values as identified by GenePop v4.0.10 (P_{HWE}) for east population. Chromosome location assignments are mapped against the zebra finch (*Taeniopygia guttata*).

Locus	Chromosome location	Chromosome location (bp)	BLAST P (N)	Nucleo-tide type	Repeat motif	Fluro-label (F)	Primer sequences (5'-3')	Optimized T_a (°C)	*A*	Exp. Allele sizes (bp)	Obs. allele sizes range (bp)	H_o	H_e	P_{HWE}	Null allele frequency
Pxa01	Tgu7	5729001	0.0	tri	$(GTT)_8$	5'-HEX	F: CAACTGATGCTGTTAGCAGATG R: AGGTTCCCAAAGTCCTTTCTC	57	5	271	260-278	0.10	0.14	0.03	0.13
Pxa02	Tgu3	7090537	5E-142	tetra	$(TAGA)_1(GTA)_1(TAGA)_2(GACA)_1$ $(TAGA)_3(GACA)_1(TAGA)_5$	5'-FAM	F: TTTCCACTTGTCCACTTCACC R: GCATCCATCCATCTATTCACC	57	9	286	273-333	0.80	0.83	0.38	0.02
Pxa03	Tgu6	7689005	0.0	tri	$(GTT)_{14}$	5'-FAM	F: CTGAGGGGAGAGGTTGTCAT R: TGGGGAAATAGTTGGAGGAG	57	9	265	246-268	0.61	0.63	0.23	0.01
Pxa04	Tgu2	3794085	3E-143	tri	$(CAA)_{10}$	5'-HEX	F: GGGAATAATGAAGCCTAAGAGG R: AACTGTGGGGCAACTAATGA	57	6	257	243-278	0.67	0.64	0.03	0.03
Pxa05	Tgu2	37430618	0.0	tri	$(CAT)_2(CAC)_1(CAT)_6$	5'-HEX	F: ATTGCTGCCATTTTCCTCTC R: GAGTGCTCTCCACACCACAC	57	2	129	121-126	0.13	0.14	0.57	0.02
Pxa06	Tgu1A	1056189	4E-107	tetra	$(CTAT)_{12}$	5'-FAM	F: GGCACTGGATGATCTTGAAG R: ATTTCCTCCTCTTGGAGCAC	57	11	158	128-171	0.88	0.82	0.77	0.04
Pxa07	Tgu3	15894209	0.0	tetra	$(CTAT)_1(CAT)_1(CTAT)_{11}$	5'-FAM	F: ACTGCACTTGTGGTGAAAGAC R: GTTTTCCTTCAGACTGGAACAG	57	6	240	201-243	0.63	0.72	0.04	0.06
Pxa08	TguZ	3112964	0.0	tetra	$(CTAT)_{20}(GCAT)_1(CTAT)_{11}$	5'-HEX	F: CAGTGCAGGACTGTGTATTATG R: GCAAAGGAAAAAGGAGAAATAG	62	11	247	168-240	0.61	0.73	0.07	0.08
Pxa09	Tgu1	7400684	1E-97	tetra	$(TAGA)_4(TA)2(TAGA)_5$	5'-FAM	F: CGTGACTCAGCACCTACAAC R: TGCACAAACAAACATTCTGTC	62	7	142	139-169	0.68	0.71	0.08	0.01
Pxa10	Tgu2	16526007	1E-127	tri	$(GTT)_{10}$	5'-HEX	F: GCTTCAGCAAACAGCATTC R: CTGCGTGAGTTCATTTCATC	57	6	262	238-265	0.45	0.42	0.25	0.04
Pxa11	Tgu1	1481243	1E-177	tetra	$(CTAT)_1(CAT)_1(CTAT)_{14}$ $(CTAAT)_1(CTAT)_4$	5'-HEX	F: GTGGCAGTGTAGCAGAGTCT R: CCATTCTTAGGCTGAGGTAAC	62	8	201	202-234	0.83	0.78	0.38	0.03
Pxa12	Tgu4	11404443	3E-75	tetra	$(CTAT)_{18}(CAT)_1(CTAT)_1$	5'-HEX	F: GCAGCACAAGTGGAACTG R: TTGAGCCAGAAGGGATAAC	57	7	259	244-270	0.83	0.75	0.61	0.06

Table 4.3. Allelic and heterozygosity patterns based on microsatellite data across the east, west, and captive Maui Parrotbill (*Pseudonestor xanthophrys*) populations. Standard errors are shown in parentheses.

	Complete Contemporary	Captive	East	West	Historical
Mean No. of alleles per locus	4.878 (0.416)	3.818 (0.519)	6.727 (0.714)	4.090 (0.609)	5.545 (0.593)
Mean No. of effective alleles per locus	2.770 (0.227)	2.512 (0.295)	3.386 (0.499)	2.413 (0.319)	3.964 (0.570)
Mean No. Private Alleles per locus		0.000 (0.000)	1.545 (0.340)	0.000 (0.000)	1.727 (0.273)
Mean No. Contemporary		0.000 (0.000)	2.273 (0.428)	0.182 (0.122)	
Shannon's Information Index	1.064 (0.096)	0.971 (0.160)	1.280 (0.168)	0.942 (0.170)	1.386 (0.170)
Observed Heterozygosity	0.574 (0.052)	0.599 (0.099)	0.618 (0.081)	0.505 (0.096)	0.428 (0.085)
Expected Heterozygosity	0.534 (0.045)	0.512 (0.081)	0.605 (0.073)	0.485 (0.082)	0.658 (0.071)
Unbiased Expected Heterozygosity	0.550 (0.046)	0.541 (0.085)	0.609 (0.073)	0.500 (0.084)	0.698 (0.077)
Fixation Index	-0.05 (0.029)	-0.16 (0.046)	0.000 (0.031)	-0.02 (0.062)	0.410 (0.086)
% of loci polymorphic	87.88% (6.06)	81.82%	100.00%	81.82%	100.00%

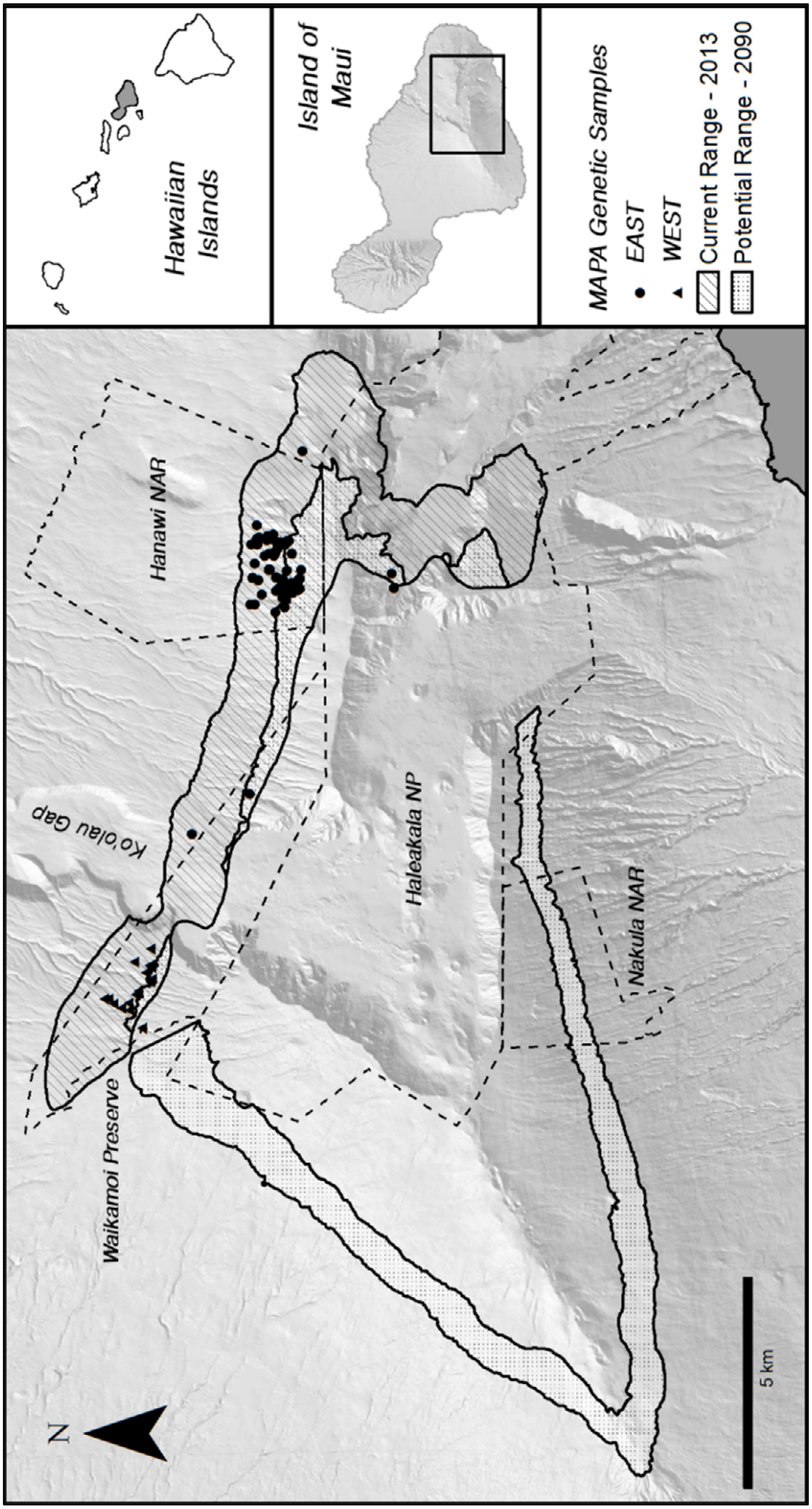
Hawaiian Islands
Island of Maui
MAPA Genetic Samples
● EAST
▲ WEST
▨ Current Range - 2013
▨ Potential Range - 2090
Hanawi NAR
Ko'olau Gap
Haleakala NP
Nakula NAR
Waikamoi Preserve
5 km
N

Figure 4.1. Map of Haleakala Volcano, eastern Maui, Hawaii, USA, with the current Maui Parrotbill (*Pseudonestor xanthophrys*) population range, the Hanawi Natural Area Reserve [managed for forest bird protection by the State of Hawaii, Division of Forestry and Wildlife (DOFAW)] and the Waikamoi Preserve (managed for forest bird protection by The Nature Conservancy Hawaii). The Nakula Natural Area Reserve on the leeward slope has been identified as a restoration priority by DOFAW as well as the most suitable location to establish a second Maui Parrotbill population. The current Maui Parrotbill range given suitable habitat is 40 km^2, showing the sampling location of all wild individuals included in the genetic analyses. The predicted range constricts to 9 km^2 by 2090 based on climate change models. The potential suitable habitat in 2090 given the same climate change models but landscape level habitat restoration would maintain 40 km^2.

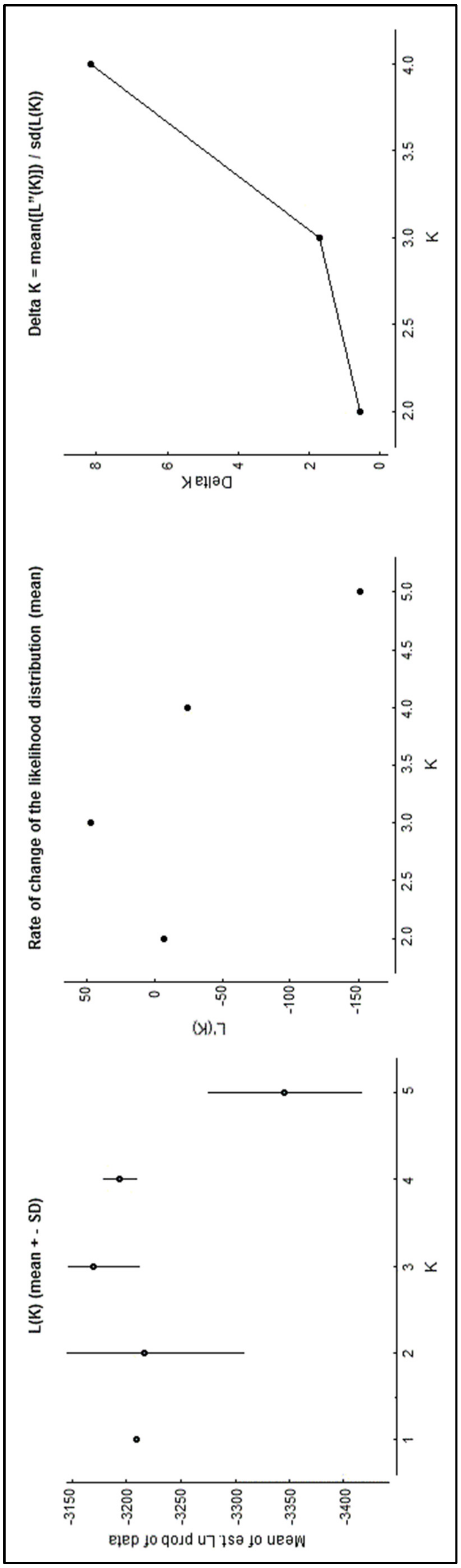

Figure 4.2. Mean likelihoods, likelihoods rate of change, and ΔK values calculated from number of genetic clusters (K = 1-5) in STRUCTURE before and after applying the Evanno et al.(2005) correction to the output in STRUCTURE HARVESTER.

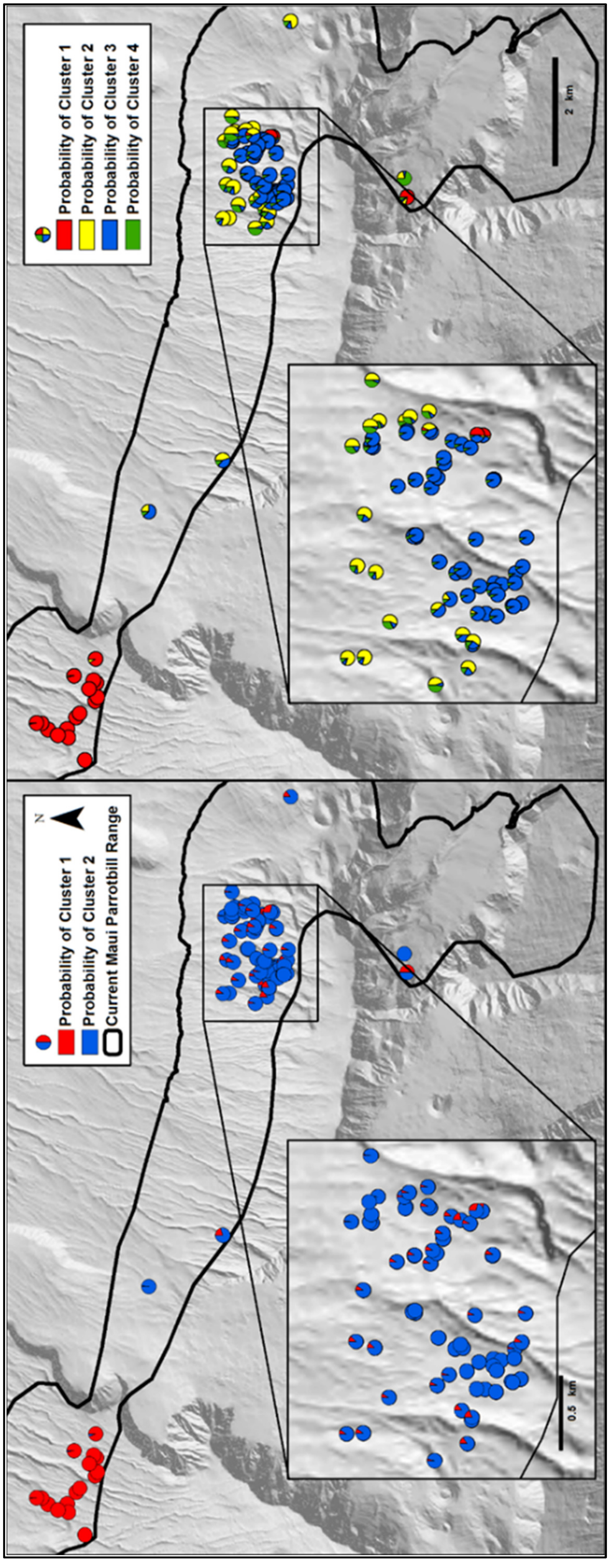

Figure 4.3. Maui Parrotbill (*Pseudonestor xanthophrys*) assignment probabilities to each population cluster

from GENELAND results of K = 2 (right) and K = 3 (left).

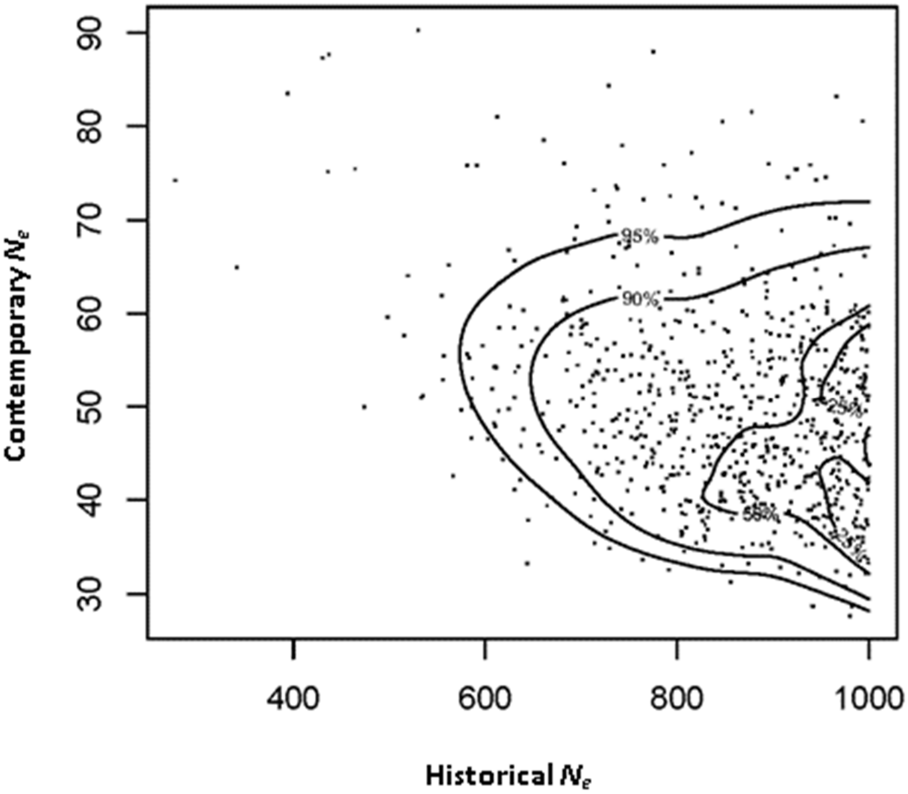

Figure 4.4. A posterior distribution of the temporal change in historical and contemporary effective population size (N_e) for the parrotbill population as derived from TMVP analyses following the methods of Beaumont (2003).

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

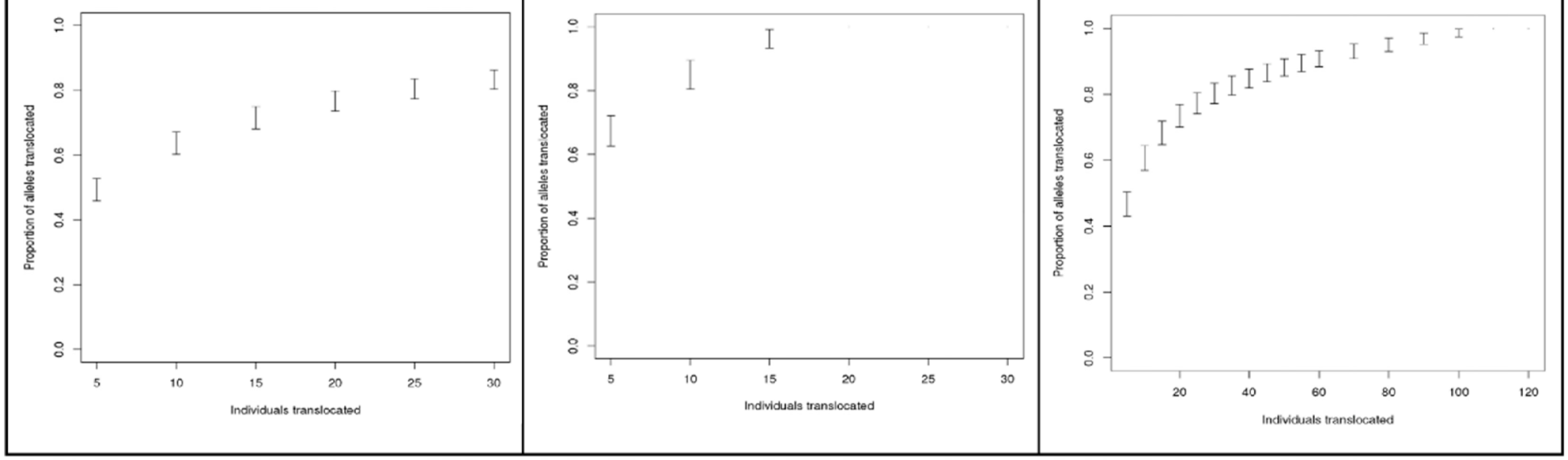

Figure 4.5. Probability values for capturing different percentages of the total genetic diversity available in the east, west and total wild Maui Parrotbill (*Pseudonestor xanthophrys*) population modelled while using different numbers of individuals in translocation efforts.


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Chapter 5 Extinction risk and reintroduction options for the endangered Maui Parrotbill (Kiwikiu, *Pseudonestor xanthophrys*)

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Keywords: extinction risk, Hawaiian Honeycreepers, population viability modelling, reintroduction, VORTEX

ABSTRACT

Extinction rates for island birds around the world have been historically high and are increasing. For forest passerines, the Hawaiian archipelago has suffered some of the highest of these extinction rates. Population viability analyses (PVA) can be used to assess risks to vulnerable populations and evaluate the relative benefits of various conservation strategies. Here we present a PVA to assess the long term viability for Maui Parrotbill (Kiwikiu, *Pseudonestor xanthophrys*), an endangered passerine on the Hawaiian island of Maui. Modeling the current demographic trajectory of the wild population, Maui Parrotbill are predicted to not persist beyond 25 years. Female mortality and fecundity are two factors driving this decline. To evaluate and compare management options involving captive rearing and translocation strategies we built a female-only age-structured, meta-population simulation model. Due to the low reproductive potential of parrotbills in captivity, the number of individuals (as many as 20% of the global population) needed to source a reintroduction from captive reared birds is unrealistic. A reintroduction model that incorporates a minimal contribution from captivity and instead translocates mostly wild individuals was found to be the most favorable option for managers. New habitat is being restored on leeward east Maui which may provide more favorable climate and habitat conditions for the species and lead increased reproductive output. Our model provides managers with benchmarks for fecundity and survival to maximize the likelihood of reintroduction success, and highlights the importance of establishing a new population in potentially favorable habitat to ensure long-term persistence of the species.

5.1 INTRODUCTION

One in eight of the world's bird species is globally threatened (BirdLife International 2014a) and extinction rates are highest on islands (Gilpin and Soulé 1986; Steadman 2006). There is a broad suite of ecological characteristics that can explain extinction risk of small populations of threatened species. Amongst these are a host of stochastic threats can that interact with deterministic factors to lead to population extinction (Shaffer 1987), including demographic, environmental, genetic, and catastrophic threats (Shaffer 1981). Together, these factors form the 'extinction VORTEX' and their interacting effects increase extinction probability (Gilpin and Soulé 1986; Soulé and Mills 1998; Mills 2007).

Population viability analysis (PVA) is an analytical tool used to measure and ultimately combat the processes that can lead to extinction, whereby data can be applied to a suite of models that combine the effects of deterministic and stochastic factors to estimate a population's probability of future persistence across specified time frames (Gilpin and Soulé 1986; Caughley 1994; Beissinger 2002). Ideally, a PVA should incorporate every aspect of a population's biology, and when correctly parameterized it can provide insights into what factors constitute the greatest threats to the population's survival (Mills 2007). While PVA is often used to quantify absolute risk of extinction, their real value is in an applied context, to examine the relative benefits of alternative management actions and estimate relative probability of extinction under different strategies (Akçakaya and Sjogren-Gulve 2000; Ellner and Fieberg 2003). The species-specific information needed to calculate a population's absolute risk of extinction with precision and to compare relative extinction risk under different management scenarios is rarely achievable for endangered species, due to their low density and often cryptic habits. However, in those instances where endangered species have been sufficiently well-studied, PVA becomes a more useful tool to conservation managers (Ralls *et al.* 2002). Indeed, predicting time to extinction under a variety of scenarios can inform conservation decisions, help guide management efforts, and prioritize management options (Clark *et al*. 1991; Cook *et al*. 2012). For example, if an endangered species is predicted to go extinct within a decade, recovery actions will need to differ substantially from those for a population predicted to decline slowly over a century. Furthermore, by comparing the model output for different scenarios, managers can evaluate which strategies are most effective to reverse the trajectory of a species' decline versus other approaches that might be capable of achieving the same goal but for which the necessary techniques are unavailable or less developed.

One species of immediate conservation concern is the Maui Parrotbill (Kiwikiu, *Pseudonestor xanthophrys*), a critically endangered Hawaiian honeycreeper endemic to Maui Nui (the islands of Maui and Molokai) in the Hawaiian Islands. The Maui Parrotbill is a feeding specialist with a parrot-like beak for extracting insect prey from bark and decaying wood (U.S. Fish and Wildlife Service 1967; Simon *et al*. 1997; IUCN 2012). Extensive demographic studies have been carried out on productivity and survival in two separate areas of the species' range (Becker *et al*. 2010; Mounce *et al*. 2013; Mounce *et al*. 2014), indicating parrotbills are long-lived, strongly monogamous passerines which can reproduce for a minimum of 15 years. Breeding pairs typically produce only one offspring per year, exhibit prolonged parental care (5-18 months), and defend multi-purpose territories averaging 6-8 ha (Simon *et al*. 2000; Mounce *et al*. 2013; Warren and Mounce 2014). Parrotbills were once abundant on the islands of Maui and Molokai (James and Olson 1991), but have undergone substantial declines since the arrival of humans approximately 800-1000 years ago (Mounce *et al*. 2015). Today the wild population comprises approximately 500-600 individuals which occupy less than 50 km^2 on windward east Maui (Scott *et al*. 1986; U.S. Fish and Wildlife Service 2006; Camp *et al*. 2009). While population-wide surveys have been unable to assess the stability of the current population (Gorresen *et al*. 2009), a spatiotemporal analysis of genetic diversity indicates a severe historical decline in population size over the past century (Mounce *et al*. 2015). Due to a lack of resistance by the native forest birds to mosquito borne diseases, such as avian malaria (*Plasmodium relictum*), forests above 1500 m elevation provides the only existing refuge for most native Hawaiian honeycreepers (Scott *et al.* 1986; Mountainspring 1987; Simon *et al.* 1997) including Maui Parrotbills. Unfortunately, avian malaria is now moving into higher elevations, coincident with increasing average temperatures in Hawaii and gradually eroding available habitat for these species (Giambelluca *et al.* 2008; Benning *et al.* 2002; Harvell *et al.* 2002). Moreover, these high elevation windward habitats are likely suboptimal for parrotbills as 1) these habitats lack a perhaps key foraging substrate, koa (*Acacia koa*) (Perkins 1903), and 2) the prevalence of nest failures, frequently attributable to severe weather, in these areas are high (U.S. Fish and Wildlife Service 2006; Becker *et al.* 2010; Mounce *et al.* 2013). The historically forested island of Maui once provided almost island-wide habitat for parrotbills including lowland and leeward (southeast) forests. Sadly, little suitable habitat exists beyond the species' current range with the exception of a few remnant forest tracts on leeward east Maui, such as those found in Nakula Natural Area Reserve (NAR). In addition to the wild population, there is a small captive flock of parrotbills (currently 15 individuals) that was established in 1997 and is managed by San Diego Zoo Global on the islands of Maui and Hawaii. Together, the captive flock and habitat restoration efforts have paved the way for a number of potential conservation strategies for this species, prompting the need for the evaluation.

We applied PVA models using detailed data from demographic, genetic, and environmental/ecological studies recently completed for this species (Mounce *et al.* 2013, 2014 and 2015), to assess long-term viability of Maui Parrotbills and evaluate conservation strategies that may be applied to increase the long-term viability of the species. We used an individual based simulation model (VORTEX) to understand key limiting factors for the current population by determining which demographic variable(s) were most influential for population growth and long-term viability. In addition, we created a custom demographic model to examine the effects of (1) different management strategies to improve productivity and survival in the species' current range, (2) removing individuals from the wild for reintroduction elsewhere, and (3) establishing an additional geographically-distinct population in the leeward forests, currently unoccupied by parrotbills, which may provide a long-term refugia for the species.

5.2 METHODS

Wild Population Viability

To explore different viability scenarios on the current wild population, we used VORTEX V10.0.7.3 (Lacy and Pollak 2014). VORTEX is a widely used and flexible simulation program that runs individual-based Monte Carlo simulations while combining the effects of deterministic values and stochastic (demographic, environmental, and genetic) events. To model the extinction processes that threaten small populations, VORTEX relies upon user-specified demographic values (including variance) and then models population dynamics as discrete, sequential events that incorporate stochasticity through random draws from probability distributions. VORTEX runs multiple simulations (set at 1000) to generate a distribution of possible fates that a population might experience under a given set of parameters. Each run steps through a series of events that describes an annual cycle (reproduction, mortality, dispersal among populations, removals, supplementation, and if necessary, truncation to the carrying capacity) (Lacy 2000; Miller and Lacy 2005). VORTEX is appropriate for modelling the parrotbill population as it was initially designed for vertebrate populations with low fecundity and long life spans. Although VORTEX has the further ability to include catastrophes in its models, we did not use this feature for simulations on Maui Parrotbills. Though there are limits to the biological and stochastic complexity that VORTEX can simulate compared to other PVA software packages, this potential shortcoming was not an issue for the analysis of the current wild population because estimates for all of the fundamental parameters were available.

Measures of viability

PVAs are limited by the quality of the input parameters available for a given species under each given scenario and do not identify absolute probabilities of extinction in a given time frame (Akçakaya and Sjogren-Gulve 2000; Reed *et al.* 2002). Therefore, it is important to evaluate a variety of quantitative measures that are available to evaluate population viability across all models rather than relying solely on the viability measures most commonly presented in PVA studies such as extinction probability, population size, and time to extinction (Pe'er *et al.* 2013). Consequently, for each model, we present mean population growth rate (stoch-*r*), mean intrinsic rate of growth (λ), net replacement rate (i.e. per generation rate of change; R_0), probability of survival (PS), mean population size from all iterations (N-all), mean population size from extant populations (N-extant), and gene diversity (GD), the expected heterozygosity in extant populations.

Base Model

Our base model was parameterized using estimates of parrotbill vital rates from Mounce *et al.* (2013, 2014, and 2015) (Table 5.1). The correlation of environmental variation (EV; defined as the annual variation in the probabilities of reproduction and survival that arise from random variation in environmental conditions) was set at zero as reproduction and survival parameters already averaged temporal variation as they were derived from long-term data sets. This base model was designed to represent the parrotbill population in its current state without incorporation of any change in threats (besides normal demographic and stochastic effects of small population size) and thus produces simulations of a probable population trajectory without any management actions.

We made four changes to this base model for subsequent scenarios. First, two of the key input parameters for these models appeared problematic for the generation of an accurate PVA. The estimate of juvenile survival presented in Mounce *et al.* (2014) was markedly lower than previously reported annual juvenile survival rates for parrotbills (0.76 ± 0.09; Vetter *et al.* 2012) and for other Hawaiian passerines (average 0.32 ± 0.03; Woodworth and Pratt 2009). Furthermore, this estimate was generated from only 10 individuals (the estimate produced by Vetter *et al.* 2012 was generated from a subset of seven of these same individuals) and had a large standard error (0.15; Mounce *et al.* 2014). Second, the estimate of annual reproductive success (ARS) presented in Mounce *et al.* (2013) was derived from the core breeding season for parrotbills (January-June). Breeding attempts for this species have been observed during 11 of 12 months. Therefore, it is probable that while this ARS estimate may capture the majority of the success in a given year, it is likely to be an underestimate of the true ARS over the entire calendar year. Third, an annual decline in carrying capacity (K) for this species in their current habitat is inevitable due to the current and future upslope movement of avian malaria and its vector due to climate change (Benning *et al.* 2002; Harvell *et al.* 2002; Giambelluca *et al.* 2008). Fourth, genetic analysis has shown that the parrotbill population is not contiguous across their range likely due to limited dispersal between two subpopulations (Mounce *et al.* 2015). To incorporate these four discrepancies in to our models, we (1) decreased juvenile mortality from 83% to 68% to reflect values found in the other Hawaiian passerines (equal to 39-44% of adult survival), (2) increased by 10% the percentage of breeding females to account for a reasonable estimate of less well-documented ARS in the months outside of January-June, (3) added a decrease in K of -1% per year to account for the influx of avian malaria resulting from climate changes (Giambelluca *et al.* 2008), and (4) divided the population into two sub-populations with an associated K for each calculated from Warren and Mounce (2014) (Modified base model in Table 5.1).

General model settings

For all individual simulations we used 1000 iterations spanning 25 years for a long-term monogamous species. Although longer time frames are more appropriate for assessing the predicted longevity of a species, for this exercise our focus was on the immediate viability risk and the effects of conservation actions that can be implemented to prevent imminent extinction. Due to persistent problems associated with introduced predators, continued loss of habitat, invasive species, and the inherent risks of a critically endangered organism, modeling population dynamics for this species on a longer timeframe would not provide any additional insight for critical management needs.

Testing demographic sensitivity

To test the demographic sensitivity of the current wild populations, all parameters were kept constant at the Modified Base Model parameter settings (Table 5.1) while one parameter in turn was manipulated (Jørgensen and Fath 2011). This sensitivity analysis was designed to identify which parameters were most sensitive to the survival of the metapopulation and to be able to compare sensitivities across parameters. To accomplish this we used a sensitivity index calculation (S_x) from Pertoldi *et al.* (2013):

$$S_x = (\Delta X/X) / (\Delta Y/Y),$$

where ΔX is the change in the observed measure of viability, and ΔY is the change in the parameter of interest.

Population Viability with Management

To evaluate and compare management options in more detail involving captive rearing and translocation strategies tailored to the recovery requirements for this species, we built a female-only age-structured, metapopulation simulation model in R 3.0.1 (The R Foundation for Statistical Computing 2013). The viability models produced using R and VORTEX were nearly identical. However, while VORTEX allowed for easily repeatable modeling of population viability, R provided a more flexible framework for simulating management scenarios. We included an immature age class and a breeding adult age class in the model to reflect known age at maturation and differential survival rates of immature and adult birds (Mounce *et al.* 2014). Future immature bird abundance was modeled as a product of the number of breeding adults (N_t^A), the fecundity rate (F_t^A) and the survival rate of young of the year (S_t^Y), as follows:

$$N_{t+1}^I = N_t^A \times F_t^A \times S_t^Y,$$

The number of adults in the future was a product of the number of adults (N_t^A) and their annual survival rate (S_t^A), plus the product of the number of immature birds (N_t^I) and their annual survival rate (S_t^I), as follows:

$$N_{t+1}^A = N_t^A \times S_t^A + N_t^I \times S_t^I.$$

Adult survival rates in the simulations were stochastic and drawn from a beta distribution where the alpha and beta shape parameters were derived from the survival estimates reported in Mounce *et al.* (2014) using the 'method of moments' calculations (see Morris and Doak 2002). As with the VORTEX model parameters described above, the juvenile survival rates from Mounce *et al.* (2014) were extremely low and based on a small sample size. Using those survival rates in our model predicted rapid and near certain extinction for the population, which seems unreasonable given the apparent population stability documented over the last 20 years based on repeated population and territory counts (Gorresen *et al.* 2009). We investigated juvenile survival estimates from other endemic Hawaiian forest bird species, but most suffer from the same low sample size issues and exhibit low survival estimates. Consistent with our VORTEX models, therefore, we set immature survival rate at 0.72 (SD 0.02) because at that rate population projections in the wild populations roughly reflected the observed population growth rates from population surveys and territory counts. We encountered the same issues with hatch-year survival rates and set the mean at 0.32 (SD 0.03). Again, these mean and standard deviations were converted to alpha and beta shape parameters for a beta distribution using the 'method of moments' calculations (see Morris and Doak 2002).

