Science for Policy

Research findings in brief

Project 4.2.3.4

Threatened Species Recovery Hub

National Environmental Science Programme

Using eDNA to track cryptic threatened burrowing petrels on Macquarie Island following pest eradication

In brief

This study used DNA from the scats and feathers from burrowing petrels on remote, sub-Antarctic Macquarie Island to determine the diversity of these seabirds on the island and to assess the use of environmental DNA (eDNA) for this type of application.

The island has undergone environmental change following eradications of invasive cats, rats, mice and rabbits, and the study examined whether eDNA can help to determine what species of burrowing petrels occur now, including looking for species that were driven to extinction.

Burrowing petrels are "cryptic" species, in that they can be difficult to detect, which makes them hard to monitor with conventional methods. The use of DNA to identify species represents a useful way to confirm and expand upon the conclusions of conventional ground searches.

We mapped occurrences of species of burrowing petrels at surveyed sites. Significantly, we located new populations of existing species, populations of returned species, and identified differences between birds of the same species from Macquarie Island and other sub-Antarctic islands.

Our research shows a successful application of DNA techniques to inventory cryptic species on remote islands in Australia. The method is especially relevant to islands in Australia and globally, where field trips are necessarily infrequent and researchers can only spend limited time.

The research was led by the Australian Government's National Environmental Science Program through the Threatened Species Recovery Hub, in collaboration with the Australian Antarctic Division, Marine Conservation Branch, and Tasmanian Parks & Wildlife, Department of Primary Industries, Parks, Water and Environment, Tasmania.

White-headed petrel burrows can exceed 2 m in length, so occupants are difficult to detect. Image: Jez Bird

Background

Sub-Antarctic Macquarie Island is undergoing environmental change following multiple successful eradications of feral animals. In 2000, cats were eradicated, and this was followed by eradications of rabbits, rats and mice in 2014. These invasive species had a negative impact on many of the native species of the island, particularly the eight species of burrowing petrels that breed there, through predation on adult birds, chicks and eggs, and destroying their nesting habitat. As a result, several species of burrowing petrel had become threatened, with some driven to extinction from the island itself and retreating to offshore rock stacks and Bishop and Clerk Islets (33km south of Macquarie Island).

Now that cats, rabbits, rats and mice have been eradicated, there is strong interest about whether populations of burrowing petrel will increase on the island and if, in fact, some species will now return to it.

Currently, Tasmanian DPIPWE Parks and Wildlife rangers and government scientists spend time on the island surveying these birds, but they can be hard to detect. Burrowing petrels are particularly difficult to monitor due to their cryptic nature: birds only return to land at night, and only at certain times of year; different species can look incredibly similar; breeding sites can be fragile and hard to access; and the burrows are often so long it is difficult to identify the occupants.

The University Of Queensland AUSTRALIA

Tasmanian Government

Australian Antarctic Program

Background (continued)

There is a suite of threatened species assessment tools that can provide valuable information about which species are present in a landscape and how they are distributed there and, importantly, whether the presence of species shows change under different environmental and anthropogenic pressures. Before this research, however, scats and feathers of unknown seabird species were not commonly used to back up with empirical evidence the inferences made from conventional ground-searching on remote islands. Such DNA analysis may be able to provide a way to identify burrowing petrel species in the region, and detect any shifts in species diversity on post-eradication Macquarie Island.

Environmental DNA (eDNA) samples can include indirect sample collections, such as water or soil, or more direct sample collections, such as tissue samples or scats to identify the main species, their prey or parasites.

Research aims

The research aimed to explore a novel method for detecting threatened burrowing petrels on post-eradication Macquarie Island. This aim involved the following:

- Developing a low-impact genetic technique for surveillance of cryptic burrow-nesting threatened species
- Determining the threatened petrel species present on Macquarie Island, including the presence of any new species
- Determining which petrel species are present in "colonies" of burrows, to ascertain whether they are single-species or multi-species colonies.

What we did

We developed and used DNA analysis techniques to ground truth field surveys of cryptic burrowing petrels on Macquarie Island, and to detect any unanticipated species which may also have returned to the island following the feral animal eradications.

