Diet breadth of a Critically Endangered owl presents challenges for management.

V. Florence Sperring[1*], Andrew R. Weeks[2,3], William Webster[1,3], Nicholas A. Macgregor[4,5], Melinda Wilson[4], Bronwyn Isaac[6], and Rohan H. Clarke[1]

[1] *School of Biological Sciences, Monash University, Victoria, Australia, 3800*
[2] *School of BioSciences, The University of Melbourne, Victoria, Australia, 3010*
[3] *EnviroDNA Pty Ltd, Brunswick, Victoria, Australia, 3056*
[4] *Parks Australia, Canberra, Australia*
[5] *Durrell Institute of Conservation and Ecology (DICE), School of Anthropology and Conservation, University of Kent, Canterbury, Kent CT2 7NR, UK*
[6] *Faculty of Science, Monash University, Victoria, Australia, 3800*

*Victoria Florence Sperring: 0432 272 188. Victoria.Sperring@monash.edu. 0000-0003-2952-971X. X handle @FlossySperring
Andrew R Weeks: 03 83442 5225. aweeks@unimelb.edu.au. 0000-0003-3081-135X.
William Webster: 0498 375 939. willwebstermyc@gmail.com
Nicholas A Macgregor: 0418 466 579. Nicholas.Macgregor@awe.gov.au. 0000-0002-7995-0230
Melinda Wilson: +6723 51491. Melinda.Wilson@awe.gov.au.
Bronwyn Isaac: 0403 386 208. Bronwyn.Isaac@monash.edu. 0000-0001-9668-5806
Rohan H Clarke: 0408 947 001. Rohan.Clarke@monash.edu. 0000-0002-6179-8402. X handle @rohanclarke01

IN REVIEW

Diet breadth of a Critically Endangered owl presents challenges for management.

Trophic interactions between threatened species complicate management. Similarly, interactions between threatened species and pest species present management challenges, given that pest control can lead to non-target impacts (e.g., trophic cascades or secondary poisoning). There are records of the Critically Endangered Norfolk Island Morepork *Ninox novaeseelandiae undulata* consuming both threatened songbirds and invasive rodents that are subject to management interventions. Despite this, the frequency at which vertebrate prey are consumed, and the dietary breadth of the morepork, remain largely unknown. We visually screened regurgitated pellets using a microscope, alongside environmental DNA (eDNA) screening of pellets and scats, to investigate the diet of the Norfolk Island Morepork. Eighty-nine pellets were collected from six owls between October 2020 and January 2021. Twenty-four pellets and 19 scats were collected from five owls between May and June 2021. All moreporks screened with eDNA metabarcoding had consumed invasive rodents. Moreporks were also found to have consumed four of five endemic songbirds and an endemic parrot, most of which are threatened. Environmental DNA metabarcoding detected more taxa overall, but visual screening identified a greater richness of Orthoptera and Coleoptera in the diet of the morepork. The frequency with which the Norfolk Island Morepork consumed rodents presents a conundrum for conservation managers. Control of invasive rodents is considered essential to support threatened songbirds, yet this same action places the Norfolk Island Morepork at risk of secondary poisoning. Urgent investigations are needed to identify effective control methods for invasive rodents that are safe for non-target species.

Key words: Anticoagulant rodenticide, secondary poisoning, eDNA, Norfolk Island Morepork, diet, conservation management.

IN REVIEW

Introduction

Understanding trophic interactions can be fundamental to species conservation and ecosystem management (Soulé, et al. 2003; Sousa et al. 2019). At a time of biodiversity crisis, as the number of threatened species increases, trophic interactions that include species of conservation concern present increasing challenges for managers (Roemer and Wayne 2003; Canale and Bernardo 2016). Island systems with a globally disproportionate number of threatened species, alongside simplified food webs and limited dietary redundancy, exemplify these challenges (Whittaker and Fernández-Palacios 2007).

The presence of invasive rodents, especially on islands, is a leading cause of biodiversity loss, and adds further to the complexity of trophic interactions (Howald, et al. 2007). Invasive rodents are typically widespread dietary generalists and have been implicated in the decline and extinction of many plant and animal species (St Clair 2011; Doherty et al. 2016, Russell et al. 2017). Programs to control or eradicate rodents are therefore common practice (Keitt et al. 2011). When successful, these rodent management programs can have substantial benefits to species and ecosystem recovery (Croll et al. 2016; Jones et al. 2016).

The management of invasive rodents routinely involves the use of anticoagulant rodenticides (Fisher et al. 2019). This approach has often been very successful (Howald et al. 2007; Wheeler et al. 2019). However, rodenticides have by necessity become increasingly toxic with longer latency periods, to maintain their effectiveness in what has become an arms race between managers and invasive rodents (Hadler and Buckle 1992; Marquez et al. 2019). Reflecting this, anticoagulant rodenticides are now characterised as either first or second-generation toxins. Both forms prevent blood clotting and cause vertebrate death through

IN REVIEW

haemorrhaging (Park et al. 1984). Second-generation baits are however 100-1000 times more toxic and have longer biological half-lives than first-generation baits (Huckle et al. 1988). Consequently, the toxins from second-generation baits remain in animal tissue for longer and are less likely to be entirely metabolised by rodents before death (Huckle et al. 1988; Erickson and Urban 2004). When a rodent that has ingested anticoagulant bait is consumed by a predator or scavenger, any secondary poisoning that occurs invariably has physiological consequences and is often fatal (Lohr and Davis 2018). Because of the markedly higher toxicity of second-generation baits and their extended environmental latency, the use of these baits increases both the prevalence of secondary poisoning and the mortality rate in non-target wildlife, compared to the effects of first-generation baits (Erickson and Urban 2004; Van den Brink et al. 2018).