Fecundity rate was incorporated as the number of female offspring fledged per breeding female and was modeled as a log-normally distributed random variable. Mean annual fecundity was set at 0.588 with a 0.15 coefficient of variation. In our model we also included a ceiling type density dependent function whereby if a specified abundance threshold (885 females) was exceeded, the fecundity rate for that year was set to 0.

We created a meta-population with implicit spatial structure to the population, by creating four separate sub-populations in the simulations. Two populations represent the existing east (Hanawi NAR) and west (TNC Waikamoi Preserve) populations (Mounce *et al*. 2015) on the windward slopes of Haleakala (Figure 5.1). Another population represents the proposed third population that will be established on the leeward slopes of Haleakala (Nakula NAR; Figure 5.1), and a fourth population represents the experimental captive breeding population that may serve as a source of individuals for release into the wild populations. The two wild populations use the projection equations described above. For the third (not yet established) reintroduced population we tested the effects of increased survival and fecundity rates on the probability of successfully establishing a wild self-sustaining population and on overall species extinction probability. We input demographic rates 5-20% higher for the third population to reflect the hypothesis that these drier forested habitats will provide higher quality habitat than the extremely wet windward rainforests, some demographic values that would more closely reflect those found in other Hawaiian honeycreepers (Woodworth and Pratt 2009). Abundance in the east wild population was initially set at 238 females, the west wild population was initially set at 53 females (we used density estimates from Camp *et al.* 2009 and extrapolated to generate population estimates across all current occupied habitat from MFBRP unpublished data), and the third wild, yet to be established population was initially set at 0.

The captive population was modelled differently from the wild populations since in captivity the birds are not subject to the same ecological processes. Instead, once established, the future abundance in captivity (N_{t+1}^{C}) is the current number of individuals (N_t^C), plus the number successfully reared (N_t^B), minus the number that died (N_t^D), which were modelled as Poisson distributed random variables with a mean of 2.0, and incorporated into the projection as follows:

$$N_t^B \sim Poission(2)$$
$$N_t^D \sim Poission(2)$$
$$N_{t+1}^C = N_t^C + N_t^B - N_t^D.$$

We set initial abundance in captive population at 7 females to reflect current conditions of the captive flock.

Movements between the sub-populations were restricted to translocations, meaning there was no natural rate of immigration between sub-populations. The projected abundance in a subpopulation was a function of natural population dynamics (as described above), and the number of individuals added to and subtracted from the population as follows:

$$N_{t+1}^{A,i} = (N_t^{A,i} \times S_t^A) + (N_t^{I,i} \times S_t^I) + \sum T_t^{j,i} - \sum T_t^{i,j}$$

where *T* indicates the number of birds moved, *i* represents the current sub-population being projected and *j* indicates the other sub-populations to or from which individuals can be translocated. The model was written in a generalized form so that birds could be moved from any sub-population to another (see Appendix C), but in our simulations management actions were limited to establishing a new sub-population and/or contributing to the small captive population. Translocations of a specific number of birds between sub-populations could be specified for a limited number of years such that if abundance in the west and east sub-populations fell below 25 or 100, respectively (excluding the captive population), removing individuals from that sub-population was prohibited. Lastly, individuals introduced to the wild from captivity are typically less successful (Fischer and Lindenmayer 2000). The fact that Maui Parrotbills will be re-established in a different habitat type increases the uncertainly regarding their survival. To reflect that uncertainty in our model, we made first year survival of captive released birds an annually varying uniformly distributed random number bounded between 0.4 and 0.9.

The reintroduction scenarios varied mainly on account of where birds are sourced, namely the east and west wild populations, and the captive population. The goal of the captive breeding program from its onset has been to develop a sustainable breeding program for the species in the event of a collapse of the wild populations and/or to act as a source for reintroduction as new habitat became available. However, the captive program has only been moderately successful. The population remains small and few birds are recruited each year. As of 2015, the captive population consisted of seven females and eight males, which together produce an average of one bird each year. Given a sex ratio of 50:50 this represents a rate of 0.07 females produced per female per year. Realistic options for sourcing birds for reintroduction from the captive population include:

a) Releasing a large proportion of the existing captive birds (e.g., 5 females and 5 males) in a single year.
b) Releasing a minimal subset of the captive population (e.g., 1 female and 1 male) over the course of a few years.

c) Augmenting the captive flock with wild birds, allowing for the release of a larger number of captive birds over the course of a few years.

A working group of researchers and managers (Maui Parrotbill Reintroduction Working Group) have developed a plan to reintroduce parrotbills to Nakula NAR over a three year period, a strategy designed to balance probability of success and the best use of resources. Data on parrotbills home range sizes has suggested that the Nakula NAR may be able to support approximately 15 parrotbill pairs in the first few years of a reintroduction program (Warren and Mounce 2014). Considering these restrictions in the total numbers of individuals the area can immediately support, we therefore tested a variety of reintroduction scenarios whereby six pairs are released each year. While there are many possible scenarios that could be tested; we selected six that we felt were realistic given current management opportunities as follows:

i. Release only captive birds currently available to establish a second population;

ii. Augment the captive flock with wild birds such that the captive flock alone would source a second population;

iii. Augment the captive flock with wild birds such that the captive flock would provide half the individuals needed for reintroduction with the other half from translocated wild individuals;

iv. Augment the captive flock with wild birds such that the captive flock would provide 1 female per year in combination with translocated wild individuals;

v. Release only captive birds currently available in combination with wild translocations to establish a second population; and,

vi. Release wild translocated individuals to establish a second population with no input from the captive population.

5.3 RESULTS

Wild Population Viability

All models predicted a negative population growth rate (Table 5.2). None of the population trajectories for the east, west, and metapopulations predicted persistence beyond 25 years (Figure 5.2, Figure 5.3A; B). The sensitivity tests identified multiple parameters as being particularly important in driving the overall population trajectory, specifically female mortality at all ages and fecundity (both the % of females producing more than one offspring and the % of females successfully producing per year; Table 5.3). Several parameters were not heavily weighted in determining the overall model results due to high levels of uncertainty surrounding them. These included initial population size, predicted annual habitat loss predicted, maximum lifespan as well as reproductive lifespan, and the age of first reproduction.

Population Viability with Management

Assuming no changes to the fecundity amongst the captive flock, sourcing the reintroduction using only captive birds would require either using those birds currently available (*i*), using six females (leaving one female in captivity), or moving 79 females from the wild into captivity to increase the captive populations' productivity (*ii*). The third option (*iii*) would still require 36 additional females to be brought into captivity to supplement the captive flock in order to source 50% of the translocations (Table 5.4). The fourth option (*iv*) requires that seven additional birds are brought into captivity so that the captive flock could consistently supply one female per year for reintroduction efforts. Population trajectories among the last three scenarios (*iv*, *v*, and *vi*) are the same as they use the same input parameters, scenarios differed in where the birds came from (Figure 5.3).

Increasing important demographic parameters under the assumption of a more beneficial environment in the mesic leeward forests results in different population trajectories (under reintroduction scenario *iv*) after the initial three-year reintroduction timeframe (Figure 5.4). All reintroduction scenario models show that a 5% increase in demographic rates may not be sufficient to maintain the reintroduced population. However, a 10% increase results in a stable or increasing leeward population and a 20% increase results in an exponential increase in this population. Using reintroduction scenario *iv*, Figure 5.4 demonstrates the effects of increased demographic rates on the fate of the reintroduced population.

5.4 DISCUSSION

The PVA models presented here further underline the perilous status of Maui Parrotbill. The precise timeline of extinction is beyond the scope of these analyses. However, the rapid decline projected by these models highlights the fact that certain aspects of the species' biology and ecology (e.g. single egg clutch, prolonged parental investment) make this species in its current state (e.g. small, contracting range, occupying potentially suboptimal habitat) highly vulnerable to rapid extinction. This modeling exercise allowed us to identify the demographic traits most limiting the species to explore potential management solutions. Herein we further identified the most promising scenarios for reintroducing the species to previously occupied leeward mesic forests. We found that a reintroduction scenario that incorporates a minimal contribution from captivity and instead translocated mostly wild individuals to be most favorable for managers.

Our population models highlight the strength and weakness of several conservation strategies that managers could implement given existing resources and capabilities in attempts to recover the wild population. Given that the population model does not reach carrying capacity, simply increasing available habitat in their current range may not have any impact on increasing the total population. Alternatively, if managers are able to augment the current habitat (e.g., threat mitigation, such as control of predators) to increase quality, and thus increase some of the more sensitive parameters (i.e. female survival) within the current populations, they may be able to increase the viability of the population. Unfortunately, for parrotbills these options appear to be quite limited for a number of reasons.

First, the forest currently occupied by parrotbills is native Hawaiian rainforest. This habitat is likely already as good as it is going to get as it is already closely protected (i.e. fenced and free of ungulates) and actively managed by the State of Hawaii and private conservation organizations. Second, there are limited options for other management interventions. Weather has been identified as a key limiting factor to reproductive success of parrotbills, with high incidence of nest failure in heavy rain events (Becker *et al.* 2010). Although weather cannot be manipulated, there have been numerous other unsuccessful attempts to manage parrotbills within their current forest habitat. These efforts have included trying to increase productivity and/or survival by providing supplemental food to wild individuals (see Appendix H), decreasing predation risk through control of invasive mammalian predators, and decreasing nest predation by protecting nest trees from mammalian predation (MFBRP unpublished data).

Our simulations and resulting extinction probabilities are limited by the precision of the demographic parameters. While this study has used the most comprehensive data available on Maui Parrotbill, there is still uncertainty in several critical parameters. The variables with the most uncertainty included initial population size, annual habitat loss as predicted through climate change models, maximum lifespan and reproductive lifespan of individual parrotbill, and age of first reproduction. The effort necessary to collect additional data for these variables would require a substantial investment (see Brinck *et al.* 2012). An alternative approach could be to model parametric uncertainty directly into our simulation models and evaluate the benefits of increased parametric precision through simulations (McGowan *et al*. 2011). However, our sensitivity analysis identified female mortality, annual reproductive success, and percentages of nests with two offspring as those parameters most responsible for driving the population changes observed - all parameters that we have high confidence in.

Without clear strategies for increasing population viability of parrotbills in existing reserves, an alternative strategy would be to establish new populations in potentially superior habitat. Historically, parrotbills may have preferred habitats containing a higher proportion of koa, a preferred food source (Perkins 1903), which tend to occur in drier, leeward areas on Maui. Furthermore, sub-fossil records show a distribution of this species across the island, not restricted to the high elevation wet windward forests where they are found currently (James and Olson 1991). Today, there are no parrotbills in koa dominated forests on Maui. We do not know whether parrotbills were historically distributed at higher densities in the wet windward forests, but it may be that these areas were always marginal habitat. Regardless, if managers do not have the tools to successfully manage this species in currently occupied habitats, then increasing the range of habitats occupied may provide a viable long-term conservation strategy. Furthermore, our models did not include possibility of catastrophic events, but having an entire global population of any species within one 40-50 km^2 area puts it at greater risk of extinction in the event of a severe hurricane or other weather event.

Given the apparent limitation of management options in currently occupied habitats, we simulated reintroduction scenarios to explore moving birds from existing populations to the leeward side of Haleakala, Nakula NAR, a drier and more mesic koa dominated habitat. Furthermore, reintroducing birds to an area where they might be able to benefit from increased survival and productivity may be key to the species long-term success. Our models evaluated moving birds from the wild, using captive bred birds, and a combination of these alternatives, building in uncertainty on immediate survival of the reintroduced birds since parrotbills have never been translocated to a new habitat before.

Using captive-bred individuals has ecological consequences such as behavioral deficiencies, high susceptibility to starvation and disease, high post-release predation rates, and overall low reintroduction success rates that have been widely documented (Curio 1996, Fischer and Lindenmayer 2000, Jule *et al.* 2008, Rantanen *et al.* 2010). Captive parrotbills have the additional disadvantage of reduced genetic variation and significant genetic differentiation compared to some wild individuals (pairwise F_{st} and R_{st} between west and captive populations [$F_{st} = 0.10$; $R_{st} = 0.16$] Mounce *et al.* 2015). Furthermore, given the low reproduction of captive parrotbills, using only captive bred birds would 1) hinder the current captive population, 2) establish a new population with genetic variation from few females (*i*), or 3) require that a very large number of wild individuals be brought into captivity (*ii* and *iii* requiring 29% or 13%, respectively, of all wild females). Without considering potential effects to the wild populations, the resources necessary to capture and care for these high numbers of a critically endangered species in captivity is unrealistic with current conservation support available in Hawaii (Leonard 2008). Conversely, if the availability of resources for this sort of hands-on management greatly improved, there may be some advantages, namely that captive birds may anchor any wild birds to the release area which would facilitate monitoring. A major obstacle in translocations of wild individuals is they often reject the habitat close to release sites and travel long distances before settling (Stamps and Swaisgood 2007), exhibiting preferences that captive individuals may not have.

Scenario *iv* models a reintroduction that incorporates a minimal contribution from captive individuals and has the advantage of potentially being among the least expensive scenarios. The ultimate monetary costs of many of the key steps involved in these scenarios remain unknown and in some cases are impossible to predict (e.g. the amount of field time required to capture 79 females (scenario *ii*)). Without these figures a cost comparison among all scenarios is not an option at this time. However, scenario *iv* calls for the least amount of effort devoted to capturing wild individuals to be added to the captive population, a benefit over *ii* and *iii*, while also making use of the investment already made toward maintaining the captive population, an advantage over *vi*. This scenario also does not deplete the already small captive population, unlike *i* and *v*, and minimizes the addition of new birds to captivity and thus the costs in maintaining the larger captive population. Scenario *iv* would also likely provide the new populations with the most solid genetic foundation considering the genetic differentiation observed between the east/captive and west wild parrotbill populations (Mounce *et al.* 2015). In order for the leeward population to be considered genetically viable (Foose 1993) birds from both the east and the west should ideally need to be incorporated in the releases; captive birds were sourced from the east population only.

We further explored scenario *iv* by looking at the reintroduced population's viability using parameter values from the current wild population (Figure 5.4, Mounce *et al.* 2013, 2014) as well as predicted trends in annual fecundity, female survivorship, and hatch-year survivorship increased by 5%, 10%, and 20%. These increased demographics were examined based on potential benefits the leeward mesic habitat may have for the species. The exact limitations of the wetter windward habitats are unknown but parrotbills in the mesic forest may have increased nest success and/or increased foraging success in the drier habitat. Parrotbills may also have reduced predation pressure in a habitat with lower invasive mammal densities (MFBRP unpublished data). Our results demonstrate that the persistence of the reintroduced population is largely predicated on there being an increase in key demographic parameters in the new and potentially favorable environment. Fortunately an increase in these demographic traits is predicted and demographic rates could be assessed through post release monitoring of individuals.

Given the importance of high demographic rates for a new leeward population, a reintroduction strategy that includes an adaptive management plan to increase demographic rates by 10-20% through management and monitoring is likely to be the most successful approach (Williams *et al*. 2007). Without having any Maui Parrotbills currently inhabiting a koa dominated landscape, it is impossible to know how individuals of this species will respond once released. Yet, the increases in demographic values for the leeward population seem justifiable given it may be more suitable habitat (Perkins 1903) and the ability to conduct active management like reforestation and predator control. However, we do not know whether this species is plastic enough in their ecology to rebound from the release of those constraints or not. Therefore, managers could use these demographic parameter values as benchmarks for the monitoring of the reintroduced population in order to have a successful and viable population in the future.

ACKNOWLEDGEMENTS

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FIGURES AND TABLES

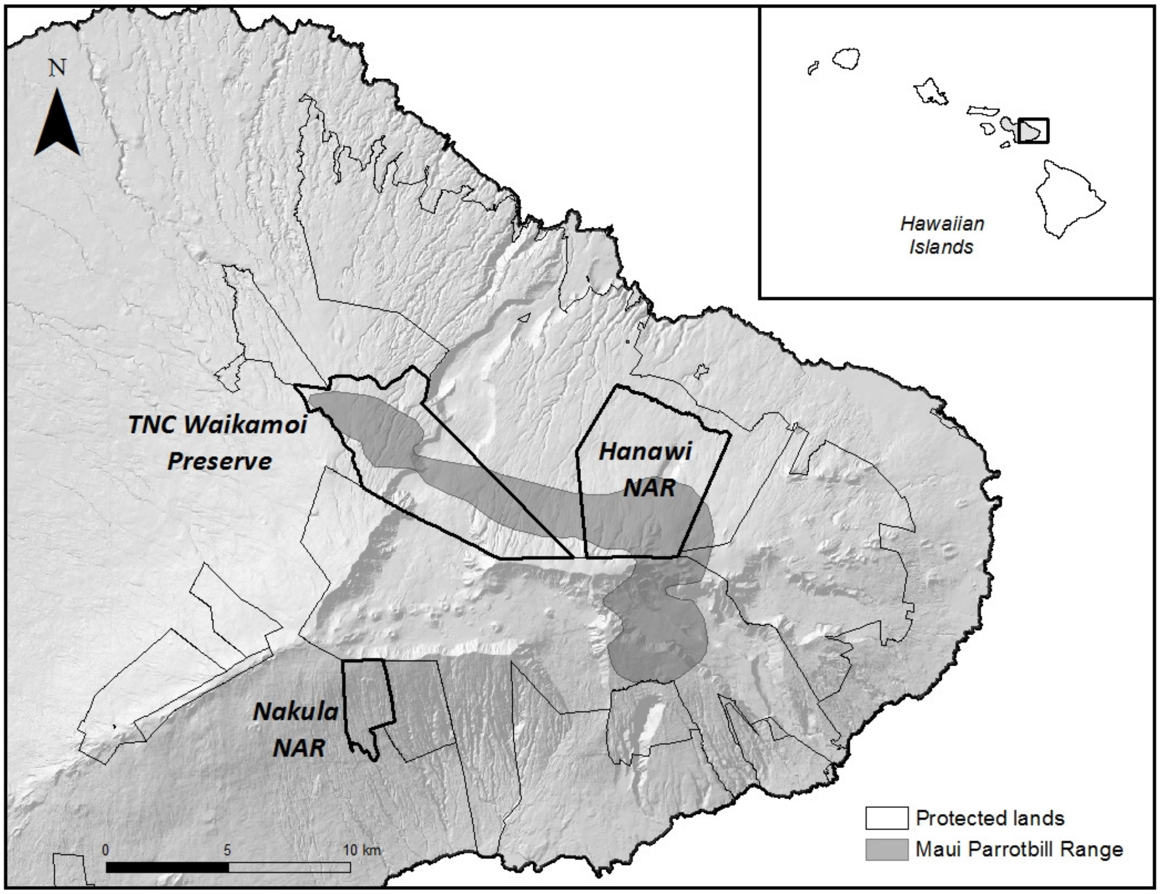

Figure 5.1. East Maui, Hawaii (Haleakala Volcano) land protections that may benefit native forest birds. The current Maui Parrotbill population range overlays the windward (northeast) reserves, Hanawi NAR and TNC Waikamoi Preserve, and the newest reserve, Nakula NAR, is shown on the leeward (southern) slope.

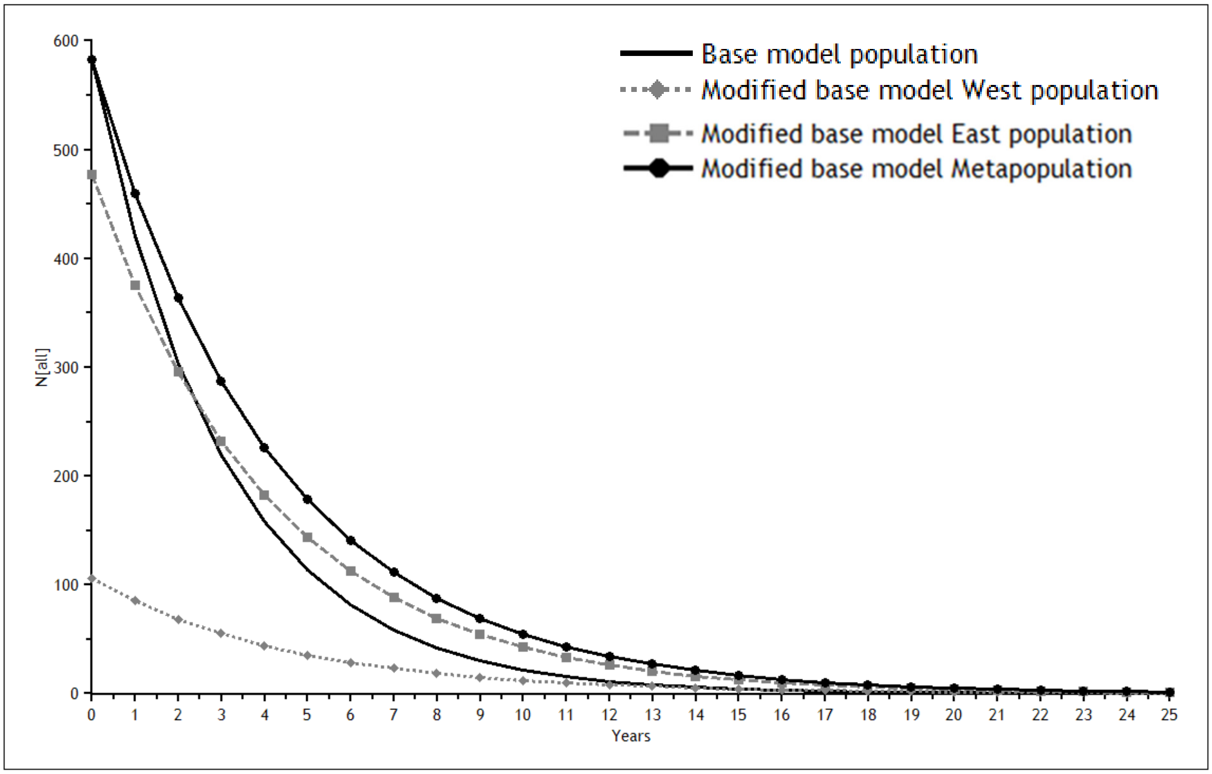

Figure 5.2. Projected mean final population sizes (N-all) for Maui Parrotbill under base and modified base models in VORTEX. Solid black line represents the "Base Model Population Metapopulation", solid black line with dot represents the "Modified Base Model Metapopulation", and broken gray line with box represents the "Modified Base Model East Population", and dotted gray line with diamond represents the "Modified Base Model West Population". Where N-all becomes zero indicates population collapse in 100% of simulations (1000 runs).

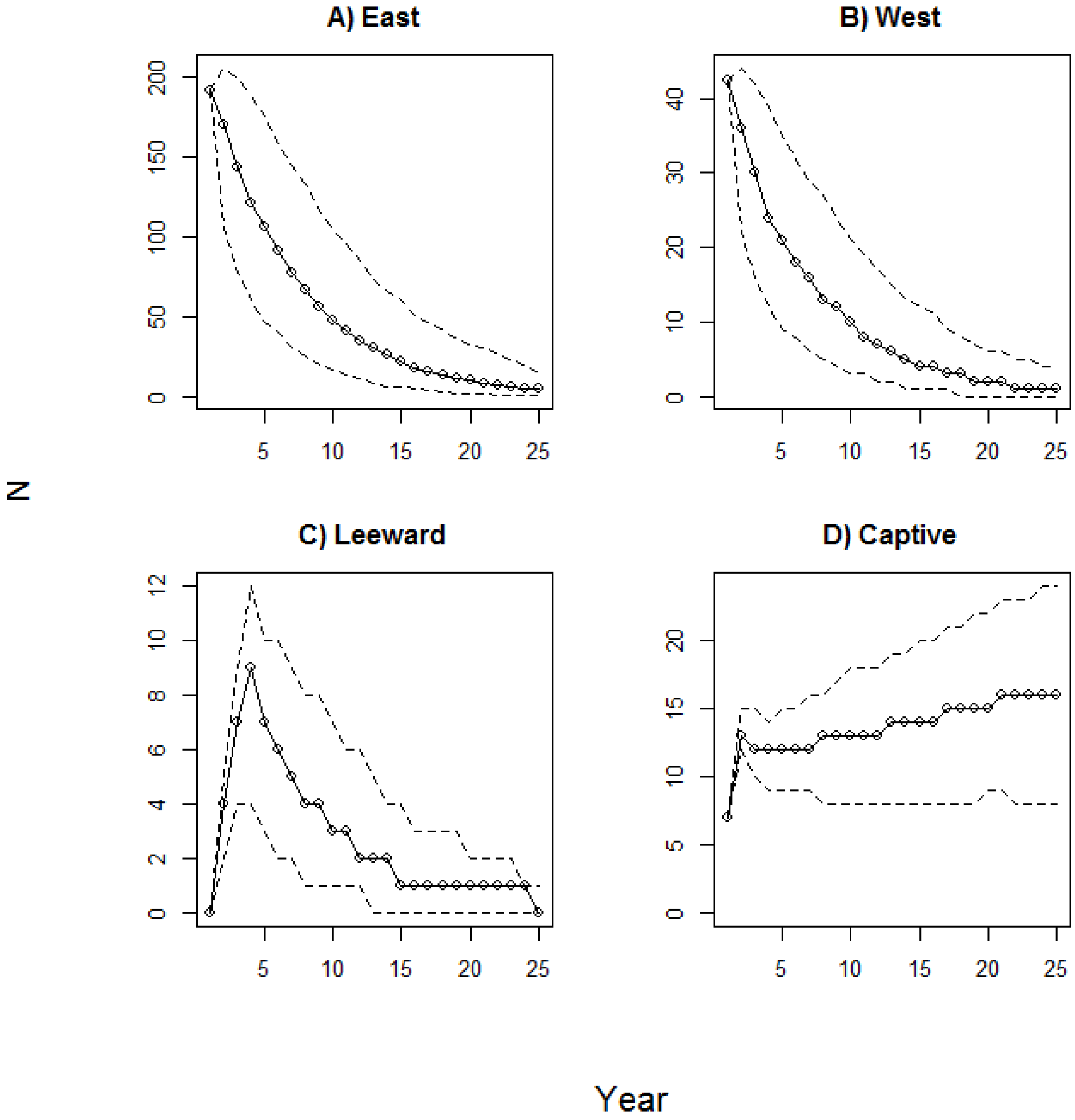

Figure 5.3. Female Maui Parrotbill population trajectories for the three existing populations (A- East, B- West, and D- Captive) and the proposed reintroduced leeward population (C- Leeward). Population estimates in for A, B, C, and D are based on a proposed three-year reintroduction scenario wherein the captive flock is augmented to source 1 female/year in combination with translocations from existing wild populations (scenario *iv*). Demographic parameters for wild populations are set to values from Mounce *et al*. 2013 and Mounce *et al*. 2014.

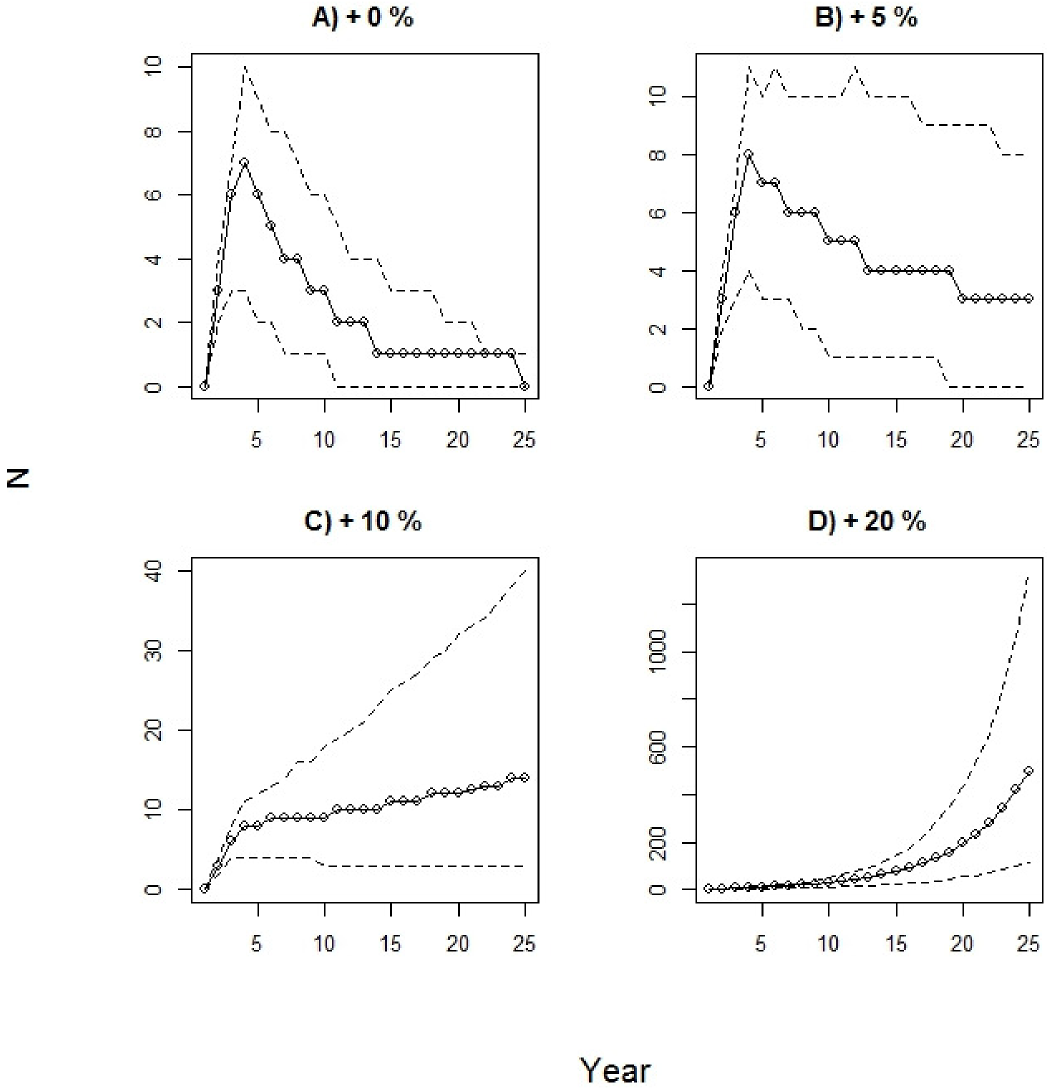

Figure 5.4. Maui Parrotbill population trajectories for the reintroduced leeward population based on reintroduction scenario *iv*. Shown first are population trajectories predicting trends with annual fecundity, female survivorship, and young of the year set as in Mounce *et al*. 2013 and Mounce *et al*. 2014 (A). Next (B, C, and D) are shown with parameters increased by 5% (B), 10% (C), and 20% (D) based on potential benefits of the leeward mesic habitat. Values shown are the number of adult females in the population.

Table 5.1. Parameter input values for the base and modified base VORTEX PVA model used for Maui Parrotbills. Input parameters derived from Mounce *et al.* 2013, Mounce *et al.* 2014, and Mounce *et al.* 2015. Values in bold highlight changes between the Base and Modified Base models.

	Base Model	**Modified Base Model**	
		East Pop (1)	West Pop (2)
Dispersal	No	**Yes**	
Age range	.	**1-5**	
Dispersing sexes	.	**M and F**	
% survival of dispersers	.	**100**	
% dispersing between pops	.	**2**	
Age of 1st offspring	2	2	
Max age of reproduction	15	15	
Max lifespan	20	20	
Max broods per year	1	1	
Max progeny per brood	2	2	
Sex ratio at birth	50	50	
% adult females breeding (EV)	46 (± 0.25)	**56 (± 0.25)**	
% 1 offspring (% 2 offspring)	95 (5)	95 (5)	
♀/♂ Mortality rates 0-1	83 (± 0.15)	**68 (± 0.10)**	
♀ Mortality rates after age 1	28 (± 0.03)	28 (± 0.03)	
♂ Mortality rates after age 1	18 (± 0.04)	18 (± 0.04)	
Catastrophes	None	None	
% ♂ in breeding pool	100	100	
Initial population size	583	477	106
Age distribution	Stable	Stable	
Carrying capacity (K) (SD due to EV)	885 (44)	724 (36)	161 (8)
Future change in K?	No	**Yes**	
Over how many years?	.	**75**	
% annual increase of decrease	.	**-1**	
Harvest	None	None	
Supplementation	None	None	
Genetics	Known	Known	

Table 5.2. VORTEX model results for the base and modified base model for the Maui Parrotbill population(s) with the viability measures of stoch-*r* (mean population growth rate), λ (mean intrinsic rate of growth), R_0 (net replacement rate), PS (probability of survival), N-all (mean population size from all iterations), N-extant (mean population size from extant populations), and GD (gene diversity).

	Base model	**Modified base model**
stoch-*r*	-0.326	-0.234
SD *r*	0.125	0.123
λ	0.725	0.791
R_0	0.105	0.241
PS	0.003	0.116
N-all	0.080	1.200
SD N-all	0.300	1.360
N-extant	2.000	3.380
SD N-extant	0.000	1.710
GD	0.413	0.516
SD GD	0.013	0.091

Table 5.3. Results of the sensitivity analysis in VORTEX for the Maui Parrotbiill metapopulation based on parameter changes in the “Modified base model.” Base model values are in grayscale in each parameter category.

Sensitivity testing index	**Model values**		**stoch-*r***	**S_x of *r***	**N- extant**
	East	West			
Base Age of 1st offspring	2		-0.247	.	2.94
Age of 1st offspring + 1 yr.	3		-0.259	0.14	2.85
Age of 1st offspring + 2 yrs.	4		-0.267	0.15	2.56
Max age of reproduction - 3 yrs.	12		-0.260	0.20	2.66
Base max age of reproduction	15		-0.247	.	2.94
Max age of reproduction + 3 yrs.	18		-0.239	0.20	3.38
Max lifespan - 5 yrs.	15		-0.252	0.06	3.12
Base max lifespan	20		-0.247	.	2.94
Max lifespan + 5 yrs.	25		-0.239	0.17	3.29
% adult females breeding - 10 % (EV)	46 (± 0.25)		-0.268	0.36	2.93
% adult females breeding - 5 % (EV)	51 (± 0.25)		-0.256	0.36	2.71
Base % adult females breeding	56 (± 0.25)		-0.247	.	2.94
% adult females breeding + 5 % (EV)	61 (± 0.25)		-0.238	0.46	3.56
% adult females breeding + 10 % (EV)	66 (± 0.25)		-0.228	0.55	3.72
% 1 offspring + 5 %	100		-0.254	0.55	3.12
Base % 1 offspring	95		-0.247	.	2.94
% 1 offspring - 5 %	90		-0.241	0.45	3.18
% 1 offspring - 10 %	85		-0.237	0.36	3.33
♀/♂ Mortality rates (0-1) - 10 %	58 (± 0.10)		-0.217	0.80	4.39
♀/♂ Mortality rates (0-1) - 5 %	63 (± 0.10)		-0.231	0.87	3.84
Base ♀/♂ Mortality rates (0-1)	68 (± 0.10)		-0.247	.	2.94
♀/♂ Mortality rates (0-1) + 5 %	73 (± 0.10)		-0.265	0.99	2.57
♀/♂ Mortality rates (0-1) + 10 %	78 (± 0.10)		-0.286	1.06	2.67
♀ Mortality rates after age 1 - 10 %	18 (± 0.03)		-0.151	1.14	15.19
♀ Mortality rates after age 1 - 5 %	23 (± 0.03)		-0.201	1.05	5.37
Base ♀ Mortality rates after age 1	28 (± 0.03)		-0.247	.	2.94
♀ Mortality rates after age 1 + 5 %	33 (± 0.03)		-0.298	1.13	2.00
♀ Mortality rates after age 1 + 10 %	38 (± 0.03)		-0.363	1.21	0.00
♂ Mortality rates after age 1 - 10 %	8 (± 0.04)		-0.235	0.04	3.89
♂ Mortality rates after age 1 - 5 %	13 (± 0.04)		-0.240	0.08	3.18
Base ♂ Mortality rates after age 1	18 (± 0.04)		-0.247	.	2.94
♂ Mortality rates after age 1 + 5 %	23 (± 0.04)		-0.256	0.16	2.73
♂ Mortality rates after age 1 + 10 %	28 (± 0.04)		-0.266	-0.20	2.51
Initial population size - 25 %	358	80	-0.246	0.01	3.43
Base initial population size	477	106	-0.247	.	2.94
Initial population size + 25 %	596	133	-0.248	0.02	3.26
Annual increase in habitat loss 0 %	0 %		-0.247	0.00	3.19
Base annual increase in habitat loss	1 %		-0.247	.	2.94
Annual increase in habitat loss + 1 %	2 %		-0.247	0.00	2.90
Annual increase in habitat loss + 3 %	4 %		-0.246	0.01	2.88

Table 5.4. The number of Maui Parrotbill needed from the east, west, and captive populations for reintroduction efforts across three-year reintroduction scenarios.

