Department of **Biodiversity, Conservation and Attractions**

GOVERNMENT OF WESTERN AUSTRALIA

Recovering Gilbert's potoroo (*Potorous gilbertii*) – a review of progress and future directions

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Recovering Gilbert's potoroo (*Potorous gilbertii*) – a review of progress and future directions

Amy Northover, Tony Friend, Stephanie Hill, Tim Button, Sarah Comer, Jackie Courtenay, Kym Ottewell, Saul Cowen, and Lesley Gibson

May 2022

Department of Biodiversity, Conservation and Attractions
Locked Bag 104
Bentley Delivery Centre WA 6983
Phone: (08) 9219 9000
Fax: (08) 9334 0498

www.dbca.wa.gov.au

Questions regarding the use of this material should be directed to:
Lesley Gibson - Program Leader, Animal Science
Biodiversity and Conservation Science
Department of Biodiversity, Conservation and Attractions
Locked Bag 104
Bentley Delivery Centre WA 6983
Email: lesley.gibson@dbca.wa.gov.au

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Acknowledgments

We acknowledge that Gilbert's potoroo conservation work was undertaken on the traditional lands and waters of the Minang, Wudjari and Njunga people of the Noongar cultural group of the southern region of Western Australia.

We would like to thank the Western Australian Government for their long-term support to the Gilbert's potoroo recovery program, allowing the commitment of permanent staff for more than 20 years and enabling strong continuity in essential recovery actions. The Australian Government has made very substantial financial contributions over the years through various programs including the Natural Heritage Trust (NHT), the Threatened Species Fund and other facets of the Natural Landcare Program (NLP). Significant support has also been provided by South Coast Natural Resource Management, directing NHT and NLP funds to this recovery program. The Gilbert's Potoroo Action Group (GPAG), Australian Geographic Society and Earth Sanctuaries Foundation have also made significant financial contributions towards the recovery of Gilbert's potoroo. We also acknowledge the enormous contribution made by many volunteers, both through GPAG and from the community at large. We express our warm thanks to the past and present members of the Gilbert's Potoroo Recovery Team, which has provided advice, support and oversight to the implementation of the recovery program.

Summary

Once thought to be extinct, Gilbert's potoroo (*Potorous gilbertii*) was rediscovered in 1994 and is now considered to be Australia's most threatened mammal. Previously abundant around Albany, only a single indigenous mainland population is known to exist on the slopes of Mount Gardner, in Two Peoples Bay Nature Reserve near Albany. Additional populations have since been established on two islands and within a fenced mainland enclosure. Since the species' rediscovery, an intensive multifaceted approach to the conservation management of Gilbert's potoroo has guided its recovery and altered the trajectory of this species from almost certain extinction. With the aim of ensuring the long-term persistence of Gilbert's potoroo by increasing the species' distribution and abundance, both in-situ and ex-situ management tools have been employed. Translocations have played a vital role in creating insurance populations for the species.

Despite major progress towards achieving recovery objectives, the future of Gilbert's potoroo remains precarious. In 2015, a bushfire destroyed approximately 90% of core potoroo habitat on Mount Gardner, and now only 1-2 individuals are believed to persist at that site. Insurance populations prevented the extinction of the species at that stage and currently represent the bulk of extant genetic diversity of the species. However, their small and fragmented nature necessitates ongoing management to adequately protect the species and prevent population declines. This review summarises the recovery actions implemented by the Department of Biodiversity, Conservation and Attractions, with advice from the Gilbert's Potoroo Recovery Team and other key stakeholders between 1994 and 2022, with particular emphasis on the scientific research that underpins our knowledge of Gilbert's potoroo and continues to inform the conservation management of this species. Recommendations are made for future actions to further improve the effective conservation of Gilbert's potoroo.

1 Introduction

Conservation of biodiversity is an ever-increasing challenge with the anthropogenic impacts of human population growth fuelling the ongoing decline and extinction of wildlife species on a global scale. The International Union for Conservation of Nature currently estimates that 1317 mammal species are threatened with extinction (IUCN, 2020). Since European settlement, Australia's mammal fauna has suffered catastrophic declines and a disproportionately high number of extinctions compared to other countries, with 34 mammal species now listed as extinct in the wild (Woinarski et al., 2019). Given that Australia is one of 17 'megadiverse' countries and 87% of Australia's terrestrial mammal fauna are endemic (Woinarski et al., 2015), it is important that the recovery of Australia's threatened species is a key priority for the conservation of global biodiversity.

Presumed to be extinct for over 100 years (Seebeck et al., 1989), Gilbert's potoroo (*Potorous gilbertii*) or ngilgyte, was rediscovered in 1994 during a field survey for the quokka (*Setonix brachyurus*) in Two Peoples Bay Nature Reserve, near Albany (Sinclair et al., 1996). Amid an extinction crisis (Rosser and Mainka, 2002), species rediscoveries such as this are a cause for celebration. While rediscoveries provide an opportunity to re-write history, most rediscovered species are classified as threatened (86% of all rediscovered mammal species; Scheffers et al., 2011) with restricted ranges and small, disjunct populations. Recovering species from the brink of extinction thus provides several challenges (see Gross, 2013) and it is likely that many rediscovered species will remain under serious threat in the absence of targeted conservation action (Fisher, 2011; Scheffers et al., 2011). For example, concerted conservation effort is estimated to have prevented the extinction of 7-16 mammal species, including Gilbert's potoroo, between 1993 and 2020 (Bolam et al., 2020).

Gilbert's potoroo is a nocturnal macropodoid marsupial (Family Potoroidae) with grey-brown fur, a slender downward curving snout, and densely furred jowls (Friend, 2008b). Adults are small, weighing between 700g and 1200g and measuring approximately 500mm in length (nose to tail tip) (Friend, 2013). The species is currently classified as Critically Endangered under the Commonwealth's *Environment Protection and Biodiversity Conservation Act 1999*, the Western Australian *Biodiversity Conservation Act 2016* [Wildlife Conservation (Specially Protected Fauna) Notice 2018, schedule 1], and by the International Union for Conservation of Nature (Woinarski and Burbidge, 2016). Gilbert's potoroo was also identified as one of the 20 priority mammal species in the Threatened Species Strategy Action Plan 2015-16 "20 mammals by 2020" (DAWE, 2016).

Following the species' rediscovery on the slopes of Mount Gardner (Start et al., 1995; Sinclair et al., 1996), a program of research to support recovery commenced. The recovery program was led by the then Department of Conservation and Land Management [now the Department of Biodiversity, Conservation and Attractions (DBCA)] with advice from the Gilbert's Potoroo Recovery Team (Friend, 2007). Initial on-ground recovery actions to: (1) protect the existing wild population and habitat within Two Peoples Bay, (2) increase knowledge of the species' ecology and population biology, and (3) search for new populations beyond Two Peoples Bay were implemented (Start and Burbidge, 1995; Courtenay et al., 1998; Courtenay and Friend, 2004). Recovery strategies simultaneously focused on the development of ex-situ reproductive and survival enhancement methods including captive breeding, artificial insemination, cross-fostering, and hand-rearing pouch young, to generate captive stock for reintroduction. Genetic studies, dietary analyses and disease investigation were carried out to address specific knowledge gaps and in response to morbidity and mortality events.