Birds of prey are particularly susceptible to secondary poisoning since rodents and other small mammals often comprise a substantial proportion of their diet. Consequently, secondary poisoning, especially where second generation baits are involved, is a key threatening process for a number of raptor species (Nakayama et al. 2019; Cooke et al. 2023). In settings where secondary poisoning is known or suspected to be occurring, a comprehensive assessment of diet can be informative for conservation managers. The traditional method to quantify the diet of birds of prey is to undertake a visual analysis of regurgitated prey remains (pellets) (Maser and Brodie 1966; Cooke et al. 2006). With the advent of environmental DNA (eDNA) techniques, traces of DNA sourced from the environment rather than directly from focal species can now also be used to detect the presence of organisms under a range of settings, including dietary screening (Cavallo et al. 2018; Quasim et al. 2018; Menning et al. 2023). eDNA techniques are

IN REVIEW

especially well suited to establishing the diet composition of birds of prey, given they pass both pellets and faeces, and frequently do so at established perching or roosting sites (Driver 1949).

The Norfolk Island Morepork *Ninox novaeseelandiae undulata* is a Critically Endangered owl restricted to Norfolk Island, with a population estimated at 25-35 individuals (Threatened Species Scientific Committee 2016; F Sperring, unpubl. data). The known diet of the Norfolk Island Morepork is based on a single pellet, prey remains from one nest-box, and incidental observations (Olsen 1997). These data suggest invertebrates are a prominent prey type, though vertebrates probably predominate in terms of prey biomass (Olsen 1996). The White Tern *Gygis alba,* at least two threatened songbirds (Norfolk Robin *Petroica multicolor* and Slender-billed White-eye *Zosterops tenuirostris),* and the introduced Polynesian Rat *Rattus exulans* have been documented as vertebrate prey (Olsen 1996). The diet of the Norfolk Island Morepork is otherwise poorly quantified, and conservation managers on Norfolk Island must largely infer dietary composition and dietary breadth by reference to the diets of the closely related New Zealand Morepork *N. n. novaeseelandiae* and (larger) Australian Boobook *N. boobook*. Both species have a generalist diet comprised largely of invertebrates, with diet composition of populations correlated with prey availability (Haw and Clout 1999; Penck and Queale 2002; Trost et al. 2008; Denny 2009).

On Norfolk Island, the invasive Black Rat *Rattus rattus* and Polynesian Rat are implicated in the decline and extinction of many threatened flora and fauna (Nance et al. 2020; Nance et al. 2023b). Invasive rodents are currently controlled intensively within protected areas to suppress their numbers and reduce direct impacts on biodiversity. Rodent control programs using toxic baits have been maintained since the 1990s, commencing with the use of first-

IN REVIEW

generation baits, and more recently transitioning to the use of second-generation baits. The control of rodents with toxic baits, especially second-generation, may pose a direct threat to the Norfolk Island Morepork population through secondary poisoning. In recognition of this as yet unquantified threat, bait deployment during the Morepork breeding season has recently been modified to use first-generation baits (Nance et al. 2023a, in review). The quantity and toxicity of second-generation baits have also been reduced through the remainder of the year. However, the potential for secondary poisoning to non-target species remains.

Here we aim to quantify the diet of the Norfolk Island Morepork to inform key conservation management actions. We utilise visual analysis of pellets, and eDNA metabarcoding of pellets and scats, to identify prey items and quantify the dietary breadth of the Norfolk Island Morepork. In doing so, we aim to quantify trophic interactions with native prey species that may benefit from rodent control, while also determining the potential threat that this same management action may pose to a Critically Endangered bird of prey.

Methods

Study site

Norfolk Island is a small oceanic island (3460 ha) in the SW Pacific. The island has largely been cleared of native vegetation for human use, with the largest area of remnant forest centred on Norfolk Island National Park (Director of National Parks 2020). Black Rats and Polynesian Rats can be abundant across the island, while House Mice tend to be more abundant within the human-modified landscape (Nance et al. 2023a, in review). Despite the relatively small size of the National Park (~460 ha), intact forest communities here support a concentration of threatened

IN REVIEW

134 species including almost all Norfolk Robins, Slender-billed White-eyes, breeding Green Parrots
135 *Cyanoramphus cookii* and Norfolk Island Moreporks (Sperring et al. 2021; Gautschi et al. 2022;
136 Nance et al. 2023b). The island is also host to a wide range of endemic invertebrates, many of
137 which are threatened (Smithers 1998; Director of National Parks 2023).