Team members collected 222 scat and 108 feather samples from Macquarie Island that were later analysed in the laboratory for DNA (Figure 1). We used these data to identify which burrowing petrel species were present on the island and mapped the locations where they were detected (see Figure 2).

We also assessed how well DNA analysis of scats and feathers can be used to determine the diversity of cryptic burrowing petrels on Macquarie Island.

We collected the scat and feather samples between November 2017 and March 2018, and again in July to November 2018. We collected the samples from either 1) breeding sites of known species; or 2) breeding sites with mixed or unknown species.

The known sites (group 1) we visited were predominantly where occurrence of white-headed petrels, grey petrels, blue petrels or Antarctic prions had been confirmed. Samples from mixed or unknown species (group 2), were collected from a range of breeding sites or during survey transects across the island. For each sample we collected, we recorded the GPS co-ordinates, date and the freshness of the scat samples, to test whether old samples still had sufficient DNA.

A considerable amount of laboratory work was necessary to investigate the genetics of these seabirds, as little work had previously been done for the species. We designed burrowing petrel primers for two gene regions. We also accessed existing genetic sequences from the families Procellariidae (prions, shearwaters, fulmarine petrels and gadfly petrels), Pelecanoididae (diving petrels), Oceanitidae (southern storm petrels) and Hydrobatidae (northern storm petrels) from online repositories.

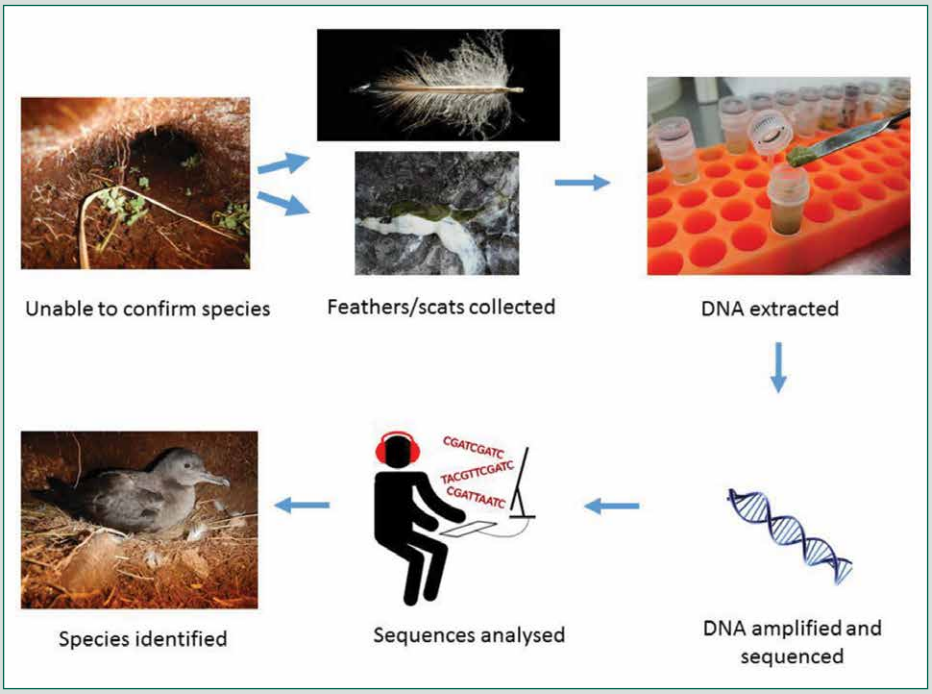

Figure 1. Workflow for DNA detection of burrowing petrels from scat and feather samples.

Key findings

The DNA that we extracted from scats and feathers on Macquarie Island to detect burrowing petrel species diversity gave valuable insights into the distribution of species around the island (see Figure 2). We detected DNA with exact matches to reference sequences for seven of the burrowing petrels species previously recorded to breed on Macquarie Island (or offshore islands). An additional two sequences could only be identified to genus (Table 1). One of the sequences closely matched both South-Georgian diving petrels (*Pelecanoides georgicus*) and common diving petrels (*P. urinatrix*) and the other sequence closely matched both fairy prion (*Pachyptila turtur*) and fulmar prion (*Pachyptila crassirostris*), with only one base pair difference in each case. This was due to the close genetic similarities between these species in this gene region.