Despite ongoing research to refine husbandry techniques and improve reproductive output, captive breeding and other reproductive technologies failed to produce sufficient young to warrant the adoption of such practices (Burbidge et al., 2001; DPaW, 2016a). Recovery initiatives subsequently focused on extending the species range through the establishment of insurance populations beyond Two Peoples Bay via translocation (Courtenay and Friend, 2004; Figure 1). Between 2005 and 2007, the first translocations of Gilbert's potoroo were undertaken to Bald Island (810 ha), 25 km east of Two Peoples Bay (Friend, 2005b). Establishment of a second mainland colony commenced in 2010 within a 380-ha enclosure, fenced to exclude foxes (*Vulpes vulpes*) and feral cats (*Felis catus*), in Waychinicup National Park, east of Albany (the Waychinicup enclosure; Friend, 2010a). In 2018, potoroos were released onto Middle Island (1080 ha) in the Recherche Archipelago, east of Esperance (Friend, 2017).

Figure 1: Location of known Gilbert's potoroo populations including the indigenous Two Peoples Bay population (yellow circle) and reintroduced populations (red triangles). Remnant vegetation is also shown.

In 2015, a severe bushfire decimated the Two Peoples Bay mainland population with only seven animals known to survive this fire. This population now has critically low numbers and is at risk of local extinction (T. Friend, pers. comm.). Despite the establishment of additional populations, it is estimated that the global population of Gilbert's potoroo is currently less than 120 individuals (T. Friend, pers. comm.). Current recovery objectives aim to: (1) ensure existing populations of Gilbert's potoroo are restored and maintained at sustainable levels and genetic diversity is maximised; (2) increase the number of populations of Gilbert's potoroo; and (3) increase the awareness of, and support for the recovery of Gilbert's potoroo (DPaW, 2016a).

The purpose of this review is to examine the recovery of Gilbert's potoroo to better understand how we can effectively and more efficiently improve their conservation status and long-term persistence. By summarising the progress against the recovery actions, and scientific research underpinning these, we aim to: (1) identify remaining knowledge gaps; (2) determine future research priorities; and (3) provide recommendations to support the ongoing recovery of Gilbert's potoroo into the future.

2 Gilbert's potoroo - from rediscovery to recovery

2.1 Protection of known populations and habitats

Habitat critical for the survival of Gilbert's potoroo includes dense, long unburnt (> 50 years) shrubland with a diverse and abundant presence of hypogeal (underground) fungi (DPaW, 2016a). Potoroos on Mount Gardner prefer *Melaleuca striata* and *M. uncinata* heath, with a dense understorey of sedges (*Lepidosperma* spp. and *Anarthria scabra*) (Friend, 2008b; DPaW, 2016a). Protection and management of this habitat, and the last indigenous population of Gilbert's potoroo, has focused on mitigating key threats such as bushfire and introduced predators [see DPaW (2016a) Section 10, for a list of the specific policies, strategies and plans that contribute to the protection of Gilbert's potoroo]. Regular monitoring has been carried out to assess the health of individuals, collect demographic data, observe population trends, and to collect samples for research purposes. Ear tissue samples have been collected from all new individuals to monitor genetic diversity (DPaW, 2017b).

While it has long-been acknowledged that inappropriate fire poses a risk to Gilbert's potoroo in the wild (Start and Burbidge, 1995), the reliance of potoroos and other fire-sensitive species on long unburnt vegetation for shelter necessitates the management of potoroo habitat in a state that contains high fuel loads. Since the 1970s, planned fire was largely excluded from Mount Gardner (although there were some small lightning-ignited fires), principally to preserve the habitat of the endangered noisy scrub-bird (*Atrichornis clamosus*) and other threatened species (Danks et al., 1996). In addition to the presence of natural firebreaks (lakes, sand patches and granite) and the maintenance of a strategic low-fuel buffer system (Danks et al., 1996), fire management strategies aimed to undertake small-scale burns to create a mosaic of fuel ages within Two Peoples Bay Nature Reserve (S.

Comer, pers. comm.) to minimise fuel load, enhance suppression capacity, and reduce the risk of high intensity fires that result in significant mortality and habitat loss (DPaW, 2016a). Managers were cognisant of the risk of losing large areas of long unburnt habitat and plans to create a fine grain mosaic in potoroo habitat were initiated in the early 2010s. Unfortunately, this work had not commenced when in November 2015 very dry conditions and a lightning storm caused a bushfire that destroyed approximately 90% of core potoroo habitat in Two Peoples Bay bringing the wild population close to extinction (De Poloni, 2015; DPaW, 2016a).

Fox control, utilising 1080 (sodium fluoroacetate) dried meat baits, has been implemented in Two Peoples Bay since 1988 (Thomas and Algar, 2002). Introduced predator control currently incorporates quarterly aerial and monthly ground baiting with 1080 baits (Probait®) to control foxes, in conjunction with targeted feral cat control (DPaW, 2016a). Sand tracks have been monitored routinely for cat and fox prints within the Waychinicup enclosure, and during fieldwork/ground baiting at other sites (DPaW, 2016b). Significant research has focused on improving methods of feral cat control with the cat specific bait Eradicat®. In 2014, the Integrated Fauna Recovery Project investigated habitat use, movement, and rates of bait uptake (Eradicat®) in radio-collared feral cats from Two Peoples Bay (Comer et al., 2020). Camera traps were also used to determine the occupancy of cats pre- and post-baiting and targeted cat trapping trials were undertaken (Comer et al., 2020). The Western Shield program and South Coast Region now integrates fox baiting with Eradicat® baiting and trapping to control feral cats at this site (DPaW, 2017a). A permanent camera trap grid is maintained on Mount Gardner to monitor introduced predators (DPaW, 2014). Emergency feral cat baiting was undertaken on Mount Gardner following the 2015 fire.

In 1996, a permanent trapping-transect based on vehicle tracks was established on Mount Gardner and now forms part of DBCA's *Western Shield* Program. In 2000, a quantitative monitoring program was formalised, whereby a four-monthly census on nine trap lines in potoroo habitat was carried out (Friend, 2001a). Additional trap lines have since been established in unburnt habitat to monitor potoroos following the 2015 fire (DPaW, 2015). Camera traps with lures have also been deployed to monitor potoroos on established trap lines (DPaW, 2016a), within unsurveyed areas on Mount Gardner (e.g., Orliac, 2015), and within remnant unburnt habitat post-fire (DPaW, 2016a).

Populations of Gilbert's potoroo on Bald Island, and within the Waychinicup enclosure, have also been monitored using established trap lines and/or monitoring grid(s) in conjunction with remote camera traps. Due to difficulty accessing Middle Island, remote camera traps have predominantly been used to monitor potoroo presence and dispersal across the island in partnership with the Tjaltjraak Rangers. Whilst introduced predators are not a current threat, monitoring for incursions, and eradication if required, is necessary to maintain the introduced predator-free status of the islands. Routine fence inspections and feral predator monitoring using remote cameras has also been undertaken within the enclosure (DPaW, 2016b; DBCA, 2021).