138 ***Owl capture and pellet collection***

139 Fieldwork was conducted on Norfolk Island from October 2020 to January 2021 (morepork
140 breeding season), and May to June 2021 (non-breeding season). Moreporks were captured using
141 mist-nets at dusk with broadcasts of morepork recordings as a lure. Individuals that exceeded a
142 mass of 100 g were fitted with a Lotek Pinpoint VHF-75 tracker (total mass 3.5 g, 1.8-2.4% of
143 total body mass consistent with ethical recommendations for logger deployment; Caccamise and
144 Hein 1985). Trackers were attached to the dorsal surface of the two central tail feathers with
145 Tesa® tape and pre-programed to emit a VHF beacon during daylight hours. In instances where
146 recapture was not possible, trackers were subsequently shed during tail moult.

147 Daytime roosts for each morepork were located using a handheld Yagi antenna and Lotek
148 Biotracker receiver unit over a period of 2-8 weeks. Cotton sheets of a neutral colour (~1.5 m x
149 1.5 m) were affixed below identified roost sites, 1-2 m off the ground. Sheets were checked
150 every one to five days and all pellets and scats were collected. Samples were stored in 70%
151 ethanol in sterile vials and held at room temperature. [Figure 1 near here]

152 Moreporks were considered to be in a pair if two individuals were using the same roost
153 during the tracking period. Where a pellet was collected in these settings, it was not possible to
154 determine from which member of the pair the sample originated, and thus paired owls were

IN REVIEW

155 treated as one sample source. Individual morepork location was classified as ‘National Park’ or
156 ‘modified landscape’ depending on the location of their roosts and territory.

157 ***Visual analysis***

158 All pellets and a small reference collection of arthropods were examined using sterile single-use
159 petri dishes under a dissecting microscope in the laboratory. Invertebrate prey remains were first
160 identified to order and vertebrate remains were identified to class where possible. Unique taxa
161 from each invertebrate order were either identified to species level or recorded with a unique
162 identifier. Identification was aided by the reference collection and the assistance of experts from
163 relevant taxonomic fields. The abundance of each prey was assessed by counting the minimum
164 possible number of individuals represented by identifiable remains.

165 ***eDNA analysis***

166 DNA was extracted from scats and pellets collected from May – June 2021 using the Qiagen
167 PowerSoil Pro DNA extraction kit (Qiagen, Clayton, Australia). All samples were collected with
168 the intention of metabarcoding; however, spring samples were lost by a third party prior to DNA
169 extraction.

170 *Metabarcoding*

171 Universal vertebrate and invertebrate assays targeting part of the 12S mitochondrial DNA gene
172 region were used to characterise vertebrates and invertebrates in the scat and pellet samples. We
173 used a two-step PCR library construction method (see McColl-Gausden et al. 2021). For
174 vertebrate analysis, the first round of PCR employed gene-specific primers (vertebrate 12S; Riaz

IN REVIEW

et al. 2011) to amplify the target region. For invertebrate analysis, the first round of PCR employed gene-specific COI (mitochondrial) primers (Zeale et al. 2011). The second round incorporated sequencing adapters and unique barcodes for each sample-amplicon combination included in the library. Negative control samples were also included during library construction. Negative controls consisted of the extraction negative as well as PCR negatives, where nuclease-free water was used in place of DNA during both rounds of PCR. Sequencing was carried out on an Illumina iSeq 100 machine using the iSeq i1 reagent kit v2 (Illumina, San Diego, CA, USA) with paired-end reads.

Vertebrate bioinformatic analysis

Following quality control filtering to remove primer sequences, truncated reads and low-frequency reads, DNA sequences were deduplicated and all unique sequences were retained and assigned a running Operational Taxonomic Unit (OTU) number. Taxonomic assignment was performed with VSEARCH software (Rognes et al. 2016), whereby each OTU cluster was assigned a species identity using a threshold of 95% by comparing against a reference sequence database. Where a species could not be assigned (i.e., the reference database was deficient and/or taxa were poorly characterised), taxonomic assignments were manually vetted by first obtaining a list of possible species through BLASTN searches against the public repository Genbank (www.ncbi.nlm.nih.gov). Species were then discounted on the basis of their geographic distribution using information from the Atlas of Living Australia (ALA). In cases where an OTU could not be adequately resolved to a single species (due to shared haplotypes for instance), it was assigned to a higher taxonomic rank. Simultaneously, in cases where there was the possibility of 'over-assignment' (assignments to species with little data corroborating their

IN REVIEW

197 occurrence in Australia), we also assigned such OTUs to a higher taxonomic level. Examples of
198 such over-assignments can occur when congeners (or confamilials) are present in Australia but
199 there is little to no genetic sequence available for individual Australian species.