There was no significant difference in the success rate of scats and feathers to detect species, with 94% and 87% success, respectively. Burrowing petrels are cryptic and difficult to survey as they burrow underground to nest and are not easy to view. Given the difficulty of monitoring such species, researchers typically make assessments based on hand searches, acoustic playback, spot-lighting and burrow-scopes. Our sampling at the established and known colonies or areas of burrows (group 1) revealed that the species thought to occur there were indeed present, providing confidence in previous survey methods.

In several instances, our research also detected burrowing petrel species at new locations. Diving petrels and fairy prions are rarely detected on Macquarie Island, with the latter previously recorded only on offshore rock stacks and Bishop and Clerk Islets. However, we found diving petrel DNA in samples from five locations around the island and fairy prion

Table 1: Burrowing petrel species detected on Macquarie Island from scat and feather DNA.

Common species (> 5 locations > 30 samples)	Less common species (<5 locations < 30 samples)
Antarctic prion, *Pachyptila desolata*	Diving petrel, *Pelecanoides sp.*
Blue petrel, *Halobaena caerulea*	Fairy prion, *Pachyptila turtur*
Sooty shearwater, *Ardenna griseus*	Soft-plumaged petrel, *Pterodroma mollis*
White-headed petrel, *Pterodroma lessonii*	Fulmar prion, *Pachyptila crassirostris* – DNA sequences indicative, but inconclusive.
	Grey petrel, *Procellaria cinerea* (winter breeder)