	Reintroduction Scenarios:	Wild Females to Captivity (Year 1)	Captive Females to Nakula (per year x 3)	East Females to Nakula (per year x 3)	West Females to Nakula (per year x 3)
i.	Release only captive birds currently available to establish a second population	0	2	0	0
ii.	Augment the captive flock with wild birds such that the captive flock would source a second population	79	6	0	0
iii.	Augment the captive flock with wild birds such that the captive flock would provide ½ the individuals needed for reintroduction with the other half from translocating wild individuals	36	3	2	1
iv.	Augment the captive flock with wild birds such that the captive flock would provide 1 female per year in combination with translocating wild individuals	7	1	3	2
v.	Release only captive birds currently available in combination with wild translocations to establish a second population	0	2	3	1
vi.	Release wild translocated individuals to establish a second population	0	0	4	2

Chapter 6 Synopsis

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6.1 SUMMARY OF KEY FINDINGS

The biodiversity of the Hawaiian Islands have suffered widespread extinctions. Consequently, they have lost over half of their native forest birds. The Maui Parrotbill (Kiwikiu; *Pseudonestor xanthophrys*) is a critically endangered Hawaiian honeycreeper endemic to the island of Maui. These birds are restricted to a single population of ~500 individuals located in remote wet rainforest on windward east Maui. This thesis is aimed at providing information needed to recover this species and to interrupt its path towards extinction. Prior to this research, parrotbills were a largely understudied species with little known about their ecology. To this end, this thesis synthesizes the best data available in order to increase our knowledge about the biology of parrotbills.

Through an intensive field effort studying fecundity, I provide estimates of annual productivity for Maui Parrotbills (46 %). I found that determining annual reproductive success by monitoring fledglings and calculating nest success through nest monitoring, both yielded similar results. More enlightening than the actual estimate, were the productivity limitations that were revealed. The limitations imposed by weather patterns on the windward slope were particularly worrisome. Parrotbill nests most often failed in heavy rain and storm events. While birds were observed to re-nest up to two additional times after failures, these events are common in the forest area to which species has been restricted to and not indicative of Hawaiian forests as a whole.

The same field efforts tracking parrotbills to estimate productivity provided data on adult and juvenile survival. These data were combined with previously collected field data to construct an 18-year encounter history for the species. A difference in survival rates between sexes was strongly supported; 0.72 ± 0.04 for adult females and 0.82 ± 0.03 for adult males. This difference may be a reflection of either reproductive costs or additional risks of incubation and brooding, such as depredation. Additionally, I found support for age- biased survival (juvenile survival = 0.17 ± 0.15; adults = 0.78 ± 0.02), but had very limited information for juveniles ($N_{juvenile}$ = 10, N_{adult} = 136).

With a population of approximately 500 individuals restricted to 50 km^2 of habitat, Maui Parrotbill are at high risk of extinction. The recovery of this species will likely require intensive hands-on management. Based on these demographic data, this can be expected to involve reintroductions to additional and/or more suitable habitat areas. A thorough understanding of all components of a species' biology, including genetic diversity and structure, are required to maximize the likelihood of success in such reintroductions.

Examining differences in genetic diversity (calculated through effective population sizes) amongst contemporary and historical parrotbill populations, this thesis highlights the impact that introduced disease had on this species just before the turn of the century. Contemporary genetic diversity in parrotbill was low (global F_{st} = 0.056), and there was a 96% reduction in genetic effective population size between contemporary and historical samples. Furthermore, measures of population differentiation (pairwise F_{st} and R_{st}) suggest a fragmented genetic structure with compromised gene flow within the global population. The distribution of the two major segments of the current population suggests that current genetic structure may be the result of a topographic barrier, an erosional depression, the Koolau Gap.

Combining this new information on Maui Parotbills, I built a comprehensive population viability model. The current population demographics predicted extinction of the species within 25 years. Among the top components driving this decline in our evaluation were female mortality and productivity.

Unfortunately for managers, techniques available to mitigate the threats to female survival and productivity in the wild are limited if not impossible.

Alternatively, there is historically occupied habitat currently being restored on leeward east Maui for the benefit of Maui Parrotbill and other imperiled Hawaiian biota on Maui. The evaluation of management options suggests that parrotbills' success and viability in this new habitat will hinge on the birds' demographic plasticity and their ability to increase productivity and survival values there. This habitat is a mesic forest that appreciates lower annual rainfall forest than the habitat in the birds' current range. While considering translocating both captive breeding and wild individuals as management options, this model provides managers with benchmark values for fecundity and survival that will need to be met amongst the new population in order for a reintroduction to be successful and sustainable.

6.2 RECOMMENDATIONS FOR CONSERVATION MANAGEMENT OF THE KIWIKIU AND OTHER ENDEMIC HAWAIIAN HONEYCREEPERS ON MAUI

The purpose of this thesis is to provide managers with the information necessary to prevent the extinction of Maui Parrotbill and to facilitate the development of practical recovery strategies and techniques. As with most Hawaiian Honeycreepers, parrotbills are surviving in a fragment of the habitat that makes up a fragment of their former historical range. The combined impacts of habitat destruction and introduced threats (i.e. mammalian predators, ungulates, invasive plants, and disease) have all drastically reduced the forest habitats available to these birds.

While habitat reclamation and restoration is possible under normal circumstances, the introduction of non-native disease, avian malaria (*Plasmodium relictum*) and its vector, mosquitoes (*Culex quinquefasciatus),* make lowland forests uninhabitable by native forest birds. There is hope for the future with some species of Hawaiian Honeycreepers showing signs of disease resistance (Woodworth *et al.* 2005; Woodworth and Pratt 2009). Unfortunately, however, the most critically endangered species in Hawaii may not have the time or the genetic diversity necessary to persist in the face of these threats. Without intervention, extinction for these critically endangered species may be imminent.

While the disease-free high elevation refugia on Maui and Hawaii Island are perhaps the best places for preserving Hawaii's avian biodiversity, the windward forests on Maui may not be the most suitable habitat. It is not clear from historical accounts of Hawaiian passerines (Perkins 1903) whether these species actually preferred drier more mesic forest areas over the wet forests they are currently inhabit; or whether the few accessible areas to which these early naturalists could observe the birds happened to be mesic forest areas. Despite the limited historical data, the demographic studies on parrotbills presented here do not predict a sustainable population in the future. The small size of the parrotbill population puts the species at a high risk of extinction and coupled with the unsustainable demographic parameters observed in the wild, almost certainly forecasts their extinction.

Management options to mitigate the threats that were found to be most limiting to the parrotbill population do not exist. Reducing predation risks to these birds would perhaps increase survival. However, the native habitat is too mountainous, fragile, and remote to make wide-scale rodent reduction a viable option for managers. Secondly, increasing nest success may also increase productivity. However, with nests failing in heavy weather, there are no management options to counteract the storm patterns in this habitat. Furthermore, the birds have not responded to management techniques that are available in this area like supplemental feeding to try to increase survival and/or productivity.

The combination of all these factors and limitations make moving these birds to a new habitat the most immediately viable option to secure their survival. Reintroductions are widely applied as a conservation tool and should be carefully implemented for Maui Parrotbill. Using the most up-to-date species biology data provided here is the first step towards a successful reintroduction. One of the biggest challenges for this species and other Hawaiian forest birds is the lack of information available about how the birds used or will use habitat areas other than those in which they are currently found. However, subfossil records confirm parrotbills existence in a wide variety of habitats (James and Olson 1991). While I might predict that these birds may demonstrate higher productivity or survival rates in areas with lower predation risk and drier weather patterns, there are no historical demographic data to evaluate this. Additionally, as discussed with the diminished genetic diversity within the species, these birds may not have the capacity to adapt to new environments in the same ways a more diverse species would.

Nonetheless, the low observed levels of genetic diversity should not prevent managers from undertaking a reintroduction of Maui Parrotbill to leeward east Maui. A well planned reintroduction can establish a population with the best genetic structure available. The models presented here provide a benchmark for how to assess the viability of the new population over time once they are on the landscape. The success of the reintroduction will largely hinge upon how plastic these birds still are in their ability to survive in novel habitats after being restricted to the wet forests for some time.

6.3 PROGRESS IN THE NEXT STEPS TO RECOVERY

Using the data presented here, I have identified that a reintroduction to historical habitat on leeward Haleakala is crucial to the long-term recovery of Maui Parrotbill. A Maui Parrotbill Reintroduction Working Group was formed in

2014 to discuss the options for reintroduction. The biggest challenge in selecting a site for a parrotbill reintroduction is that there are no pristine high elevation native forests remaining on Maui outside of the 40-50 km^2 area where the species currently exists. However, a remnant strip of degraded mesic forest still exists on leeward Maui. The Nakula Natural Area Reserve (NAR) on leeward east Maui has been selected as the site for a trial reintroduction in 2017.

Many steps are currently underway to prepare the Nakula NAR for parrotbills by 2017. The first was landscape level fencing and ungulate removal. The native habitat within the reserve is degraded and is expected to require significant restoration before it will be suitable to support a viable parrotbill population. Maui Forest Bird Recovery Project initiated an experimental restoration experiment in 2012 to identify the most efficient and effective method(s) of restoring forest in an approximate 170-ha section of Nakula (see Appendix G). In addition to these experimental plots, landscape-level planting has begun. This is focused especially on planting corridors to connect existing vegetation across the landscape. Many challenges lay ahead for both restoration efforts and reintroduction planning. These include, but are not limited to, controlling weeds, sourcing seeds, herbicide restrictions in a forested landscape, and applying the experimental restoration results to the larger Nakula-Kahikinui area. Despite these challenges, the Maui Parrotbill Reintroduction Working Group has begun to draft a plan for the reintroduction in 2015.

6.4 FUTURE RESEARCH

There will be future research needed on demographics, behavior, and particularly habitat use by the new reintroduced Maui Parrotbill population before we can assure their viability and sustainability into the future. In addition, other areas of Maui, particularly on the leeward and western slopes of Haleakala, should be identified as possible restoration areas to provide more high elevation forest habitats. Hopefully, the lessons learned in the Nakula NAR restoration project will be able to be applied to larger landscape areas of Maui in the future and increase the habitat available for parrotbills and other native honeycreepers on Maui.

While this reintroduction is the next step needed to diminish an immediate extinction risk, it is focused on a single species. There is very limited ecosystem restoration implemented in Hawaii. With so many forest birds already lost, their ecosystem functions have also been lost. Unless these functions are replaced by an introduced non-native species, these ecosystems will never be restored to their original health. Hawaii should invest research into looking at designing translocations outside of species' historical ranges for the conservation of functional Hawaiian forests. Furthermore, the long term viability of high elevation forests on many Hawaiian Islands is unknown. With climate change and diseases encroaching at higher elevations, we need to also look at designing translocations outside of species historical ranges if we are going to conserve them in the future.

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Appendices

Appendix A. Supplementary materials for Chapter 3: Management implications derived from long term re-sight data: annual survival of the Maui Parrotbill (*Pseudonestor xanthophrys*)

Table A.1. Capture histories for male and female Maui Parrotbills 1994-2011

Ages: ASY = After Second Year bird, AHY = After Hatch Year bird, HY = Hatch Year bird

Sexes: F = Female, M = Male, U = Unknown

Site: HR3 = Home Range 3 or more eastern portion of study site, FSB = Frisbee Meadows or more western portion of study site

/* MAPA Data, Recaptures and Resights, 17 occasions, 2 groups
Group 1=Males Group 2=Females */

/*1181-80089	1/8/2011	ASY	F	HR3	*/000000000000000001	0	1
/*1181-80096	1/9/2011	ASY	F	HR3	*/000000000000000001	0	1
/*1371-04807	7/14/1994	AHY	F	FSB	*/110000000000000000	0	1
/*1371-04808	7/16/1994	AHY	M	FSB	*/111000000000000000	1	0
/*1371-04809	7/20/1994	ASY	M	FSB	*/100000000000000000	1	0
/*1371-04810	7/30/1994	AHY	F	FSB	*/110000000000000000	0	1
/*1371-04811	8/2/1994	AHY	M	FSB	*/111100000000010000	1	0
/*1371-04812	8/18/1994	AHY	M	FSB	*/110111110000000000	1	0
/*1371-04813	9/5/1994	AHY	F	FSB	*/110100000000000000	0	1
/*1371-04814	9/7/1994	AHY	M	FSB	*/100000000000000000	1	0
/*1371-04815	2/15/1995	ASY	M	FSB	*/011010000000000000	1	0
/*1371-04816	2/15/1995	AHY	F	FSB	*/011000000000000000	0	1
/*1371-04817	10/5/1994	ASY	F	FSB	*/111100000000000000	0	1
/*1371-04818	2/15/1995	ASY	M	FSB	*/011100000100000000	1	0
/*1371-04819	2/16/1995	ASY	M	FSB	*/011100000000000000	1	0
/*1371-04820	2/22/1995	ASY	F	FSB	*/010000000000000000	0	1
/*1371-04821	7/1/1995	AHY	F	FSB	*/011000000000000000	0	1
/*1371-04822	8/13/1995	AHY	F	FSB	*/011110000000000000	0	1
/*1371-04823	9/6/1995	AHY	F	FSB	*/011100000000000000	0	1
/*1371-04824	9/21/1995	AHY	M	FSB	*/011100000000000000	1	0
/*1371-04825	9/21/1995	AHY	F	FSB	*/010000000000000000	0	1
/*1371-04826	11/1/1995	AHY	M	FSB	*/011100000000000000	1	0
/*1371-04828	1/29/1996	ASY	F	FSB	*/001110000000000000	0	1

/*1371-04829	3/11/1996	AHY	F	FSB	*/001000000000000000	0	1
/*1371-04830	7/1/1996	AHY	F	FSB	*/001000000000000000	0	1
/*1371-04831	2/19/1997	AHY	M	FSB	*/000100000000000000	1	0
/*1371-04903	3/31/1996	ASY	M	HR3	*/001000000000000000	1	0
/*1371-04906	4/3/1996	ASY	M	HR3	*/001100000000000000	1	0
/*1371-04907	4/3/1996	SY	M	HR3	*/001000000000000000	1	0
/*1371-04915	2/17/1997	ASY	M	HR3	*/000110000000000000	1	0
/*1371-04916	2/17/1997	ASY	F	HR3	*/000100000000000000	0	1
/*1371-04918	2/20/1997	ASY	F	HR3	*/000100000000000000	0	1
/*1371-04919	2/20/1997	ASY	M	HR3	*/000100000000000000	1	0
/*1371-04920	2/20/1997	SY	M	HR3	*/000100000000000000	1	0
/*1371-04921	2/20/1997	ASY	M	HR3	*/000100000000000000	1	0
/*1371-04922	2/20/1997	AHY	F	HR3	*/000100000000000111	0	1
/*1371-04929	12/11/1997	AHY	M	HR1	*/000100000000000001	1	0
/*1371-04932	12/14/1997	AHY	M	HR1	*/000100000100000010	1	0
/*1371-04933	1/13/1998	SY	F	HR3	*/000010000000000000	0	1
/*1371-04934	1/13/1998	AHY	F	HR3	*/000011000000000000	0	1
/*1371-04937	2/6/1998	AHY	M	HR3	*/000011010100000000	1	0
/*1371-04938	2/6/1998	AHY	F	HR3	*/000010000000000000	0	1
/*1371-04939	2/8/1998	AHY	M	HR3	*/000010000000000101	1	0
/*1371-04940	2/11/1998	SY	F	HR3	*/000011000000000000	0	1
/*1371-04941	2/21/1998	AHY	F	HR3	*/000011000000000000	0	1
/*1371-04942	2/21/1998	AHY	M	HR3	*/000011010011000000	1	0
/*1371-04944	4/4/1998	AHY	F	HR3	*/000010000000000000	0	1
/*1371-04947	6/12/2001	AHY	M	FSB	*/000000000000000100	1	0
/*1371-04948	1/8/2002	SY	F	HR3	*/000000010000000000	0	1
/*1371-04949	1/11/2002	ASY	M	HR3	*/000000010000000000	1	0
/*1371-04950	1/11/2002	ASY	F	HR3	*/000000010111000000	0	1
/*1371-04951	1/14/2002	ASY	F	HR3	*/000000010000000000	0	1
/*1371-04952	1/14/2002	ASY	M	HR3	*/000000010111110000	1	0
/*1371-04953	1/14/2002	SY	F	HR3	*/000000010000000000	0	1
/*1371-04954	1/25/2002	ASY	F	HR3	*/000000010110000000	0	1
/*1371-04955	2/5/2002	AHY	F	HR3	*/000000010110000000	0	1
/*1371-04956	2/4/2003	SY	M	HR1	*/000000000100000000	1	0
/*1371-04959	5/27/2003	AHY	M	HR3	*/000000000100000000	1	0
/*1371-04960	10/22/2003	AHY	M	HR3	*/000000000111111111	1	0

```
/*1371-04961  10/24/2003  AHY  M  HR3  */000000000100000000  1  0
/*1371-04962  10/25/2003  AHY  F  HR3  */000000000111111100  0  1
/*1371-04963  10/26/2003  AHY  F  HR3  */000000000110100000  0  1
/*1371-04964  10/31/2003  SY   F  HR3  */000000000100000000  0  1
/*1371-04967  3/12/2004   SY   F  HR3  */000000000010000000  0  1
/*1371-04968  3/12/2004   SY   M  HR3  */000000000011110000  1  0
/*1371-04969  3/13/2004   SY   F  HR3  */000000000010000000  0  1
/*1371-04970  3/13/2004   ASY  M  HR3  */000000000010000000  1  0
/*1371-04971  3/13/2004   ASY  F  HR3  */000000000011111111  0  1
/*1371-04976  11/18/2006  AHY  F  FSB  */000000000000110000  0  1
/*1371-04977  11/18/2006  AHY  M  FSB  */000000000000100000  1  0
/*1371-04978  12/4/2006   AHY  M  FSB  */000000000000100000  1  0
/*1371-04979  1/17/2007   ASY  M  HR3  */000000000000010000  1  0
/*1371-04980  1/17/2007   ASY  F  HR3  */000000000000011010  0  1
/*1371-04981  1/20/2007   ASY  F  HR3  */000000000000010000  0  1
/*1371-04982  1/20/2007   ASY  M  HR3  */000000000000011111  1  0
/*1371-04983  2/3/2007    AHY  F  HR3  */000000000000011110  0  1
/*1371-04984  2/4/2007    SY   M  HR3  */000000000000010001  1  0
/*1371-04985  2/4/2007    SY   F  HR3  */000000000000010000  0  1
/*1371-04986  5/4/2007    SY   F  HR3  */000000000000010110  0  1
/*1371-04987  5/4/2007    ASY  M  HR3  */000000000000010110  1  0
/*1371-04988  11/17/2006  AHY  M  FSB  */000000000000100000  1  0
/*1371-04989  5/21/2003   AHY  M  HR3  */000000000100110110  1  0
/*1401-47511  11/16/2007  AHY  F  FSB  */000000000000010000  0  1
/*1401-47512  11/16/2007  AHY  M  FSB  */000000000000011000  1  0
/*1401-47513  11/18/2007  AHY  M  FSB  */000000000000010110  1  0
/*1401-47514  11/19/2007  AHY  M  FSB  */000000000000011000  1  0
/*1401-47515  11/30/2007  AHY  F  FSB  */000000000000011100  0  1
/*1401-47516  12/1/2007   AHY  M  FSB  */000000000000011100  1  0
/*1401-47517  12/2/2007   AHY  M  FSB  */000000000000011111  1  0
/*1401-47518  2/27/2008   SY   F  FSB  */000000000000001000  0  1
/*1401-47519  2/27/2008   AHY  F  FSB  */000000000000001000  0  1
/*1401-47520  4/24/2008   ASY  M  HR3  */000000000000001000  1  0
/*1401-47521  4/25/2008   AHY  M  HR3  */000000000000001110  1  0
/*1401-47523  2/24/2008   ASY  F  HR3  */000000000000001100  0  1
/*1401-47525  4/30/2008   AHY  F  FSB  */000000000000001101  0  1
```

/*1401-47528	12/7/2008	AHY	F	FSB	*/000000000000001100	0	1
/*1401-47530	12/7/2008	AHY	M	FSB	*/000000000000001000	1	0
/*1401-47531	12/9/2008	AHY	M	FSB	*/000000000000001111	1	0
/*1401-47532	12/9/2008	AHY	F	FSB	*/000000000000001100	0	1
/*1401-47533	1/30/2009	AHY	M	HR3	*/000000000000000110	1	0
/*1401-47534	1/30/2009	AHY	F	HR3	*/000000000000000111	0	1
/*1401-47535	1/27/2009	AHY	M	HR3	*/000000000000000111	1	0
/*1401-47536	2/16/2009	AHY	F	FSB	*/000000000000000100	0	1
/*1401-47537	3/19/2009	AHY	M	HR3	*/000000000000000110	1	0
/*1401-47539	8/17/2009	AHY	F	HR3	*/000000000000000100	0	1
/*1401-47540	4/16/2009	AHY	F	FSB	*/000000000000000111	0	1
/*1401-47541	4/16/2009	ASY	M	FSB	*/000000000000000111	1	0
/*1401-47548	11/24/2009	AHY	F	HR3	*/000000000000000110	0	1
/*1401-47549	12/4/2009	AHY	F	FSB	*/000000000000000111	0	1
/*1401-47550	12/4/2009	AHY	F	FSB	*/000000000000000111	0	1
/*1401-47551	12/8/2009	SY	M	FSB	*/000000000000000100	1	0
/*1401-47552	1/14/2010	AHY	F	HR3	*/000000000000000011	0	1
/*1401-47553	1/16/2010	ASY	M	HR3	*/000000000000000010	1	0
/*1401-47555	1/26/2010	ASY	F	FSB	*/000000000000000011	0	1
/*1401-47556	1/26/2010	ASY	M	FSB	*/000000000000000010	1	0
/*1401-47557	1/27/2010	ASY	M	FSB	*/000000000000000011	1	0
/*1401-47558	1/31/2010	ASY	M	FSB	*/000000000000000011	1	0
/*1401-47559	2/21/2010	ASY	M	FSB	*/000000000000000011	1	0
/*1401-47560	2/23/2010	ASY	M	FSB	*/000000000000000011	1	0
/*1401-47561	2/23/2010	ASY	M	FSB	*/000000000000000011	1	0
/*1401-47566	7/26/2010	AHY	M	FSB	*/000000000000000011	1	0
/*1401-47587	3/2/2011	AHY	M	HR3	*/000000000000000001	1	0
/*1541-80268	6/17/2011	ASY	M	FSB	*/000000000000000001	1	0
/*1541-80269	6/19/2011	ASY	M	FSB	*/000000000000000001	1	0
/*1541-80270	6/19/2011	ASY	F	FSB	*/000000000000000001	0	1
/*1541-80271	6/22/2011	ASY	M	FSB	*/000000000000000001	1	0
/*1791-16901	5/3/2011	ASY	F	HR3	*/000000000000000001	0	1
/*1791-16902	6/14/2011	AHY	F	HR3	*/000000000000000001	0	1
/*1791-16904	6/15/2011	ASY	M	HR3	*/000000000000000001	1	0
/*1821-17705	3/3/2011	SY	M	FSB	*/000000000000000001	1	0
/*1871-17701	2/27/2011	ASY	M	FSB	*/000000000000000001	1	0

```
/*1871-17702  3/1/2011    ASY  F  FSB  */000000000000000001  0  1
/*1871-17703  3/2/2011    ASY  M  FSB  */000000000000000001  1  0
/*1871-17704  3/2/2011    ASY  M  FSB  */000000000000000001  1  0
/*1871-17708  3/15/2011   AHY  F  FSB  */000000000000000001  0  1
/*1871-17709  4/28/2011   ASY  M  FSB  */000000000000000001  1  0
```

Table A.2. Capture histories for adult and juvenile Maui Parrotbills 1994-2011

```
/* MAPA Data, Recaptures and Resights, 17 occasions, 2 groups
 Group 1=HY Group 2=Adult */

/*1181-80089  1/8/2011    ASY  F  HR3  */000000000000000001  0  1
/*1181-80096  1/9/2011    ASY  F  HR3  */000000000000000001  0  1
/*1371-04807  7/14/1994   AHY  F  FSB  */110000000000000000  0  1
/*1371-04808  7/16/1994   AHY  M  FSB  */111000000000000000  0  1
/*1371-04809  7/20/1994   ASY  M  FSB  */100000000000000000  0  1
/*1371-04810  7/30/1994   AHY  F  FSB  */110000000000000000  0  1
/*1371-04811  8/2/1994    AHY  M  FSB  */111100000000010000  0  1
/*1371-04812  8/18/1994   AHY  M  FSB  */110111110000000000  0  1
/*1371-04813  9/5/1994    AHY  F  FSB  */110100000000000000  0  1
/*1371-04814  9/7/1994    AHY  M  FSB  */100000000000000000  0  1
/*1371-04815  2/15/1995   ASY  M  FSB  */011010000000000000  0  1
/*1371-04816  2/15/1995   AHY  F  FSB  */011000000000000000  0  1
/*1371-04817  10/5/1994   ASY  F  FSB  */111100000000000000  0  1
/*1371-04818  2/15/1995   ASY  M  FSB  */011100000100000000  0  1
/*1371-04819  2/16/1995   ASY  M  FSB  */011100000000000000  0  1
/*1371-04820  2/22/1995   ASY  F  FSB  */010000000000000000  0  1
/*1371-04821  7/1/1995    AHY  F  FSB  */011000000000000000  0  1
/*1371-04822  8/13/1995   AHY  F  FSB  */011110000000000000  0  1
/*1371-04823  9/6/1995    AHY  F  FSB  */011100000000000000  0  1
/*1371-04824  9/21/1995   AHY  M  FSB  */011100000000000000  0  1
/*1371-04825  9/21/1995   AHY  F  FSB  */010000000000000000  0  1
/*1371-04826  11/1/1995   AHY  M  FSB  */011100000000000000  0  1
/*1371-04827  1/26/1996   HY   U  FSB  */001000000000000000  1  0
/*1371-04828  1/29/1996   ASY  F  FSB  */001110000000000000  0  1
/*1371-04829  3/11/1996   AHY  F  FSB  */001000000000000000  0  1
```

/*1371-04830	7/1/1996	AHY	F	FSB	*/00100000000000000	0	1
/*1371-04831	2/19/1997	AHY	M	FSB	*/00010000000000000	0	1
/*1371-04903	3/31/1996	ASY	M	HR3	*/00100000000000000	0	1
/*1371-04906	4/3/1996	ASY	M	HR3	*/00110000000000000	0	1
/*1371-04907	4/3/1996	SY	M	HR3	*/00100000000000000	0	1
/*1371-04908	4/29/1996	HY	F	HR1	*/00110000000000000	1	0
/*1371-04915	2/17/1997	ASY	M	HR3	*/00011000000000000	0	1
/*1371-04916	2/17/1997	ASY	F	HR3	*/00010000000000000	0	1
/*1371-04918	2/20/1997	ASY	F	HR3	*/00010000000000000	0	1
/*1371-04919	2/20/1997	ASY	M	HR3	*/00010000000000000	0	1
/*1371-04920	2/20/1997	SY	M	HR3	*/00010000000000000	0	1
/*1371-04921	2/20/1997	ASY	M	HR3	*/00010000000000000	0	1
/*1371-04922	2/20/1997	AHY	F	HR3	*/00010000000000111	0	1
/*1371-04923	5/21/1997	HY	M	HR3	*/00010000000000000	1	0
/*1371-04929	12/11/1997	AHY	M	HR1	*/00010000000000001	0	1
/*1371-04932	12/14/1997	AHY	M	HR1	*/00010000010000010	0	1
/*1371-04933	1/13/1998	SY	F	HR3	*/00001000000000000	0	1
/*1371-04934	1/13/1998	AHY	F	HR3	*/00001100000000000	0	1
/*1371-04937	2/6/1998	AHY	M	HR3	*/00001100110000000	0	1
/*1371-04938	2/6/1998	AHY	F	HR3	*/00001000000000000	0	1
/*1371-04939	2/8/1998	AHY	M	HR3	*/00001000000000101	0	1
/*1371-04940	2/11/1998	SY	F	HR3	*/00001100000000000	0	1
/*1371-04941	2/21/1998	AHY	F	HR3	*/00001100000000000	0	1
/*1371-04942	2/21/1998	AHY	M	HR3	*/00001100101100000	0	1
/*1371-04944	4/4/1998	AHY	F	HR3	*/00001000000000000	0	1
/*1371-04947	6/12/2001	AHY	M	FSB	*/00000000000000100	0	1
/*1371-04948	1/8/2002	SY	F	HR3	*/00000000100000000	0	1
/*1371-04949	1/11/2002	ASY	M	HR3	*/00000000100000000	0	1
/*1371-04950	1/11/2002	ASY	F	HR3	*/00000000111100000	0	1
/*1371-04951	1/14/2002	ASY	F	HR3	*/00000000100000000	0	1
/*1371-04952	1/14/2002	ASY	M	HR3	*/00000000111111000	0	1
/*1371-04953	1/14/2002	SY	F	HR3	*/00000000100000000	0	1
/*1371-04954	1/25/2002	ASY	F	HR3	*/00000000111000000	0	1
/*1371-04955	2/5/2002	AHY	F	HR3	*/00000000111000000	0	1
/*1371-04956	2/4/2003	SY	M	HR1	*/00000000010000000	0	1
/*1371-04959	5/27/2003	AHY	M	HR3	*/00000000010000000	0	1

```
/*1371-04960  10/22/2003   AHY   M    HR3   */000000000111111111      0    1
/*1371-04961  10/24/2003   AHY   M    HR3   */000000000100000000      0    1
/*1371-04962  10/25/2003   AHY   F    HR3   */000000000111111100      0    1
/*1371-04963  10/26/2003   AHY   F    HR3   */000000000110100000      0    1
/*1371-04964  10/31/2003   SY    F    HR3   */000000000100000000      0    1
/*1371-04966  3/10/2004    HY    M    HR3   */      00000000010000000 1    0
/*1371-04967  3/12/2004    SY    F    HR3   */000000000010000000      0    1
/*1371-04968  3/12/2004    SY    M    HR3   */000000000011110000      0    1
/*1371-04969  3/13/2004    SY    F    HR3   */000000000010000000      0    1
/*1371-04970  3/13/2004    ASY   M    HR3   */000000000010000000      0    1
/*1371-04971  3/13/2004    ASY   F    HR3   */000000000011111111      0    1
/*1371-04975  6/10/2006    HY    U    HR3   */000000000000100000      1    0
/*1371-04976  11/18/2006   AHY   F    FSB   */000000000000110000      0    1
/*1371-04977  11/18/2006   AHY   M    FSB   */000000000000100000      0    1
/*1371-04978  12/4/2006    AHY   M    FSB   */000000000000100000      0    1
/*1371-04979  1/17/2007    ASY   M    HR3   */000000000000010000      0    1
/*1371-04980  1/17/2007    ASY   F    HR3   */000000000000011010      0    1
/*1371-04981  1/20/2007    ASY   F    HR3   */000000000000010000      0    1
/*1371-04982  1/20/2007    ASY   M    HR3   */000000000000011111      0    1
/*1371-04983  2/3/2007     AHY   F    HR3   */000000000000011110      0    1
/*1371-04984  2/4/2007     SY    M    HR3   */000000000000010001      0    1
/*1371-04985  2/4/2007     SY    F    HR3   */000000000000010000      0    1
/*1371-04986  5/4/2007     SY    F    HR3   */000000000000010110      0    1
/*1371-04987  5/4/2007     ASY   M    HR3   */000000000000010110      0    1
/*1371-04988  11/17/2006   AHY   M    FSB   */000000000000100000      0    1
/*1371-04989  5/21/2003    AHY   M    HR3   */000000000100110110      0    1
/*1401-47511  11/16/2007   AHY   F    FSB   */000000000000010000      0    1
/*1401-47512  11/16/2007   AHY   M    FSB   */000000000000011000      0    1
/*1401-47513  11/18/2007   AHY   M    FSB   */000000000000010110      0    1
/*1401-47514  11/19/2007   AHY   M    FSB   */000000000000011000      0    1
/*1401-47515  11/30/2007   AHY   F    FSB   */000000000000011100      0    1
/*1401-47516  12/1/2007    AHY   M    FSB   */000000000000011100      0    1
/*1401-47517  12/2/2007    AHY   M    FSB   */000000000000011111      0    1
/*1401-47518  2/27/2008    SY    F    FSB   */000000000000001000      0    1
/*1401-47519  2/27/2008    AHY   F    FSB   */000000000000001000      0    1
/*1401-47520  4/24/2008    ASY   M    HR3   */000000000000001000      0    1
```

/*1401-47521	4/25/2008	AHY	M	HR3	*/0000000000000001110	0	1
/*1401-47523	2/24/2008	ASY	F	HR3	*/0000000000000001100	0	1
/*1401-47525	4/30/2008	AHY	F	FSB	*/0000000000000001101	0	1
/*1401-47526	9/12/2008	HY	M	FSB	*/0000000000000001000	1	0
/*1401-47527	12/5/2008	HY	U	FSB	*/0000000000000001000	1	0
/*1401-47528	12/7/2008	AHY	F	FSB	*/0000000000000001100	0	1
/*1401-47530	12/7/2008	AHY	M	FSB	*/0000000000000001000	0	1
/*1401-47531	12/9/2008	AHY	M	FSB	*/0000000000000001111	0	1
/*1401-47532	12/9/2008	AHY	F	FSB	*/0000000000000001100	0	1
/*1401-47533	1/30/2009	AHY	M	HR3	*/0000000000000000110	0	1
/*1401-47534	1/30/2009	AHY	F	HR3	*/0000000000000000111	0	1
/*1401-47535	1/27/2009	AHY	M	HR3	*/0000000000000000111	0	1
/*1401-47536	2/16/2009	AHY	F	FSB	*/0000000000000000100	0	1
/*1401-47537	3/19/2009	AHY	M	HR3	*/0000000000000000110	0	1
/*1401-47539	8/17/2009	AHY	F	HR3	*/0000000000000000100	0	1
/*1401-47540	4/16/2009	AHY	F	FSB	*/0000000000000000111	0	1
/*1401-47541	4/16/2009	ASY	M	FSB	*/0000000000000000111	0	1
/*1401-47547	11/24/2009	HY	U	HR3	*/0000000000000000100	1	0
/*1401-47548	11/24/2009	AHY	F	HR3	*/0000000000000000110	0	1
/*1401-47549	12/4/2009	AHY	F	FSB	*/0000000000000000111	0	1
/*1401-47550	12/4/2009	AHY	F	FSB	*/0000000000000000111	0	1
/*1401-47551	12/8/2009	SY	M	FSB	*/0000000000000000100	0	1
/*1401-47552	1/14/2010	AHY	F	HR3	*/0000000000000000011	0	1
/*1401-47553	1/16/2010	ASY	M	HR3	*/0000000000000000010	0	1
/*1401-47555	1/26/2010	ASY	F	FSB	*/0000000000000000011	0	1
/*1401-47556	1/26/2010	ASY	M	FSB	*/0000000000000000010	0	1
/*1401-47557	1/27/2010	ASY	M	FSB	*/0000000000000000011	0	1
/*1401-47558	1/31/2010	ASY	M	FSB	*/0000000000000000011	0	1
/*1401-47559	2/21/2010	ASY	M	FSB	*/0000000000000000011	0	1
/*1401-47560	2/23/2010	ASY	M	FSB	*/0000000000000000011	0	1
/*1401-47561	2/23/2010	ASY	M	FSB	*/0000000000000000011	0	1
/*1401-47566	7/26/2010	AHY	M	FSB	*/0000000000000000011	0	1
/*1401-47587	3/2/2011	AHY	M	HR3	*/0000000000000000001	0	1
/*1541-80268	6/17/2011	ASY	M	FSB	*/0000000000000000001	0	1
/*1541-80269	6/19/2011	ASY	M	FSB	*/0000000000000000001	0	1
/*1541-80270	6/19/2011	ASY	F	FSB	*/0000000000000000001	0	1

/*1541-80271	6/22/2011	ASY	M	FSB	*/000000000000000001	0	1
/*1791-16901	5/3/2011	ASY	F	HR3	*/000000000000000001	0	1
/*1791-16902	6/14/2011	AHY	F	HR3	*/000000000000000001	0	1
/*1791-16904	6/15/2011	ASY	M	HR3	*/000000000000000001	0	1
/*1791-16905	6/16/2011	HY	U	HR3	*/000000000000000001	1	0
/*1791-16906	6/15/2011	HY	U	HR3	*/000000000000000001	1	0
/*1821-17705	3/3/2011	SY	M	FSB	*/000000000000000001	0	1
/*1871-17701	2/27/2011	ASY	M	FSB	*/000000000000000001	0	1
/*1871-17702	3/1/2011	ASY	F	FSB	*/000000000000000001	0	1
/*1871-17703	3/2/2011	ASY	M	FSB	*/000000000000000001	0	1
/*1871-17704	3/2/2011	ASY	M	FSB	*/000000000000000001	0	1
/*1871-17708	3/15/2011	AHY	F	FSB	*/000000000000000001	0	1
/*1871-17709	4/28/2011	ASY	M	FSB	*/000000000000000001	0	1

Table A.3. Maui Parrotbill individuals included in the MARK analyses 1994-2011. Banded represents birds marked during that year, re-sighted includes both observed and recaptured individuals.