200 *Invertebrate bioinformatic analysis*

201 Amplicon pools were first demultiplexed based on the unique barcodes which identified
202 individual samples. Reads R1 and R2 from the paired-end sequencing were merged using the
203 fastq-mergepairs function in VSEARCH (Edgar 2016), retaining only merged reads flanked by
204 matches to the gene-specific COI primers. Following quality control filtering to remove primer
205 sequences, truncated reads and low-frequency reads, DNA sequences were deduplicated and all
206 unique sequences were retained and assigned a running OTU number. Taxonomic assignment
207 was performed with VSEARCH's SINTAX algorithm (Edgar 2016). Each OTU was assigned a
208 taxonomic identity using a threshold of 80% by comparing against the MIDORI2 reference
209 sequence database (Leray et al. 2022), supplemented by BLAST searches. In cases where OTUs
210 could not be adequately assigned to a species, it was assigned to the lowest taxonomic rank
211 possible.

212 *Data filtering*

213 Low level detections that were likely to represent contamination events from a natural source
214 were excluded by removing detections with less than 50 sequencing reads (Dully et al. 2021).
215 Taxa detected with an adult body length <10 mm were also excluded (as possible secondary
216 detections) as moreporks are expected to mostly consume prey larger than this threshold (Denny
217 2009).

IN REVIEW

Data analysis

We compared detections from both analysis methods (eDNA metabarcoding and visual analysis of the same pellets) and sample types (eDNA analysis of scats and pellets) to determine prey taxa richness per owl. Specifically, we investigated taxa richness within avifauna, rodents, and various invertebrate orders. The difference in richness (Δ richness) between analysis methods, and sample types was calculated per owl for each taxonomic group. The minimum, maximum, and average Δ richness was then determined per taxonomic group.

Using visual analysis outputs, Fisher's exact tests were run to compare the proportion of rodents detected in pellets between seasons and habitat. We also ran a Fisher's exact test to compare the proportion of vertebrate and invertebrate detections between eDNA sample types. All analyses were implemented in R and significance was tested at the 0.05 level (R Core Team 2023).

Results

Samples were collected from seven Norfolk Island Moreporks across Norfolk Island, with each bird utilising two or three day-roosts (Fig. 1). Two owls occurred as a pair and two owls occupied territories entirely outside of the National Park. A total of 24 regurgitated pellets (2-7 per owl) and 19 scats (2-6 per owl) were collected in autumn from five owls. Eighty-nine pellets (5-25 per owl) were collected in spring from six owls.

Vertebrate consumption

Using eDNA metabarcoding, prey taxa richness for each owl was between two and five vertebrate taxa after pooling all available pellets and scats for each owl (Table 1). All Norfolk

IN REVIEW

239 Island Moreporks were found to have consumed rodents over sampling periods that spanned just
240 five to 39 days. Rodents were also detected in 44% of all samples, with a more refined
241 identification to *Rattus sp.* detected in 32% of samples. House Mice were consumed by two
242 owls. The proportion of pellets that contained rodents did not differ between season or habitat
243 ($\chi^2(1, 113) = 0.016, p = 0.90$ and $\chi^2(1, 113) < 0.001, p = 1$ respectively; visually screened pellets
244 only). [Table 1 near here]

245 The eDNA metabarcoding analysis detected Green Parrot in the pellets of one owl and
246 *Zosterops sp.* (either the Silvereye *Z. lateralis* or the Near Threatened Slender-billed White-eye)
247 was detected as prey for four of five moreporks, and in 28% of all samples screened. Visual
248 analysis was unable to identify bird taxa below avian order that had been consumed as prey. The
249 contents of a nest box that supported a breeding pair of moreporks in 2019 included bird bands
250 (issued by the Australian Bird and Bat Banding Scheme) that had been placed on a Slender-
251 billed White-eye and a Norfolk Robin, indicating both species had been brought to the nest box
252 as prey during that period.

253 ***Invertebrate consumption***

254 Using all samples screened with metabarcoding, invertebrate prey richness was between three
255 and seven taxa per owl (Table 1). Araneae (spiders) were consumed by all owls. All owls also
256 consumed at least two of seven Lepidoptera taxa (moths and butterflies) taken as prey.
257 Stylommatophora (land snails and slugs) were detected in three samples from one owl occupying
258 territory entirely within the National Park. Visual analysis of pellets identified Coleoptera
259 (beetles), Orthoptera (grasshoppers and crickets) and Lepidoptera in the diet of moreporks (Table

IN REVIEW

2). Coleoptera were found in 75% of pellets in autumn and 84% of pellets in spring while Orthoptera were found in 92% of pellets in autumn and 62% in spring. [Table 2 near here]

Comparison of methods

Metabarcoding of pellets and scats identified 25 taxa in the diet of moreporks in the single season that was screened using this technique. By contrast, visual screening of pellets identified just nine taxa across combined breeding and non-breeding seasons. Where the same pellet was screened using both methods, each vertebrate taxa that was identified visually was also detected using eDNA metabarcoding. Metabarcoding detected more species of bird that were consumed as prey and visual analysis detected more species/taxa of Orthoptera and Coleoptera that were consumed as prey (Figure 2a). [Figure 2 near here].

Richness for each taxonomic group and total taxa richness was similar for analyses of pellets and scats (pellets = 19 taxa (n = 15), scats = 17 (n = 19)) (Figure 2b). At a finer taxonomic resolution, the screening of pellets detected significantly more vertebrate taxa, while the screening of scats detected significantly more invertebrate taxa ($p = 0.048$) (vertebrate richness: scats = 4, pellets = 11. Invertebrate richness: scats = 13, pellets = 8).