Predation by female southern carpet pythons (*Morelia spilota imbricata*) has been implicated as a threat to the Gilbert's potoroo (Arnall, 2015; Pearson and Friend, 2016). Within the Waychinicup enclosure, it has been estimated that at least four adult potoroos are taken by pythons each year (Pearson, 2018). Potoroos recently introduced from Bald Island are the most likely to be predated (DPaW, 2016b). It is unknown whether this reflects predator naivety in island-bred potoroos (e.g., Blumstein and Daniel, 2005) or merely their vulnerability in the weeks following release into unfamiliar habitat. Targeted conservation action to reduce the incidence of python predation in the enclosure by opportunistic removal of large pythons was initiated by DBCA in 2014 (Friend, 2014), and potoroo numbers have grown and remained relatively stable since then; although recovery of this population may be associated with other factors, such as rainfall. A project to develop new methods to locate female pythons for removal from the enclosure was conducted between 2017 and 2021 (D. Pearson et al., unpublished data). Carpet python predation has also been reported sporadically in wild potoroos on Mount Gardner (Pearson and Friend, 2016), but their influence on the population there is unknown.

Dieback disease, caused by *Phytophthora cinnamomi*, was initially considered to be a threat to Gilbert's potoroo as it can alter the floristic composition and structure of vegetation, and cause death of host plants of dependent mycorrhizal fungi on which Gilbert's potoroo relies (Start and Burbidge, 1995; Courtenay and Friend, 2004). The disease was first discovered in the nature reserve in the early 1980s (Hart, 1983), and dieback mapping undertaken between 1989 and 1992 revealed that *Phytophthora* infestation was widespread (Danks et al., 1996). Hygiene practices to reduce the spread of disease were in place prior to the rediscovery of Gilbert's potoroo (Courtenay and Friend, 2004). Following the species' rediscovery in *Phytophthora*-free areas on Mt Gardner, re-mapping of dieback occurrence began in 1995 to identify and protect disease-free sites (Gillen, 1995). Research to investigate the effect of dieback on the fungal diet of bush rats (*Rattus fuscipes*) in Waychinicup National Park found that there was no significant difference in the diversity of hypogeal fungi between dieback and non-dieback affected areas, though sporocarp abundance and the number of bush rats were lower in dieback affected areas (Whelan, 2003). While potoroos were first detected in *Phytophthora*-free areas on Mount Gardner (Courtenay and Friend, 2004), healthy potoroo colonies have since been identified in *Phytophthora*-infected sites (Friend, 2005b). Dieback is no longer considered to be a threat to the potoroo; though hygiene practices, including visitor management to restrict access to areas of high conservation value for a number of species, have been maintained to reduce the spread of disease (DPaW, 2016a).

2.2 Ecology and population biology

Understanding the unique biological and ecological requirements of the highly secretive Gilbert's potoroo has involved long-term, intensive field studies utilising a combination of trapping and radiotracking. Twenty-four-hour radio-tracking and, more recently, GPS-loggers have also been used to accurately record the movements of individuals, and collect additional location data (e.g., nocturnal activity; Friend et al., 2018). Over the years, DBCA scientists have developed a good understanding of the species' biology and ecology, which has assisted with searches for, and the establishment of, new populations. The microhabitat preferences of Gilbert's potoroo on Mount Gardner were first examined using a modified spool-and-line tracking technique (Vetten, 1996). This study showed that potoroos utilised a range of vegetation ecotones, including dense scrub for diurnal shelter and protection from predators, and open areas for nocturnal foraging. Intensive radiotracking surveys (using specialised tail transmitters) have since been, and continue to be, undertaken to evaluate home range, spatial organisation, habitat use and population dynamics, as well as to monitor reproduction, study the dispersal and survival of young, investigate population declines, and monitor individuals following translocation (see Friend, 2001b; Friend, 2003b; Friend, 2008a; Orliac, 2015; DPaW, 2016a; Friend and Hill, 2020).

Radiotracking has been used to determine causes of death in adult and subadult potoroos, although further study is required to determine causes of mortality in juveniles. Analysis of trapping data showed that 60-80% of pouch young at Two Peoples Bay did not reach maturity (Friend, 2008b). Monitoring of habitat use by potoroos in burnt versus unburnt vegetation on Mount Gardner is ongoing to investigate post-fire recolonisation, along with resampling vegetation transects sampled by Vetten (1996) to investigate how the structure of the vegetation is recovering post-fire (supported by the Gilbert's Potoroo Action Group - GPAG).

2.3 Searches for new populations

Wide-ranging hair trap (arches and tubes) surveys have been conducted along the south coast region in long unburnt, dense heathland to search for new populations of Gilbert's potoroo (e.g., Wayne, 1995; Friend, 2003a). Hair preservation and analysis techniques (Brunner and Coman, 1974) were acquired under the tutelage of Hans Brunner, and a local mammal hair reference collection created. A specialist hair identification laboratory was established in 2004, incorporating use of the Hair ID program (Triggs and Brunner, 2002). Hair trap surveys were conducted by DBCA personnel under a project coordinated by the Denmark Environment Centre, with the assistance of GPAG and other trained volunteers. In response to the detection of putative potoroo hair, follow-up trapping was undertaken. Surveys carried out from Pallinup River west to Augusta failed to detect any new populations (DPaW, 2016a) and there have been no specific searches for new populations since 2008.

2.4 Captive breeding

Within two months of rediscovery, a captive breeding colony was established at Two Peoples Bay with the intent of establishing an insurance population and to provide stock for future translocations. A captive management plan (Courtenay, 1998a) and draft husbandry manual (Courtenay, 1998b) for Gilbert's potoroo were developed and implemented. Despite the colony being maintained for 15 years, only eight young survived to independence between 1995 and 2001, with most young produced within two years of establishment (Courtenay, 1997a; DPaW, 2016a). Collaborative research aimed to determine the cause of poor reproductive success in captivity.

Remote video monitoring assessed mating behaviour as part of a detailed behavioural study (Burke, 1998). While attempted matings were observed, none of these appeared to be successfully completed, although a new pouch young was produced during the monitoring period. Cyclic reproductive activity was investigated by monitoring levels of faecal oestradiol-17b and progestogens (Stead-Richardson et al., 2010). Clear reproductive patterns could not be obtained, though one female displayed evidence of cycling. Faecal cortisol levels were also examined to determine whether stress may be suppressing reproductive output (Stead-Richardson et al., 2010). However, cortisol levels were lower in captive compared to wild potoroos; and it was concluded that stress was not a factor limiting reproduction in captivity.

Several dietary studies were undertaken in the early years to enable a more detailed understanding of the diet of wild potoroos (Bougher, 1998; Nguyen 2000; Nguyen et al., 2005). Nutrient analysis of hypogeal fungi (Friend, 2004b) was also undertaken to inform improvement of the artificial captive diet. This diet, which was developed in consultation with Healesville Sanctuary and adapted from that used to maintain the long-nosed potoroo (LNP) (*Potorous tridactylus*) in captivity (Courtenay, 1998b), was gradually modified based on new research. For example, following the diagnosis of renal oxalosis in captivity (see below), potoroos were fed a low-oxalate diet (Forshaw et al., 2017). From 2001, hypogeal fungi harvested from the wild were added to the captive diet at about 5-10% of total food intake (Friend, 2004b).