Year	Banded birds	Re-sighted birds	Total Encounters
1994	9	0	9
1995	11	7	18
1996	8	12	26
1997	11	15	36
1998	9	10	22
1999	0	13	13
2000	0	4	6
2001	1	0	1
2002	8	4	12
2003	8	10	21
2004	6	12	19
2005	0	7	7
2006	5	7	12
2007	16	8	24
2008	12	11	23
2009	13	21	35
2010	10	23	33
2011	19	16	33
Total	146	180	326

Table A.4. Goodness of fit results for all tests runs in Program RELEASE (within Program MARK).

	x^2	df	p-value
Sex-Specific Model			
TEST 1	15.046	29.000	0.985
TEST 2	34.895	22.000	0.040
TEST 3	16.425	35.000	0.997
TEST 2 + TEST 3	51.320	57.000	0.687
Age-Specific Model			
TEST 1	5.238	7.000	0.631
TEST 2	40.592	13.000	0.000
TEST 3	15.572	22.000	0.837
TEST 2 + TEST 3	56.164	35.000	0.013

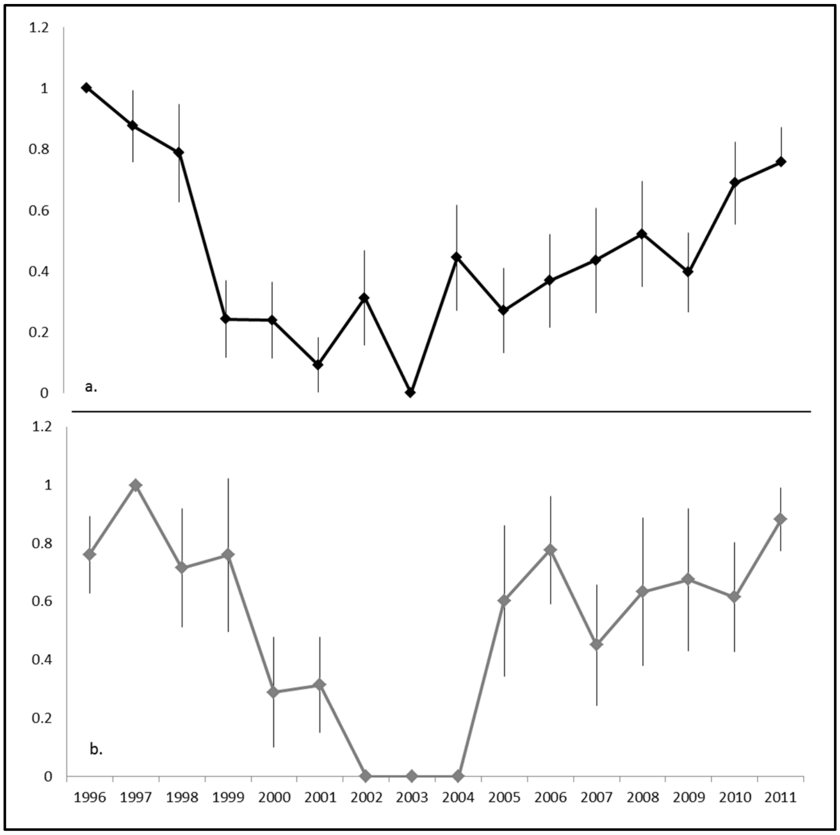

Figure A.1. Detection probability for male (a) and female (b) adult Maui Parrotbills over time. Error bars indicate standard error for each year 1996-2011. In model $\Phi_{sex.}\rho_{sex}$ model (QAICc weight = 0.000), males had a detection probability of 0.457 (± 0.043) and females 0.666 (± 0.060).

Appendix B. Supplementary materials for Chapter 4: Spatial genetic architecture of the critically-endangered Maui Parrotbill (*Pseudonestor xanthophrys*): management considerations for reintroduction strategies

Table B.1. Historical Maui Parrotbill museum sample information from American Museum of Natural History, New York, USA (AMNH), Museum of Comparative Zoology, Cambridge, MA, USA (MCZ), Cambridge University Museum of Zoology, Cambridge, UK (CMZ), British Museum of Natural History, London, UK (BMNH UK), Bernice Pauahi Bishop Museum, Honolulu, USA (BMNH US), and Smithsonian National Museum of Natural History, Washington DC, US (USNM). Museum specimens were collected across the period from 1892-1901. All specimens were presumed to be collected from the western-most region of the parrotbill's range on Haleakala as per collectors' notes on locations.

Museum	Sample ID	Date of Collection	Collector	Location	Age	Sex
AMNH	193408	05/1896	Perkins	Haleakala	AHY	Male
AMNH	193409	6/17/1901	Henshaw	Olinda	AHY	Female
AMNH	453556	10/1896	Palmer	Haleakala	AHY	Male
AMNH	453557	08/4/1892	Palmer		AHY	Male
AMNH	453558	08/3/1892	Palmer		AHY	Male
AMNH	453559	08/16/1892	Palmer		AHY	Male
AMNH	453560	08/15/1892	Palmer		AHY	Female
AMNH	453561	08/3/1892	Palmer		AHY	Female
AMNH	453562	08/03/1892	Palmer		AHY	Unknown
AMNH	453563		Palmer		AHY	Female
MCZ	134719	1894	Perkins		AHY	Male
MCZ	47905	1896	Perkins		AHY	Male

CMZ	27/DRE/9/a/1	1894	Perkins		AHY	Female
CMZ	27/DRE/9/a/2	1896	Perkins		AHY	Male
CMZ	27/DRE/9/a/3	1896	Perkins		AHY	Female
CMZ	27/DRE/9/a/4	1896	Perkins		HY	Male
CMZ	27/DRE/9/a/5	1896	Perkins		SY	Male
BMNH UK	1939.12.9.53	1892	Rothschild		AHY	Male
BMNH UK	1939.12.9.57	08/05/1892	Rothschild		HY	Unknown
BMNH US	95.7.20.170	1894	Perkins		AHY	Male
BMNH US	95.7.20.171	1894	Perkins		AHY	Female
BMNH US	97.10.28.22	1896	Perkins		AHY	Female
BMNH US	97.10.28.23	1896	Perkins		AHY	Male
BMNH US	97.10.28.24	1896	Perkins		SY	Female
BMNH US	97.10.28.25	1896	Perkins		AHY	Male
USNM	177971	1901	Henshaw	Ukulele	AHY	Male
USNM	177972	1901	Henshaw	Ukulele	AHY	Female

Table B.2. Average allelic and private allelic richness using rarefaction technique over all loci. Includes results for 10, 20, 50, and 110 genes based on microsatellite data across the east (*n*=110), west (*n*=19), and captive (*n*=11) Maui Parrotbill (*Pseudonestor xanthophrys*) populations.

Allelic Richness	**10 genes**	**20 genes**	**50 genes**	**110 genes**
Captive	3.27	3.82	3.83	3.83
East	3.74	4.71	5.83	6.66
West	3.04	3.72	4.08	4.08
Private Allelic Richness				
Captive	0.47	0.28	0.09	0.00
East	0.71	0.89	1.47	2.10
West	0.45	0.43	0.35	0.26

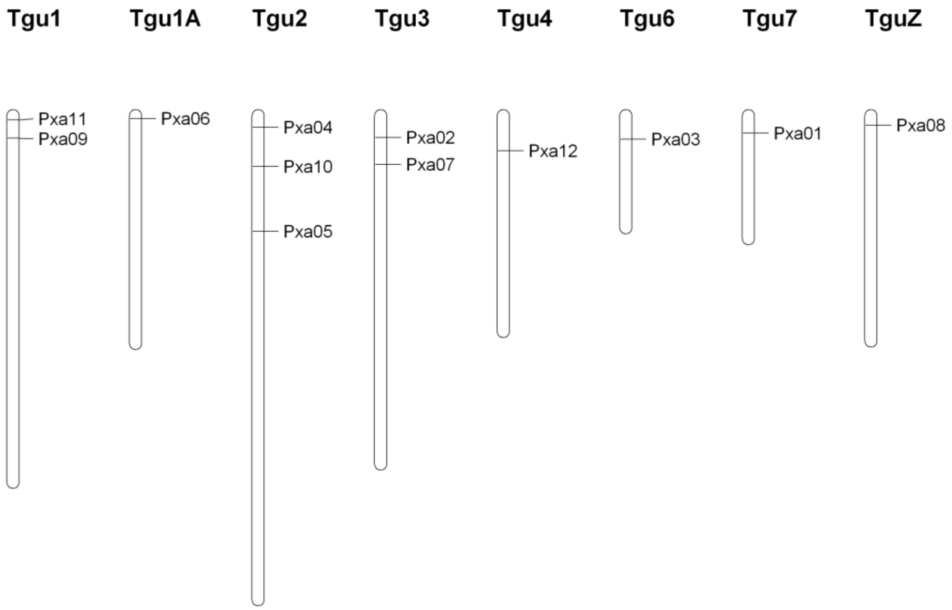

Figure B.1. Chromosome locations in the zebra finch (*Taeniopygia guttata*) genone of the 12 microsatellite loci characterized in the Maui Parrotbill (*Pseudonestor xanthophrys*) compiled by the National Library of Medicine, http://www.ncbi.nlm.nih.gov/blast/ Blast.cgi?PAGE_TYPE=BlastSearch&PROG_DEF=blastn&BLAST_PROG_DEF=megaBlast&BLAST_SPEC=OGP__59729__12898).

Appendix C. Supplementary materials for Chapter 5: Using population viability analysis to model extinction risk and recovery options for a critically endangered Hawaiian honeycreeper: Maui Parrotbill (Kiwikiu, *Pseudonestor xanthophrys*)

MAPA PVA Model Code in Program R

```
It = 1000
yrs=25

#Model parameters for Pops 1 & 2 (East & West)
F=0.588        #Annual fecundity ((0.95*1)+(0.05*2)
SDF=F*0.15     #SD Annual fecundity
AS=0.72        #Adult female survival from Mounce et al. 2014
SDAS=0.02      #SD Adult female survival
IS=0.72        #Immature (1 year old) survival from Mounce et al. 2014
SDIS=0.02      #SDImmature survival
YS=0.32        #Woodworth et al. 2009
SDYS=0.03      #Woodworth et al. 2009

#Max and Min survival of newly released (captive released or translocated)
RSmax=0.9
RSmin=0.4
RS=matrix(runif(yrs*It,RSmin,RSmax),It,yrs)

#Model parameters for pop 3 (shown are 5% increase in demographics)
```

```
F3=0.631        #Annual fecundity ((0.95*1)+(0.05*2), boosted 5%
SDF3=F3*0.15 #SD Annual fecundity
AS3=0.78        #Adult female survival from Mounce et al. 2014 boosted 5%
SDAS3=0.02      #SD Adult female survival
IS3=0.78        #Immature (1 year old) survival from Mounce et al. 2014 boosted 5%
SDIS3=0.02      #SDImmature survival
YS3=0.37        #Woodworth et al. 2009 boosted 5%
SDYS3=0.03      #Woodworth et al. 2009

# Alpha shape parameter for each iteration to be used in annual variable selection process
aAS = AS*((AS*(1-AS)/SDAS)-1)
bAS = (1-AS)*((AS*(1-AS)/SDAS)-1)
aIS = IS*((IS*(1-IS)/SDIS)-1)
bIS = (1-IS)*((IS*(1-IS)/SDIS)-1)
aYS = YS*((YS*(1-YS)/SDYS)-1)
bYS = (1-YS)*((YS*(1-YS)/SDYS)-1)
aF = log(F)-1/2*SDF
bF = log((SDF^2)/(F^2) + 1)

aAS3 = AS3*((AS3*(1-AS3)/SDAS3)-1)
bAS3 = (1-AS3)*((AS3*(1-AS3)/SDAS3)-1)
aIS3 = IS3*((IS3*(1-IS3)/SDIS3)-1)
bIS3 = (1-IS3)*((IS3*(1-IS3)/SDIS3)-1)
aYS3 = YS3*((YS3*(1-YS3)/SDYS3)-1)
bYS3 = (1-YS3)*((YS3*(1-YS3)/SDYS3)-1)
aF3 = log(F3)-1/2*SDF3
```

```
bF3 = log((SDF3^2)/(F3^2) + 1)


CD = 0.25 #mean number of deaths in the captive population (CD = captive deaths)

CN = 0.5 #mean number of successful rearings in the captive population (CN = captive nubes)


YM = 3 #Max years of management (e.g. translocation or captive releases)

M12 = matrix(0,It,yrs) #Move individuals from pop 1 to pop 2

M13 = matrix(3,It,yrs) #Move individuals from pop 1 to pop 3

M21 = matrix(0,It,yrs) #Move individuals from pop 2 to pop 1

M23 = matrix(2,It,yrs) #Move individuals from pop 2 to pop 3

M31 = matrix(0,It,yrs) #Move individuals from pop 3 to pop 1

M32 = matrix(0,It,yrs) #Move individuals from pop 3 to pop 2

M1c = matrix(4,It,yrs) #Move individuals from pop 1 to captivity

M2c = matrix(3,It,yrs) #Move individuals from pop 2 to captivity

M3c = matrix(0,It,yrs) #Move individuals from pop 3 to captivity

Mc1 = matrix(0,It,yrs) #Move individuals from captivity to pop 1

Mc2 = matrix(0,It,yrs) #Move individuals from captivity to pop 2

Mc3 = matrix(1,It,yrs) #Move individuals from captivity to pop 3


#Limits management to years less than the Max

M12[,(YM+1):yrs]=0       #Move individuals each year up until the max allowed

M13[,(YM+1):yrs]=0

M21[,(YM+1):yrs]=0

M23[,(YM+1):yrs]=0

M31[,(YM+1):yrs]=0

M32[,(YM+1):yrs]=0
```

```
M1c[,2:yrs]=0    #Move individuals in year 1 only

M2c[,2:yrs]=0

M3c[,(YM+1):yrs]=0

Mc1[,(YM+1):yrs]=0

Mc2[,(YM+1):yrs]=0

Mc3[,(YM+1):yrs]=0


#Data arrays for stochastic iterations

A=matrix(0,It,yrs)

I=matrix(0,It,yrs)

Y=matrix(0,It,yrs)

f=matrix(0,It,yrs)

A3=matrix(0,It,yrs)

I3=matrix(0,It,yrs)

Y3=matrix(0,It,yrs)

f3=matrix(0,It,yrs)

MLam1=matrix(0,It,yrs)

MLam2=matrix(0,It,yrs)

MLam3=matrix(0,It,yrs)

MLamc=matrix(0,It,yrs)

et =matrix(0,It,yrs)

e1 =matrix(0,It,yrs)

e2 =matrix(0,It,yrs)

e3 =matrix(0,It,yrs)

ec =matrix(0,It,yrs)
```

```
#data arrays fors abundance projections
Na1=matrix(0,It,yrs) #number of adults in region 1
Nj1=matrix(0,It,yrs) #number of adults in region 1
Na2=matrix(0,It,yrs)#number of adults in region 2
Nj2=matrix(0,It,yrs)#number of adults in region 2
Nj3=matrix(0,It,yrs)#number of adults in region 3
Na3=matrix(0,It,yrs)#number of adults in region 3
Nc=matrix(0,It,yrs)#number of adults in captivity
Na1i = 238.5 #initial number of birds in pop 1
Na2i = 53 #initial number of birds in pop 2
Na3i = 0 #initial number of birds in pop 3
Nci = 7 #initial number of birds in captivity

#Begin Stochastic simulation
for(i in 1:It){
for(j in 1:yrs){
#Parameter values for pop 1&2
A[i,j]=rbeta(1,aAS,bAS)
I[i,j]=rbeta(1,aIS,bIS)
Y[i,j]=rbeta(1,aYS,bYS)
f[i,j]=rlnorm(1,aF,bF)
#Parameter values for pop 3
A3[i,j]=rbeta(1,aAS3,bAS3)
I3[i,j]=rbeta(1,aIS3,bIS3)
Y3[i,j]=rbeta(1,aYS3,bYS3)
f3[i,j]=rlnorm(1,aF3,bF3)
```

```
#Abundance projection split by separate populations
#projects current years crop of juvenile age class
if(j==1) Nj1[i,j]=round(Na1i*.2) else Nj1[i,j]=round((Na1[i,j-1]*f[i,j-1])*Y[i,j-1])
#projects current years crop of adult age class, include translocation and captive releases
if(j==1) Na1[i,j]=round(Na1i*.8) else Na1[i,j]= round(((Na1[i,j-1]*A[i,j-1])+(Mc1[i,j-1]+M21[i,j-1]+M31[i,j-1])*RS[i,j])+((Nj1[i,j-1]-(M1c[i,j-1]+M12[i,j-1]+M13[i,j-1]))*I[i,j-1]))
#sets any abundance less than 1 to extinct
if (Na1[i,j]<1) Na1[i,j]=0
#resets fecundity to 0 if abundance exceeds 885 (carrying capacity)
if (Na1[i,j]>885) f[i,j]=0
#restrict management actions to 0 movements if abundance is below 100
if (Na1[i,j]<100) M12[i,j] = 0 else M12[i,j]=M12[i,j]
if (Na1[i,j]<100) M13[i,j] = 0 else M13[i,j]=M13[i,j]
if (Na1[i,j]<100) M1c[i,j] = 0 else M1c[i,j]=M1c[i,j]

if(j==1) Nj2[i,j]=Na2i*.2 else Nj2[i,j]=round((Na2[i,j-1]*f[i,j-1])*Y[i,j-1])
if(j==1) Na2[i,j]=Na2i*.8 else Na2[i,j]= round(((Na2[i,j-1]*A[i,j-1])+(Mc2[i,j-1]+M12[i,j-1]+M32[i,j-1])*RS[i,j])+((Nj2[i,j-1]-(M2c[i,j-1]+M21[i,j-1]+M23[i,j-1]))*I[i,j-1]))
if (Na2[i,j]<1) Na2[i,j]=0
if (Na2[i,j]>885) f[i,j]=0
if (Na2[i,j]<25) M21[i,j] = 0 else M21[i,j]=M21[i,j]
if (Na2[i,j]<25) M23[i,j] = 0 else M23[i,j]=M23[i,j]
if (Na2[i,j]<25) M2c[i,j] = 0 else M2c[i,j]=M2c[i,j]

if(j==1) Nj3[i,j]=Na3i*.2 else Nj3[i,j]=round((Na3[i,j-1]*f3[i,j-1])*Y3[i,j-1])
```

```
if(j==1) Na3[i,j]=Na3i*.8 else Na3[i,j]= round(((Na3[i,j-1]*A3[i,j-1])+(Mc3[i,j-1]+M13[i,j-1]+M23[i,j-1])*RS[i,j])+((Nj3[i,j-1]-(M3c[i,j-1]+M31[i,j-1]+M32[i,j-1]))*I3[i,j-1]))

if (Na3[i,j]<1) Na3[i,j]=0

if (Na3[i,j]>885) f[i,j]=0

if (Na3[i,j]<100) M31[i,j] = 0 else M31[i,j]=M31[i,j]

if (Na3[i,j]<100) M32[i,j] = 0 else M32[i,j]=M32[i,j]

if (Na3[i,j]<100) M3c[i,j] = 0 else M3c[i,j]=M3c[i,j]


#To add births and deaths to the captive population we used a random poisson distributed function and there is not carrying capacity

if(j==1) Nc[i,j]=Nci else if(Nc[i,j-1]==0) Nc[i,j]=round(0+(M1c[i,j-1]+M2c[i,j-1]+M3c[i,j-1])-(Mc1[i,j-1]+Mc2[i,j-1]+Mc3[i,j-1])) else Nc[i,j]=round(Nc[i,j-1]+rpois(1,CN)-rpois(1,CD)+(M1c[i,j-1]+M2c[i,j-1]+M3c[i,j-1])-(Mc1[i,j-1]+Mc2[i,j-1]+Mc3[i,j-1]))

if (Nc[i,j]<1) Nc[i,j]=0

if (Nc[i,j]<20) Mc1[i,j] = 0 else Mc1[i,j]=Mc1[i,j]

if (Nc[i,j]<20) Mc2[i,j] = 0 else Mc2[i,j]=Mc2[i,j]

if (Nc[i,j]<5) Mc3[i,j] = 0 else Mc3[i,j]=Mc3[i,j]


if(j>1)MLam1[i,j]=Na1[i,j]/Na1[i,j-1]

if(j>1)MLam2[i,j]=Na2[i,j]/Na2[i,j-1]

if(j>1)MLam3[i,j]=Na3[i,j]/Na3[i,j-1]

if(j>1)MLamc[i,j]=Nc[i,j]/Nc[i,j-1]


#Calculate quasi extinction probability

if ((Na1[i,j]+Na2[i,j]+Na3[i,j]) < 10)  et[i,j]=1

if (Na1[i,j] < 10)  e1[i,j]=1

if (Na2[i,j] < 10)  e2[i,j]=1
```

```
if (Na3[i,j] < 10)  e3[i,j]=1

if (Nc[i,j] < 2)  ec[i,j]=1

}}


#Calculate median population size and 95% quantiles for each population

MNa1=apply(Na1,2,median)

LBNa1=apply(Na1,2,quantile, probs=(.025))

UBNa1=apply(Na1,2,quantile, probs=(.95))

MNa2=apply(Na2,2,median)

LBNa2=apply(Na2,2,quantile, probs=(.025))

UBNa2=apply(Na2,2,quantile, probs=(.95))

MNa3=apply(Na3,2,median)

LBNa3=apply(Na3,2,quantile, probs=(.025))

UBNa3=apply(Na3,2,quantile, probs=(.95))

MNc=apply(Nc,2,median)

LBNc=apply(Nc,2,quantile, probs=(.025))

UBNc=apply(Nc,2,quantile, probs=(.95))


#calculate median population growth

median(MLam1[,2:yrs])

median(MLam2[,2:yrs])

median(MLam3[,2:yrs])

median(MLamc[,2:yrs])


#Calculate and print quasi extinction for each region and captivity

set = apply(et,2,sum)
```

```
pet = set/It
pet

se1 = apply(e1,2,sum)
pe1 = se1/It
pe1

se2 = apply(e2,2,sum)
pe2 = se2/It
pe2

se3 = apply(e3,2,sum)
pe3 = se3/It
pe3

sec = apply(ec,2,sum)
pec = sec/It
pec
```

Appendix D. European Molecular Biology Laboratory deposits

Accession#: LM993639
Status: not confidential
Description: Pseudonestor xanthophrys mitochondrial partial D-loop
LM993639; SV 1; linear; genomic DNA; STD; VRT; 252 BP.

08-AUG-2014 (Rel. 121, Created)
08-AUG-2014 (Rel. 121, Last updated, Version 1)

Pseudonestor xanthophrys mitochondrial partial D-loop

Pseudonestor xanthophrys
Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
Testudines + Archosauria group; Archosauria; Dinosauria; Saurischia;
Theropoda; Coelurosauria; Aves; Neognathae; Passeriformes; Passeroidea;
Fringillidae; Drepanidinae; Pseudonestor.
Mitochondrion

1-252
Mounce H.;

Submitted (25-JUL-2014) to the INSDC.
Dr. Jim Groombridge, Maui Forest Bird Recovery Project, 2465 Olinda Road,
96768, USA.

Mounce H.L., Raisin C., Swinnerton K.J., Leonard D.L., Wickenden H.,
Groombridge J.J.;
"Spatial genetic architecture of the critically-endangered Maui Parrotbill
(Pseudonestor xanthophrys): management considerations for reintroduction
strategies";
Unpublished.

MD5; db95fcd47c0cdd21852b4eaf478b0310.

Key Location/Qualifiers

source 1..252
/organism="Pseudonestor xanthophrys"
/organelle="mitochondrion"
/mol_type="genomic DNA"
/country="USA:Hawaii"
/isolation_source="Blood"
/collection_date="08-Oct-2009"
/sex="Male"
/db_xref="taxon:64813"
D-loop <1..>252

Sequence 252 BP; 80 A; 91 C; 36 G; 45 T; 0 other;
acgaacagcc caaacattat ctccaaaacg gacctcatac ggccaataca cccaccagag
acattcttgt ttcaggtacc atatagccca aatgctccta cctacagcca agccgcaagc
gtcacccaaa gacccaggaa cttacctact ataccccaaa cccaaccaag gaaacgaggg
atgtcccagt acacctttgc attcccctag accactgaat tcgcccacct cctaggcaag
attctcctcc aa

Accession#: LM993640
Status: not confidential
Description: Pseudonestor xanthophrys mitochondrial partial D-loop
LM993640; SV 1; linear; genomic DNA; STD; VRT; 250 BP.

08-AUG-2014 (Rel. 121, Created)
08-AUG-2014 (Rel. 121, Last updated, Version 1)

Pseudonestor xanthophrys mitochondrial partial D-loop

Pseudonestor xanthophrys

Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
Testudines + Archosauria group; Archosauria; Dinosauria; Saurischia;
Theropoda; Coelurosauria; Aves; Neognathae; Passeriformes; Passeroidea;
Fringillidae; Drepanidinae; Pseudonestor.
Mitochondrion

1-250
Mounce H.;

Submitted (25-JUL-2014) to the INSDC.
Dr. Jim Groombridge, Maui Forest Bird Recovery Project, 2465 Olinda Road,
96768, USA.

Mounce H.L., Raisin C., Swinnerton K.J., Leonard D.L., Wickenden H.,
Groombridge J.J.;
"Spatial genetic architecture of the critically-endangered Maui Parrotbill
(Pseudonestor xanthophrys): management considerations for reintroduction
strategies";
Unpublished.

MD5; 6471aaa6f02172b6b9b011171799a096.

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Key            Location/Qualifiers

source          1..250
               /organism="Pseudonestor xanthophrys"
               /organelle="mitochondrion"
               /mol_type="genomic DNA"
               /country="USA:Hawaii"
               /isolation_source="Blood"
               /collection_date="16-Apr-2009"
               /sex="Female"
               /db_xref="taxon:64813"
D-loop          <1..>250
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Sequence 250 BP; 79 A; 90 C; 36 G; 45 T; 0 other;
gaacagccca aacattatct ccaaaacgga cctcatacgg ccaatacacc caccagagac
attcttgttt caggtaccat atagcccaaa tgctcctacc tacagccaag ccgcaagcgt
cacccaaaga cccaggaact tacctactat accccaaacc caaccaagga aacgagggat
gtcccagtac acctttgcat tcccctagac cactgaattc gcccacctcc taggcaagat
tctcctccaa

Accession#: LM993641
Status: not confidential
Description: Pseudonestor xanthophrys mitochondrial partial D-loop
LM993641; SV 1; linear; genomic DNA; STD; VRT; 131 BP.

08-AUG-2014 (Rel. 121, Created)
08-AUG-2014 (Rel. 121, Last updated, Version 1)

Pseudonestor xanthophrys mitochondrial partial D-loop

Pseudonestor xanthophrys
Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
Testudines + Archosauria group; Archosauria; Dinosauria; Saurischia;
Theropoda; Coelurosauria; Aves; Neognathae; Passeriformes; Passeroidea;
Fringillidae; Drepanidinae; Pseudonestor.
Mitochondrion

1-131
Mounce H.;

Submitted (25-JUL-2014) to the INSDC.
Dr. Jim Groombridge, Maui Forest Bird Recovery Project, 2465 Olinda Road,
HI 96768, USA.

Mounce H.L., Raisin C., Swinnerton K.J., Leonard D.L., Wickenden H., Groombridge J.J.;
"Spatial genetic architecture of the critically-endangered Maui Parrotbill (Pseudonestor xanthophrys): management considerations for reintroduction strategies";
Unpublished.

MD5; 75289cd5f0aab6ba6b4601faf0fce329.

Key Location/Qualifiers

source 1..131
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/organelle="mitochondrion"
/mol_type="genomic DNA"
/country="USA:Hawaii"
/isolation_source="Blood"
/collection_date="13-May-2009"
/sex="Male"
/db_xref="taxon:64813"
D-loop <1..>131

Sequence 131 BP; 40 A; 49 C; 18 G; 24 T; 0 other;
tcacccaaag acccaggaac ttacctacta taccccaaac ccaaccaagg aaacgaggga
tgtcccagta cacctttgca ttcccctaga ccactgaatt cgcccacctc ctaggcaaga
ttctcctcca a

Accession#: LM993642
Status: not confidential
Description: Pseudonestor xanthophrys microsatellite DNA locus Pxa01
ID LM993642; SV 1; linear; genomic DNA; STD; VRT; 819 BP.
public

08-AUG-2014 (Rel. 121, Created) 08-AUG-2014 (Rel. 121, Last updated, Version 0)

Pseudonestor xanthophrys microsatellite DNA locus Pxa01

Pseudonestor xanthophrys
Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
Testudines + Archosauria group; Archosauria; Dinosauria; Saurischia;
Theropoda; Coelurosauria; Aves; Neognathae; Passeriformes; Passeroidea;
Fringillidae; Drepanidinae; Pseudonestor.

1-819
Mounce H.;
Submitted (26-JUL-2014) to the INSDC.
Dr. Jim Groombridge, Maui Forest Bird Recovery Project, 2465 Olinda Road,
96768, USA.

MD5; 3f200caea7d107e9c1d3a6cbc7a4d120.
Key Location/Qualifiers
source 1..819
/organism="Pseudonestor xanthophrys"/chromosome="7"/mol_type="genomic
DNA"/country="USA:Hawaii, Hanawi NAR"/collection_date="2001"/tissue_type="blood"
/db_xref="taxon:64813"/repeat_region 223..244/rpt_type=TANDEM/rpt_unit_seq="GTT"
/satellite="microsatellite:Pxa01"

Sequence 819 BP; 229 A; 162 C; 195 G; 219 T; 14 other;

```
 ctgaggtaaa aaagccattc agtgacaggc cacataaagc attttatgac agaaaacaaa        60
 ggagcaggtc tttcatccaa agagtggagg aggcagaatg ccttgaaggt atggtccatg       120
 tatgtgcaac tgatgctgtt agcagatgca aaaaaaaaac ttagagtatc agccctagaa       180
 ttctatggta tttcgtgttg ctattttact tagtattatt attgttgttg ttgttgttgt       240
 tgttggtttt gctgctattg ttgtggttag tagatatgat gtgctagata ttgtccatta       300
 gtagaaaaaa tggatgagga aaaaagtgca gtgcaggctt ttatgtgcaa gacaattcaa       360
 ggtaagtagc ataatagaga aaggactttg ggaacctcat gggcgaggcc agggaggact       420
 tgtaggctgc tacaggagaa cctgaacagt tgaaattggg atagcagatg taaaaggggt       480
 gggtaaggca aaagagaatc cttttcttaa tgcctccata aaacagtcca ttgccagagt       540
```

```
gtttttctgt tgcactgcag acagttcgtc tctggaccct gaagtctcca aggcaaatgc      600
ctatggttca ctggctgcct gtcctctgtt cagtacaacc acatctcctc cccctgaagg      660
ctggccttac gccatcccca agcngttngc tccccgttga actgtnccaa nggttnccct       720
ggaacaanaa attccaaaag nctttgnnat tgnccctggc nagggtccga acttcctaaa       780
aaaggaattc cccccggggt taacccnaan ncctcccaa                           819
```

Accession#: LM993643

Status: not confidential

Description: Pseudonestor xanthophrys microsatellite DNA locus Pxa02

ID LM993643; SV 1; linear; genomic DNA; STD; VRT; 671 BP.

public

08-AUG-2014 (Rel. 121, Created) 08-AUG-2014 (Rel. 121, Last updated, Version 0)

Pseudonestor xanthophrys microsatellite DNA locus Pxa02

Pseudonestor xanthophrys
Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
Testudines + Archosauria group; Archosauria; Dinosauria; Saurischia;
Theropoda; Coelurosauria; Aves; Neognathae; Passeriformes; Passeroidea;
Fringillidae; Drepanidinae; Pseudonestor.

1-671
Mounce H.;
Submitted (26-JUL-2014) to the INSDC.
Dr. Jim Groombridge, Maui Forest Bird Recovery Project, 2465 Olinda Road,
96768, USA..

MD5; db5b2d91822845b713072b0f8e324bf3.
Key Location/Qualifiers
source 1..671
/organism="Pseudonestor xanthophrys"/chromosome="3"/mol_type="genomic
DNA"/country="USA:Hawaii, Hanawi

NAR"/collection_date="2001"/tissue_type="blood"/db_xref="taxon:64813"/repeat_region 249. .280/rpt_type=TANDEM/rpt_unit_seq="TAGA"/satellite="microsatellite:Pxa02"

```
Sequence 671 BP; 190 A; 136 C; 134 G; 211 T; 0 other;
 aatacacaaa atccccctct tggaggccca cctctaagtg ttctatgcac cccaattctc       60
 catttgccaa gccatgggat gcactgggtt tagtggatga gccacttatg gcagcctaaa      120
 tatttgaaaa cttctgtttt tttcttttt tcccagattt tttcagtacc cctttccact      180
 tgtccacttc accagtcaga tgttcttgtt ccctctattt tttttctgga tgaggcaaga      240
 catgagagat agatgataga tagacagata gatagataga cagatagata gatagataga      300
 taatatatct gtatcatcta caatggtatt tatctggaga gatatccatg catttatatc      360
 tatatataaa gagagatgga tggatggatg atggatggat gggtgggtgg atggatggat      420
 aaatgtatta ataaatgggt gaatagatgg atggatgcaa taccacagca gagactttgc      480
 acatggtctt acaacatcta tatcttaata tgtccttgga ctgagtgcca gaccttttcc      540
 tgcttctcca tattcctgcc tcagcagtca tcccgagaca agaaacctgc attttgaccc      600
 ctctatacct tcagaaacag atttagagat aatttcagcc tcccaaacag ttgcagaggt      660
 gcttctcttg c                                                           671
```

Accession#: LM993644

Status: not confidential

Description: Pseudonestor xanthophrys microsatellite DNA locus Pxa03

ID LM993644; SV 1; linear; genomic DNA; STD; VRT; 643 BP.

public

08-AUG-2014 (Rel. 121, Created) 08-AUG-2014 (Rel. 121, Last updated, Version 0)

Pseudonestor xanthophrys microsatellite DNA locus Pxa03

Pseudonestor xanthophrys
Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
Testudines + Archosauria group; Archosauria; Dinosauria; Saurischia;
Theropoda; Coelurosauria; Aves; Neognathae; Passeriformes; Passeroidea;
Fringillidae; Drepanidinae; Pseudonestor.

1-643
Mounce H.;
Submitted (26-JUL-2014) to the INSDC.
Dr. Jim Groombridge, Maui Forest Bird Recovery Project, 2465 Olinda Road,
96768, USA.