Discussion

Here, we quantify the prey frequency and dietary breadth of the Critically Endangered Norfolk Island Morepork. Of particular note, molecular screening of pellets and scats revealed that all moreporks consumed invasive rodents. In the presence of an ongoing rodent control program, these results highlight a conundrum for conservation managers at this site. The control of invasive rodents is considered essential for the recovery of threatened species on Norfolk Island,

IN REVIEW

yet this same management program poses a genuine threat through secondary poisoning to the tiny remaining Norfolk Island Morepork population.

The rodent control conundrum

Almost half of all dietary sampling events collected during the non-breeding period and screened using metabarcoding contained rodents (44%), and there was no significant difference in the proportion of rodent-positive samples between seasons or habitat using visual analysis. Rodents are well known in the diet of congeneric small hawk-owls that occur in both Australia and New Zealand; however, the frequency of consumption often varies between populations and time of year (Haw et al. 2001; Penck and Queale 2002; Denny 2009). Elsewhere, consumption of rodents has been posited to be largely influenced by two factors: resource requirements for breeding, and prey abundance (Olsen 2012). Invertebrate abundance is often lower in winter, which is thought to cause an increase in the uptake of rodent consumption (Stephenson 1998; Trost et al. 2008). However, during spring, the elevated energetic requirements associated with breeding can also lead to an increase in the consumption of vertebrates (Olsen 2012). Here we suggest the consistent prevalence of rodents in the diet of the Norfolk Island Morepork may best be explained by the documented high abundance and year-round and island-wide availability of rodents (Nance et al. 2023a, in review).

Given the frequent consumption of rodents, and the use of second-generation rodenticides across Norfolk Island, secondary poisoning can be inferred to pose a genuine risk to the Norfolk Island Morepork. There is a growing body of research suggesting that secondary impacts of rodenticides are greater than previously thought, and elsewhere the incidence of secondary poisoning for boobooks and moreporks that consume rodents is well documented (Stephenson et

IN REVIEW

al. 1999; Lohr 2018l; Cooke et al. 2023). Locally, the only known Norfolk Island Morepork chick that hatched between 2011 and 2019 died due to suspected secondary poisoning in the nest box, and an adult with symptoms of secondary poisoning (possibly Alphachloralose poisoning associated with feral chicken control; F Sperring, unpubl. data) was rehabilitated in 2021. Other possible consequences of secondary poisoning could include reduced hatching success as a result of reduced egg size and shell thickness (Porter and Wiemeyer 1969; Fry and Toone 1981). For the protection of Norfolk Island Moreporks, further exploration of owl-safe rodent control methods should be considered a priority action.

While Norfolk Island Moreporks will likely benefit from a reduction in the use of second-generation rodenticides, these same toxins are currently considered essential to suppress rodent populations and reduce nest predation of threatened songbirds (Nance et al. 2023b). Invasive rodents on Norfolk Island are implicated in the extinction of many bird species and, are responsible for the majority of nest failures in the endemic songbirds that persist (Nance et al. 2020; Nance et al. 2023b). Nest survival of threatened songbirds is higher in areas with more intensive baiting, emphasizing the importance of the current control methods (Nance et al. 2023b). Elsewhere, second-generation baits have proven capacity to control and eradicate rodents, with positive outcomes for threatened species that rodents prey on (Hadler and Buckle 1992; Gillespie and Bennett 2017). While other rodent control methods are evolving, there remains limited evidence that rodents can be suppressed to low levels without the use of second-generation baits (Howald et al. 2007; Gronwald and Russell 2022; DIISE 2023). Thus, the dependence of Norfolk Island songbirds on a program that utilises second-generation toxins may continue for some time.

IN REVIEW

Given the breadth of the threat that invasive rodents pose to biota on Norfolk Island, their management must remain an ongoing priority. Therefore, effective rodent control strategies that simultaneously minimise the risk to moreporks warrant urgent exploration. Firstly, a non-toxic control measure currently being trialled on Norfolk Island is the use of self-resetting kill traps (e.g., Goodnature A24 or AT220 traps). While this technique is not uniformly successful, these traps have had some success in areas with large rodent populations (Peters et al. 2014; Gronwald and Russell 2022). Secondly, olfactory misinformation may also be used to manipulate rodent behaviour to disregard unrewarding cues, such as the smell of songbird nests, thereby minimising the predation risk (Price and Banks 2012). Finally, a promising toxicant under investigation is cholecalciferol (vitamin D3), which raises blood calcium levels causing death through heart failure (Hix et al. 2012). This toxin has proven to be effective for rodent control and shows promising results for a reduced risk of secondary poisoning (Eason et al. 2000; Noh et al. 2023). Each of these approaches have the potential to reduce the impact of rodents, while minimising the risk to non-target species. However further research and development is required.