Disease investigation was undertaken in response to the deaths of five captive individuals from renal oxalosis between 1997 and 2003 (Vaughan, 2008; Forshaw et al., 2017). Analysis of the captive diet and vegetation within the pens indicated that oxalate levels were negligible, thus eliminating dietary intake as a primary cause (Friend, 2005a). Excess glycolate was detected in the urine, which suggests a disorder of oxalate metabolism, though the absence of oxalate-degrading bacteria in the gut of captive animals was also a possibility (Forshaw et al., 2017). All individuals were closely related, and an inherited disorder of oxalate metabolism was suspected (Forshaw et al., 2017). Renal oxalosis has been detected at low prevalence (one confirmed case amongst 16 animals sampled in 2004) in wild potoroos at Two People's Bay (Friend, 2005a). In 2016, a wild potoroo that was being held in captivity following the 2015 fire also succumbed to this disease (DPaW, 2016b).

Balanoposthitis, an inflammatory condition of the penis and prepuce, with associated crusting, ulceration and preputial discharge was first identified in captive potoroos in 1997. A study that sought to determine the significance of this disease and its potential impact on reproductive success in captivity (Vaughan, 2008), identified a significant association between balanoposthitis and *Treponema* sp. infection, raising concern of dyspareunia (painful intercourse) and subsequent negative impacts on reproduction (Vaughan-Higgins et al., 2011). Balanoposthitis however, was frequently observed in wild potoroos without apparent reproductive consequence at a population level (i.e., continued recruitment of young; Friend, 2009). The presence of *Treponema* sp. infection also had no impact on white blood cell morphology and minimal significance on blood values (Vaughan, 2008), thus the clinical significance of balanoposthitis remains questionable.

Over time captive husbandry methods continued to evolve as DBCA sought to optimise breeding success (see Courtenay, 1997a; Courtenay, 1997b; Friend, 2004a). Genetic management of the colony was initially informed by genealogy and mean kinship values (Courtenay, 1998b) however pairings were largely unsuccessful. Various housing arrangements were trialled to address potential issues with behavioural incompatibility (e.g., mate preference) and overcrowding (Courtenay, 1997a; 1998b). To induce reproduction in non-breeding long-term captive animals, a 14-ha enclosure was constructed in 2007 on private land (Ryedene farm) 25 km east of Albany (DEC, 2007). The release of non-breeding captive potoroos into this semi-natural environment failed to stimulate breeding, but at least demonstrated their ability to survive in *Eucalyptus marginata-Allocasuarina fraseriana* woodland (Bougher and Friend, 2009a). Hygiene practices and animal handling protocols were also modified to minimise the risk of disease and reduce stress (Courtenay, 1997a; 1998b), and improved methods for monitoring and record keeping were implemented (e.g., Courtenay, 1997c). Unfortunately, the cause(s) of poor reproductive success in captivity could not be identified, and it was hypothesised that dietary and/or husbandry inadequacies for this highly specialised species may have reduced breeding frequency over time (DPaW, 2016a).

2.5 Assisted reproduction

In response to the poor success of conventional captive breeding, assisted reproductive and survival enhancement technologies were trialled to enhance reproductive output. Research into the development of artificial insemination (AI) techniques was undertaken in collaboration with Perth Zoo (Friend, 2003a). Initial trials investigated electroejaculation, under general anaesthesia, to collect semen from *P. tridactylus*, with the view of refining the AI protocol prior to the application of this technique in Gilbert's potoroo. In 2001, two captive potoroos were relocated to Perth Zoo for AI trials, however both died within 8 months of transfer. Stress associated with relocation and frequent handling may have contributed towards the death of these animals, given the documented intolerance of Gilbert's potoroo to handling and disturbance (Friend, 2004a). A regime of hand-rearing and regular handling was thus recommended to produce more tolerant individuals for future AI research (Friend, 2004a). Two late-stage pouch young were taken from wild females and hand-reared to trial this technique of transferring wild young into captivity (Friend, 2007). This method, however, proved to be extremely labour intensive. Fortunately, the success of the Bald Island translocations by wild-to-wild transfer of adult animals between 2005 and 2007 (see below) precluded the need for other methods to produce stock for translocation.

Between 2002 and 2009, cross-fostering trials were undertaken in collaboration with Dr David Taggart of the Royal Zoological Society of South Australia. Cross-fostering refers to the transfer of pouch young from a target species (donor) to a surrogate mother of a different taxon (recipient) and has been used successfully in macropodoid marsupials to increase reproductive output (Taggart et al., 2010). For Gilbert's potoroo, research carried out by Dr Taggart's group prior to cross-fostering this species investigated the survival rate of cross-fostered LNP pouch young to LNP, woylies (*Bettongia penicillata*) and boodies (*B. lesueur*); LNP had the highest success rate (Friend, 2008a; Taggart et al., 2010). Isolation trials (using LNP) were also conducted to determine the optimum temperature, humidity, and maximum transfer time for pouch young (D. Taggart., unpublished data; see also Crowley et al. 1988). Growth rates and the effect of methods of reattachment were investigated in cross-fostered LNP (Taggart et al., 2010), and milk composition was compared between the LNP and Gilbert's potoroo (unpublished data).

Initial trials between 2002 and 2003 (n=3) involved the transfer of pouch young from wild Gilbert's potoroo to captive LNP in South Australia, however pouch young did not survive beyond 4 weeks post-transfer, presumably due to prolonged isolation time between pouches (DPaW, 2016a). To reduce transfer time between pouches, a local colony of LNP was established near Albany in Western Australia. Subsequent trials between 2006 and 2009 (n=6) resulted in two individuals surviving to independence (Friend, 2008a). In 2009, the cross-fostering program ceased due to a lack of funding.

2.6 Genetics

Following the rediscovery of Gilbert's potoroo, allozyme electrophoresis and sequence analysis of the cytochrome b gene were used to evaluate phylogenetic relationships between extant potoroo species, reaffirming that Gilbert's potoroo was a separate species (Sinclair and Westerman, 1997). Chromosome morphology was likewise examined to infer taxonomic and evolutionary relationships among *Potorous* taxa; major similarities were identified between Gilbert's potoroo and *P. tridactylus* (Sinclair et al., 2000). Genetic variation in Gilbert's potoroo was also examined in detail by Sinclair (1998), including collaborative research that explored genetic variation in microsatellite and mitochondrial DNA. This study detected evidence of a significant genetic bottleneck, consistent with a recent demographic decline, highlighting concern for the long-term survival of Gilbert's potoroo (Sinclair et al., 2002).

Genetic research will continue to play an important role in the management of Gilbert's potoroo, with the potential to inform the selection of individuals for translocation. Given that remaining populations are small and geographically isolated, genetic augmentation is necessary to actively maintain genetic diversity, and founder representativeness over the long term (DBCA, 2020). DBCA in association with GPAG has been undertaking a population genetic analysis of Gilbert's potoroo using both 'historical' early post-rediscovery samples from Two Peoples Bay (1999 onwards) and 'contemporary' recently collected samples from all sites up to 2021 (Balaam, 2020; J. Courtenay, pers. comm.) to: (1) evaluate the current genetic diversity of the species' relative to historical records to investigate whether there has been genetic erosion over time; and (2) determine how effective translocations have been at capturing and preserving genetic diversity (K. Ottewell, pers. comm.). Ultimately, this research will reveal how genetic diversity is currently distributed across each of the populations at the individual and population level, thus informing future management requirements and enabling the targeted selection of individuals for translocation if aiming to minimise kinship in founder individuals.