MD5; f51646bc11d5c2767bcc40c217f93015.
Key Location/Qualifiers
source 1..643
/organism="Pseudonestor xanthophrys"/chromosome="6"/mol_type="genomic DNA"/country="USA:Hawaii, Hanawi NAR"/collection_date="2001"/tissue_type="blood" /db_xref="taxon:64813"/repeat_region 521..563/rpt_type=TANDEM/rpt_unit_seq="GTT"/satellite="microsatellite:Pxa03"

Sequence 643 BP; 150 A; 132 C; 140 G; 221 T; 0 other;

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 tctggagagt ttctgctgtt tttcttcacc cctctttctt taacaagaag aaaaaaagta        60
 gggctttgga agattttttt tgagcaaaac aacctttcaa gctgaatctg tgtttatagt       120
 tggaactgat ctatgctgag ccccagcacc ctatgctaaa ccacgtgtgc aaaaggacct       180
 cattatcatg tactgtgggt ggatgaaatg gctcccaaca cacctccctg ccccagggct       240
 ctgctcagat gtgagagtat catccatcac gttctgttgc ctctgtgttt gcagtcttgg       300
 tgaatctgca cagacattag gactgttact tcacatataa ttgtggctaa agtacccaga       360
 aaccctaatt ggtttctgag gggagaggtt gtcatggatt ttgtagagcc aagagggacc       420
 ttcataatca cacattgttc tgttctgtat aattcggatt ggagagtttg atcagtacct       480
 tctgctttat ttaacttttt gtgactaggg caggacctga cgttgttgtt gttgttgttg       540
 ttgttgttgt tgttgttgtt gttttgcatt aataattttc ctgagatgat ggaaaatgca       600
 ctgcttgtcc ttctgaccat ctcctccaac tatttcccca cag                         643
```

Accession#: LM993645
Status: not confidential
Description: Pseudonestor xanthophrys microsatellite DNA locus Pxa04
ID LM993645; SV 1; linear; genomic DNA; STD; VRT; 557 BP.
public

08-AUG-2014 (Rel. 121, Created) 08-AUG-2014 (Rel. 121, Last updated, Version 0)

Pseudonestor xanthophrys microsatellite DNA locus Pxa04

Pseudonestor xanthophrys
Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
Testudines + Archosauria group; Archosauria; Dinosauria; Saurischia;
Theropoda; Coelurosauria; Aves; Neognathae; Passeriformes; Passeroidea;
Fringillidae; Drepanidinae; Pseudonestor.

1-557
Mounce H.;
Submitted (26-JUL-2014) to the INSDC.
Dr. Jim Groombridge, Maui Forest Bird Recovery Project, 2465 Olinda Road,
96768, USA.

DR MD5; 38dcb47b3132bd424a0344eeeba6c9bb.
Key Location/Qualifiers
source 1..557
/organism="Pseudonestor xanthophrys"/chromosome="2" /mol_type="genomic DNA"
/country="USA:Hawaii, Hanawi NAR" /collection_date="2001"/tissue_type="blood"
/db_xref="taxon:64813"/repeat_region 217..247 /rpt_type=TANDEM /rpt_unit_seq="CAA"
/satellite="microsatellite:Pxa04"

Sequence 557 BP; 180 A; 129 C; 106 G; 142 T; 0 other;
gcactgaata ttaacctaca tattcaacag gtgaactttc ccatttcaga agggaataat 60
gaagcctaag aggccaaatg ttctggaaaa aagcacaaca aacccaattg tttacccaaa 120
catgcccaga gagatgacat ttaaattgtt tattttcagc ctgtttaaaa tgtctaaatt 180
agcattcaaa ttgtattgac cactgtgatg gggaaaacaa caacaacaac aacaacaaca 240
acaacaaatc ctgaagatct ggatgttaac aaatagcaga agccaatttc attagttgcc 300
ccacagttca gcatctgatc acactggggc ttccaggcat ccaaagggct gtcgctcaga 360
gcctgacctg tcactgggtt ctggctacca ggccaccatc tggtttatga taccagtggc 420
tctaacacct ccagattttg gtgccctcct ctggagcacg cttacagtct aatgcagccc 480
agacatgcag atgtgggtat gtcagatatg caatctgatc aatgcttttc caaaattcca 540

taaaatgatg tagtgag 557

Accession#: LM993646

Status: not confidential

Description: Pseudonestor xanthophrys microsatellite DNA locus Pxa05

ID LM993646; SV 1; linear; genomic DNA; STD; VRT; 687 BP.

public

08-AUG-2014 (Rel. 121, Created) 08-AUG-2014 (Rel. 121, Last updated, Version 0)

Pseudonestor xanthophrys microsatellite DNA locus Pxa05

Pseudonestor xanthophrys
Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
Testudines + Archosauria group; Archosauria; Dinosauria; Saurischia;
Theropoda; Coelurosauria; Aves; Neognathae; Passeriformes; Passeroidea;
Fringillidae; Drepanidinae; Pseudonestor.

1-687
Mounce H.;
Submitted (26-JUL-2014) to the INSDC.
Dr. Jim Groombridge, Maui Forest Bird Recovery Project, 2465 Olinda Road,
96768, USA.

MD5; ccc3273b14a53f30ef1021f99850d794.
Key Location/Qualifiers
source 1..687
/organism="Pseudonestor xanthophrys"/chromosome="2"/mol_type="genomic DNA"/country="USA:Hawaii, Hanawi NAR"/collection_date="2001"/tissue_type="blood"
/db_xref="taxon:64813"/repeat_region 130..157 /rpt_type=TANDEM/rpt_unit_seq="CAT"
/satellite="microsatellite:Pxa05"

Sequence 687 BP; 161 A; 162 C; 141 G; 223 T; 0 other;

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gagagcatat gttcttgagc agttaatcct tgcacaggct taggcaggaa aagaaaaatg        60
aattgtatat gctatgacta ttggagttga attatgtatt tagtgtgatt gctgccattt       120
tcctctcaat catcatcacc atcatcatca tcatcatcac ctccctccct gctcctgcca       180
tgcctcatcc tcctggatgt acatctccag gcccatgtgt ggtgtggaga gcactctgac       240
tcctttgccc gtggactcac taacgcttct ctcccaggca gtgagaatgc cttgtcacac       300
ttgaaccaca agtggattct ggattctccc gcttggagtt catggatcta acctggcagc       360
caccaatctg tatgtcttct gtaaatgatc ctccaagggt ttacctgtag cagtgctgtc       420
atgctcccag gaataatcac aaggtttgtg ttcattttg tagctcttta ttctgtgatg       480
tgcccttgaa acccgtgagg ctccaacttt gtctataaat cttgttaaaa gggtgtgggt       540
tgcctgttcc acacagcaaa aaaagcaatt tttaagttat ttagatacta ttcatcttct       600
ccctgaagcc tggtcaaaac ttctgtttca acattgtcct ttgttataga gtgaggaatg       660
gtggcaattt tcctgcatgt gctgtgg                                           687
```

Accession#: LM993647

Status: not confidential

Description: Pseudonestor xanthophrys microsatellite DNA locus Pxa06

ID   LM993647; SV 1; linear; genomic DNA; STD; VRT; 480 BP.

public

08-AUG-2014 (Rel. 121, Created) 08-AUG-2014 (Rel. 121, Last updated, Version 0)

Pseudonestor xanthophrys microsatellite DNA locus Pxa06

Pseudonestor xanthophrys
Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
Testudines + Archosauria group; Archosauria; Dinosauria; Saurischia;
Theropoda; Coelurosauria; Aves; Neognathae; Passeriformes; Passeroidea;
Fringillidae; Drepanidinae; Pseudonestor.

1-480
Mounce H.;
Submitted (26-JUL-2014) to the INSDC.
Dr. Jim Groombridge, Maui Forest Bird Recovery Project, 2465 Olinda Road,

96768, USA.

DR MD5; d8d296d3eda052317bf1f56b17e9216c.
Key Location/Qualifiers
source 1..480
/organism="Pseudonestor xanthophrys"/chromosome="1A"/mol_type="genomic DNA"/country="USA:Hawaii, Hanawi NAR"/collection_date="2001"/tissue_type="blood" /db_xref="taxon:64813"/repeat_region 367..415/rpt_type=TANDEM /rpt_unit_seq="CTAT" /satellite="microsatellite:Pxa06"

Sequence 480 BP; 114 A; 86 C; 119 G; 161 T; 0 other;

```
 gggtctcacc tcacttgtca cttctgtaaa ctgggagtga acttgactga aagttttaca       60
 tgggcaggta ttgacagaac aagggtgttt tgaagtttta aagggtgttt taagcgtttt      120
 aaactaggaa aggagacatt cagattggtt attaaccaga aatacttctg agggtggtga      180
 ggtcctggca cagggtgctt agacaagttg tggctgttcc tggaagtgtc caaggccagg      240
 ttggacattt gggcttggag caacctggga tggtggaagg tgtccctgcc catggcaggg      300
 ggtggcactg gatgatcttg aagatcaaac tattgtctga ttttgagaac tttatctatc      360
 tgtctgtcta tctatctatc tatctatcta tctatctatc tatctatcta tctatcttat      420
 ctctctcttt ctctctctct agtgctccaa gaggaggaaa taactctgat tatgttgttt      480
```

Accession#: LM993648
Status: not confidential
Description: Pseudonestor xanthophrys microsatellite DNA locus Pxa07
ID LM993648; SV 1; linear; genomic DNA; STD; VRT; 643 BP.
public

08-AUG-2014 (Rel. 121, Created) 08-AUG-2014 (Rel. 121, Last updated, Version 0)

Pseudonestor xanthophrys microsatellite DNA locus Pxa07

Pseudonestor xanthophrys
Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
Testudines + Archosauria group; Archosauria; Dinosauria; Saurischia;
Theropoda; Coelurosauria; Aves; Neognathae; Passeriformes; Passeroidea;

Fringillidae; Drepanidinae; Pseudonestor.

1-643
Mounce H.;
Submitted (26-JUL-2014) to the INSDC.
Dr. Jim Groombridge, Maui Forest Bird Recovery Project, 2465 Olinda Road, 96768, USA.

MD5; 2c124f2fdced76f7c53b4ad1010914fb.
Key Location/Qualifiers
source 1..643
/organism="Pseudonestor xanthophrys"/chromosome="3"/mol_type="genomic DNA"
/country="USA:Hawaii, Hanawi NAR"/collection_date="2001"/tissue_type="blood"
/db_xref="taxon:64813"/repeat_region 60..111/rpt_type=TANDEM/rpt_unit_seq="CTAT"
/satellite="microsatellite:Pxa07"

Sequence 643 BP; 192 A; 127 C; 124 G; 200 T; 0 other;

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aacaccagga ctgcacttgt ggtgaaagac ttctcattag agaaaagtat tatatctatt        60
ctatcatcta tctatctatc tatctatcta tctatctatc tatctatcta tatgaataat       120
gtactatggg gatttcagaa gaagattttg ctttgaagca ctccaaaatc attgatataa       180
agagacattt ttgaaaaaag gctctgtctg tacaatattt aacttttctg ttccagtctg       240
aaggaaaaca ttaatgaata aaaatattat ttccaagcat caaactgaag tacatagcag       300
aattggctgg agcacttgac acagtccatg ttcttcaggt tttgtagaat cagttctagg       360
aaacaaaccc attcacaatg gctaggacag gttggaagag acagatcacc aacacattaa       420
cttgttcttt tcccttctac ttatcagtaa gactactgtg atgaccctac cagtgtcctc       480
ctggactgcc ctgtccccat agcaaggagg ttgagtggag aatccctgtg ctgacttgcg       540
tcctctgtgg gcatcttttc ttctgcagga tctggatgct tacagattaa atttggtggg       600
agtaactctc ttagggtagg acatagttac gagccggaca gag                         643
```

Accession#: LM993649
Status: not confidential
Description: Pseudonestor xanthophrys microsatellite DNA locus Pxa08
ID LM993649; SV 1; linear; genomic DNA; STD; VRT; 733 BP.
public

08-AUG-2014 (Rel. 121, Created) 08-AUG-2014 (Rel. 121, Last updated, Version 0)

Pseudonestor xanthophrys microsatellite DNA locus Pxa08

Pseudonestor xanthophrys
Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
Testudines + Archosauria group; Archosauria; Dinosauria; Saurischia;
Theropoda; Coelurosauria; Aves; Neognathae; Passeriformes; Passeroidea;
Fringillidae; Drepanidinae; Pseudonestor.

1-733
Mounce H.;
Submitted (26-JUL-2014) to the INSDC.
Dr. Jim Groombridge, Maui Forest Bird Recovery Project, 2465 Olinda Road,
96768, USA.

MD5; a68aa617e1b484d65847c7f04f84495a.
Key Location/Qualifiers
source 1..733
/organism="Pseudonestor xanthophrys" /chromosome="Z" /mol_type="genomic DNA"
/country="USA:Hawaii, Hanawi NAR" /collection_date="2001" /tissue_type="blood"
/db_xref="taxon:64813"/repeat_region 393..521 /rpt_type=TANDEM /rpt_unit_seq="CTAT"
/satellite="microsatellite:Pxa08"

Sequence 733 BP; 214 A; 171 C; 101 G; 247 T; 0 other;

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 cttctactaa ggaaccatct ttaattggag acagagggga ggttttttgt gctgaactct        60
 ttcccacccc ataagcaacc attgtacaac tgcaaaactc agaaaattta cccagaaact       120
 ttgtgtagaa actttgtcca ggcacacctg gacagacaga cacacaccct cacctgctct       180
 cttgatgtca acaaatttcc agcatcttcc agagagaagt ttgagccagt ccaaaactcc       240
 ttacagagga tgtactgggg aatttactag aagaaccagg aaagccaatt ttctatacat       300
 ccatgggccc ctacccctga cagtgcagga ctgtgtatta tgactacagg ccatgatcac       360
 acatgtgtca ctggaggcat gggtagtgat tatctatcta tctatctatc tatctatcta       420
 tctatctatc tatctatcta tctatctatc tatctatcta tctatctatc tatgcatcta       480
```

```
tctatctatc tatctatcta tctatctatc tatctatcta tgcatctatc taatctcttc      540
tctctctatt tctccttttt cctttgcaat tttcctctca tgggatgatc ataaacacca      600
gaatggctat agatcccttt tacaatcctt acagtttttt ccagatagat ttcaaagcaa      660
tttctcaaat aaaattaatt ttaatataat ttagatagag taatttgttt taagacatcc      720
caaagaatct gtt                                                         733
```

Accession#: LM993650
Status: not confidential
Description: Pseudonestor xanthophrys microsatellite DNA locus Pxa09
ID LM993650; SV 1; linear; genomic DNA; STD; VRT; 462 BP.
public

08-AUG-2014 (Rel. 121, Created) 08-AUG-2014 (Rel. 121, Last updated, Version 0)

Pseudonestor xanthophrys microsatellite DNA locus Pxa09

Pseudonestor xanthophrys
Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
Testudines + Archosauria group; Archosauria; Dinosauria; Saurischia;
Theropoda; Coelurosauria; Aves; Neognathae; Passeriformes; Passeroidea;
Fringillidae; Drepanidinae; Pseudonestor.

1-462
Mounce H.;
Submitted (26-JUL-2014) to the INSDC.
Dr. Jim Groombridge, Maui Forest Bird Recovery Project, 2465 Olinda Road,
96768, USA.

MD5; 2ac28a4f0ac216741a0b06a3b0da6872.
Key Location/Qualifiers
source 1..462
/organism="Pseudonestor xanthophrys" /chromosome="1" /mol_type="genomic DNA"
/country="USA:Hawaii, Hanawi NAR" /collection_date="2001" /tissue_type="blood"

/db_xref="taxon:64813"/repeat_region   110..150 /rpt_type=TANDEM /rpt_unit_seq="TAGA"
/satellite="microsatellite:Pxa09"

Sequence 462 BP; 133 A; 123 C; 113 G; 93 T; 0 other;

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 gctgcgagag ggctgctgcc tgagcagcct gcttccctgc cttccctgaa ctgcctcagc       60
 acccacgtga ctcagcacct acaacatggt tagaaacata aatacacaga tagatagata      120
 gatagatata tagatagata gatagataga tatggtaagc aaatgcactg cgcctatgca      180
 ctgatggaca gaatgtttgt ttgtgcacct ggcattctga tgcacgaaca gccacgaggc      240
 gtggcattgg cacgctgagg caatgagtca gggcaccact ggtgaagcaa agcccacagc      300
 ctggcaacac agcgagttgg acaactagca gcatcggcac catgacagca tcacggcgtc      360
 attcaggttg gagaagactt aggaggtcac tgagtccaac tgttatccca ggactgtcaa      420
 gtccaacact aaaccacgtc cctcactgcc acttctacac ag                         462
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Accession#: LM993651
Status: not confidential
Description: Pseudonestor xanthophrys microsatellite DNA locus Pxa10
ID   LM993651; SV 1; linear; genomic DNA; STD; VRT; 799 BP.
public

08-AUG-2014 (Rel. 121, Created) 08-AUG-2014 (Rel. 121, Last updated, Version 0)

Pseudonestor xanthophrys microsatellite DNA locus Pxa10

Pseudonestor xanthophrys
Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
Testudines + Archosauria group; Archosauria; Dinosauria; Saurischia;
Theropoda; Coelurosauria; Aves; Neognathae; Passeriformes; Passeroidea;
Fringillidae; Drepanidinae; Pseudonestor.

1-799
Mounce H.;
Submitted (26-JUL-2014) to the INSDC.
Dr. Jim Groombridge, Maui Forest Bird Recovery Project, 2465 Olinda Road,

96768, USA.

DR MD5; 396f44e41b5a371f6ff0f511ed91f1d7.
Key Location/Qualifiers
source 1..799
/organism="Pseudonestor xanthophrys" /chromosome="2" /mol_type="genomic DNA"
/country="USA:Hawaii, Hanawi NAR" /collection_date="2001"
/tissue_type="blood"/db_xref="taxon:64813"/repeat_region 384..423 /rpt_type=TANDEM
/rpt_unit_seq="GTT"/satellite="microsatellite:Pxa10"

Sequence 799 BP; 229 A; 177 C; 160 G; 233 T; 0 other;

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gtctaattac acataaccta gttctgactt atttggcatg tttatgctgc ctcctgatcc       60
agcaccacac agccagcatg tgcaggactg agtcccacaa gcagccctga ttcccagaca      120
cctcaaggtc ttagtgaggc tgacaaaacg ctgcatcccc ctaagcatct ctcaggctca      180
gatcacctct gtgcagaagt ccaggggact gatggggaag ggtttctgtg aggcaaagta      240
ggaggaagaa accagcccca ctggttaggt gatcaccaac atgaactgac tcaatccttt      300
aaaacactgt gcttcagcaa acagcattcc ttgaccctcc tcttaaccct cagtgttaga      360
gagtgatttt tttttttttt ttttgttgtt gctgttgttg ttgttgttgt tgttgttgtt      420
gttgaaaaag ctcttgacca aacctgagaa gtcttcaggg aaaaggtgat ttggcttttc      480
aaataacttt ttttcataac tcctctctga ctgtcagttt agtttattta tatcataaat      540
caaaaggtta aagatgaaat gaactcacgc agagaattac ttaaagactc atagcaacat      600
catcagtact ttatacaaaa gccaaaaaat cagcaaaaca ttaattttaa gtgtgtagac      660
aaataagtta ttttagccag taatcagaag agaattccac ctccataaaa gcttgcatgc      720
ctgcaggtcg actctagagg atccccgggt accgagctcg aattcactgg ccgtcgtttt      780
acaacgtcgt gactgggaa                                                   799
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Accession#: LM993652
Status: not confidential
Description: Pseudonestor xanthophrys microsatellite DNA locus Pxa11
ID LM993652; SV 1; linear; genomic DNA; STD; VRT; 542 BP.
public

08-AUG-2014 (Rel. 121, Created) 08-AUG-2014 (Rel. 121, Last updated, Version 0)

Pseudonestor xanthophrys microsatellite DNA locus Pxa11

Pseudonestor xanthophrys
Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
Testudines + Archosauria group; Archosauria; Dinosauria; Saurischia;
Theropoda; Coelurosauria; Aves; Neognathae; Passeriformes; Passeroidea;
Fringillidae; Drepanidinae; Pseudonestor.

1-542
Mounce H.;
Submitted (26-JUL-2014) to the INSDC.
Dr. Jim Groombridge, Maui Forest Bird Recovery Project, 2465 Olinda Road,
96768, USA.

MD5; 8db518c3885dd5d3f9f925b62ecc2d2b.
Key Location/Qualifiers
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 aatctacctg tctatctatc tatcgtccat ctatttccat gtaagttgta gttctatgaa       240
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 atcagtggct gctagaagaa gccatcaaat tgtgggcagg tatttcaatc cagagaatct       420
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ag 542

Accession#: LM993653

Status: not confidential

Description: Pseudonestor xanthophrys microsatellite DNA locus Pxa12

ID LM993653; SV 1; linear; genomic DNA; STD; VRT; 759 BP.

public

08-AUG-2014 (Rel. 121, Created) 08-AUG-2014 (Rel. 121, Last updated, Version 0)

Pseudonestor xanthophrys microsatellite DNA locus Pxa12

Pseudonestor xanthophrys
Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
Testudines + Archosauria group; Archosauria; Dinosauria; Saurischia;
Theropoda; Coelurosauria; Aves; Neognathae; Passeriformes; Passeroidea;
Fringillidae; Drepanidinae; Pseudonestor.

1-759
Mounce H.;
Submitted (26-JUL-2014) to the INSDC.
Dr. Jim Groombridge, Maui Forest Bird Recovery Project, 2465 Olinda Road,
96768, USA.

MD5; 883ddef7ce7050834383448daba4f011.
Key Location/Qualifiers
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Sequence 759 BP; 212 A; 179 C; 146 G; 222 T; 0 other;

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gagcaaaagt atttccagca acaagaacaa aaggttttta ttcccaagca tttcgagtga 720

gaggcccctg ccagcaaggc accaaggcag ccgcgggag

Appendix E. MAPA sample histories for genetic analyses

Table D.1 Individual histories for all Maui Parrotbill (*Pseudonestor xanthophrys*) samples used in genetic analyses

MAPA Samples Collected							
Label	**Sample Type**	**Band Number**	**Color Bands**	**Date**	**Location**	**Age**	**Sex**
MAPA001	Blood/Feather	1401-47543	AL/BL, BL/YE	10/8/2009	WAIK	AHY	M
MAPA002	Blood/Feather	1401-47542	AL/BK, BK/YE	10/2/2009	WAIK	AHY	M
MAPA003	Blood/Feather	1401-47539	AL/BL, BL/RD	8/17/2009	HR3	AHY	F
MAPA004	Blood/Feather	1401-47538	AL/GR, BL/YE	5/13/2009	HR2	AHY	M
MAPA004	Blood/Feather	1401-47538	AL/GR BL/YE	5/13/2009	HR2	AHY	M
MAPA005	Blood/Feather	1401-47541	GR/AL,BK/YE	4/16/2009	FSB	ASY	M
MAPA005	Blood/Feather	1401-47541	GR/AL, BK/YE	4/16/2009	FSB	ASY	M
MAPA006	Blood/Feather	1401-47540	GR/BK,AL/YE	4/16/2009	FSB	AHY	F
MAPA007	Blood/Feather	1401-47537	BK/AL, BL/RD	3/19/2009	HR3	AHY	M
MAPA008	Blood/Feather	1371-04971	GR/YE, RD/AL	3/18/2009	HR3	ASY	F
MAPA008	Blood/Feather	1371-04971	GR/YE, RD/AL	3/13/2004	HR3	ASY	F
MAPA009	Blood/Feather	1401-47536	RD/AL, BL/BL	2/16/2009	FSB	AHY	F
MAPA010	Blood/Feather	1401-47533	RD/AL,RD/BL	1/30/2009	HR3	AHY	M
MAPA011	Blood/Feather	1401-47534	AL/RD,RD/GR	1/30/2009	HR3	AHY	F
MAPA012	Blood/Feather	1401-47535	AL/BL,YE/RD	1/27/2009	HR3	AHY	M
MAPA013	Blood/Feather	1401-47532	AL/GR,GR/BK	12/9/2008	FSB	AHY	F
MAPA014	Blood/Feather	1401-47531	BK/YE,AL/YE	12/9/2008	FSB	AHY	M
MAPA015	Blood/Feather	1401-47528	BL/YE,BL/AL	12/7/2008	FSB	AHY	F
MAPA016	Blood/Feather	1401-47530	YE/BL,AL/YE	12/7/2008	FSB	AHY	M
MAPA017	Blood/Feather	1401-47526	AL/RD, YE/BK	9/12/2008	FSB	HY	M
MAPA018	Feather	1401-47512	AL/BL, YE/BK	11/16/2007	FSB	AHY	M

MAPA019	Blood/Feather	1401-47516	AL/BL, BK/RD	12/1/2007	FSB	AHY	M
MAPA020	Blood/Feather	1401-47525	AL/BL, GR/YE	4/30/2008	FSB	AHY	F
MAPA021	Feather	1401-47521	YE/YE, RD/AL	4/25/2008	HR3	AHY	M
MAPA022	Blood/Feather	1401-47520	YE/RD, BL/AL	4/24/2008	HR3	ASY	M
MAPA023	Blood/Feather	1401-47544	AL/GR , RD/GR	10/22/2009	WAIK	AHY	F
MAPA024	Blood/Feather	1401-47545	AL/BL, YE/GR	10/28/2009	WAIK	AHY	F
MAPA025	Blood/Feather	1401-47546	AL/GR, GR/YE	10/28/2009	WAIK	AHY	M
MAPA026	Blood/Feather	1371-04982	BL/BL, BL/AL	1/20/2007	HR3	ASY	M
MAPA026	Blood/Feather	1371-04982	BL/BL, BL/AL	11/23/2009	HR3	ASY	M
MAPA027	Blood/Feather	1371-04983	BL/YE, AL/RD	2/3/2007	HR3	AHY	F
MAPA027	Blood/Feather	1371-04983	BL/YE, AL/RD	11/24/2009	HR3	AHY	F
MAPA028	Blood/Feather	1401-47548	AL/GR, GR/RD	11/24/2009	HR3	AHY	F
MAPA029	Blood/Feather	1371-04989	GR/GR, RD/AL	11/24/2009	HR3	ASY	M
MAPA030	Blood/Feather	1401-47550	AL/BL, BL/GR	12/4/2009	FSB	AHY	F
MAPA031	Blood/Feather	1401-47549	AL/BK, BK/BL	12/4/2009	FSB	AHY	F
MAPA032	Blood/Feather	1401-47551	AL/E, GR/RD	12/8/2009	FSB	SY	M
MAPA033	Blood/Feather	1401-47552	RD/GR, GR/AL	1/14/2010	HR3	AHY	F
MAPA034	Blood/Feather	1401-47553	GR/AL, GR/BK	1/16/2010	HR3	ASY	M
MAPA035	Blood/Feather	1401-47554	GR/YE, AL/BL	1/21/2010	WAIK	ASY	M
MAPA036	Blood/Feather	1401-47555	RD/YE, AL/BL	1/26/2010	FSB	ASY	F
MAPA037	Blood/Feather	1401-47556	RD/BL, AL/BL	1/26/2010	FSB	ASY	M
MAPA038	Blood/Feather	1401-47557	GR/AL, WH/GR	1/27/2010	FSB	ASY	M
MAPA039	Blood/Feather	1401-47558	AL/RD, GR/GR	1/31/2010	FSB	ASY	M
MAPA040	Blood/Feather	1401-47559	GR/BK, GR/AL	2/21/2010	FSB	ASY	M
MAPA041	Blood/Feather	1401-47560	YE/BK, BL/AL	2/23/2010	FSB	ASY	M
MAPA042	Blood/Feather	1401-47561	YE/BK, AL/BK	2/23/2010	FSB	ASY	M
MAPA043	Blood/Feather	1371-04922	GR/AL, WH/BK	2/23/2010	FSB	ASY	M
MAPA044	Blood/Feather	1401-47562	AL/GR, BL/RD	4/22/2010	WAIK	SY	F
MAPA045	Blood/Feather	1401-47563	GR/AL, BL/WH	4/27/2010	WAIK	ASY	M
MAPA046	Blood/Feather	1401-47564	GR/BL, BL/AL	5/17/2010	KIPA	SY	F

MAPA047	Blood/Feather	1401-47565	BL/RD, AL/GR	5/18/2010	KIPA	ASY	M
MAPA048	Feather	1401-47566	GR/GR, AL/RD	7/26/2010	FSB	AHY	M
MAPA049	Feather	1401-47511	YE/AL, YE/YE	11/16/2007	FSB	AHY	F
MAPA050	Feather	1401-47513	GR/BK, BL/AL	11/18/2007	FSB	AHY	M
MAPA051	Feather	1401-47514	AL/BK, GR/GR	11/19/2007	FSB	AHY	M
MAPA052	Feather	1401-47515	AL/BK, RD/BK	11/30/2007	FSB	AHY	F
MAPA053	Feather	1401-47517	AL/RD, RD/YE	12/2/2007	FSB	AHY	M
MAPA054	Feather	1401-47518	RD/GR, AL/GR	2/27/2008	FSB	SY	F
MAPA055	Feather	1401-47523	BK/AL, RD/RD	2/24/2008	HR3	ASY	F
MAPA056	Feather	1401-47527	RD/AL,BL/GR	12/5/2008	FSB	HY	U
MAPA057	Feather	1401-47547	AL/BL, BK/YE	11/24/2009	HR3	HY	U
MAPA058	Blood/Feather	Captive SB#1	.	7/6/2010	KBCC	ASY	M
MAPA059	Blood/Feather	Captive SB#20	.	4/24/2010	KBCC	ASY	M
MAPA060	Feather	1371-04981	YE/AL, YE/BL	1/20/2007	HR3	ASY	F
MAPA061	Feather	1371-04987	GR/AL, BK/RD	5/4/2007	HR3	ASY	M
MAPA062	Feather	1371-04979	GR/BK, AL/BL	1/17/2007	HR3	ASY	M
MAPA063	Feather	1371-04978	AL/RD, RD/BK	12/4/2006	FSB	AHY	M
MAPA064	Feather	1371-04977	.	11/18/2006	FSB	AHY	M
MAPA065	Feather	1371-04985	AL/BK, BL/RD	2/4/2007	HR3	SY	F
MAPA066	Feather	1371-04986	AL/RD, GR/RD	5/4/2007	HR3	SY	F
MAPA067	Feather	1371-04976	YE/RD, YE/AL	11/18/2006	FSB	AHY	F
MAPA068	Feather	1371-04980	BK/AL, BK/GR	1/17/2007	HR3	ASY	F
MAPA069	Feather	1371-04984	GR/YE, BK/AL	2/4/2007	HR3	SY	M
MAPA070	Feather	1371-04830	AL/RD, WH/BL	6/1/1996	FSB	AHY	F
MAPA071	Feather	1371-04962	GR/GR,AL./GR	5/11/2006	HR3	AHY	F
MAPA072	Feather	1401-47519	BK/BK,BL/AL	2/27/2008	FSB	AHY	F
MAPA073	Blood	1371-04951	BK/RD, AL/BK	1/14/2002	HR3	ASY	F
MAPA074	Blood	1371-04954	AL/RD, OR/WH	1/25/2002	HR3	ASY	F
MAPA075	Blood	1371-04952	AL/WH, GR/RD	1/14/2002	HR3	ASY	M
MAPA076	Blood	1371-04948	BK/AL, BL/GR	1/8/2002	HR3	SY	F

MAPA077	Blood	1371-04937	BL/RD, AL/WH	12/12/2003	ECF	ASY	M
MAPA078	Blood	Captive SB#3	.	6/15/2011	MBCC	.	F
MAPA079	Blood	Captive SB#10	.	6/15/2011	MBCC	.	M
MAPA080	Blood	Captive SB#18	.	6/15/2011	MBCC	.	M
MAPA081	Blood	Captive SB#11	.	6/15/2011	MBCC	.	F
MAPA082	Feather (Pins)	1181-80096	AL/WH, WH/YE	1/9/2011	HR3	ASY	F
MAPA083	Blood	1371-04960	AL/GR, BL/BL	1/9/2011	HR3	ASY	M
MAPA084	Blood	1181-80089	BL/RD, YE/AL	1/8/2011	HR3	ASY	F
MAPA085	Blood	1401-47586	RD/AL, RD/GR	2/15/2011	WAIK	ASY	F
MAPA086	Blood	1401-47587	AL/BK, RD/BL	3/2/2011	HR3	ASY	M
MAPA087	Blood	1871-17701	YE/GR, AL/RD	2/27/2011	FSB	ASY	M
MAPA088	Blood	1871-17702	YE/AL, GR/RD	3/1/2011	FSB	ASY	F
MAPA089	Blood	1871-17703	YE/GR, AL/WH	3/2/2011	FSB	ASY	M
MAPA090	Blood	1871-17704	WH/AL, BL/BK	3/2/2011	FSB	ASY	M
MAPA091	Blood	1871-17705	GR/BK, AL/GR	3/3/2011	FSB	SY	M
MAPA092	Blood	1871-17708	GR/BL, AL/BL	3/15/2011	FSB	AHY	F
MAPA093	Blood	1791-16901	RD/RD,RD/AL	5/3/2011	HR3	ASY	F
MAPA094	Blood	1871-17709	YE/BL, YE/AL	4/28/2011	FSB	ASY	M
MAPA095	Blood	1371-04939	WH/AL, WH/RD	5/19/2011	FSB	ASY	M
MAPA096	Blood	1541-80237	GR/WH, YE/AL	5/30/2011	WAIK	ASY	M
MAPA097	Blood	1541-80243	GR/AL, RD/YE	5/30/2011	WAIK	HY	.
MAPA098	Blood	Captive SB#9	.	6/15/2011	MBCC	.	F
MAPA099	Blood	1791-16902	RD/BL, AL/RD	6/14/2011	HR3	AHY	F
MAPA100	Feather (Pins)	1791-16905	BL/BK, AL/GR	6/16/2011	HR3	HY	.
MAPA101	Blood	1791-16906	YE/GR, AL/YE	6/15/2011	HR3	SY	.
MAPA102	Blood	1541-80271	YE/AL, YE/GR	6/22/2011	FSB	ASY	M
MAPA103	Blood	1541-80268	AL/RD, YE/RD	6/17/2011	FSB	ASY	M
MAPA104	Blood	1791-16904	WH/GR, RD/AL	6/15/2011	HR3	ASY	M
MAPA105	Blood	1541-80270	RD/AL, BL/BK	6/19/2011	FSB	ASY	F
MAPA106	Blood/Feather	1541-80269	GR/AL, RD/BK	6/19/2011	FSB	ASY	M

MAPA107	Blood	Captive SB#15	.	7/26/2011	KBCC	.	F
MAPA108	Blood	Captive SB#17	.	7/26/2011	KBCC	.	M
MAPA109	Blood	1791-16907	BL/YE, YE/AL	8/24/2011	WAIK	ASY	F
MAPA110	Blood	Captive SB#12	.	.	KBCC	ASY	F
MAPA111	Blood	Captive SB#4	.	.	KBCC	ASY	F
MAPA112	Tissue	.	.	5/12/2011	FSB	nestling	.
MAPA113	Blood slide	.	.	2001	HR3	.	.
MAPA119	Blood	1871-17927	BL/AL, YE/BK	9/1/2011	WAIK	AHY	M
MAPA120	Blood	1871-17928	RD/YE, AL/YE	9/2/2011	WAIK	AHY	F
MAPA121	Blood	1871-17935	GR/AL, RD/GR	.	WAIK	AHY	M
MAPA122	Blood	1871-17958	AL/GR, RD/RD	9/19/2011	WAIK	AHY	M
MAPA123	Blood	1871-17957	GR/AL, RD/BL	9/18/2011	WAIK	AHY	F
MAPA124	Blood	1791-16909	YE/BL, AL/BK	10/7/2011	WAIK	AHY	M
MAPA125	Blood	1401-47568	AL/YE, RD/RD	10/23/2011	WAIK	AHY	M
MAPA201	Toe pad	AMNH #193408	.	05/1896	Perkins	.	M
MAPA202	Toe pad	AMNH #193409	.	6/17/1901	Henshaw	.	F
MAPA203	Toe pad	AMNH #453556	.	10/1896	Palmer	.	M
MAPA204	Toe pad	AMNH #453557	.	8/4/1892	Palmer	.	M
MAPA205	Toe pad	AMNH #453558	.	8/3/1892	Palmer	.	M
MAPA206	Toe pad	AMNH #453559	.	8/16/1892	Palmer	.	M
MAPA207	Toe pad	AMNH #453560	.	8/15/1892	Palmer	.	F
MAPA208	Toe pad	AMNH #453561	.	8/3/1892	Palmer	.	F
MAPA209	Toe pad	AMNH #453562	.	8/03/1892	Palmer	.	.
MAPA210	Toe pad	AMNH #453563	.	.	Palmer	.	F
MAPA211	Toe pad	BMNH 95.7.20.170	.	1894	Perkins	.	M
MAPA212	Toe pad	BMNH 1939.12.9.57	.	8/5/1892	Rothschild	HY	M?
MAPA213	Toe pad	USNM 177972	.	1901	Henshaw		F
MAPA214	Toe pad	MCZ 47905	.	1896	Perkins	.	M
MAPA215	Toe pad	MCZ 134719	.	1894	Perkins	.	M
MAPA216	Toe pad	USNM 177971	.	1901	Henshaw	.	M

MAPA217	Toe pad	BMNH 1939.12.9.53	.	1892	Rothschild	.	M
MAPA218	Toe pad	BMNH 95.7.20.171	.	1894	Perkins	.	F
MAPA219	Toe pad	BMNH 97.10.28.22	.	1896	Perkins	.	F
MAPA220	Toe pad	BMNH 97.10.28.23	.	1896	Perkins	.	M
MAPA221	Toe pad	BMNH 97.10.28.25	.	1896	Perkins	.	M
MAPA222	Toe pad	BMNH 97.10.28.24	.	1896	Perkins	.	M
MAPA223	Toe pad	CMZ 27/DRE/9/a/1	.	1894	Perkins	.	M
MAPA224	Toe pad	CMZ 27/DRE/9/a/2	.	1896	Perkins	.	M
MAPA225	Toe pad	CMZ 27/DRE/9/a/3	.	1896	Perkins	.	F
MAPA226	Toe pad	CMZ 27/DRE/9/a/4	.	1896	Perkins	HY	M
MAPA227	Toe pad	CMZ 27/DRE/9/a/5	.	1896	Perkins	.	M
MAPA228	Toe pad	BBM 241		.	.	.	.
MAPA229	Toe pad	BBM 4094		.	.	.	.