There are also some more ambitious strategies that may be worthy of consideration, notwithstanding considerable logistical, financial, and social challenges. These include the development of a predator-free enclosure encompassing the National Park and surrounding forested areas, or an island-wide rodent eradication (Howald et al. 2007). Future techniques such as gene drive technology may also provide a more species-specific, toxin-free eradication option (Leitschuh et al. 2017; Prowse et al. 2017). However, projected timelines to success might extend across multiple decades and consequently render this approach inviable. Finally, more intensive restoration efforts on nearby Phillip Island, which is rodent free but largely devoid of

IN REVIEW

347 forested areas, may present an alternative pathway, or a complementary strategy, to increase the
348 resilience of both morepork and songbird populations (Director of National Parks 2020).

349 Norfolk Island exemplifies the importance of an integrated, ecosystem-wide approach for
350 threatened species management. We provide evidence that the Norfolk Island Morepork
351 consumes four of five endemic songbirds (two of which are threatened), as well as the
352 Endangered Green Parrot. In a scenario where rodent abundance has been substantially reduced,
353 a dietary shift of Norfolk Island Moreporks to other more abundant species is likely (Denny
354 2009). The effective management of anthropogenic threats to endemic birds, namely of invasive
355 mammals and habitat loss, should ensure that these same populations are robust to natural
356 predation by moreporks (Salo et al. 2007; Gautschi et al. 2022). Additional goals for ecological
357 restoration might include the return of nocturnally active reptile species and a species of giant
358 centipede from Phillip Island to Norfolk Island (though this may be contingent on extensive
359 rodent control or rodent eradication). The reptile species, and probably the giant centipede,
360 appear to have been extirpated from the main island as a result of predation by invasive rodents,
361 and were likely to have featured prominently in the diet of the Norfolk Island Morepork prior to
362 human settlement (Director of National Parks 2020; Olsen 2012). The incremental loss of
363 invertebrates and small vertebrates from Norfolk Island, which is largely attributable to the
364 arrival of invasive rodents, highlights a history of dietary simplification and prey switching for
365 the Norfolk Island Morepork. Effective threatened species management relies on an ecosystem-
366 wide approach with an understanding of trophic interactions.

IN REVIEW

Methods comparison

Here we further validate the effectiveness of eDNA metabarcoding of regurgitated pellets to identify prey (Benamane et al. 2019; Miller-Brown 2022). While total taxa richness was similar between pellets and scats, scat analysis detected significantly more invertebrate taxa and pellet analysis detected more vertebrate taxa. Feathers, bones, and fur are indigestible and must be regurgitated (Smith and Richmond 1972), whereas soft bodied invertebrates are likely to pass through the digestive system (Hill and Lill 1998). Consequently, taxon-specific digestive processes may influence the residual eDNA in different sample types.

Relative to visual analysis, eDNA identified three times as many prey taxa, with particular improvements to identification of soft-bodied prey. For example, Stylommatophora, Diptera, and Araneae were detected only through metabarcoding. Stylommatophora (land snails and slugs), are particularly interesting as putative prey of the Norfolk Island Morepork as these have not previously been identified in the diet of moreporks or boobooks. Our results are consistent with previous studies highlighting the value of eDNA analysis for a more comprehensive and refined assessment of dietary composition and breadth, relative to traditional methods (Sousa et al. 2019; Hoenig et al. 2022).

Visual analysis identified more Coleoptera and Orthoptera taxa than metabarcoding. This may be explained by incompleteness of the reference sequence database (Beng and Corlett 2020) which did not contain many of the invertebrate species' endemic to Norfolk Island. Since reference sequences are often available only for a few genes for most species, targeted marker regions cannot always accurately resolve all groups, even to higher taxonomic levels (Liu et al. 2017; Beng and Corlett 2020). A more complete sequence database would likely improve

IN REVIEW

identification of these orders. In settings where trophic relationships may directly inform management considerations, we highlight the value of applying multiple screening strategies to investigate species diet.

Conclusion

During both the breeding and non-breeding season, Norfolk Island Moreporks consumed invasive rodents that were subject to intensive control measures using toxic baits. This clearly places a Critically Endangered owl at risk of secondary poisoning, with some anecdotal evidence that this is indeed occurring. While first-generation baits may provide a safer alternative for the Norfolk Island Morepork, available evidence indicates that such baits are less capable of supressing rodent numbers. The conundrum for managers is that the current rodent baiting program on Norfolk Island is known to improve nesting success of endemic songbirds. This management challenge therefore requires a considered response to prevent the loss of songbirds, while minimising the threat of secondary poisoning to moreporks. We recommend managers urgently explore novel approaches and innovative technologies to control rodents effectively and safely. Our study highlights the interconnectedness of systems through food webs and that a targeted understanding of trophic interactions can be essential to inform effective whole-of-system conservation actions.

IN REVIEW

Geolocation information

This work was carried out on Norfolk Island -29.030116, 167.953828.