In 2021, the Gilbert's potoroo genome was sequenced by DNA Zoo at the University of Western Australia (Pauletto, 2021) as an in-kind contribution to GPAG's State NRM Community Stewardship small grant. Chromosome length (2n = 12, 14) was greater than that previously reported by Sinclair (2000) (2n = 12, 13) due to the discovery of an additional X chromosome in males (P. Kaur, pers. comm.). The availability of a reference genome for Gilbert's potoroo should facilitate further research into aspects of functional genetic diversity, such as understanding immunogenetic diversity and underlying genetic basis of disease, that may assist in future management of the species.

2.7 Diet

Gilbert's potoroo is a specialist mycophagist, with sporocarps (fruiting bodies/truffles) of hypogeal fungi comprising over 90% of its diet (Bougher, 1998; Nguyen, 2000; Nguyen et al., 2005). As fungi are critical for the survival of Gilbert's potoroo (DPaW, 2016a), a significant amount of research has focused on diet. Faecal samples collected between 1994 and 1998 were examined and the fungal spore-types found in them were described (Bougher, 1998). This was the first study to identify the highly specialised dietary strategy of Gilbert's potoroo and demonstrated the diversity of fungi being consumed. In 1998, quantitative and opportunistic searches for hypogeal fungi were conducted near known potoroo habitat in Two Peoples Bay for the purposes of nutritional analysis and to identify plant-fungi associations (Syme, 2004). Fungal specimens were compared to those previously identified in potoroo faeces by Bougher (1998).

A larger study (Nguyen, 2000), in which faecal analyses indirectly examined the diet of Gilbert's potoroo from three sites in Two Peoples Bay, identified 44 different fungal spore-types in faeces (Nguyen et al., 2005). Subsequent studies have identified a diverse range of fungi from other sites, and varying species richness in potoroo diet between sites. For instance, 27 fungal spore-types were isolated from potoroo faeces on Bald Island, and up to 14 spore-types were identified in potoroo faeces collected from Ryedene farm (Bougher and Friend, 2009a). It has also been found that the number of spore-types detected in faeces tends to increase over time following translocation (Bougher and Friend, 2009a; Friend et al., 2018). On occasion, potoroos have also been documented to consume epigeal (above ground) fungi (e.g., during translocation trials on Middle Island; Friend et al., 2018), although this may reflect difficulty in finding hypogeal fungi at a new site. The fungal spore-types detected in potoroo faeces are listed in Bougher (1998), Nguyen (2000), and Bougher and Friend (2009a).

Importantly, knowledge regarding the abundance and diversity of hypogeal fungi present within a site is vital for determining its suitability for the establishment of new populations. Methods to enhance detection of hypogeal fungi and thus determine site suitability (e.g., greater understanding of plant-fungi associations) need to be refined. Investigation into how food resources differ between burnt and unburnt habitat post-fire (Syme et al., unpublished data) is part of an ongoing State NRM funded GPAG project.

2.8 Disease

Disease investigation has been undertaken in response to morbidity and mortality events, particularly for captive animals. Disease screening has also been carried out prior to translocation. The infectious and non-infectious agents that have been documented in Gilbert's potoroo are summarised in Appendix 1. Renal oxalosis and balanoposthitis (as discussed previously) represent the most significant health conditions reported to date, while other disease agents have been encountered sporadically. A comprehensive study of the health and disease status of Gilbert's potoroo was conducted by Vaughan (2008), with particular focus on determining the prevalence of parasitic disease, cryptococcosis, toxoplasmosis and *Treponema* sp. infection in potoroos. Haematological, serum biochemical and urinalysis reference values were established for Gilbert's potoroo during this study (Vaughan-Higgins et al., 2009). The cytological characteristics of blood cells from Gilbert's potoroo were previously described by Clark (2004).

Vaughan (2008) identified the presence of various pathogens in wild and captive potoroos. With the exception of *Cryptococcus neoformans* (Vaughan et al., 2007), *Mycobacterium avium intracellulare* (Vaughan, 2008) and potentially treponemes (Vaughan-Higgins et al., 2011), most pathogens were clinically insignificant. Parasites for instance, have been commonly identified in healthy wild potoroos (Vaughan 2008; Austen, 2015). Within the captive colony, parasites were comparatively rare, as all potoroos were prophylactically treated with topical ivermectin (0.2mg/kg) and sprayed with pyrethrin, N-Octyl-bicycloheptene and Piperonyl butoxide (Fido's free-itch concentrate® diluted 2ml to 200ml water) upon entering captivity, and routinely every three months thereafter (Vaughan, 2008). Comprehensive parasite-screening has not been undertaken in Gilbert's potoroo and other cohabiting species across all sites. A qualitative disease risk assessment for Gilbert's potoroo and quokka was carried out prior to the Bald Island translocation (Friend, 2005b; Swan, 2005). Due to the lack of knowledge and quantitative data regarding disease in Gilbert's potoroo and cohabiting wildlife, potoroos receive prophylactic anti-parasitic drug treatment (e.g., Revolution® and Ivomec®; Friend, 2017) prior to translocation and typically undergo a period of quarantine to reduce the risk of disease and eliminate hypogeal fungal spores from their system (Friend, 2010a). Time in captivity also reduces the likelihood of *Phytophthora* propagule transmission into *Phytophthora*-free sites (e.g., Bald Island; Friend, 2005b).

2.9 Translocations

Since 2005, translocations have been a focus of Gilbert's potoroo conservation initiatives. Field research to determine the specific habitat and dietary requirements of Gilbert's potoroo, in conjunction with detailed knowledge of habitat use and social structure (see examples above), has guided the successful establishment of two insurance populations (Bald Island and the Waychinicup enclosure). The short-term success of the most-recent translocation to Middle Island was established through a trapping survey in April 2021, during which three founders and three island-born individuals were captured, as well as other evidence of breeding (Friend and Cochrane, 2021). Following the bushfire that placed the original Two Peoples Bay population at risk of local extinction, the value of establishing insurance populations was fully realised. Not only did the establishment of such populations save the species from immediate extinction (De Poloni, 2015), but these populations contain the extant genetic diversity of the species, and it is anticipated that future reintroductions will facilitate the recovery of potoroos on Mount Gardner (DBCA, 2020). Future recovery initiatives also aim to re-establish populations of Gilbert's potoroo at other locations on the mainland (DPaW, 2016a; DBCA, 2020).

Methods to assess habitat suitability continue to be refined, but suitability is largely dependent on critical habitat attributes such as fungal availability, appropriate vegetation structure and floristics, and the abundance of shelter (Friend, 2005b). As fungi are essential for the persistence of Gilbert's potoroo, the diversity and abundance of hypogeal fungi is the most important determinant of suitable habitat (Bougher and Friend, 2009a). Threat mitigation also plays a vital role, particularly for mainland populations (Friend, 2010b; DPaW, 2016a). Trial translocations to Mermaid Point on the mainland in 2010 and Michaelmas Island in 2016 both failed due to unforeseen predation pressure by foxes (DPaW, 2016a) and insufficient food availability (Friend, 2017), respectively. The latter translocation was undertaken as an emergency measure following the 2015 fire (Friend et al., 2016). After the emergency relocation of seven surviving potoroos from Mount Gardner to captivity, it was recommended (based on a previous trial; Friend, 2016c) that potoroos be relocated to Michaelmas Island in the short-term, given that the remaining Two Peoples Bay habitat could probably only support 2-3 individuals, and there was concern about observed declines at other sites (Bald Island and the Waychinicup enclosure; see Figure 2) (Friend, 2016a). The failure of the Mermaid Point translocation may have been associated with predator naivety given that potoroos were sourced from predator-free Bald Island, and five of the six individuals were predated within a week of release (DPaW, 2016a). However, as the animals had settled in an area with little dense cover, exposure to predators was high.