Appendix F. Maui Alauahio haplotype diversity

INTRODUCTION

The endangered Maui Parrotbill (Kiwikiu; *Psuedonestor xanthophyrs*) and Maui Alauahio (*Paroreomyza montana)* are two extant insectivorous honeycreepers endemic to the island of Maui. Both have suffered severe range contractions from the destruction of habitat and the compounding impacts of exotic species and diseases (Scott *et al.* 1986). Avian malaria has decimated low and mid-elevation bird populations throughout the islands and is a primary threat because of its potential rise in elevation (Benning *et al.* 2002).

Currently, 421 (209-674 with 95% CI) Maui Parrotbills and 55,262 (52,729-57,921 with 95% CI) Alauahios persist within 40-50 km^2 of native forest (Brinck *et al.* 2011). Differences in life history traits may be responsible for the difference in abundance of these species, which have suffered similar range contractions and are susceptible to the same threats within their habitat.

Maui Parrotbill (left) and Maui Alauahio (right).

The low population number for Maui Parrotbills suggests that genetic factors may be increasing their risk of extinction as populations with low diversity are susceptible to the impacts of inbreeding depression, which increases their susceptibility to novel diseases (Frankham *et al.* 2002). The evolution of a resistance to malaria is essential for the long-term survival of Hawaii's honeycreepers, and a high genetic diversity will likely facilitate this evolution (Kilpatrick 2006; Foster *et al.* 2007). We investigated the mitochondrial DNA diversity in Maui Parrotbills and Maui Alauahios using control region sequence data.

METHODS

Study Sites and Sample Collection

Maui Parrotbills and Maui Alauahios were sampled from Hanawi Natural Area Reserve, Kipahulu Valley (Haleakala National Park) and Waikamoi Preserve (The Nature Conservancy) (Figure E.1). Birds were caught with mist-nets and blood samples were collected from the brachial vein.

mtDNA Extraction and Amplification

Genomic DNA was extracted from blood samples using an ammonium acetate precipitation method (Nicholls *et al.* 2000). We used the control-region primers LCRL1 (5'-CGCTATGACCCTCCACGAA-3') and HCR1045 (5'- GAGACGACCTTATCCGCAAA-3') (Tarr 1995) for Maui Parrotbills and L16743 (5'-TTCTCCGAGATCTACGGCCT-3') (Tarr 1995) and CH1 (5'-CCAATAGCGCAAAAGAGCAA-3') (Marthinsen *et al.* 2008) for Maui Alauahios. PCR products were sequenced by Macrogen Genomics and Source BioScience.

The first 10 DNA extractions were sequenced off both the forward and the reverse primers and showed no differences in base calls between the two. Subsequent samples were sequenced on the forward primer only. Chromatographs were edited using FinchTV (Geospiza Inc.). Sequences were aligned in ClustalX Version 2 (Larkin *et al.* 2005). Samples with rare polymorphisms were sequenced twice by Macrogen Inc. and Source BioScience. Standard DNA polymorphism and genetic differentiation measures were calculated in DnaSP Version 4.00 (Rozas *et al.* 2003).

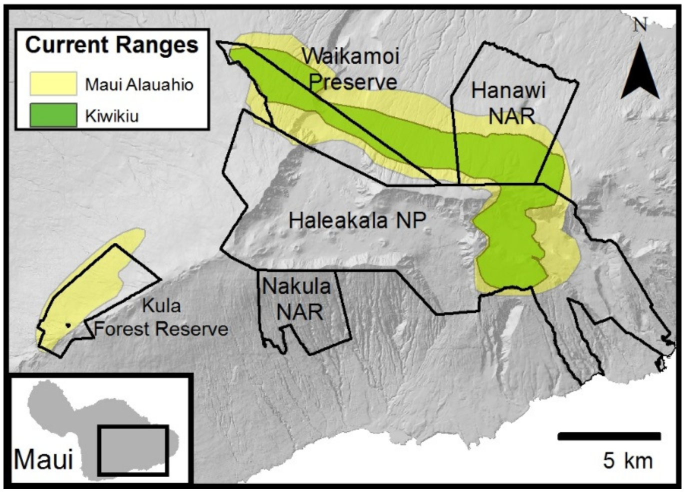

Figure E.1*. Current ranges of the Maui Parrotbill and the Maui Alauahio overlaying East Maui's protected areas.*

RESULTS

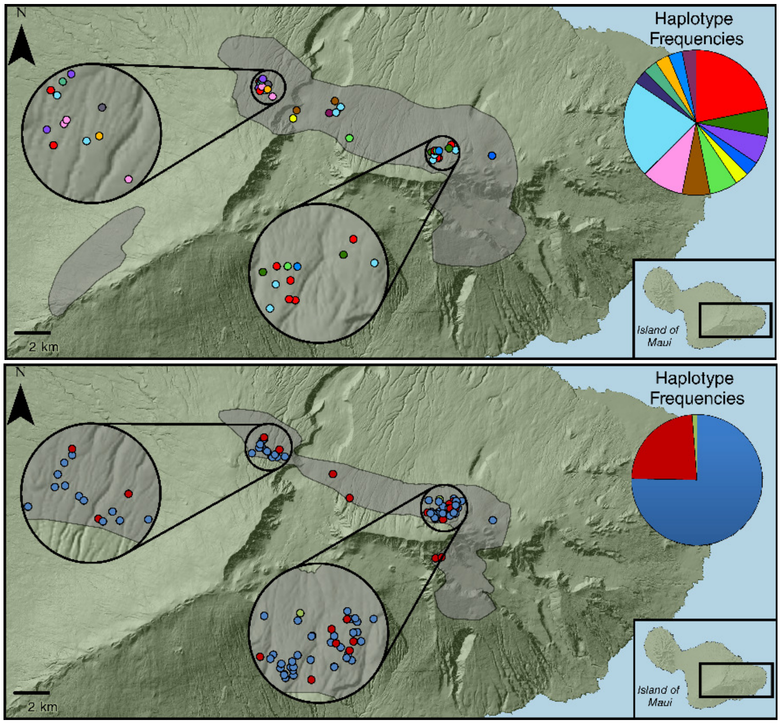

Figure E.2*: Locations of sampled Maui Parrotbills and Maui Alauahios and the distribution of unique haplotypes, each represented by a different color. The pie chart displays the proportion of the sampled birds expressing each of these haplotypes.*

I sequenced 74 Maui Parrotbills across the population. These data defined 3 haplotypes (A, B, and C) of 667 bp in length. While haplotypes A and B were found in all 3 parts of the populations, haplotype C was only found in one individual. Haplotype diversity (*Hd*) was 0.365, nucleotide diversity (π) 0.001.

I sequenced 32 Maui Alauahios within the range of Maui Parrotbills. These data defined 14 haplotypes of 519 bp in length. There was wide variation in base pair changes for Alauahios. (*Hd*) was 0.901 and (π) was 0.006.

DISCUSSION

Maui Parrotbills have a much lower genetic diversity than Maui Alauahios. Although this is expected due to their smaller population size, the high level of diversity in Alauahio and the comparison between these two species was surprising. An earlier evolutionary divergence, a lesser degree of specialization and a higher fecundity are likely responsible for the larger population and the higher level of genetic diversity in Alauahio. The small population size and low genetic diversity of Maui Parrotbills makes the reestablishment of a second wild population a high priority for their recovery (USFWS 2006).

In the radiation of the Hawaiian honeycreepers, Maui Alauahio diverged 1.18 million years earlier than Maui Parrotbill (Lerner *et al.* 2011). Mitochodrial DNA is theorized to mutate at a mean rate of about 2% sequence divergence per million years, so this earlier divergence may have benefitted Alauahio some, but other factors must have contributed (Lovette 2004).

Maui Parrotbills, with their powerful hooked bill, are more specialized, and do not persist in the exotic forests occupied by Alauahios. This suggests a higher level of adaptability and behavioral plasticity in Alauahios. A higher fecundity also may help Alauahios maintain higher densities and a larger population than Maui Parrotbills (Simon *et al.* 1997, Baker and Baker 2000).

Evidence suggests that Maui Parrotbills have saturated their available habitat and that young disperse to lower elevations where they are more susceptible to avian malaria (MFBRP unpubl.) An expansion of habitat would allow an increase in population and genetic diversity and this may facilitate the evolution of a resistance to malaria. This resistance has been demonstrated in Hawaii Amakihis (*Hemignathus virens)* on the island of Hawaii (Foster *et al.* 2007).

Restoration efforts have begun in Nakula Natural Area Reserve (NAR). Nakula NAR lies in Kahikinui Forest Reserve on the leeward side of Haleakala. Fossil evidence suggests that Maui Parrotbills and Alauahios once persisted there (Scott *et al.* 1986, Baker and Baker 2000). Managers plan to translocate Maui Parrotbills to this new habitat within five years.

Although the impacts of founder effects may be severe for Maui Parrotbills, their translocation to the expanded range may be their only hope for increasing populations and genetic diversity. Maui Alauahios have a greater potential to develop a resistance to malaria and will become even greater if introduced to the new habitat because of their higher diversity.

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Appendix G. Leeward Haleakala Experimental Restoration Plan

Protocols for Restoration Trials in the Nakula Natural Area Reserve

This report was generated for internal use and for distribution to partners of Maui Forest Bird Recovery Project, State of Hawaii Division of Forestry and Wildlife and American Bird Conservancy by Chris Farmer, David Leonard, and Hanna Mounce

NAKULA NATURAL AREA RESERVE

The Nakula Natural Area Reserve (NAR; 614 ha) on the leeward slope of Haleakalā is continuous with the 925 ha Kahikinui Forest Reserve (FR) (Figure 1). The dramatic elevation change across the NAR (> 1,700 vertical m in 4 km) and the corresponding moisture gradient has compressed several native habitats into a relatively small area. Nakula NAR has numerous small gullies, which provide moist, shady habitat and support several rare plant species in what is now a harsh, dry environment. These gulches also protect native plants from ungulates. Leeward koa forests on the slopes of the larger Hawaiian volcanoes are unique in that they depend largely on precipitation and fog drip from clouds created by convection and diurnal heating. They differ markedly from koa forests on the windward slopes of the islands.

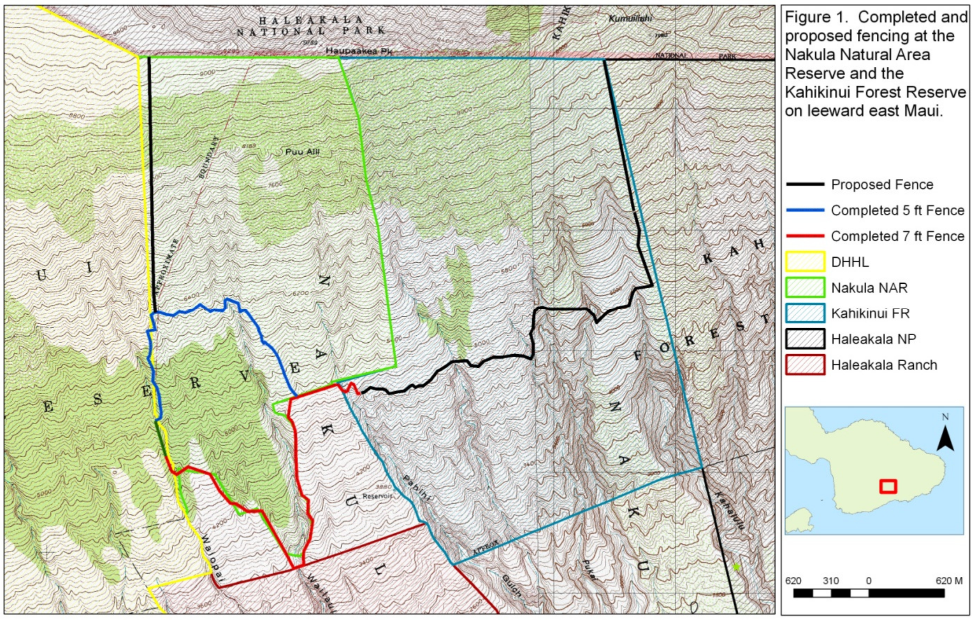

Figure 1. Location of Nakula NAR, Kahikinui FR, and existing and proposed fence lines.

Between the top of the NAR at 2,830 m and approximately 2,461 m a relatively intact Pūkiawe / ʻŌhelo Dry Subalpine Shrubland (plant community classifications follow Gagne and Cuddihy 1999) predominates and is characterized by a dense pūkiawe shrubland matrix, interspersed with native grass and fern patches. Although feral goats and pigs are present, their impact on this community has been minimal.

Between 2,461 m and 2,000 m, goats have mostly denuded the native vegetation. However, remnants of ʻŌhiʻa Subalpine Dry Forest and Māmane Subalpine Dry Forest persists in some of the larger gulches, or in areas where the underlying substrate has resisted erosion.

Below the temperature inversion layer at about 2,000 m ungulates have mostly converted the Koa / ʻŌhiʻa Montane Mesic Forest to an open grassland dominated by non-native pasture grasses. Prior to the invasion of ungulates, many rare plants occurred in this community. At the upper reaches of this community, a dry subtype of this forest exists, with a koa canopy and an understory of tall ʻaʻaliʻi shrubs. As moisture increases with decreasing elevation, species diversity and tree size increase, and this community is best represented between 1,077 m and 1,385 m elevation. This portion of the NAR has many gulches and cliff faces, which provide protected microhabitats, as well as springs and seeps that feed intermittent streams. In areas inaccessible to ungulates, a diverse assemblage of native ferns and understory plants persist.

Below 1,077 m elevation, moisture decreases, and the vegetation grades into a severely degraded remnant of what was once a diverse assemblage of dryland trees; classified loosely as Olopua Montane Mesic Forest.

A 150 ha parcel between 1,108 and 1,785 m has been fenced, and ungulates will be removed in the fall of 2012 (Figure 2). This area historically supported a koa-ʻōhiʻa montane mesic forest, which declined markedly between 1890 and 1930 due to feral ungulates (Hosmer 1912). The area is now mostly pasture dominated by kikuyu grass (*Pennisetum clandestinum*) with widely scattered native trees, although a diverse assemblage of native ferns and other understory plants are present in areas inaccessible to ungulates. This area receives over 1,000 mm of rainfall annually, with 70% falling between November and March. Temperatures rarely fall below 8° C (Minyard et al. 1994). Site access is by helicopter or a rugged 4.8 km hike.

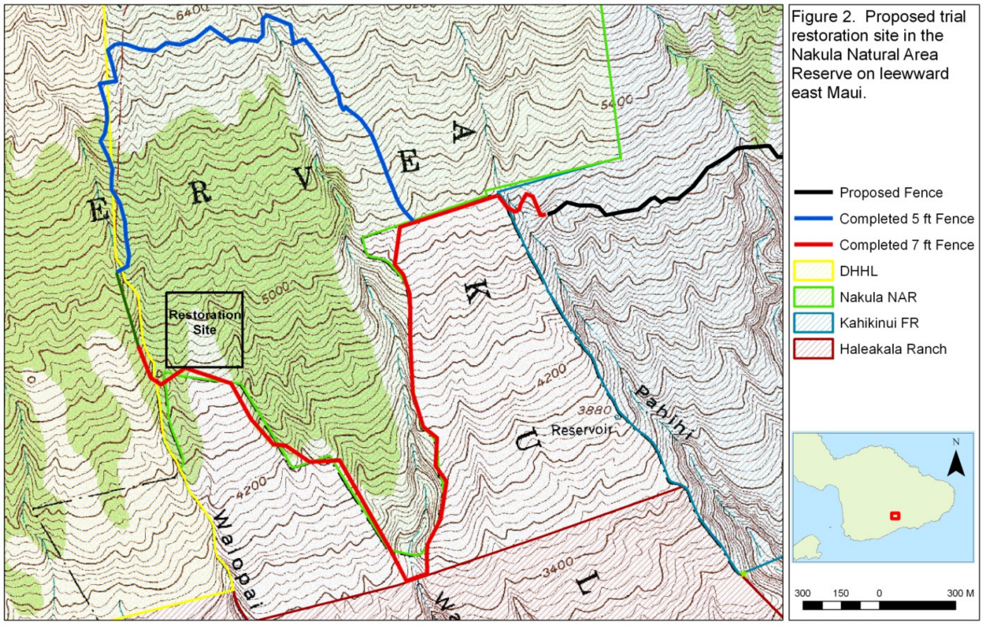

Figure 2. Location of the trial restoration site within the Nakula NAR.

This plan outlines experimental trials that will be conducted within the restoration area (Figure 2, delineated by the red and blue completed fencelines) to determine cost-effective protocols for the eventual restoration of native forest within the entire Nakula NAR. The preliminary data from these trials will be incorporated into an overall Nakula Restoration Plan that will expand and implement these results to the entire NAR. The ultimate goal is to restore the ecosystem so that the landscape is capable of supporting a self-sustaining Maui Parrotbill population.

MAUI PARROTBILL

The endangered Maui Parrotbill (*Pseudonestor xanthophrys*) numbers ~500 individuals and is restricted to ~40 km^2 of high elevation ‘ōhi‘a (see Table 1 for scientific names) forest on windward east Maui. Their current distribution is an artifact of habitat loss and the past and current distribution of alien diseases (e.g., avian malaria [*Plasmodium relictum*]) and alien disease vectors (i.e., mosquitoes; USFWS 2006). However, this habitat is likely suboptimal partly because winter storms are a significant cause of nest failure (Becker et al. 2010). Historically parrotbills occurred in mesic koa forest and this may be the species' preferred habitat (Perkins 1903). Restoring remnant koa forest on leeward east Maui in the

Nakula NAR and the Kahikinui FR and establishing a second population is a high priority recovery action (USFWS 2006). Leeward east Maui is drier than windward habitats and storm frequency is lower and storms are less severe (i.e., more suitable; Mawdsley et al. 2009). In addition, leeward areas support few mosquitoes and once restored will provide additional high elevation, disease-free forest, which is critical as climate change will facilitate the upward movement of malaria (Benning et al. 2002). Restoring degraded habitat for rare species is a common sense approach to addressing climate change (Hunter et al. 2010, Lindenmayer et al. 2010). Creating an additional population and increasing the number of individuals, could facilitate the development of disease resistance (Kilpatrick 2006) and robust populations, well distributed across suitable habitat, are most likely to persist despite climate change (Schwartz et al. 2006).

Table 1. Maui Parrotbill food plants, important canopy, or midstory species, the difficulty of successfully growing large numbers of individuals (1 easiest, 5 most difficult), and the number of months required for seedlings to mature in SC10 dibble tubes.

Common name	Scientific name	MAPA food plant	Difficulty	SC10
ʻAʻaliʻi	*Dodonaea viscosa*	No	1	10
Ākala	*Rubus hawaiensis*	Yes	1	5
Alani	*Melicope clusiifolia*	Yes	3	10
Kanawao	*Broussaisia arguta*	Yes	5	na
Kāwaʻū	*Ilex anomala*	Yes	4	8
Koa	*Acacia koa*	Yes	2	5
Kōlea	*Myrsine lessertiana*	Yes	1	6
Mamaki	*Pipterus albidus*	No	2	5
Māmane	*Sophora chrysophylla*	No	1	10
ʻŌhelo	*Vaccinium calycinum*	Yes	5	na
ʻŌhiʻa	*Metrosideros polymorpha*	Yes	3	10
ʻŌlapa	*Cheirodendron trigynum*	Yes	3	9
Pilo	*Coprosma* spp.	Yes	1	6
Pūkiawe	*Styphelia tameiameiae*	Yes	5	na

NAKULA RESTORATION TRIALS

The entire Nakula NAR and 376 ha of the Kahikinui FR will be fenced by February 2014. What is learned from these trials may have some applicability to the Kahikinui FR, but additional methods will likely have to be developed to restore the most degraded areas. Trials will focus on dominant canopy (e.g., koa, ʻōhiʻa) and subcanopy (māmane, ʻaʻaliʻi) species as well as important Maui Parrotbill food plants. Seed availability, germination and growing success will limit the species available for outplanting. Initially, the nine species that are easiest to germinate and grow in the nursery (Table 1, species with a difficulty ranking of 1–3) will be attempted. Seeds of the remaining species will be collected opportunistically and provided to Native Nursery, LCC (1267 Naʻalae Road, Kula, HI 96790; the nursery with which the state has a standing contract) so that propagation protocols can continue to be refined. Additional seeds from all species will be collected, processed, and stored with the Maui

Forest Bird Recovery Project (MFBRP) until ready to be used for seed scatter trials. A weather station that collects daily temperature highs and lows and rainfall will be installed on-site.

Plot placement

All the plots will be placed in open areas (i.e., no canopy), and with a relatively even slope (i.e., not super steep or dead-flat) at approximately 1,500–1,600 m elevation (Figure 2). The plots will be in the large, relatively open grassy area in the central western section of the parcel.

Plot locations for the three experimental trials (outplanting, seed scatter, and natural regeneration) will be determined during the July and October seed collection trips. Plots to quantify natural regeneration will be monitored as soon as the area is ungulate-free. Seed scattering within plots will be initiated as soon as sufficient quantities of seeds have been collected and the area is ungulate-free. Treatments will be applied to outplanting plots based on the availability of seedlings. All plots will be permanently marked and GPS coordinates collected.

Outplanting

To develop outplanting protocols that maximize survival and minimize costs, twenty-seven 10 x 15 m plots will be established (Table 2). Three replicates of three treatments will be deployed for nine species (see below): 1) No treatment, living grass (i.e., Control; Treatment P1), 2) grass killed with herbicide (i.e., Treatment P2), and 3) grass killed with herbicide cleared with a weed-eater or mattock (i.e., Treatment P3). The species mix outplanted may change based on seedling availability.

Details of treatments

Treatment 2 (herbicide) will use a mixture of two non-restricted use pesticides, 0.8% Honcho Plus (glyphosphate; EPA Reg. No. 524-454) and 0.15% Polaris AC (imazapyr; EPA Reg. No. 228-570). These will be combined with and 0.5% Can-Hance surfactant, and applied 80–110 days prior to outplanting at a rate of about 757.1 liters/ha. These herbicides have been used effectively at Ulupalakua Ranch, Maui (J. Leary pers. comm.) and Pu‘u Mali, Hawai‘i Island (R. Stephens, pers. comm.) to prepare sites dominated by exotic grasses for restoration. For Treatment 3, an approximately 0.5 m^2 area will be cleared with a weed-eater for each seedling.

Planting of seedlings

Seedlings will be planted using a planting stick (ʻōʻō) or pick. Staff from Leeward Haleakala Watershed Restoration Partnership will provide instructions on proper planting techniques prior to the first planting trip. One hundred and fifty seedlings will be planted, at 1 m spacing, in each plot. Kōlea, māmane, and mamaki (species group 1) will be planted at nine plots; ʻaʻaliʻi, ākala, and ʻōhiʻa (species group 2) will be planted at nine plots; and koa, ʻōhiʻa, and pilo (species group 3) will be planted at nine plots. The remaining species (alani, kanawao, kāwaʻū, ʻōhelo, and pūkiawe) were not selected due to germination difficulty and low seed availability (Table 1). ʻŌlapa seeds were collected and planned for planting group 3. However, poor germination meant that the species had to be replaced by ʻōhiʻa in this planting group. Poor germination also meant that fewer ākala will be ready for planting; 38 individuals / plot instead of 50 / plot. As a result additional ʻaʻaliʻi and ʻōhiʻa will be planted per plot in group 2, 56 / species/ plot. In planting group 3, 28 ʻōhiʻa / plot will be planted, taking the place of the planned ʻolapa, and 61 koa and pilo / plot will be planted to make up the difference. All the seedlings cannot be planted at once, so each group will be planted in alternate, staggered weeks. In each treatment 1,350 seedlings will be planted (Table 2). Seedlings will be planted systematically, alternating species for subsequent identification and monitoring. Blue-X tree shelters will be installed on 20 randomly selected seedlings / species / plot for species with ≥ 50 individuals per plot and ½ of plants will receive shelters for species with < 50 individuals per plot; 60 tree shelters per plot in G1, 59 shelters per plot in G2, and 54 shelters per plot in G3. Survival of all seedlings per species per plot will be tracked throughout the experiment. Survival will be assessed at 6, 12, 18, and 24 mo. The following information will be collected and examined for an effect on survival: slope, aspect, presence of shelter, rainfall, and temperature. This information will allow subsequent planting protocols and locations to be fine-tuned to increase the survival of seedlings.

	Species Group 1				Species Group 2				Species Group 3				
	Kl[1]	Sc	Pa	Subtotal	Dv	Rh	Mp	Subtotal	Ak	Mp	Cs	Subtotal	TOTAL
Treatment 1 (control)													
Replicate Plot 1	50	50	50	150	56	38	56	150	61	28	61	150	450
Replicate Plot 2	50	50	50	150	56	38	56	150	61	28	61	150	450
Replicate Plot 3	50	50	50	150	56	38	56	150	61	28	61	150	450
Subtotal	150	150	150	450	168	114	168	450	183	84	183	450	1350
Treatment 2 (herbicide)													
Replicate Plot 1	50	50	50	150	56	38	56	150	61	28	61	150	450
Replicate Plot 2	50	50	50	150	56	38	56	150	61	28	61	150	450
Replicate Plot 3	50	50	50	150	56	38	56	150	61	28	61	150	450
Subtotal	150	150	150	450	168	114	168	450	183	84	183	450	1350
Treatment 3 (herbicide + cleared)													
Replicate Plot 1	50	50	50	150	56	38	56	150	61	28	61	150	450
Replicate Plot 2	50	50	50	150	56	38	56	150	61	28	61	150	450
Replicate Plot 3	50	50	50	150	56	38	56	150	61	28	61	150	450
Subtotal	150	150	150	450	168	114	168	450	183	84	183	450	1350
TOTAL	450	450	450	1350	504	342	504	1350	549	252	549	1350	4050

Table 2. Number of seedlings per replicated plot, per treatment, and overall in outplanting trials. Twenty individuals of each species will receive Blue-X tree shelters for all species with ≥ 50 individuals per plot and ½ of plants will receive shelters for species with < 50 individuals per plot; 60 tree shelters per plot in G1, 59 shelters per plot in G2, and 54 shelters per plot in G3. Each plot will be 10 x 15 m.

1 Kl = Kōlea, Sc = Māmane, Pa = Mamaki; Dv = ‘A‘ali‘i, Rh = Ākala, Mp = ‘Ōhi‘a; Ak = Koa, Cs = Pilo.

Outplanting Corridors

Uneven germination between species has meant that fewer than 50 individuals / species / plot will be available for planting, as discussed above. However, for other species far more individuals germinated than expected resulting in more individuals ready for planting than are necessary for the experimental plots. Due to the value of these seedlings that were sourced from within the study site, one or more “planting corridors” will be established running along contours, effectively connecting drainages. These corridors will serve as repositories for any “extra” seedlings not required for the restoration trial experiment. Priority of “extra” seedlings will be given to tree canopy plots and then the planting corridors. Plants will be planted at 3 m intervals throughout the corridor in a systematic manner among species. Planting locations will be prepared with herbicide in the same fashion as the herbicide treatment outplanting plots. Survival and height will be recorded for all individuals planted in corridors.

Seed Scatter

To evaluate the efficacy of seed scatter, sixteen 5 x 10 m plots will be established. Four replicates of four treatments will be deployed: 1) No treatment; living grass (i.e., Control; Treatment S1), 2) grass killed with herbicide (i.e., Treatment S2), 3) grass killed with herbicide and removed with a rake (i.e., Treatment S3), 4) plot scarified with a mattocks, and no herbicide applied (i.e., Treatment S4). The more intensive treatments (S3, S4) are not feasible to conduct over the entire 614 ha NAR, but the results will indicate the maximum restoration potential at the site. The results will also indicate the practicality and utility of creating small, restored “habitat islands” across the landscape. These restored “islands” could serve as seed sources, and also potentially make the microclimate more hospitable for seedling establishment and growth. These changes could serve to jump start the restoration across the NAR, and increase the effectiveness of the less labor-intensive techniques (e.g., S2). A mix of all available native species’ seeds, but most likely the 14 species in Table 1, will be hand broadcast over the area and gently raked as appropriate. The amount of seed per species broadcasted will be carefully documented. The exact mix and amount of seeds will depend on the amount of seed collected, but the minimum threshold will be 320 seeds per species (20 seeds per plot, 16 scatter plots). The seed plots will be searched every 3 mo. for seedlings, and an appropriate number of seedlings will be marked and tracked to determine germination and survival. We will request that Native Nursery provides photographs of seedlings of all species or staff will visit the nursery to become familiar with the species. See below for protocols for treating seeds prior to scatter.

Seed Collection and Growing Seedlings

Seeds will be collected on site, or as near to the site as practical. As many seeds as possible will be collected following the protocols outlined below. All seeds will be germinated and grown by Native Nursery. Seeds for scatter will be processed and stored with MFBRP.

Natural Regeneration

To evaluate the presence of a seed bank and the rate of natural recruitment, twenty-four 10 x 10 m plots will be established. These plots also will provide data on the number of seedlings expected to germinate if no seeds were scattered). Six replicates (Table 3) of four treatments will be monitored: 1) No treatment; living grass (i.e., Control; Treatment R1), 2) grass killed with herbicide (i.e., Treatment R2), 3) grass killed with herbicide and removed with a rake (i.e., Treatment R3), 4) plot scarified with a mattocks and no herbicide applied (i.e., Treatment R4). Similar to the seed scatter trials, the more labor intensive treatments (R3, R4) will indicate the maximum restoration potential of these techniques, and their potential utility in creating smaller, restored “islands” across the greater landscape. To examine distance effects from mature koa trees, the edge of plots will be placed 5 and 25 m from the edge of the crown of living koa trees. Three plots per distance per treatment will be deployed. Plots will be searched semi-annually for recruitment and an appropriate number of seedlings will be flagged to track survival and to determine factors associated with germination (i.e., distance from mother tree, seed bed [mineral or organic]).

Table 3. Number of natural regeneration plots for each treatment (R1–4).

	5 m[1]	25 m
Treatment R1. control	3	3
Treatment R2. herbicide	3	3
Treatment R3. herbicide + cleared	3	3
Treatment R4. scarified	3	3

[1] Distance from the edge of the crown of the largest living koa tree in the area.

Tree canopy

To evaluate the effect of growing under the canopy of mature trees, eighteen mature, living koa trees will be selected as “tree canopy plots”. Plot borders will be defined by the drip-line of selected trees. Trees will be non-randomly selected to reduce variation in plot area but an effort will be made to select trees throughout the restoration trial area at the same elevation as the other treatment plots. These plots will provide data on the potential benefits of the comparatively more mesic microhabitat that exists below the drip-line of mature trees. Four replicates of four treatments will be applied: 1) No treatments; living grass (i.e. Control; Treatment T1), 2) grass killed with herbicide (i.e. Treatment T2), 3) grass killed with herbicide and removed with a weed-eater and/or rakes (i.e. Treatment T3), 4) aboveground grass removed with a weed-eater (i.e. Treatment T4). Two additional plots will be sprayed with fertilizer (i.e. Fertilizer, Treatment T5) to potentially promote growth and stimulate the natural seed bank. Plots in Treatments T1-T4 will be divided in half creating two equal sections with respect to canopy cover and slope. Half of each plot will receive outplantings (in a similar manner to the outplanting plots) and the other half will not (similar to natural regeneration plots). Dividing plots in half will help account for variation in plot size and slope. All outplantings will be flagged. The fertilizer plots will not receive outplantings. The following information will be collected and examined for an effect on survival: slope, aspect, diameter at breast height of plot tree, height of plot tree, plot area, rainfall, and temperature.

Timeline

Seed collecting trips

Based on documented phenology (Lamoureux 1973, Medeiros 1998, Berlin et al. 2000) seed collecting trips to Nakula are scheduled for July 2012, October 2012, and January 2013. However, additional trips will be scheduled based on information from the field regarding seed availability.

Outplanting

The timing of outplanting will be dependent on having a sufficient number of seedlings, but are tentatively scheduled to start in September 2013. Planting should proceed as soon as possible, although the driest months (June–August) should be avoided.

Seed Scatter

Seed scatter should be conducted several times during the winter rainy season, but will be dependent on having sufficient seeds.

Natural Regeneration

Monitoring the natural regeneration within the plots will begin as soon as the plots are established and the majority of the ungulates are removed, e.g., fall 2012.

Seed collection and treatment protocols

Alvin Yoshinga (2001, 2007, 2010) has written extensively about seed collection, preparation and storage for native Hawaiian plants. We will be following his recommended protocols for most species.

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Appendix H. Supplemental Feeding Trials for Wild Maui Parrotbills

2012 and 2013 Experiments with Developing and Using Supplemental Feeders for Kiwikiu (Maui Parrotbill; *Psuedonestor xanthophrys*): Potentials for translocation efforts and increasing productivity

This report was generated for internal use and to share with partners at Maui Forest Bird Recovery Project by Hanna L. Mounce and Laura K. Berthold

Introduction

The Kiwikiu is a critically endangered insectivorous Hawaiian honeycreeper, with a population of ~500 individuals found only on the windward side of east Maui (Simon *et al*. 1997). Lack of habitat due to invasive species destruction and the presence of avian malaria and non-native predators are some of the reasons why Kiwikiu are endangered.

Additionally, recent research has indicated that productivity may be inadequate to increase the population, and available habitat may decrease with climate change. Two management strategies that could be critical towards recovery are:

1) Determining a method of population management that will increase reproductive output

2) Expanding available habitat through restoration and invasive species management in addition to creating a second population through reintroductions on the leeward side of east Maui

Providing supplemental food to birds has been found to increase reproductive output by boosting clutch size, number of breeding attempts, nestling weight, and the number of independent young produced, e.g. Song Sparrows (*Melospiza melodi*) (Arcese and Smith 1988) and Florida Scrub Jay (*Aphelocoma coerulescens*) (Schoech *et al*. 2007). Supplemental food has also been used when reintroducing a population, such as the endangered Hihi (*Notiomystis cincta*) in New Zealand (Castro *et al*. 2003).

During the 2012 and 2013 breeding seasons, we experimented with providing supplemental food to closely monitored Kiwikiu breeding pairs. If Kiwikiu use supplemental feeding stations this could increase productivity of the existing population and assist with the re-establishment of a second population.

Materials and Methods

Kiwikiu pairs were located and monitored February through June in 158 ha of The Nature Conservancy's Waikamoi Preserve, between 1600 and 1900 m in elevation (Fig. H.1). Feeding stations were set up in April of 2012 and February of 2013 based on pair activities.

We provided commercially raised mealworms on a feeder tray situated 1 m above ground in 2012 and 2.5 m above the ground in 2013 (Fig. H.2). The feeding apparatus was designed to be rat-proof and to slowly dispense mealworms over time. Stations were monitored with remote trail cameras (Reconyx PC800 HyperFire Professional Semi-Covert IR) and visited every few days for maintenance.

Since Maui Alauahio (*Paroreomyza montana*, MAAL) and Kiwikiu (MAPA) forage together, we attempted to lure both species to the stations with playback and bird decoys. We also camouflaged half of the feeders with native vegetation.

Results

2012

Six feeder stations were installed in 2012 (Figure H.1, Table H.1). Five were located in an area where pairs with a hatch-year (HY) regularly foraged and one where a pair was nest building. Cameras were set up at four of six feeders.

The only species detected using the feeders were Red-billed Leiothrix (*Leiothrix lutea*, RBLE). Rats were also captured on camera but were never successful at getting onto the feeder trays.

When playback was used, Alauahio would chip (contact call) above the feeder but would not visit it.

2013

Six feeder stations were installed in 2013 (Figure H.2). Each were set out as a pair of feeders in areas of high MAPA activity. Cameras were set up at all feeders.

The only species detected using the feeders were Red-billed Leiothrix.