Acknowledgements

We pay our respect to the Norf'k Ailen Kaunsl' Eldas and extend our recognition to all Pitkern descendants who call Norf'k home. We acknowledge the contribution of Parks Australia and Norfolk Island National Park, Norfolk Island Regional Council, and the Norfolk Island community for their cooperation and assistance during this research. Particular thanks go to Nigel Greenup, Margaret Christian, Mark Hallam and Jolene Oliver. Rohan Bilney and the CSIRO Insect Collections team provided invaluable assistance with visual taxa identification. We thank Anthony van Rooyen for eDNA metabarcoding. Penny Olsen and Allie Nance provided valuable feedback on the manuscript. This research was funded through the National Environmental Science Program and by Director of National Parks. All research was approved by Parks Australia and conducted under Monash University Animal Ethics approval 2021-25691. FS was supported by an Australian Government Research Training Program Scholarship. The authors report there are no competing interests to declare.

IN REVIEW

References

Benamane, A. H., Bissati-Bouafia, S., Amrani, K., Pansu, J., Pompanon, F., Doumandji, S., et al. (2019). Diet of Barn Owl (*Tyto alba*) determination from regurgitated pellets in southeastern Algeria, coupling the classical approach with the eDNA analysis. *International Journal of Sciences and Research* **75**, 61-78. doi:10.21506/j.ponte.2019.2.5

Beng, K. C. and Corlett, R. T. (2020). Applications of environmental DNA (eDNA) in ecology and conservation: opportunities, challenges and prospects. *Biodiversity and Conservation* **29**, 2089-2121. doi:10.1007/s10531-020-01980-0

Caccamise, D. F. and Hedin, R. S. (1985). An aerodynamic basis for selecting transmitter loads in birds. *The Wilson Bulletin* **97**, 306-318.

Canale, G. R. and Bernardo, C. S. S. (2016). Predator-prey interaction between two threatened species in a Brazilian hotspot. *Biota Neotropica* **16**, e0059. doi:10.1590/1676-0611-bn-2015-0059

Cavallo, C., Chiaradia, A., Deagle, B. E., McInnes, J. C., Sánchez, S., Hays, G. C., et al. (2018). Molecular analysis of predator scats reveals role of salps in temperate inshore food webs. *Frontiers in Marine Science* **5**, 381. doi:10.3389/fmars.2018.00381

Cooke, R., Wallis, R., Hogan, F., White, J. and Webster, A. (2006). The diet of Powerful Owls (*Ninox strenua*) and prey availability in a continuum of habitats from disturbed urban fringe to protected forest environments in south-eastern Australia. *Wildlife Research* **33**, 199-206. doi:10.1071/WR05058

IN REVIEW

441 Cooke, R., Whiteley, P., Death, C., Weston, M. A., Carter, N., Scammell, K., et al. (2023). Silent
442 killers? The widespread exposure of predatory nocturnal birds to anticoagulant rodenticides.
443 *Science of the Total Environment* **904**, 166293. doi:10.1016/j.scitotenv.2023.16629

444 Croll, D. A., Newton, K. M., McKown, M., Holmes, N., Williams, J. C., Young, H. S., et al.
445 (2016). Passive recovery of an island bird community after rodent eradication. *Biological*
446 *Invasions* **18**, 703-715. doi:10.1007/s10530-015-1042-9

447 Denny, K. M. (2009). The diet of moreporks (*Ninox novaeseelandiae*) in relation to prey
448 availability, and their roost site characteristics and breeding success on Ponui Island, Hauraki
449 Gulf, New Zealand. Masters Thesis, Massey University, New Zealand.

450 DIISE. (2023). The Database of Island Invasive Species Eradications. Available at
451 http://diise.islandconservation.org/. [Accessed July 2023].

452 Director of National Parks (2020). 'Norfolk Island National Park and Norfolk Island Botanical
453 Garden Management Plan 2020.' Director of National Parks, Canberra.

454 Director of National Parks (2023). 'Norfolk Island Region Threatened Species Recovery Plan.'
455 Director of National Parks, Canberra. In draft.

456 Doherty, T. S., Glen, A. S., Nimmo, D. G., Ritchie, E. G. and Dickman, C. R. (2016). Invasive
457 predators and global biodiversity loss. *Proceedings of the National Academy of Sciences* **113**,
458 11261-11265. doi:10.1073/pnas.1602480113

459 Driver, E. C. (1949). Mammal remains in owl pellets. *The American Midland Naturalist* **41**, 139-
460 142.

IN REVIEW

461 Dully, V., Wilding, T. A., Mühlhaus, T. and Stoeck, T. (2021). Identifying the minimum
462 amplicon sequence depth to adequately predict classes in eDNA-based marine biomonitoring
463 using supervised machine learning. *Computational and Structural Biotechnology Journal* **19**,
464 2256-2268. doi:10.1016/j.csbj.2021.04.005

465 Eason, C. T., Wickstrom, M., Henderson, R., Milne, L. and Arthur, D. (2000). Nontarget and
466 secondary poisoning risks associated with cholecalciferol. *New Zealand Plant Protection* **53**,
467 299-304. doi:10.30843/nzpp.2000.53.3699

468 Edgar, R. C. (2016). SINTAX: a simple non-Bayesian taxonomy classifier for 16S and ITS
469 sequences. *biorxiv*, 074161. doi:10.1101/074161

470 Erickson, W. and Urban, D. (2004). Potential risks of nine rodenticides to birds and nontarget
471 mammals: a comparative approach. US Environmental Protection Agency, Office of Prevention,
472 Pesticides and Toxic Substances, Washington.