The process of establishing new populations of Gilbert's potoroo via translocation has involved: (1) extensive research to determine the suitability of the host environment (e.g., access; geology, geomorphology, and soils; climate; fire history; vegetation floristics and associations; presence of endemic vertebrate fauna; Friend, 2017) and similarity to known potoroo habitat; (2) preliminary site evaluations (often seasonal) to visually assess the habitat, and search for hypogeal fungi (see Bougher et al., 2008), and hair arch surveys to confirm the presence of other mammals if required (Friend et al., 2005); (3) short-term trial translocations of a few individuals to test suitability; and (4) a full-scale translocation. A detailed translocation proposal requires approval prior to each translocation.

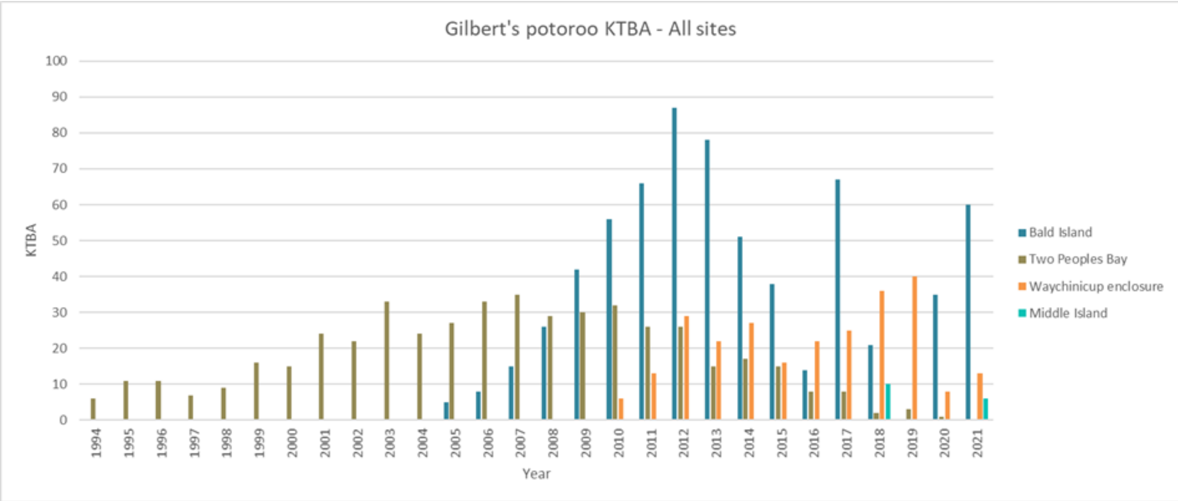

Figure 2: The number of individuals known to be alive (KTBA) over time (years), for each Gilbert's potoroo population. Note: Bald Island was not surveyed in 2019; Middle Island was not surveyed in 2019 or 2020. No potoroos were trapped or detected on cameras at Two People's Bay in 2021.

Trial translocations have involved the release of 'pioneer potoroos' which are intensively monitored by radio-tracking and trapping (Friend et al., 2005), and more recently GPS loggers (Friend, 2016b, 2016c; Friend et al., 2018). Both radio-tracking and GPS logging can assist with the identification of preferred habitat and nesting sites, which can then be targeted in subsequent releases. Trapping has been used to closely monitor the health, weight, and body condition of individuals post-release to determine whether success criteria are met (e.g., Friend, 2017). Searches for diggings and faecal analysis have been used to assess the ability of animals to find food and determine the variety of hypogeal fungi being consumed (Bougher and Friend, 2009b; Friend et al., 2018). Bougher and Friend (2009a) recommend sampling hypogeal fruiting bodies in conjunction with faecal analysis for monitoring fungal diversity due to the complimentary nature of the two techniques.

To indirectly assess the availability of hypogeal fungi within potential translocation sites, studies have investigated the use of indicator species for determining the presence of fungi. The bush rat is known to consume hypogeal fungi (Whelan, 2003); thus, bush rat faeces were analysed to indirectly evaluate habitat on Michaelmas Island in 2016 (Friend, 2016c; 2017). Although hypogeal fungi were detected in bush rat faeces, and trial translocation potoroos accessed a variety of fungal species (albeit at lower numbers compared to bush rats within Two Peoples Bay), the results of such investigations require cautious evaluation, as the larger group of translocated potoroos failed to find enough food (Friend, 2016b; 2017). Evaluating the diet of cohabiting species is also important for determining the likelihood of competition between species. The diet of extant quokkas and introduced potoroos was compared on Bald Island (Friend, 2006). Quokkas were found to consume hypogeal fungi in winter, but consumption was minimal during summer when fungal abundance was lower, so it was concluded that competition for food with Gilbert's potoroo was unlikely (Friend, 2006).

Climate change should also be considered when selecting future release sites. Climate change is recognised as an extreme threat to the persistence of Gilbert's potoroo (Gilfillan et al., 2009), with increasing temperatures and reduced rainfall predicted to increase the frequency and intensity of bushfire events (Hope et al., 2015). A drying climate is also likely to reduce the availability of hypogeal fungi on which Gilbert's potoroo rely (DPaW, 2016a). Below-average rainfall is thought to have been associated with an observed decline in potoroo numbers on Bald Island and within the Waychinicup enclosure between 2012 and 2016, although reduced resource availability on Bald Island (which may be inextricably linked to reduced rainfall or a result of the population exceeding carrying capacity), and carpet python predation within the enclosure may have also been contributing factors (DPaW, 2017b; DPaW, 2016a). Given the reliance of Gilbert's potoroo on hypogeal fungi, a greater understanding of the impact of climate change on the diversity and abundance of hypogeal fungi is warranted. Species distribution modelling (e.g., Molloy et al., 2020) may assist with the selection of bioclimatically suitable habitat.

2.10 Education, citizen science and community involvement

In 2002, a not-for-profit, volunteer community group, known as the Gilbert's Potoroo Action Group (GPAG) was incorporated (see https://potoroo.org/). The three key objectives of GPAG include fundraising, education, and volunteer recruitment to assist with the recovery of Gilbert's potoroo. GPAG has provided invaluable support to DBCA through the provision of volunteers and by securing funds for the implementation of recovery actions (Friend, 2007). GPAG has been successful in obtaining several large grants, one of the most recent, through Western Australia's State NRM program, is supporting four citizen science projects in collaboration with DBCA (see GPAG 2020a, 2020b, 2020c; Friend and Hill, 2020). Importantly, GPAG continues to increase awareness of the plight of Gilbert's potoroo by engaging the community and encouraging their involvement in conservation activities (Friend, 2007). Gilbert's potoroo also features in an environmental education package "What's in your backyard?" (Marr, 2020), which uses GPAG as an example of "Science as a Human Endeavour", bringing scientists, land managers and volunteers together to conserve Gilbert's potoroo. In 2021, DBCA secured the support of the Esperance Tjaltjraak Rangers to assist with potoroo monitoring on Middle Island. The Tjaltjraak Ranger program is part of a State Government initiative to create jobs and training and community development opportunities for Aboriginal people.