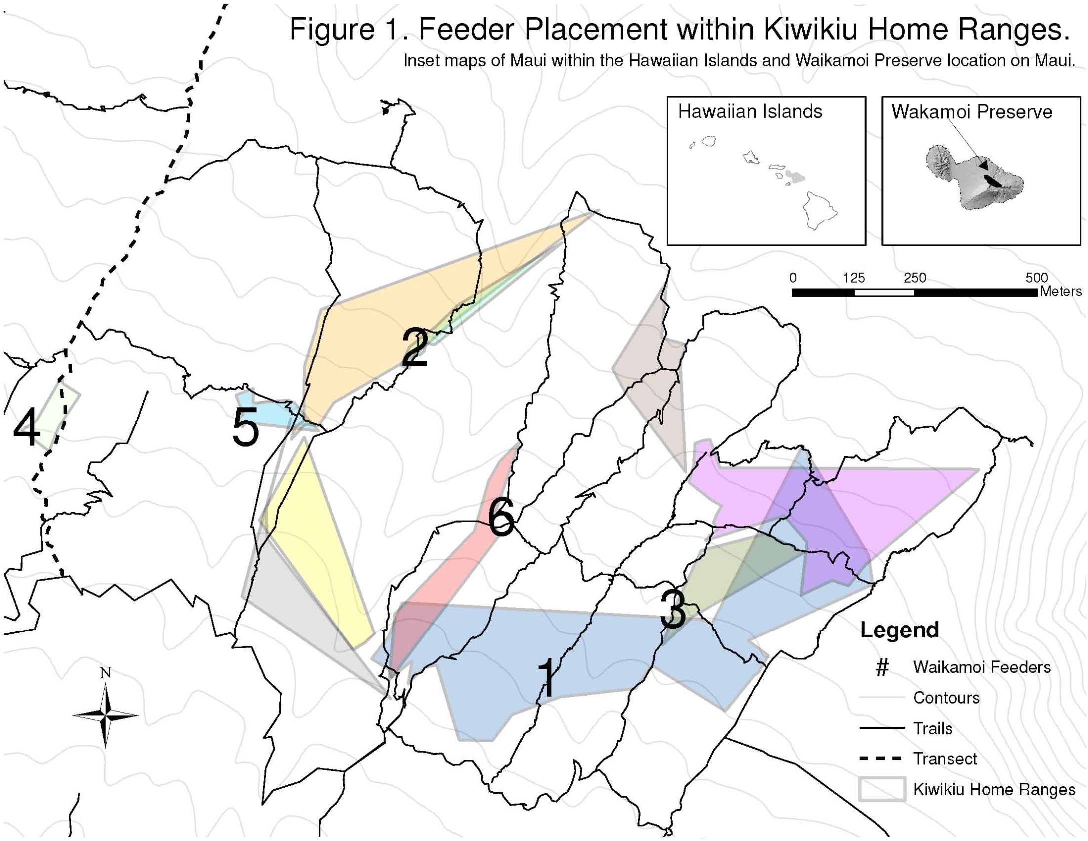

Figure H.1. Locations of feeders and pairs targeted in 2012.

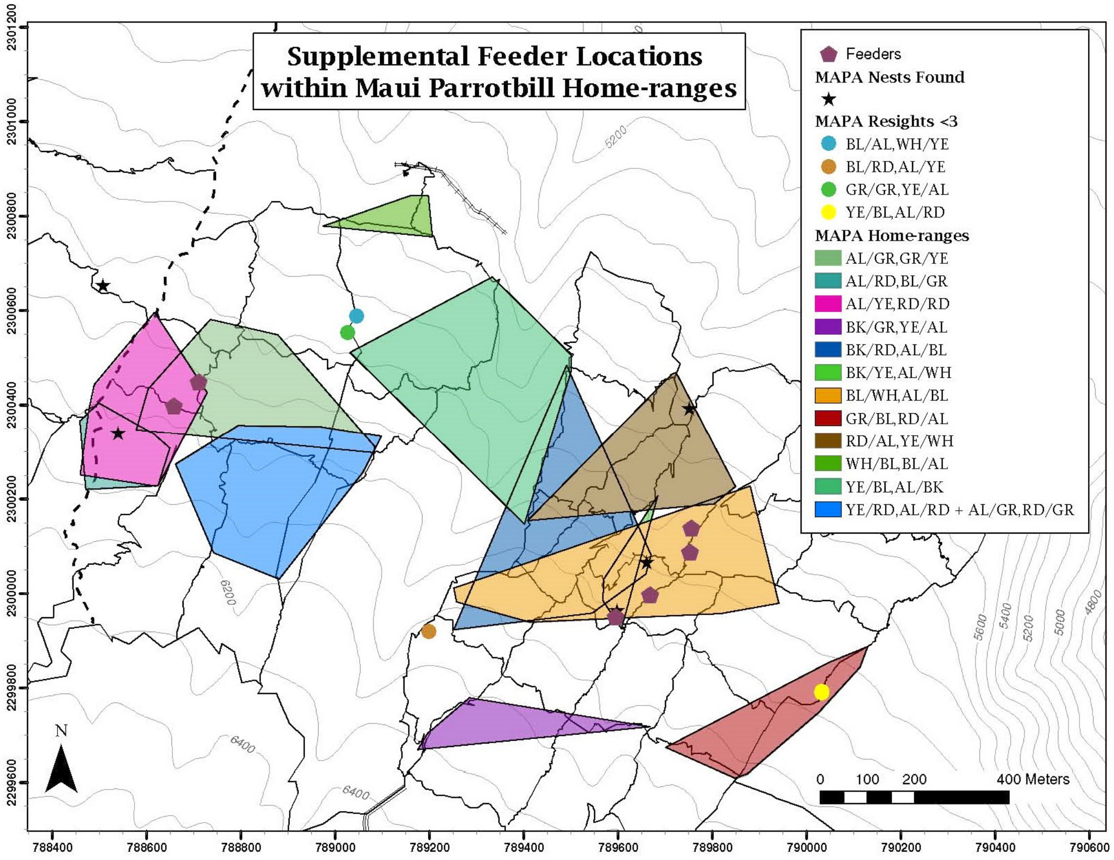

Figure H.2. Locations of feeders and pairs targeted in 2013.

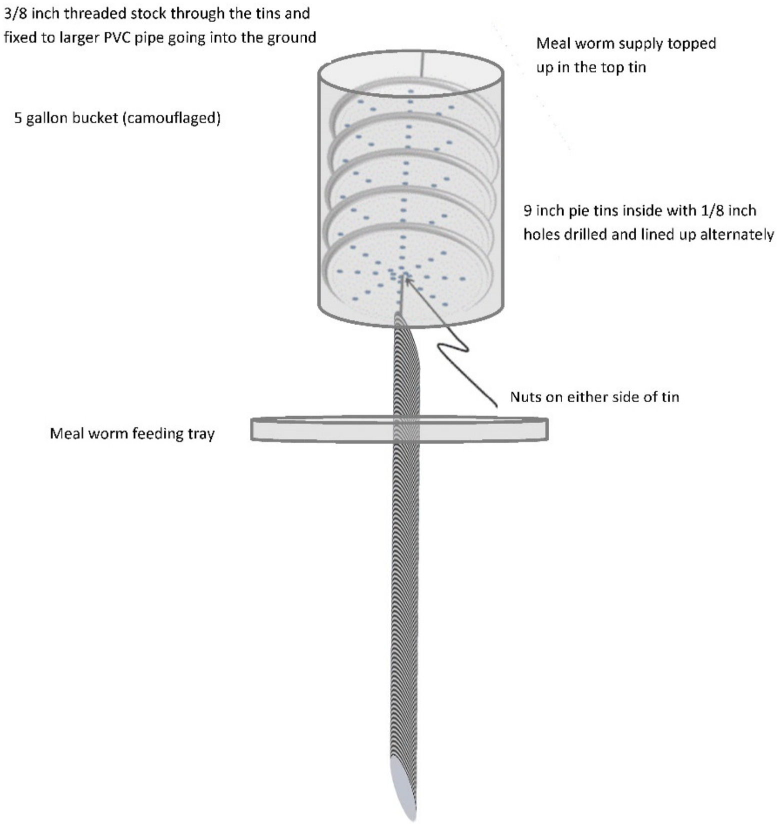

Figure H.3. Maui Parrotbill supplemental feeder design.

Figure H.4. Maui Parrotbill supplemental feeders installed in the field.

Discussion

Wild Kiwikiu have never visited the supplemental feeding stations; however, it can take time for target birds to find and use supplemented arthropod food (Podolsky *et al*. 2004). Even though we attempted to place the feeders where Kiwikiu were foraging, pairs typically forage throughout their homes ranges, which are fairly large (average ~5 ha/pair in the core of their population) (MFBRP unpublished data). It is possible that because of this, they did not find the feeders in the time that they were available. In order to increase the chances of Kiwikiu finding these feeders, in 2013 we positioned the feeders in a more clustered arrangement, but it did not change the results we observed. After the 2012 season, we also thought that more experimentation could be done with making the feeders look more natural, such as making the feeder appearance mimic a preferred plant. This was heavily focused on in 2013 again with no difference in the observed results.

Red-billed Leiothrix forage low in the understory, have higher densities, and have smaller home ranges (3 ha) (Male *et al*. 1998), which may have pre-disposed them to discovering the feeders before native species. Leiothrix pose several problems in that they quickly remove all the mealworms, may chase off other birds from the feeder, and may transmit avian disease and/or parasites via the feeder. To dissuade leiothrix from using the feeders after 2012 we increased the height of the station above the common feeding height of leiothrix but still in the foraging range of Kiwikiu but this did not discourage the leothrix use of the feeders. Another alternative might be to try the feeders outside the core of the leiothrix breeding season (April-August) (Male *et al*. 1998), but this would also be attempting to supplement Kiwikiu outside of their breeding season as well.

We would also like to work with captive Kiwikiu and perhaps released captive birds could “teach” wild birds to use the stations. When this feeder design was installed in an aviary with captive Kiwikiu, they used the feeder immediately. This would be advantageous in designing the reintroduction protocols for Kiwikiu to leeward Haleakala.

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Appendix I. Progress in Nakula Experimental Restoration Efforts

2013-2014 Results of Experimental Restoration Efforts in Nakula NAR

This report was created by Hanna Mounce and Chris Warren for distribution to Maui Forest Bird Recvoery Project Partners and financial sponsors (American Bird Conservancy and State of Hawaii Division of Forestry and Wildlife)

Outplanting Monitoring – May 2014

Monitoring protocols were designed to assess the density, diversity and survival of woody plant species ≥ 15 cm in height. The presence of key non-native species is also being recorded to assess the threat of invasive species. Initial six-month survival rates were very high with 97% of planted individuals surviving 6 months post-planting. All species had survival rates above 97% with the exception of mamaki (92%) and akala (87%). See Figure 1 for survival rates per species. Blue-X tree shelters were retained on 87% of plants where they were installed; the remainder had blown off. However, a fair number of retained shelters malfunctioned wherein they blew down but stayed on the plant. We recorded the number of malfunctioning shelters that likely had killed or were likely to kill the plant and these shelters were removed. These accounted for 11% of all shelters installed. Approximately 25-50% of remaining shelters needed adjusting (e.g. adding additional stake) per plot. Additional statistics per plot type have not yet been analyzed.

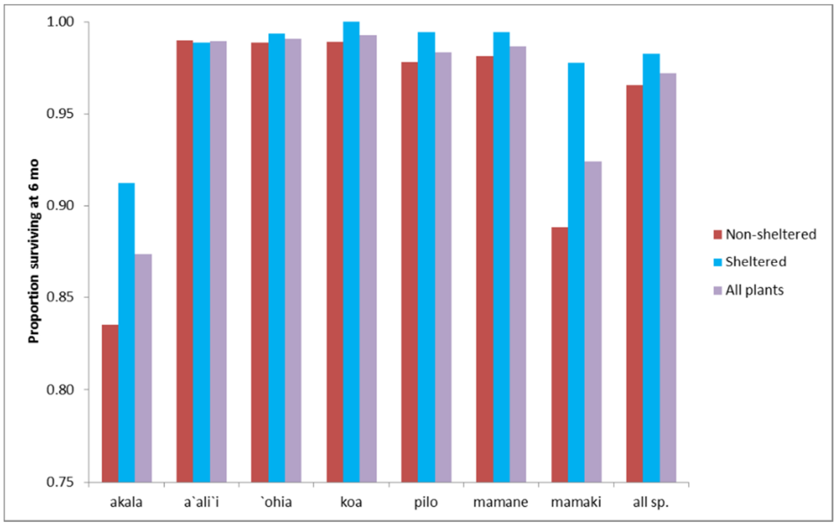

Figure 1. Six month out-planting plot survival statistics

Natural Regeneration Monitoring - July 2014

As of July 2014 the MFBRP restoration experiment entered the 24-month mark since plots were assigned and the 12-month mark for monitoring the first plots in which treatments were applied (natural regeneration treatment plots). In July, we monitored 40 restoration plots including all plots of the natural regeneration and seed scatter plots. This represented the 6-month monitoring (first round) for seed scatter plots and 12-month monitoring (second round) for natural regeneration plots. Within these two plot types, four treatments were applied; control, herbicide, herbicide and weed-whack and ground scarification.

At the 12-month mark since treatment application in the natural regeneration plots we are able to make some *preliminary* conclusions about the outcomes of the four treatments applied to these plots; control, herbicide-only, herbicide + weed-whack, and ground scarification. It is important to note that long-term success of the different treatments may change as the plots mature, particularly in regards to grass re-growth.

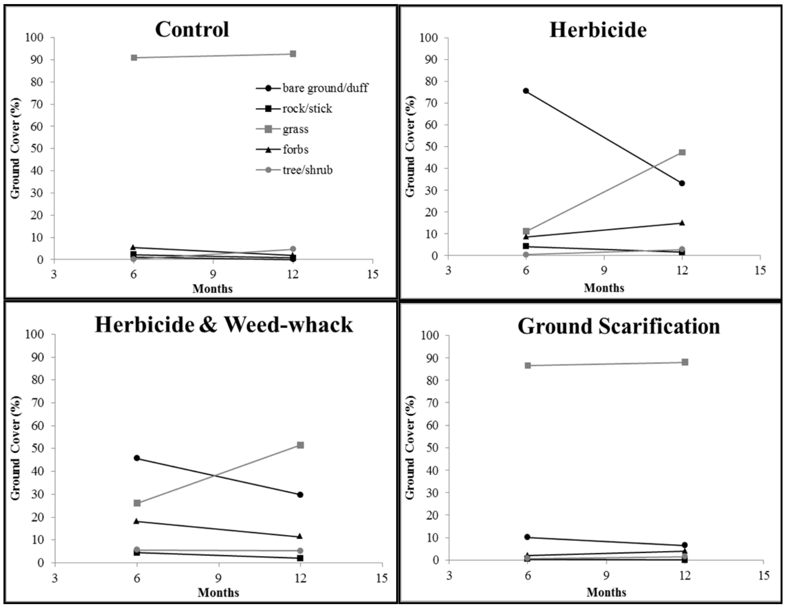

***Figure 2**. Ground cover of natural regeneration plots. This figure shows ground cover of natural regeneration plots at the 6- and 12-month marks.*

We recorded cover as the percentage (out of 100%) each of five categories covered each entire plot (10 m × 10 m). (Notes: 1) Rock/stick and tree/shrub make up a very small proportion of the ground cover at even the 12-month mark. Tree/shrub will likely remain somewhat low because each tree/shrub generally does not cover a large area and therefore there would need to be a very large number of mature trees/shrubs to add up to *much cover. 2) Grass cover recovered similarly in the herbicide and herbicide + weed-whack treatments, rebounding to 50% cover by 12-months. All plots likely had ~90% grass cover prior to treatment application. 3) Forbs (mainly non-native annuals) generally do not amount to a large proportion of the ground cover but appear to be increasing in the herbicide-only treatment. This includes anecdotal evidence that some weedy species, e.g. Cirsium vulgare, are increasing in the herbicide-only treatment and single plants can account for a fair percentage of ground cover. 4) The ground scarification method used (described below) was insufficient in suppressing the overall dominance of grass within experimental plots. By 6-months, the first monitoring time period, these plots showed little to no difference compared to the control plots in overall ground cover patterns. By 12-months the exposed topsoil sections were still visible but were quickly being overtopped by grass, shading most of these sections if not overgrowing them entirely.*

Overall, the herbicide in combination with weed-whacking treatment within the natural regeneration plots has produced the largest number of seedlings by far.

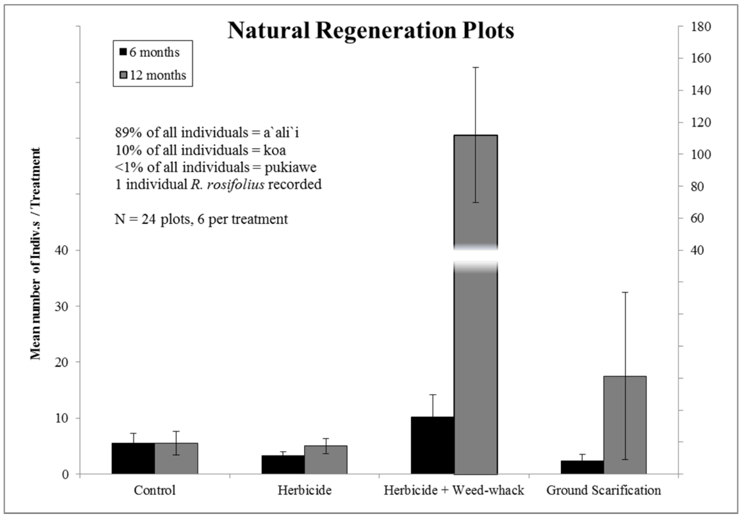

Figure 3*. Mean number of individual plants per treatment. This figure compares the number of individual wood plants (>=15 cm in height) present within the natural regeneration treatment experimental plots at the 6- and 12-month marks. Note that the y-axis scales differently at 40. [Notes: 1) Herbicide-only and ground scarification (without herbicide) did not result in a higher number of individual plants than the control treatment. 2) Herbicide + Weed-whack treatment plots had significantly more individual plants present at the 6-month mark and vastly more at the 12-month mark. 3) The vast majority (89%) of recorded individual plants measured were a`ali`i (Dodonea viscosa). Most of the remaining plants recorded besides a`ali`i were koa with a very small minority being pukiawe and thimbleberry. 4) The large standard error indicated in the 12-month monitoring of the ground scarification treatment is largely due to a single plot. This plot,*

R06, was the first to receive the scarification treatment. Originally, the plan had been to use a rototiller to remove the grass mat but this plan was scrapped after it became clear that using a rototiller out there was not going to be possible. The alternative strategy was to physically remove the grass from the plots by hand with pulaskis and rakes. This was done on R06 but this method took about 8 hrs to complete this one plot. Because of this we moved to the strategy used on all other scarification plots in the natural regeneration and seed scatter plots wherein we manually removed grass mats in 1-m^2 sections throughout the plot with a pulaski, exposing the topsoil within a total of ~25% of the plot. The number of seedlings in R06 is much higher than all other scarification plots. This may suggest that the physical removal of grass, exposing the topsoil provides the biggest benefit to germinating native plants. The results from the herbicide + weed-whack treatment may indicate that the addition of herbicide may help suppress grass re-growth after the physical removal of the grass biomass. This may explain the large difference between the herbicide-only and herbicide + weed-whack treatments.]

By 12-months the average number of seedlings in this treatment was ~ 20 × the number of seedlings in the control and herbicide-only treatments. Average height of these seedlings in the herbicide + weed-whack plots is shorter than those in the other treatments.

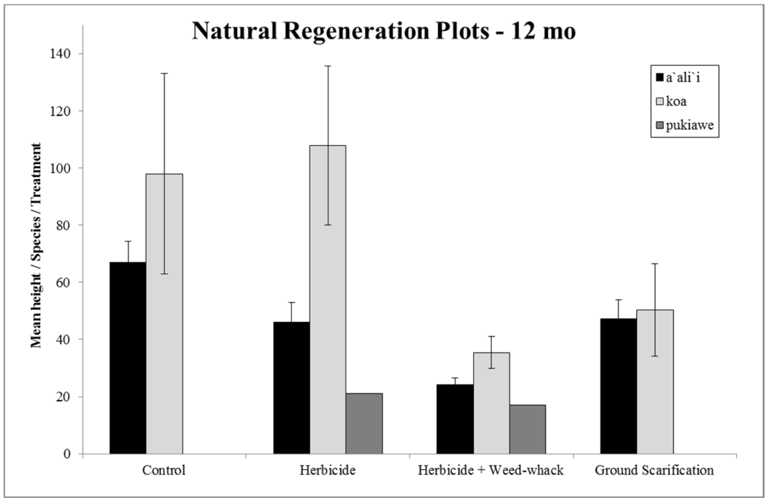

Figure 4*. The mean height of plants per species per treatment. This figure shows the height of recorded woody plants (>=15cm) within natural regeneration treatment experimental plots at the 12-month mark. Error bars represent ± SE. No error bars are present on pukiawe averages as these represent single individuals and no variance could be calculated. [Notes: 1) The comparatively shorter average in the herbicide + weed-whack treatment is likely the result of three main factors: i. Plants in the control, herbicide-only, and ground scarification plots may be older than 12-months as these plants were not disturbed by treatment application. We observed very little herbicide-related death of*

woody plants in the herbicide-only plots. ii. All individuals recorded in the herbicide + weed-whack treatment present at the time of the treatment application were cut down during weed-whacking. Thus, all recorded individuals represented in the above figure are not older than 12-months. iii. We recorded a much higher percentage of small seedlings (15-18 cm) in the herbicide + weed-whack treatment which likely drew the average down. In total 16% of all a`ali`i recorded were 15-18 cm in size and all but one individual were recorded in the herbicide + weed-whack treatment (20% of all a`ali`i in the H + WW treatment). This is reflected in part by the average number of individuals in the previous figure. 2) Many (most?) koa seedlings appear to be root-shoots, thereby benefiting from the resources of a mother tree. The heights attained from root-shoot individuals undoubtedly are greater than those germinating from seed. Thus, the heights in all treatments represented here are likely taller than seeded individuals. 3) We did not record average grass height during monitoring. In retrospect this may have been smart as we could then speak more directly to what heights need to be achieved in a native seedling to not be suppressed by future grass growth. Comparative ground cover (shown in following figure) shows that grass suppression in terms of soil coverage is approximately equal for the herbicide-only and the herbicide + weed-whack treatments. However, anecdotally the grass height appears to be shorter in the herbicide + weed-whack treatment. In most places grass height in this treatment is < 15 cm. Thus, an average height of >20 cm shown in all species has allowed the seedlings at 12-months to achieve a height greater than the grass. This may change as exponential grass growth continues.]

This is likely the result of the fact that all plants were cut down during the weed-whack application while plants present in the other plots were largely unaffected by treatment application. Additionally, 20% of seedlings recorded in the herbicide + weed-whack treatment were between 15-18 cm in height. This size class was largely absent in the other treatments. Therefore the average height of seedlings in the herbicide + weed-whack plots was drawn down by the presence of many, small seedlings.

Across all plots we have only recorded four woody species to date; a`ali`i (*Dodonea viscosa*, 89% of all seedlings recorded), koa (*Acacia koa*, 20%), pukiawe (*Styphelia tameiameiae*, <1%), and thimbleberry (*Rubus rosifoilus*,1 individual). The lack of diversity by 12-months in the natural regeneration plots is a bit concerning and may indicate a depauperate seed bank within much of Nakula. However, in many areas outside the plots we have noted the presence of seedling growth of pilo (*Comprosma foliosa*), ohelo (*Vaccinium reticulatum*) and others largely in gulches (pilo) and eroded areas (ohelo) free from grass. This may indicate that the poor seed bank may be restricted to the most heavily grazed areas now dominated by grasses, also where our experimental plots are located.

The relative abundance of naturally regenerating a`ali`i seedlings even in the control plots may indicate that <u>significant outplanting of this species is not the most pressing restoration need in Nakula</u>. It seems likely that this of all species has the ability to recover on its own to a large degree. We should focus our outplanting efforts and resources toward adding more diversity to the area.

Despite the observed regeneration of koas in Nakula, outplanting this species may still be needed as many (most?) seedlings recorded in our plots appear to be root-shoots (Figure 5). Outplanting genetically distinct individuals will greatly increase the genetic diversity in the area and make the species more robust and disease/pest resistant. We do not know the number of koa genets in Nakula at present and the number of root-shoots and low seed sets that we have observed in the last two years may indicate that this number may be low. This highlights the need for outplantings of individuals sourced from the largest number of genets possible.

We have yet to observe **<u>any</u>** natural regeneration in the form of seedlings, in and outside experimental plots, of many important native tree and shrub species that are common within Nakula. This includes ohia (*Metrosideros polymorpha*), kolea (*Myrsine lessertiana*), and iliahi (*Santalum haleakalae*, much rarer than others). Many mature ohias and some koas are showing signs of lateral growth from the trunks. This may increase the “bushiness” of these species and add shade to the understory. Extremely limited regeneration of kawau (*Ilex anomala*) and olapa (*Cheirodendron trigynum*) has been observed and is highly localized in gulches. Therefore it is very important to the overall diversity and health of the forest that these species continue to be outplanted.

Additionally, we monitored 1,291 seedlings planted between 10/08/14 and 01/25/14 for survival, representing 7-9 month survival data (Figure 5). These plants form a corridor connecting existing vegetation and are additional to the restoration plots. Overall survival was high at 87% for all 10 species combined. All spp. showed 7-9-mo survival ~ 90% except mamaki (43.9%) and kawau (81.8%).

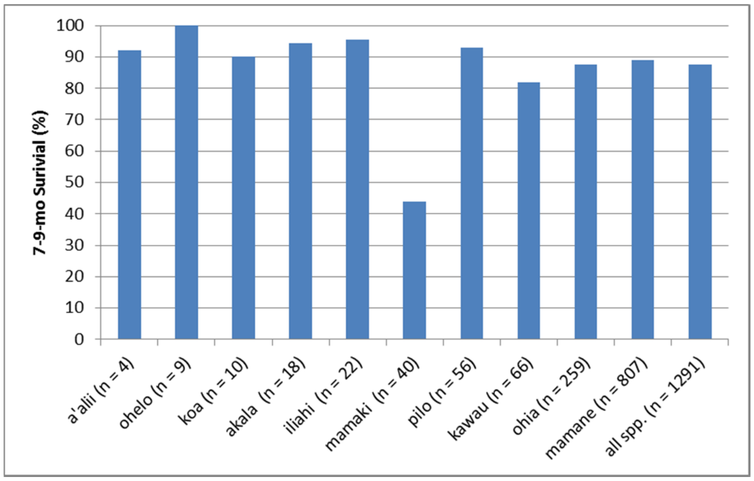

Figure 5. Seven-nine month survival of corridor plantings.

Appendix J: Home range Patterns of Maui Parrotbill and Maui `Alauahio: Implications for Proposed Translocations Efforts

Christopher C. Warren, Peter J. Motyka, and Hanna L. Mounce

Manuscript under review with Journal of Wildife Management, March 2015.

ABSTRACT Once occupying a variety of habitats on the islands of Maui and Moloka`i, the critically endangered Maui Parrotbill (Kiwikiu; *Pseudonestor xanthophrys*) is now restricted to native, wet forest on the windward slopes of east Maui above 1200 m in elevation. Parrotbills are restricted to this fraction of their former range mainly through habitat loss and disease. Continued range contraction is expected. In order to prevent extinction, reintroducing parrotbills to historically occupied native, mesic forest on the leeward slopes of Haleakalā is considered a critical recovery action. Managers have selected the newly established Nakula Natural Area Reserve (NAR) as the site for reintroduction, and restoration efforts are currently underway to support this goal. It is also expected that other extirpated species, including the endemic Maui `Alauahio (*Paroreomyza montana*), may recolonize these forests naturally as the habitat improves.We estimated home range size (area) of parrotbills and `alauahios at three sites within the birds' current ranges to provide a measure of area required by individuals of these species. We then used these estimates to calculate the potential abundance of both species in five planned restoration areas where future populations may occur on leeward Haleakalā. We calculated home ranges using minimum convex polygons and kernel density estimators from resighting data of color-banded birds from 2007-2014. Parrotbill home ranges were estimated to be between 8.48 ± 1.18 ha and 8.76 ± 1.05 ha (± SE) depending on estimation technique and `alauahio home ranges were between 0.8 ± 0.08 ha and 0.95 ± 0.08 ha. These estimates are the first to be derived from such a large dataset and date range for these species. The relative homogeneity of home range sizes among study sites may support the use of this metric, estimated in the species' current ranges, to predict potential abundance on leeward Haleakalā. Though we do not know how these species

will behave in the new habitat, the estimates of home range size presented here provide guidance in planning the reintroduction of parrotbills to Nakula NAR.

KEY WORDS Home range, Hawaii, Kernel density estimators, Maui `alauahio, Maui Parrotbill, Minimum convex polygon, *Paroreomyza montana*, Potential Abundance, *Pseudonestor xanthophrys*, Translocation

INTRODUCTION

As in the entire Hawaiian archipelago, the native avifauna of the island of Maui has suffered widespread extinctions and range contractions (Warner 1968, Scott et al. 1986, Pratt et al. 2009). Of the more than 20 species of forest passerine known to have existed prior to the arrival of humans (James and Olson 1991) only six species remain. Of these six, three are endemic to Maui and two are federally listed as endangered. The global wild populations of the endemic species, Maui Parrotbill (Kiwikiu; henceforth parrotbill; *Pseudonestor xanthophrys*), Maui `Alauahio (henceforth `alauahio; *Paroreomyza montana*), and `Ākohekohe (*Palmeria dolei*), are restricted to a single strip of native forest (the largest tract remaining) on Haleakalā Volcano in east Maui above 1200 m in elevation (85 $km^2$) with the exception of a small, relictual population of `alauahio in Kula Forest Reserve (FR; henceforth Kula) (Figure J.1). Continued range contraction is expected for these species driven by habitat destruction and disease (Benning *et al*. 2002, U.S. Fish and Wildlife Service 2006). Establishing a second population of parrotbills, the most critically endangered of the three, to once occupied habitat on leeward Haleakalā is considered the highest priority for the long-term persistence and viability of the species (U.S. Fish and Wildlife Service 2006). A leeward population of parrotbills will provide protection from loss due to stochastic events in the single extant population, increased genetic diversity for the species as a whole, and the drier leeward habitat may provide some refuge from disease-carrying mosquitos.

Planning a reintroduction of this kind requires a good understanding of the expected ecology of the organism within the release site (Griffith et al. 1989, Seddon et al. 2007, International Union for Conservation of Nature 2013). An estimate of home range size (Burt 1943), the area that an individual requires for survival and reproduction, allows conservation managers to estimate the number of individuals that may be supported within a reserve (i.e. carrying capacity) and may provide a benchmark for success of translocation efforts. To estimate home range in birds requires observations of individuals across an extended time period. Spot mapping (or territory mapping) was designed to investigate local densities of a species by marking known locations of individuals from repeated visits to a site (Verner 1985, Bibby et al. 1992). Spot mapping is a robust method for estimating home range that produces fine-scale, spatially explicit estimates of space use and density within localized areas (Verner 1985). Even if an individual temporarily emigrates from its home range, the repeated surveys capture the core area(s) and thus presumably the most important area(s) to that individual. Using marked individuals for these methods, such as through color-banding, allows an observer to confidently track individuals across extended time periods providing estimates of both among and within individual variation in home range size.

In 2011, the State of Hawaii Division of Land and Natural Resources established Nakula Natural Area Reserve (NAR; henceforth Nakula) on leeward Haleakalā in part for the protection of the parrotbill and has been designated as the reintroduction site for the species (Division of Forestry and Wildlife 2010; Figure 1). The habitat is classified as mesic forest with a canopy dominated by koa (*Acacia koa*), \`a\`ali\`i (*Dodonea viscosa*) and \`ōhi\`a (*Metrosideros polymorpha*). Although koa is not present in much of the current parrotbill range, some of the earliest observations of this species noted a strong affinity for koa (Henshaw 1902, Perkins 1903) suggesting that mesic, koa-dominated forests may have been a preferred habitat for the species. Subfossils also show the species to have been historically present within the Kahikinui region (leeward Haleakalā west of the Kaupō Gap) (James and Olson 1991). Unfortunately, the remaining native mesic forest has been greatly denuded as a result of heavy grazing by ungulates. Restoration efforts are currently in place within a 170-ha fenced, ungulate-free area of Nakula in preparation for the reintroduction. Complete restoration of the area is expected to take decades but portions of the area are considered currently suitable for a small population of parrotbills until more of the forest regenerates and/or are restored. However, the number of individual parrotbills that can be supported within this area is unknown. Although the habitat composition and structure within Nakula will likely remain different in many aspects from the habitat that the species currently occupies, estimates of space use from the current range provide a baseline estimate of the amount of area required per individual and therefore the number of individuals this area may support. This method of predicting space use for conservation efforts has been previously conducted for multiple species including (red squirrels; Rodriguez and Andren 1999) (Eurasian lynx; Schadt et al. 2002).

The total population of parrotbills has been estimated at 500-600 individuals (502 ± 116 [Scott et al. 1986], 590 ± 208 [Camp et al. 2009]) and occurs at low density compared to sympatrics throughout its range (Scott et al. 1986, Camp et al. 2009, Brinck et al. 2011). Little is known about how much area is required for an individual parrotbill to survive and reproduce (Simon et al. 1997). Using the best data available at the time, Pratt et al. (2001) estimated home range size (area) of the parrotbill to be 2.26 ha based on a limited sample size of individuals (n = 7) in one study site at the core of the species' range (Hanawi NAR; also included in the present study). Herein we utilized similar methods to include seven years of study and a second study site at the western edge of the species' range. Although the entire range of parrotbills is approximately 50 km^2 on the windward slopes of east Maui (Simon et al. 1997), Mounce et al. (2015) found evidence suggesting that the Ko`olau Gap, a large erosional depression in the center of the species' range, acts as an east-west dispersal barrier shaping the genetic population structure of the species. This genetic variation, combined with variation in habitat and climate across the species' range, suggests that the biology of the species may also vary spatially.

Additionally, we estimated home range size for the `alauahio; another endemic insectivore that shares many habitats and foraging substrates with parrotbills. The global population of `alauahi is significantly larger than that of parrotbills (>55,000 [Brinck et al. 2012]) and the population on windward Haleakalā may be increasing (Camp et al. 2009). Home range size of the species is reported to be 1–2 ha on windward Haleakalā (Baker and Baker 2000). `Alauahio were likely extirpated from the Kahikinui region sometime before 1980 and no definitive contemporary records exist for the species in this region. However, subfossil evidence shows the species to have been historically present (James and Olson 1991). Few surveys have been conducted in the leeward region and the status and distribution of the species beyond its current known range remains unknown. However, `alauahios are known to exist in some marginal habitats (e.g. scrubland, non-native forest) and may have the potential to recolonize Kahikinui as the habitat improves (Scott et al 1986). The abundance of `alauahios in the small, disjunct population in Kula is unknown but this represents the closest known population of the species to the restoration areas of Kahikinui (approximately 4 km).

STUDY AREA

We investigated home range size of parrotbills and `alauahios at two and three study sites, respectively, within Hanawi NAR (henceforth Hanawi; 20°44’N, 156°7’W), The Nature Conservancy’s Waikamoi Preserve (henceforth Waikamoi; 20°46’N, 156°13’W), and Kula (26°42’N, 156°18’W) in east Maui, Hawaii, USA (Figure J.1). Both Hanawi and Waikamoi contained some of the most pristine remaining native forest on Maui; primarily dense, montane rainforests dominated by `ōhi`a and `ōlapa (*Cheirodendron trigynum*; Jacobi 1989). Kula in contrast was dominated by non-native tree species including various conifers (Families: Pinaceae and Cupressaceae), eucalyptus (*Eucalyptus spp.*), and tropical ash (*Fraxinus uhdei*). Hanawi is situated in the eastern portion of both parrotbill and `alauahio ranges while Waikamoi is situated at the western edge of their ranges (Figure J.1). Rainfall was greatest in Hanawi receiving ≥

10,000 mm on average each year, moderate in Waikamoi at ≥ 2,000 mm per year, and comparatively low in Kula with around 900 mm each year (Giambelluca et al. 2013).

The protected lands on leeward Haleakalā that have been identified as areas for forest restoration fall within three land management units, Nakula (20°41’N, 156°13’W), Kahikinui FR (20°41’N, 156°12’W), and State of Hawaii Department of Hawaiian Home Lands (henceforth Hawaiian Home Lands)–Kahikinui unit (20°40’N, 156°15’W) (Figure 1). It is unclear what proportion of these areas is currently suitable to support these species or how long it may take to be restored to high quality native forest bird habitat. The time-scale associated with protecting these areas varies as by size of management unit and the associated costs of fencing and restoring each section. Consequently each section of habitat will become available to native forest bird species at different times. On account of the different timelines associated with each unit, we estimated the number of parrotbills and `alauahios in areas equivalent to the sizes of five management units (Figure J.1, Table J.2).