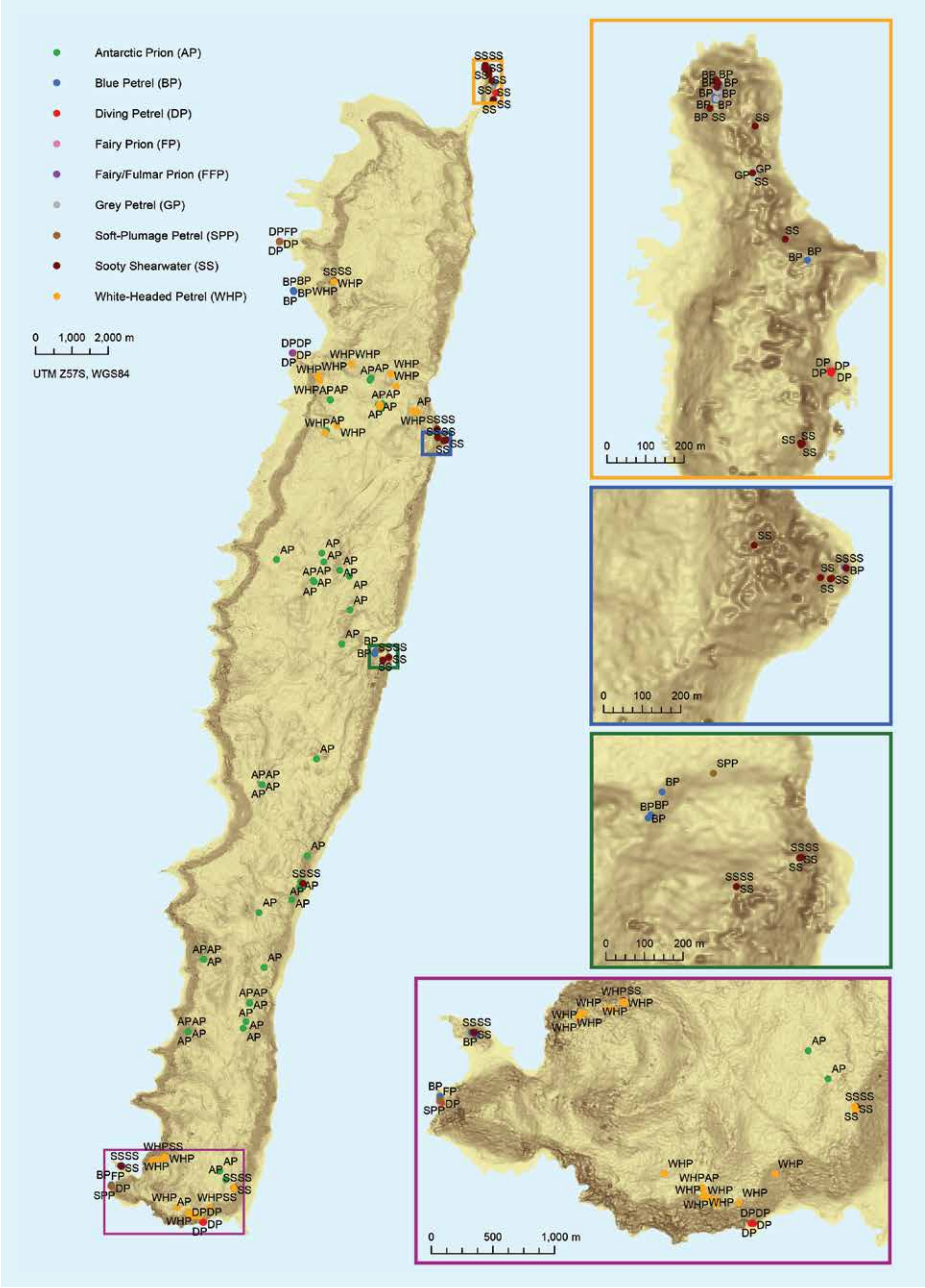

Figure 2. Sampling locations on Macquarie Island in 2017–18 where burrowing petrel species identification was confirmed using DNA from scats and feathers. NB: we couldn't distinguish between fulmar and fairy prion DNA in two samples.

Acknowledgements

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DNA (or fulmar prion) from three locations. Two rock stacks were "hotspots" of burrowing petrel activity, with at least four burrowing petrel species detected on each. One of these rock stacks likely provided a refuge to burrowing petrel species while rats, mice and rabbits existed on Macquarie Island, as it is not connected to the main island. However, the presence of four petrel species on a second connected rock stack, which was previously accessible to invasive vertebrates, is encouraging and provides the first evidence in recent years of fairy prions on the main island.

As well as identifying the various species of burrowing petrel, this study highlighted potential genetic differences between individuals found in Macquarie island populations and specimens previously collected from individuals of the same species of bird on other sub-Antarctic islands.

Finally, while DNA sampling indicates the presence of species rather than confirming their breeding activity, the technique is a valuable tool that complements other methods of assessing the presence of burrowing petrels. This work demonstrates the value of DNA methods in confirming species' identity when the birds cannot be otherwise identified, as well as being a pilot study that identifies species' presence and provides key locations for follow-up census work. The samples also provide high-quality DNA for further reference, and may offer scope for future studies that use other markers to look at the population genetics of burrowing petrels on Macquarie Island and other sub-Antarctic islands.

Implications

The research project provides vital information for post-eradication monitoring and post-eradication fauna management on Macquarie Island. It has confirmed the presence of seven burrowing petrel species and one genus known to breed on the island, including those that had been lost from the island prior to invasive animal eradications.

The project tested and confirmed the efficacy of current survey techniques for threatened burrowing seabirds on the island, and the value of DNA methods using both scats and feathers in assessing the diversity of species and their distribution. The results indicate the value of incorporating the DNA methods developed into future studies of burrowing petrels to complement ground surveys.

Further work with a range of genetic markers may help resolve any species uncertainty (e.g., diving petrels), as well as population genetics (e.g., white-headed petrels).

The DNA methods and markers developed in this project are highly relevant for surveys of cryptic species on other islands in Australia and globally that are remotely located, such as Heard Island, and where field trips are consequently infrequent and/or where resources, logistics and access limited.

Our findings can also be used to help inform future eradication projects on other islands, where managers, funders and researchers are required to anticipate ecosystem responses to eradication.

Grey petrels leave feathers and scats outside their burrows that can be used to identify the occupants. Image: Jez Bird

Further reading

McInnes, J., & Shaw, J. et al. Detecting cryptic species: Using eDNA to track threatened burrowing petrels following an island eradication. In prep.

Further Information

Justine Shaw – j.shaw6@uq.edu.au

Threatened Species Recovery Hub

National Environmental Science Programme

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