3 Gilbert’s potoroo – ongoing recovery

Targeted conservation action informed by scientific research has supported the recovery of Gilbert’s potoroo in the 28 years since the species’ rediscovery. Considerable progress has been made towards achieving many of the recovery actions outlined in the 2016 Recovery Plan (DPaW, 2016a), and the establishment of new populations via translocation has been instrumental in preventing the extinction of Gilbert’s potoroo (NESP TSRH, 2019; Bolam et al., 2020). Despite this progress, the conservation status of the species is yet to be downgraded and it is still considered to be at risk of extinction in the next 20 years (Geyle et al., 2018). Current population estimates (2022) indicate that only 1-2 animals persist on Mount Gardner, and less than 120 individuals are known to be alive on islands and within the Waychinicup enclosure (T. Friend, pers. comm.). Bald Island supports the largest population and is assumed to contain most of the genetic diversity for the species (DBCA, 2020).

Given the small and fragmented nature of the remaining populations and evidence of a significant genetic bottleneck (Sinclair et al., 2002), ongoing intensive management is likely to be required to conserve and promote the long-term persistence of viable populations of Gilbert’s potoroo into the future. For a species with such a critically low population size, ongoing monitoring of all populations is important to: (1) determine population trends and swiftly respond to threats (e.g., disease, predation); (2) manage the species to maintain genetic representativeness; and (3) facilitate the collection of data to inform ongoing research and management. Importantly, we highlight the need for consistent, long-term monitoring (e.g., mark-recapture studies) of all populations to closely monitor population trends and readily detect hazards when they arise. Determining an appropriate and cost-effective monitoring strategy for each population is needed, with clear articulation of time commitments.

In 2020, a Population Management and Translocation Strategy was drafted in consultation with the Gilbert’s Potoroo Recovery Team (DBCA, 2020). Using an adaptive management approach, the overall objective of this strategy is to “conserve and promote the long-term persistence of viable populations of Gilbert’s potoroo”. Alongside population recovery strategies, integrated genetic management will play an important role in the recovery of the species going forward, particularly with regard to optimising the selection of individuals for translocation and ongoing genetic monitoring of insurance and wild populations to achieve the specific objectives outlined in this strategy.

We now have a clearer understanding of the species’ habitat requirements, including ecological and biological needs, and have begun to refine the logistics of translocating and establishing new populations. To continue to support recovery of the Gilbert’s potoroo into the future, we have identified a number of recommendations for consideration.

3.1 Recommendations

3.1.1 Recovery of the Two Peoples Bay population

To prevent the local extinction of the only remaining indigenous Gilbert's potoroo population, supporting the post-fire recovery at Two Peoples Bay remains a major priority. A population management strategy (DBCA, 2020) aims to ensure this population "is recovering and considered secure, with a trajectory to pre-2015 bushfire numbers (>25 independent individuals by 2030)". Recent monitoring at this site has indicated that to a limited degree, potoroos are now using the 2015 burnt habitat, and translocations from Bald Island and/or the Waychinicup enclosure to supplement this critically small population are planned for 2022/2023. Monitoring of both source populations is also required to ensure these populations can be sustainably harvested. Intensive post-release monitoring is recommended to determine the role of possible predator naivety in the survival of these animals (e.g., will exposure to carpet pythons within the enclosure be advantageous to survival post-release, compared to island-bred potoroos with no prior exposure to either native or introduced predators?), as well as the effectiveness of ongoing threat management such as introduced predator control and fire management.

3.1.2 Establishment of the Middle Island population

Results of recent monitoring have shown that the potoroos translocated to Middle Island are persisting and breeding, with three founders and three new individuals detected in April 2021 (Friend and Cochrane, 2021). However, the long-term viability of this population is yet to be established. Ongoing monitoring is vital to determine the outcome of this translocation. Genetic supplementation of this population at an early stage, when it is most likely to be effective, should be considered.

3.1.3 Identify additional mainland sites for translocation

Identification of potential mainland sites for future translocation is a further priority. A structured decision-making process that prioritises potential mainland sites has been proposed. Refining methods to better determine habitat suitability and investigating the impact of climate change on fungal abundance and diversity (see below) is recommended to promote the successful establishment and long-term resilience of translocated populations. The ability to implement effective integrated predator control is also required for population persistence (DPaW, 2016a). Fire management around suitable habitat aimed at protecting the dense vegetation preferred by potoroos will require long-term planning to mesh with broader management priorities. Other requirements necessary for the successful establishment of mainland populations need to be determined, including effective threat mitigation strategies.

3.1.4 Refine methods to better determine habitat suitability

One of the most important determinants of habitat suitability for Gilbert's potoroo is the abundance and diversity of hypogeal fungi. As such, refining techniques to better detect this food source would assist with identifying potential translocation sites. Radio tracking individual potoroos to further elucidate fine scale habitat use may also assist with identifying plant-fungi associations. To systematically evaluate habitat suitability at potential translocation sites, we propose the implementation of a structured decision-making protocol. The development of molecular tools to better understand dietary complexity should assist with identifying a minimum requirement for food availability.

3.1.5 Investigate the impacts of climate change

With a drying climate predicted to reduce the availability of hypogeal fungi (as modelled for the northern bettong *Bettongia tropica* by Bateman et al., 2011), climate change poses a potential threat to the persistence of Gilbert's potoroo. There is already some evidence to suggest an association between a drying climate and reduced food availability for Gilbert's potoroo, with observed declines coinciding with years of below average rainfall (DPaW, 2017b). As such, selection of future translocation sites may benefit from a consideration of the influence of climate change on the abundance and diversity of fungi (i.e., are there species of mycorrhizal fungi that are more tolerant of a drying climate?). Implementing Climate Change Vulnerability Assessments, as per the IUCN SSC guidelines (Foden and Young, 2016), may assist with identifying and mitigating other potential impacts of climate change on Gilbert's potoroo. Maintenance of genetic diversity should also be prioritised to promote the species' resilience to climate change.

3.1.6 Reducing mortality

Action to reduce mortality events is critical for a species in such low abundance. Ongoing research to better understand the influence of carpet python predation, which appears to have limited population growth of potoroos within the Waychinicup enclosure and may constrain their recovery on Mount Gardner, is recommended. Given the associated mortality and apparent hereditary nature of renal oxalosis, investigating the prevalence of this disease is also recommended. Now the Gilbert's potoroo genome has been sequenced, it may be possible to begin to identify genetic markers for the disease (K. Ottewell, pers. comm.), thus enabling individual disease-screening to better inform the selection of individuals for translocation (i.e., targeting disease-free animals when establishing new populations). Radio tracking is likely to continue to play an important role in determining other causes of mortality.

3.1.7 Other considerations

The prophylactic use of anti-parasitic drugs during translocation requires consideration. While treatment may be justified on the basis that there is a lack of knowledge regarding the parasites infecting Gilbert's potoroo and other cohabiting species, future research could focus on parasite-screening to elucidate whether antiparasitic treatment is necessary. With evidence to suggest that parasite conservation during translocation may enhance host immunity and improve translocation outcomes (Pizzi, 2009; McGill et al., 2010; Boyce et al., 2011; Rideout et al., 2016), parasite conservation may be important for a species such as Gilbert's potoroo with low genetic diversity, which may be more susceptible to disease (Wait et al., 2017). Furthermore, susceptibility to disease upon re-exposure could be an issue for Gilbert's potoroo, as parasite loss is likely to have occurred during the establishment of island populations. Immunological naivety in island-bred potoroos may enhance vulnerability to infection and adversely impact health and/or survival in circumstances where potoroos encounter host-specific parasites that have evolved to exploit them (e.g., island to mainland translocations) (McGill et al., 2010; Almberg et al., 2012).