METHODS

Observation Data

Maui Forest Bird Recovery Project (MFBRP) conducted intensive spot mapping surveys (Verner 1985, Bibby et al. 1992) for parrotbills and `alauahios annually from 2007-2011 in Hanawi, 2012-2014 in Waikamoi and 2013-2014 in Kula. At each site individuals were captured and fitted with a unique combination of colored leg bands. Each year from 1 February to 1 July three to seven observers systematically searched study sites and recorded locations of all color-banded individuals encountered, using handheld Global Positioning System (GPS) units in Universal Transverse Mercator (UTM) coordinates.

The difficulty in traversing the terrain, as well as the sensitivity of the forest to disturbance, forced observers to stay largely on established trails in Hanawi and Waikamoi. Extensive trail systems allowed for comprehensive coverage of each study site with minimal damage to the forest. Observers were not as limited to trails within the non-native forests of Kula. However, banding efforts and spot mapping were concentrated in areas with the highest densities of `alauahios. Areas covered were 184 ha in Waikamoi, 133 ha in Hanawi, and 220 ha in Kula. Survey effort was similar across all three study sites and averaged 2,504.7 ± 480.5 survey hours per year (survey effort recorded 2010-2014).

Home Range Metrics

In general, despite the high survey effort, sample size of resight points per individual per year was low for the use of estimating home ranges (parrotbill = 7.23 ± 0.93 resights/bird/yr, `alauahio = 5.38 ± 0.47 resights/bird/yr). The low number of observations per individual is to be expected for rare and low density species. As sample size of observations per individual may influence the size and shape of a home range we chose to use two methods to delineate home ranges; minimum convex polygons (MCP) (Mohr 1947, Hayne 1949) and kernel density estimators (KDE) (Worton 1989) (Figure J.2).

Both MCP and KDE use a set of repeated observations to estimate a home range area. These methods predict areas where an animal was likely to occur during the survey period based on proximity to known locations (Bibby et al. 1992). In this way each observation is used as an index of the movement patterns of an individual animal. Outlying points (i.e. resighting points separated from the main cluster) in this case may represent two possibilities: 1) an individual travelling outside its core range or 2) an artifact of uneven survey effort within the localized area (e.g. wide-spread trails). In this example using KDE limits the impact of outlying points on the metrics of a given home range by weighting contours by frequency of occurrence thereby targeting the core area(s) of a home range. An MCP for the same individual would incorporate all points and the space between as part of the home range, thereby accounting for potential missed observations in the interstitial space between the apparent main cluster of observations and an outlier due to uneven survey effort. For these reasons we chose to estimate home ranges using both MCP and KDE to obtain two estimates based on these different techniques.

We restricted delineation of home ranges to individuals with sufficient data for accurate home range construction; ≥ 10 resights per year (28.7% of resighted parrotbill and 20.8% of resighted `alauahio) (Pratt et al. 2001). Some individuals were resighted more than once within a given day and these were only included if the bird was resighted ≥ 15 minutes after and/or was seen ≥ 50 m between consecutive points. To reduce the influence of single days on the size of home ranges we also restricted our analyses to individuals resighted on a minimum of three days. Only one `alauahio was removed from our analyses based on this parameter. In rare cases (*n* = 10) a single outlying resight point was excluded from construction of an `alauahio home range. This was done only for single points that were clearly the result of a GPS error or a band misidentification resulting in a point distantly disjunct (e.g. > 1 km) from the main cluster of resight points for an individual (e.g. outside the study site). One additional `alauahio was excluded from all analyses because the loss of an outlier point meant that they no longer met the 10 resight minimum. No parrotbill individuals were excluded from analyses due to the date minimum or outlying points.

We estimated MCP and KDE home ranges of both species in Geospatial Modeling Environment version 0.7.2.0 (Beyer 2012) using the ‘genmcp’ and ‘kde’ tools (Appendix J.1). We used smoothed cross validation to estimate bandwidth and a raster cell size of 10 per recommendations of Beyer (2012). We estimated 100% MCP home ranges rather than eliminating a certain proportion of outer points because we were interested in an estimate of the entire area an individual utilized during the study period. We chose to delineate 50%, 75% and 90% KDE isopleths (contour intervals) for each individual. The isopleths contain a percentage of the volume of the contour raster created by the KDE. In effect these represent different levels of confidence in the size of a home range, 50% being the most conservative and 90% being the most liberal. The 90% isopleths ultimately contain the largest areas within an approximated home range that do not contain observation points.

Pair Home Ranges

As parrotbills are known to be socially monogamous (Simon et al. 1997), an estimate of the area used by a mated pair of individuals may be more appropriate for conservation planning. This follows the assumption that a mated pair would occupy a smaller amount of habitat than the sum of two unrelated individuals. Pairing status and identity was recorded for all banded parrotbill within each study site each year. We compared home ranges between paired individuals and estimated a combined home range for each known pair within each year. We compared home range size and overlap between known paired individuals where both individuals were resighted ≥ 10 times within a year. This analysis was not conducted for `alauahios as pairing status was not recorded for this species.

To estimate pair home ranges we clipped (‘Clip’ tool in ArcMap 10.0 [Environmental Systems Research Institute, Inc., Redlands, CA]) the MCP and 70% KDE home range polygon of each individual of a pair by their respective mate’s home range polygons. We then added the shared (overlapped), male- and female-only areas of each pair to estimate a collective pair home range. From this result we calculated the area and proportion of overlap among pairs’ home ranges. We then compared the sizes of the pair home ranges to the home ranges of the same paired individuals independent of their mates. Estimating home range size for pairs provided a way to adjust mean home range size for all individuals as if we had been able to measure home range for all mated pairs. Although pairing status is not always established for all individuals within a study site each year, unpaired adult parrotbills were exceptionally rare during these spot mapping surveys (MFBRP unpublished data). All individuals for which a home range was estimated in this study were known to be paired with either a banded or unbanded bird, justification for adjusting individual home range size to pair home ranges for use in predicting the number of parrotbills that may inhabit a given area.

Estimating Potential Abundance

We calculated an adjusted mean home range size for all parrotbill home ranges as though they were all paired by multiplying the mean home range size of all individuals (H_i) by the quotient of mean pair home range size (H_p) and mean individual home range size of the known paired individuals (H_m; i.e the individuals used to estimate H_p). Home range size could not be adjusted to pair home range size for `alauahios because pair identity was not recorded for this species. However, `alauahios are highly gregarious and live in small family groups usually consisting of a male, female and two to four sub-adults (often helpers at the nest) (Baker and Baker 2000). This may mean that every `alauahio home range may actually represents approximately three individuals, so we calculated the number of individuals that could potentially occupy an area as though each home range was equivalent to three individuals, a "family home range".

We estimated the potential abundance of both species across a range of habitat sizes equivalent to the sizes of five planned restoration units within the elevational range of parrotbills (1200 – 2150 m, Camp et al. 2009), Nakula 1 (162 ha), Nakula 2 (98 ha), Nakula 3 (120 ha), Kahikinui (264 ha), and Hawaiian Home Lands (1052 ha). These areas are all contiguous and differ only in administration and/or restoration timeline.

To estimate the potential number of individual parrotbills and `alauahios ($\widehat{N}$) that may inhabit restoration areas in the future we divided area ($A$) relevant to size of available habitat on leeward Haleakalā by the adjusted pair home range of parrotbills (Equation J.1) and the individual home range size of `alauahios (Equation J.2).

$$2\left(\frac{A}{H_i\left(\frac{H_p}{H_m}\right)}\right) = \widehat{N}$$

Equation J.1

$$3\left(\frac{A}{H_i}\right) = \widehat{N}$$

Equation J.2.

Statistical Analysis

To assess the effect of the number of resight points and resight dates on home range size we performed separate linear regressions for these factors and species. To test for variation in home range size between study sites we performed separate repeated measures analyses of variance (ANOVA) for each species. We did this using linear mixed effects modeling blocking for individual bird ID followed by Type III ANOVA in R 3.0.1 (The R Foundation for Statistical Computing, Vienna, Austria). For the analysis of parrotbill home range we included site and sex as fixed factors. Determining sex of `alauahios was not possible in the field unless birds were in breeding condition. As a result only a small subset of individual `alauahios were of known sex and therefore sex was not included as a factor of home range for this species in these analyses. Only site was included as a fixed factor in the `alauahio models. We used a two-tailed t-test to compare the percentage home range overlap between parrotbill mates.

RESULTS

Of the 223 parrotbills and 1287 `alauahios banded by MFBRP from 1992–2014, 51.5% and 56.7% were resighted between 2007 and 2014, respectively. After excluding individuals with < 10 observations and < 3 observation dates, 33 parrotbill and 152 `alauahio individuals were available for analyses. We were able to calculate home ranges for an average of 5 (± 3.2 SD) (range 0 [2010] to 10 [2011]) parrotbills and 26.4 (± 22.1) (range 10 [2007 & 2012] to 75 [2014]) `alauahios per year. We estimated home range size of 17 parrotbills from Hanawi and 16 from Waikamoi. We estimated home range size for 59, 54, and 39 `alauahios from Hanawi, Waikamoi and Kula study sites, respectively. Minimum known age of individuals included in analyses ranged from 2-10 years old in parrotbills and 1-10 years old in `alauahios. We were able to estimate the home ranges of a total of 19 male and 14 female parrotbills. A total of eight parrotbill pairs were available for analysis of pair home range.

Of the 33 parrotbill individuals analyzed, we repeatedly measured home range size of five individuals (15%) (i.e. more than one year) and two of these individuals were measured in three separate years. Repeatedly measured `alauahios accounted for 27.6% of individuals (*n* = 42) and 7% of individuals (*n* = 12) were measured more than two years. We were able to estimate a home range for a single individual `alauahio in Hanawi in five separate years from 2007–2011.

`Alauahio MCP home range size was not correlated with the number of resight points (*R* = 0.08) or the number of resighting dates (*R* = 0.17) per year. Home range size of `alauahio calculated by KDE was also not correlated to the number of resights (*R* = 0.09) or number of resighting dates (*R* = 0.006). In contrast, MCP-described parrotbill home range size was positively correlated with both the number of resight points (*R* = 0.36, *P* < 0.001) and resighting dates (*R* = 0.30, *P* < 0.001). However, parrotbill home ranges measured with KDE were not correlated with the number of resights (*R* = 0.05) or resight dates (*R* = 0.01). As a result all means are presented unweighted except MCP home ranges of parrotbills presented as an average weighted by the number of resights per individual.

Maui Parrotbill Home range Size and Overlap

Mean MCP home range of parrotbill was 8.76 ± 1.05 (SE) ha and ranged from 0.45–31.23 ha across all years and study sites (Figures J.3A, J.4A). Mean KDE home range of parrotbill was 4.69 ± 0.69 ha among 50% isopleths and 16.39 ± 2.21 ha among 90% isopleths. Of the 70% isopleths mean home range size was 8.48 ± 1.18 ha. This contour level captured the most resight points while also minimizing the amount of "extrapolated" area beyond the cluster of observation points. Outlier home ranges (> 2 × SD) using all methods were rare (5% of home ranges) (Figure J.3A). Parrotbill home range size did not vary among study sites or sex among MCPs or at any KDE contour levels (Table J.1, Figure J.4A).

Parrotbill pairs share home ranges to a large degree; sharing an average of 74.84 ± 9.35% (MCP) or 64.6 ± 6.91% (KDE) of their home range with their mate's home range.

Males and females overlapped their mate’s home ranges to the same degree (MCP: t = 0.63, P = 0.548; KDE: t = 0.70, P = 0.508). The mean pair home range size was 15.48 ± 3.74 ha (MCP) and 17.62 ± 4.03 ha (KDE). The mean area of the additive pair home range was between 20.75 % (MCP) and 44.69% (KDE) larger than the mean home range size of the individuals included in the pair analysis. The adjusted pair home range size of parrotbill pairs using data from all individuals was 10.21 ha (MCP) and 11.54 ha (KDE).

Maui `Alauahio Home Range Size

Mean home range size of `alauahio was 0.8 ± 0.08 (SE) ha and ranged from 0.02–9.08 ha using the MCP method across all years and study sites (Figures J.3B, J.4B). Mean KDE home range of `alauahios was 0.52 ± 0.04 ha among 50% isopleths and 1.86 ± 0.15 ha among 90% isopleths. Among 70% isopleths, the contour producing most realistic home ranges, mean home range was 0.95 ± 0.08 ha and ranged from 0.3 to 8.87 ha (Figure J.3B, J.4B). As in parrotbills, outlier home ranges were rare (4%) (Figure J.3B). Home range size of `alauahios varied among study sites based on the MCP method and the 50% and 90% isopleths of KDE home ranges but not the 70% isopleths (Table J.1). Using the MCP method `alauahio home ranges were significantly larger in Hanawi than the other two sites (Waikamoi: t = 3.51, $p < 0.001$; Kula: t = 3.42, $P < 0.001$). Home ranges in Waikamoi and Kula did not differ (t = -0.24, P = 0.811).

Estimating Abundance on Leeward Haleakalā

To estimate the number of parrotbill and `alauahio individuals on leeward Haleakalā we used the mean home range size based on MCP and 70% KDE polygons.

Table J.2 presents the predicted number of non-overlapping home ranges within areas equivalent to the size of five highlighted restoration areas. By size of area we predict that between 33.3–37.7 pairs or 66.7–75.3 individual parrotbills and 405.3–481.6 `alauahio family groups or 1,215.9–1,441.8 individual `alauahio could exist in Nakula (sections 1–3), identified as the first reintroduction site, depending on home range estimation technique. We also predict that the fenced section of Hawaiian Home Lands will support an additional 182.3–206 parrotbills and 3,325.6–3,951.4 `alauahios. Although the forest in the Kahikinui section (outside of the small section fenced in as part of Nakula 2) has the longest restoration timeline, this area may support an additional 41.3–46.7 parrotbills and 753.1–894.8 `alauahios.

DISCUSSION

For decades, the Kahikinui region of Maui has been identified as an area in need of conservation and restoration for both watershed health and the preservation of threatened and endangered organisms (Scott et al. 1986, Division of Forestry and Wildlife 2010). Preparation for the planned reintroduction of parrotbills to the area has provided the impetus for significant restoration efforts in Nakula NAR. Concurrently, large sections of contiguous areas in Hawaiian Home Lands and Kahikinui FR have been fenced (or soon will be) and restoration actions are underway. Together these areas contain the majority of the remaining forest on leeward Haleakalā, providing a large area for reintroduced parrotbills and dispersing individuals to occupy. Before the reintroduction can proceed, however, conservation managers require an estimate of the number of individual parrotbills the area can support to plan the number and density of released individuals. To this end we estimated the home range size of parrotbills and the `alauahios in their current ranges. By considering these estimates measure of the amount of area individuals or pairs require we extrapolated this space requirement to areas equivalent to restoration zones to estimate the number of individuals that can potentially be supported by the Kahikinui region. While much remains unknown as to how these species will behave in this new habitat, the estimates presented here provide a method to broadly predict the potential abundance of these species in areas that may be occupied in the future, critical for conservation planning and recovery of these species.

One of the challenging aspects of designing a reintroduction plan for parrotbills, similar to other Hawaiian birds, is the relative paucity of published information about the biology and behavioral ecology of this species. The first estimates of parrotbill abundance were presented in 1986 (Scott et al.) and the first active nest was not described until 1993 (Van Gelder). While robust demographic and behavioral information is crucial to designing a successful reintroduction, collecting these data is challenging for a species that even historically was described as “local and rare” (Rothschild 1900) and exists at low densities throughout its native habitat (Scott et al. 1986, Brinck et al. 2011). An estimate of home range size and potential abundance throughout the recovery region (U.S. Fish and Wildlife Service 2006) is a key piece of this information. The previous estimate of home range size by Pratt et al. (2001) was based on a limited number of individuals at a single study site. Our 8.76 ha estimate of individual parrotbill home range size was much larger than the estimate provided by Pratt et al. (6.67 ha based on 100% MCP, 2.26 ha based on 80% MCP), likely a result of the increased sample size and/or the inclusion of a second study site.

We found no difference in parrotbill home range size between the two study sites. While both sites are dominated by the same native tree species, Hanawi receives significantly more annual rainfall compared to Waikamoi (Giambelluca et al. 2013) and the structure and composition of the plant community differs slightly between the two sites. This result may indicate little variation or plasticity in home range size of the species in response to variation in habitat characteristics. However with only two sites containing some of the best remaining habitat for the species, our data have limited capacity to speak to the overall variation that may exist throughout the entire species’ range (including marginal habitat) or may have existed historically (including other habitat types no longer available).

The relative abundance of `alauahios masks the threats that the species faces. As in parrotbills, limited behavioral and demographic information is available for `alauahios and the overall distribution of the species beyond the range described here remains in question. The current study presents the first estimates of home range size based on such large sample sizes across both of these species’ ranges. We estimated home range size of `alauahios at 0.8–0.95 ha, similar to the 1–2 ha reported by Baker and Baker (2000). However, these authors reported home range size to be smaller in wet native forest where we found the opposite pattern; home range size was largest at our wettest native forest site. The structure of heterogeneity in `alauahio home range size throughout its range does not fall along an apparent rainfall or habitat gradient. Rainfall amounts generally decrease from an east to west direction within the area encompassing these three study sites (Giambelluca et al. 2013). Home range size was largest in Hanawi and smallest in Waikamoi, both dominated by native forest, with the non-native-dominated Kula site having intermediate home range size. The data suggest that several factors likely influence home range size of `alauahios and that the species can exist in a wide range of habitats and climates. Waikamoi may present more favorable conditions for a variety of reasons allowing individuals to maintain smaller home ranges.

The habitat on leeward Haleakalā differs markedly from the habitat that either species currently inhabits (e.g. koa- rather than ohia-dominant canopy) and no information exists on habitat utilization for these species outside their current ranges. For the purpose of reintroduction planning we need to predict the number of individuals that may inhabit an area. In order to do this using these data we make two important assumptions. 1) We assume no overlap among individual home ranges. Although home ranges do overlap to an unknown degree, assuming no overlap means that predictions of abundance for a given size of habitat are conservative; greater overlap equals higher potential abundance. 2) We assume no variation in home range size as a function of habitat. We tested this hypothesis indirectly by sampling both species at multiple study sites throughout their ranges. The actual future populations will be influenced by home range overlap and habitat consistency within conservation areas. These estimates represent a maximum number of individuals given 100% saturation of suitable, equivalent habitat within Nakula and surrounding areas and a minimum number of individuals from the perspective of home range overlap.

Home range size in the leeward habitat will undoubtedly be driven in part by food resource availability (Schoener 1971). Both parrotbills and `alauahios are primarily insectivorous, gleaning or extracting insects from tree and shrub branches (Simon et al. 1997, Baker and Baker 2000). While insect density and diversity per stem is similar in Nakula to that in Hanawi, stem density is lower in Nakula (Peck et al. 2015) thereby reducing food resource density. Additionally, the historic observations of the parrotbills' preference for koa as a foraging substrate (Henshaw 1902, Perkins 1903) may indicate a qualitative benefit to a habitat dominated by koa. Our assumption that home range size will be similar to that within the current range may hold particularly if qualitative differences in food resources ("preferred" habitat) balance out the reduction in quantity of resources (stem density).

MANAGEMENT IMPLICATIONS

While the habitat across the Kahikinui region varies in quality and is in various stages of restoration, this region has perhaps the greatest potential to increase the range and population size of many of the rarest birds on Maui (U.S. Fish and Wildlife Service 2006). Based on home range size and the sizes of restoration areas our analyses show the potential to increase the size of parrotbills range by 33% and the overall population by 58–66%. Of most concern to the preservation of all native passerines on Maui, global climate change is predicted to allow disease-carrying mosquitos to breed at higher elevations, thereby reducing the current ranges of these species (Benning et al. 2002). If we are to mitigate loss of habitat due to a rising "mosquito line" more habitat must be restored at higher elevations outside of the current ranges of these species. If enough habitat is restored at high elevations around Haleakalā Volcano, theoretically parrotbills may maintain a similar range and population size as exists today well into the future regardless of the climate-change-influenced habitat contractions. Establishing new populations of these species in Kahikinui is the first step toward protecting them. Given the time scale of habitat loss due to disease prevalence combined with the length of time it takes to fully restore forested habitats, attention should also be given to restoring additional available lands at high elevation beyond Kahikinui, particularly the western slopes of Haleakalā.

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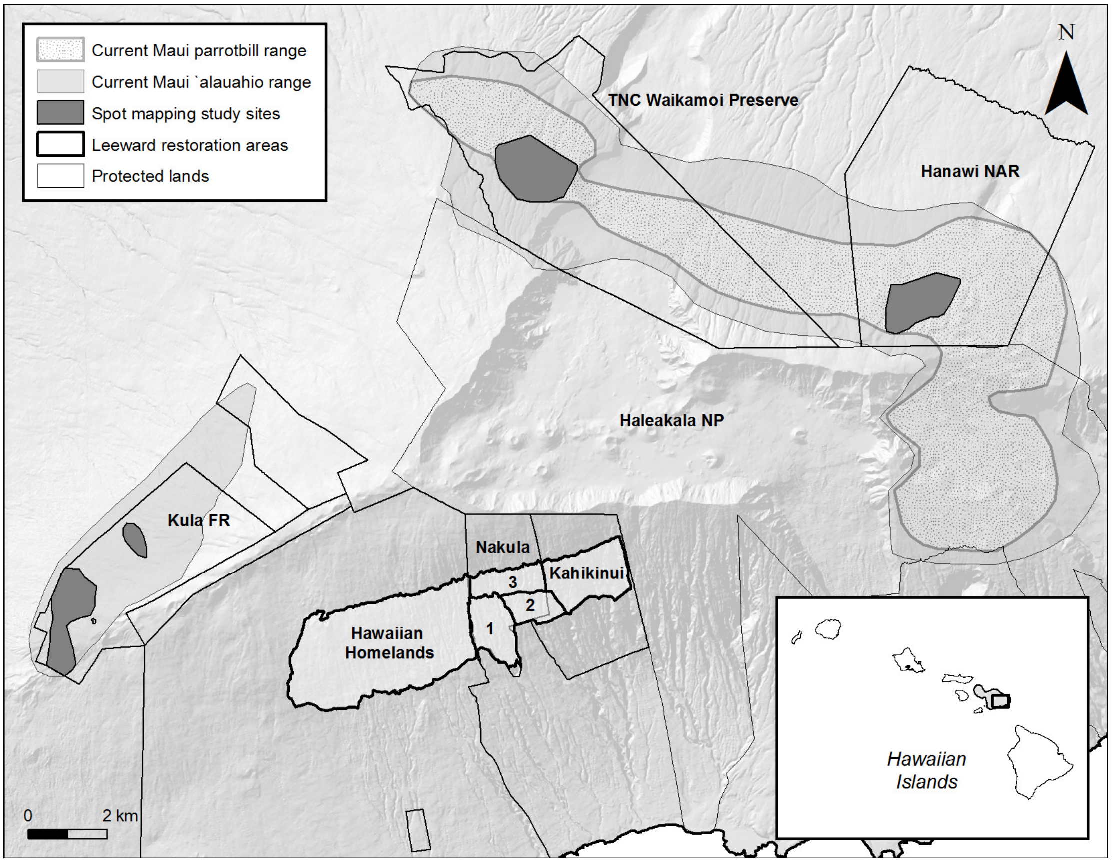

Figure J.1. Study sites where home ranges of Maui Parrotbill and Maui `Alauahio were examined as well as protected lands for future populations of both species. Highlighted are the five conservation areas within the elevational range of parrotbills where potential abundance was predicted.

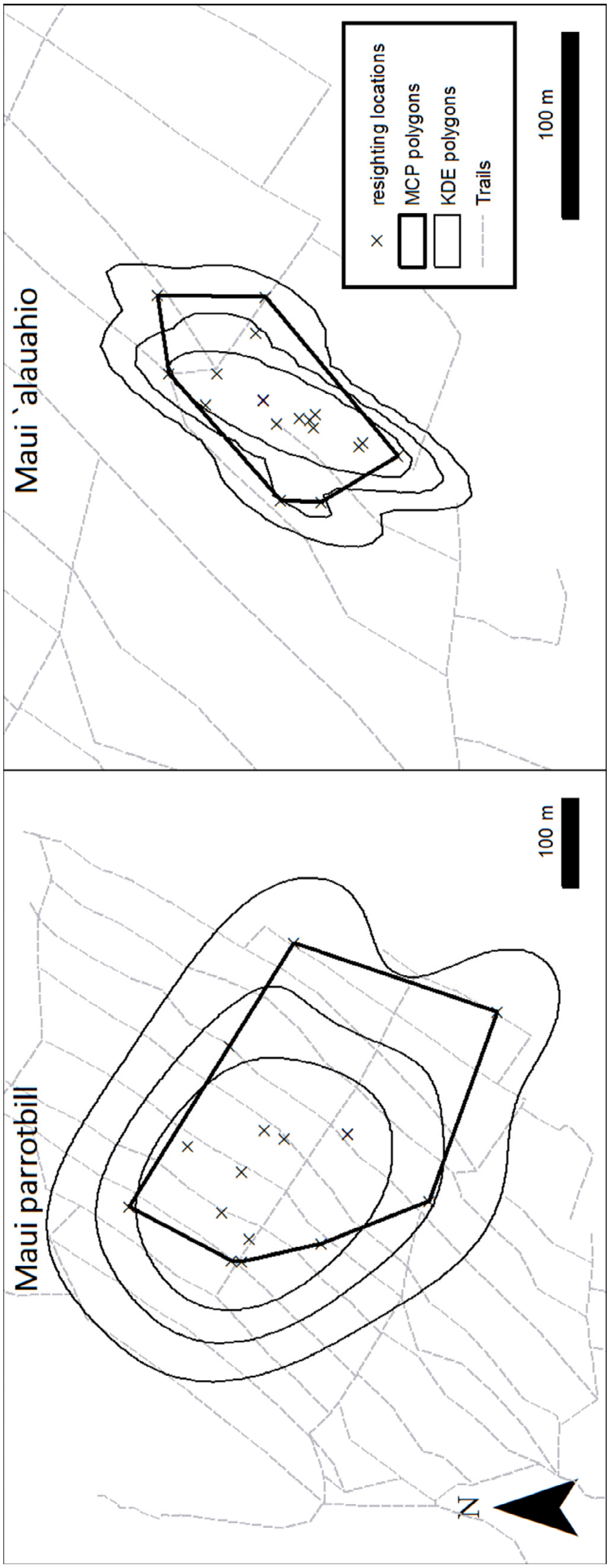

Figure J.2. Examples of home ranges of individual (A) Maui Parrotbill and (B) Maui `Alauahio for a single year constructed using minimum convex polygons (MCP) and kernel density estimators (KDE). Each KDE home range is shown with 50%, 70% and 90% isopleths. Scale differs between panels.

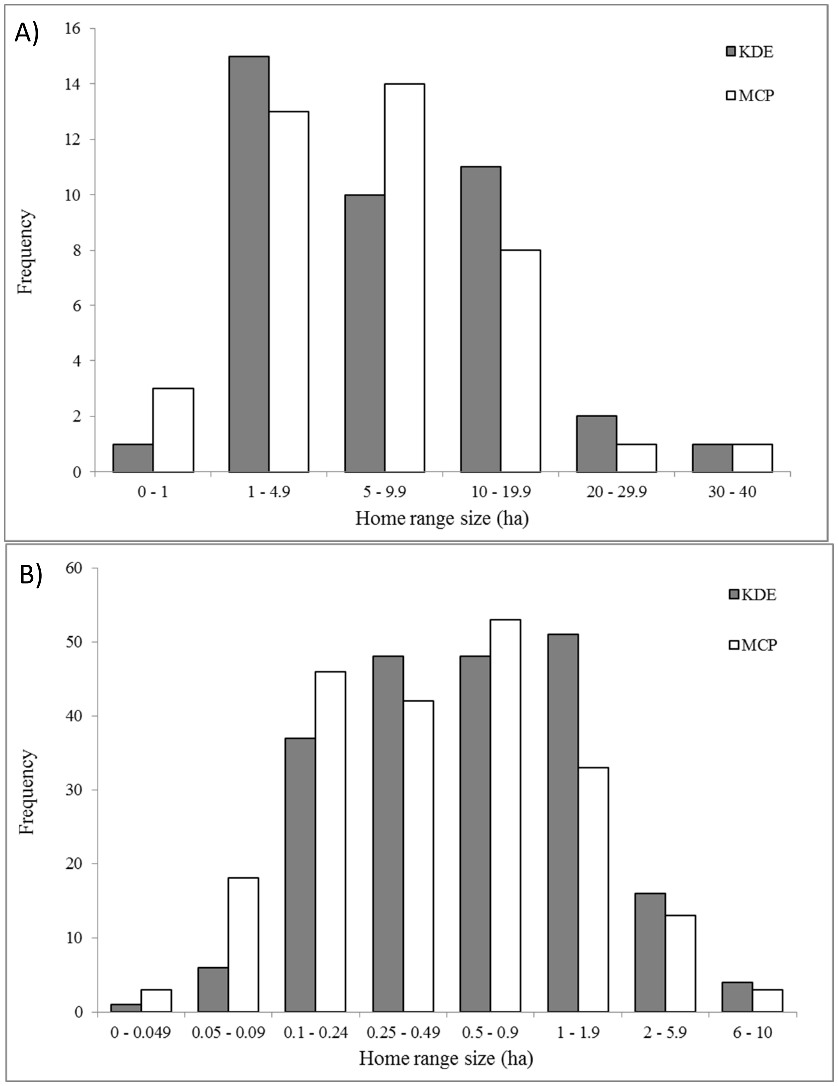

Figure J.3. Histogram showing frequencies of minimum convex polygon (MCP) and 70% isopleth kernel density estimated (KDE) home range sizes for (A) Maui Parrotbill and (B) Maui `Alauahio. Axes differ between panels.

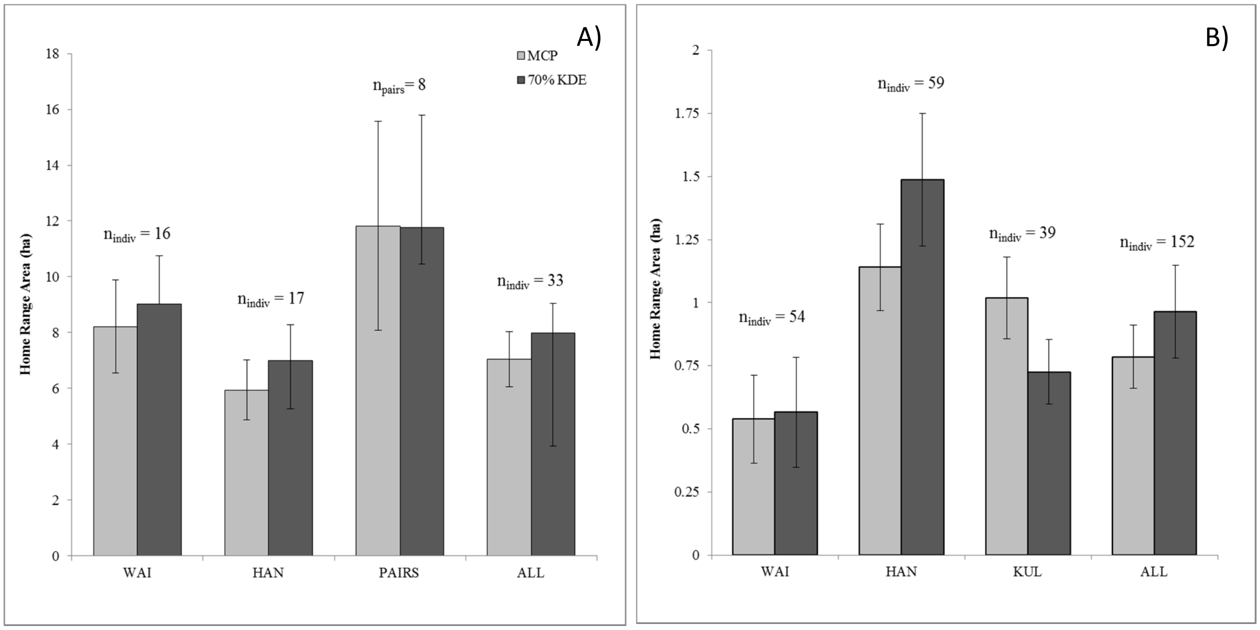

Figure J.4. Mean home range size per study site. (A) Mean Maui Parrotbill home range in WAI, KUL, all sites and pairs (all sites). (B) Mean Maui `Alauahio home ranges at the three study sites. Axes differ between panels.

Table J.1. Analysis of Variance Type III results for Maui `Alauahio and Maui Parrotbill. Fixed factors included were site for `alauahios and site and sex for parrotbills. Results are given for Kernel Density Estimator (KDE) and Minimum Convex Polygon (MCP) home ranges.

	Maui parrotbill									Maui `alauahio		
	Site			Sex			Site × Sex			Site		
	F	*P*	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*	df
50% KDE	0.98	0.323	1,1	0.10	0.754	1,1	0.19	0.667	1,1	7.29	0.026	1,2
70% KDE	0.90	0.342	1,1	0.06	0.808	1,1	0.11	0.744	1,1	4.47	0.107	1,2
90% KDE	0.78	0.376	1,1	0.03	0.853	1,1	0.03	0.867	1,1	43.88	≤ 0.001	1,2
MCP	0.23	0.633	1,1	0.04	0.850	1,1	0.22	0.638	1,1	17.04	≤ 0.001	1,2

Table J.2. Predicted abundance of Maui Parrotbill and Maui `Alauahio within habitat equivalent to the sizes (ha) of five restoration segments on leeward Haleakalā, Maui, USA (Figure 1). Managing agencies are Department of Hawaiian Home Lands (DHHL) and Division of Forestry and Wildlife (DOFAW). Restoration segments are listed based on approximate restoration timeline, Nakula 1 having the shortest timeline. Predictions are based on estimated pair home range size of 10.21 ha (MCP) and 11.54 ha (KDE) for parrotbills and individual home range size of `alauahios of 0.8 ha (MCP) and 0.95 ha (KDE). The percentage increase in total population size is based on a global population size of 500 parrotbills and 55000 `alauahios.

				MAPA individuals ($\hat{N}$)		MAAL individuals ($\hat{N}$)	
Name	Managing Agency	Protected Area	Year fenced	MCP	KDE	MCP	KDE
Nakula 1	NARS	162.32	2012	31.8	28.1	609.6	513.0
Nakula 2	NARS	98.01	2015[a]	19.2	17.0	368.1	309.8
Hawaiian Homeland	DHHL	1052.21	2015[a]	206.0	182.3	3951.4	3325.6
Nakula 3	NARS	120.72	TBD	23.6	20.9	453.4	381.6
Kahikinui	FR	238.26	TBD	46.7	41.3	894.8	753.1
Total		1671.53		327.3	289.7	6277.2	5283.0
% increase of Total Population				65.5%	57.9%	11.4%	9.6%

APPENDIX J.1. GEOSPATIAL MODELLING CODE.

The full code used to create of minimum convex polygons and kernel density estimated polygons in Geospatial Modelling Environment; adapted from Beyer (2012). Prior to these analyses we created separate shapefiles containing only individuals with ≥ 10 resight points. These analyses were run for each species separately by year. *n* = individuals for a given year.

```
setwd(in="file", out="outfile");
splitdataset(in="resight_shapefile.shp", uidfield="ANIMID", outws="outfile",
prefix="ANIM");
for(i in 1:n){
genmcp(in=paste("ANIM",i,".shp"), out=paste("mcp_","ANIM","#year#",i,".shp"))
};
for(i in 1:n){
kde(in=paste("ANIM",i,".shp"), out=paste("kde","scv","#year#","ANIM",i,".img"),
bandwidth="SCV", cellsize=10)
};
for(i in 1:n){
isopleth(in=paste("kdescv#year#ANIM",i,".img"),
out=paste("iso#year#_ANIM",i,".shp"), quantiles=c(0.5,0.7,0.9),
poly=paste("isopoly#year#_ANIM",i,".shp"))
};
for(i in 1:n){
addarea(in=paste("mcp_","ANIM","#year#",i,".shp"), area="AREA_ha",
areaunits="hect")
};
for(i in 1:n){
addarea(in=paste("isopoly#year#_ANIM",i,".shp"), area="AREA_ha",
areaunits="hect")
};
for(i in 1:n){
```

```
addcodedfield(in=paste("mcp_","ANIM","#year#",i,".shp"), field="ANIMID", fieldtype="SHORT", constant=i)

};

for(i in 1:n){

addcodedfield(in=paste("isopoly#year#_ANIM",i,".shp"), field="ANIMID", fieldtype="SHORT", constant=i)

}
```