4 Conclusion

Recovering a species from the brink of extinction requires long-term, targeted conservation action, as has been demonstrated in the case of Gilbert's potoroo. We advocate an adaptive management approach informed by science, to maximise the probability of recovery. For Gilbert's potoroo, closely monitored translocations, combined with intensive management of habitat, have effectively increased the population trajectory, and prevented the species' extinction. Recovery of Gilbert's potoroo into the future requires ongoing management, informed by genetic analysis, to conserve genetic diversity and promote species' resilience. Recommendations for future directions include promoting the post-fire recovery of the Mount Gardner population, establishment of the Middle Island population, selection of a third mainland translocation site, refining methods to better determine habitat suitability, investigating the potential impacts of climate change, reducing mortality, and evaluating whether island/enclosure-bred potoroos have reduced anti-predator behaviour. Consideration of comprehensive parasite screening and determining the prevalence of renal oxalosis is also recommended. Most importantly, a consistent long-term monitoring regime will facilitate the collection of data to inform and support the recovery of Gilbert's potoroo.

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Personal Communication Reference Affiliations

Sarah Comer	Department of Biodiversity, Conservation and Attractions Regional Ecologist (South Coast)
Jackie Courtenay	Gilbert's Potoroo Action Group (GPAG) Communications Coordinator
Tony Friend	Department of Biodiversity, Conservation and Attractions Research Associate
Parwinder Kaur	University of Western Australia Director, DNA Zoo Australia
Kym Ottewell	Department of Biodiversity, Conservation and Attractions Senior Research Scientist

Appendices

Appendix 1: Infectious and non-infectious agents documented in Gilbert's potoroo (*Potorous gilbertii*)

Infectious			**Agent**	**References**
Parasites	**Endoparasites**	Haemoparasites	*Breinlia macropi*	Vaughan, 2008
			Theileria gilberti n. sp.	Lee, 2004; Lee et al., 2009; Austen, 2015; Barbosa et al., 2019
			Theileria worthingtonorum	Barbosa et al., 2019
			Trypanosoma copemani n. sp.	Austen et al., 2009; Austen et al., 2015
		Protozoa	Coccidian oocysts	Vaughan, 2008
			Eimeria spp. oocysts	Vaughan, 2008
			Eimeria potoroi	Swan, 2005
			Toxoplasma gondii	Vaughan, 2008
		Nemotodes	*Ophidascaris robertsi*	Vaughan, 2008
			Potostrongylus temperatus	Vaughan, 2008
			Strongyle spp. eggs/larvae	Vaughan, 2008
			Trichuris sp.	Vaughan, 2008
			Unidentified microfilaria	Austen, 2015
			Unidentified nematode eggs/larvae	Vaughan, 2008
			Unidentified nematode sp.	Vaughan, 2008
	Ectoparasites	Fleas	*Pygiopsylla* spp.	Vaughan, 2008
			Stephanurus dasyurii	Lee, 2004; Vaughan, 2008; Lee et al., 2009; Austen, 2015
		Mites	*Cytostethum* spp. mite	Vaughan, 2008
			Trombiculidae family	Vaughan, 2008
		Lice	Unidentified species	Vaughan, 2008
		Ticks	*Ixodes australiensis*	Lee, 2004; Vaughan, 2008; Lee et al., 2009; Austen, 2015
			Ixodes fecialis	Lee, 2004; Vaughan, 2008; Lee et al., 2009
			Ixodes myrmecobii	Vaughan, 2008
			Unidentified *Ixodes* spp.	Vaughan, 2008
Fungi			*Cryptococcus neoformans*	Vaughan et al., 2007
Bacteria			*Actinobacillus* sp.	Vaughan-Higgins et al., 2011
			Bacillus sp.	Vaughan, 2008
			Bacteroides fragilis	Vaughan, 2008
			Bacteroides melaninogenicus	Vaughan, 2008
			Bacteroides thetaiotaomicron	Vaughan, 2008
			Bacteroides sp.	Vaughan, 2008
			Bifidobacterium sp.	Vaughan, 2008
			Brackiella oedipus	Vaughan, 2008
			Brachyspira-like spirochaetes	Vaughan, 2008
			Clostridium glycolicum	Vaughan, 2008
			Corynebacterium pilosum	Vaughan, 2008
			Coryneform bacilli	Vaughan, 2008
			Enterobacter aerogenes	Vaughan, 2008
			Enterobacter cloacae	Vaughan, 2008
			Enterobacter faecalis	Vaughan, 2008
			Enterobacter vulneris	Vaughan, 2008
			Enterococcus sp.	Vaughan, 2008

Infectious	**Agent**	**References**
	Escherichia coli	Vaughan, 2008
	Escherichia coli (non-haemolytic)	Vaughan, 2008
	Escherichia coli (haemolytic)	Vaughan, 2008
	Eubacterium lentum	Vaughan, 2008
	Fusobacterium sp.	Vaughan, 2008
	Helcococcus sp.	Vaughan, 2008
	Klebsiella oxytoca	Vaughan, 2008
	Lactobacillus sp.	Vaughan, 2008
	Mycobacterium avium-intracellulare	Vaughan, 2008
	Non-fermentative gram-negative rods	Vaughan, 2008
	Pantoea sp.	Vaughan, 2008
	Pasteurella sp.	Vaughan-Higgins et al., 2011
	Porphyromonas asaccharolytica	Vaughan, 2008
	Prevotella sp.	Vaughan, 2008
	Proteus mirabilis	Vaughan, 2008
	Proteus vulgaris	Vaughan, 2008
	Pseudomonas aeruginosa	Vaughan, 2008
	Pseudomonas sp.	Vaughan, 2008
	Rickettsia spp. (within *I. australiensis*)	Vaughan, 2008
	Serratia liquefaciens	Vaughan, 2008
	Serratia marcescens	Vaughan, 2008
	Staphylococcus aureus	Vaughan, 2008
	Staphylococcus epidermidis	Vaughan, 2008
	Staphylococcus sp. (coagulase-positive)	Vaughan, 2008
	Staphylococcus sp. (coagulase-negative)	Vaughan, 2008
	Streptococcus sp. (α-haemolytic)	Vaughan, 2008
	Streptococcus sp. (ß-haemolytic)	Vaughan, 2008
	Streptococcus sp. (non-haemolytic)	Vaughan, 2008
	Treponema sp.	Vaughan-Higgins et al., 2011
	Unidentified coliforms	Vaughan, 2008
	Unidentified gram-negative rods	Vaughan, 2008
	Waddlia chondrophila	Bodetti et al., 2003; Vaughan, 2008
Viruses	Novel papillomavirus or BPCV-like virus strain	Benevides, McWhorter and Woolford; unpublished data
Non-infectious	**Agent**	**References**
Neoplasia	Erythroid myelosis	Vaughan, 2008
	Malignant sarcoma	Vaughan, 2008
	Skin haemangioma	Vaughan, 2008
	Squamous cell carcinoma	Vaughan, 2008