473 Fisher, P., Campbell, K. J., Howald, G. R. and Warburton, B. (2019). Anticoagulant
474 rodenticides, islands and animal welfare accountancy. *Animals* **9**, 919. doi:10.3390/ani9110919

475 Fry, D. M. and Toone, C. K. (1981). DDT-induced feminization of gull embryos. *Science* **213**,
476 922-924. doi:10.1126/science.7256288

477 Gautschi, D., Heinsohn, R., Crates, R., Macgregor, N. A., Wilson, M. and Stojanovic, D. (2022).
478 Utilization of modified and artificial nests by endemic and introduced parrots on Norfolk Island.
479 *Restoration Ecology* **30**, e13586. doi:10.1111/rec.13586

IN REVIEW

480 Gillespie, R. and Bennett, J. (2017). Costs and benefits of rodent eradication on Lord Howe
481 Island, Australia. *Ecological Economics* **140**, 215-224. doi:10.1016/j.ecolecon.2017.05.007

482 Gronwald, M. and Russell, J. (2022). Behaviour of invasive ship rats, *Rattus rattus*, around
483 Goodnature A24 self-resetting traps. *Management of Biological Invasions* **13**, 479-493.
484 doi:10.3391/mbi.2022.13.3.02

485 Hadler, M. R. and Buckle, A. P. (1992). Forty-five years of anticoagulant rodenticides--past,
486 present and future trends. Paper presented at the Proceedings of the Vertebrate Pest Conference.

487 Haw, J. and Clout, M. (1999). Diet of morepork (*Ninox novaeseelandiae*) throughout New
488 Zealand by analysis of stomach contents. *Notornis* **46**, 333-345.

489 Haw, J., Clout, M. and Powlesland, R. (2001). Diet of moreporks (*Ninox novaeseelandiae*) in
490 Pureora Forest determined from prey remains in regurgitated pellets. *New Zealand Journal of*
491 *Ecology* **25**, 61-67.

492 Hill, F. R. and Lill, A. (1998). Diet and roost site characteristics of the Christmas Island Hawk-
493 Owl *Ninox natalis*. *Emu* **98**, 227-233. doi:10.1071/MU98031

494 Hix, S., Aylett, P., Shapiro, L., MacMorran, D., Eason, C., Sam, S., et al. (2012). Low-dose
495 cholecalciferol bait for possum and rodent control. *New Zealand Journal of Agricultural*
496 *Research* **55**, 207-215. doi:10.1080/00288233.2012.665806

497 Hoenig, B. D., Snider, A. M., Forsman, A. M., Hobson, K. A., Latta, S. C., Miller, E. T., et al.
498 (2022). Current methods and future directions in avian diet analysis. *The Auk* **139**, 1-28.
499 doi:10.1093/ornithology/ukab077

IN REVIEW

Howald, G., Donlan, C. J., Galvan, J. P., Russell, J. C., Parkes, J., Samaniego, A., et al. (2007). Invasive rodent eradication on islands. *Conservation Biology* **21**, 1258-1268. doi:10.1111/j.1523-1739.2007.00755.x

Huckle, K. R., Hutson, D. H. and Warburton, P. A. (1988). Elimination and accumulation of the rodenticide flocoumafen in rats following repeated oral administration. *Xenobiotica* **18**, 1465-1479. doi:10.3109/00498258809042269

Jones, H. P., Holmes, N. D., Butchart, S. H., Tershy, B. R., Kappes, P. J., Corkery, I., et al. (2016). Invasive mammal eradication on islands results in substantial conservation gains. *Proceedings of the National Academy of Sciences* **113**, 4033-4038. doi:10.1073/pnas.1521179113

Keitt, B., Campbell, K., Saunders, A., Clout, M., Wang, Y., Heinz, R., et al. (2011). The global islands invasive vertebrate eradication database: a tool to improve and facilitate restoration of island ecosystems. Paper presented at the Island invasives: eradication and management, IUCN, Gland, Switzerland.

Leitschuh, C. M., Kanavy, D., Backus, G. A., Valdez, R. X., Serr, M., Pitts, E. A., et al. (2017). Developing gene drive technologies to eradicate invasive rodents from islands. *Journal of Responsible Innovation* **5**, S121-S138. doi:10.1080/23299460.2017.1365232

Leray, M., Knowlton, N. and Machida, R. J. (2022). MIDORI2: A collection of quality controlled, preformatted, and regularly updated reference databases for taxonomic assignment of eukaryotic mitochondrial sequences. *Environmental DNA* **4**, 894-907. doi:10.1002/edn3.303

IN REVIEW

Liu, S., Yang, C., Zhou, C. and Zhou, X. (2017). Filling reference gaps via assembling DNA barcodes using high-throughput sequencing—moving toward barcoding the world. *GigaScience* **6**, gix104. doi:10.1093/gigascience/gix104

Lohr, M. T. (2018). Anticoagulant rodenticide exposure in an Australian predatory bird increases with proximity to developed habitat. *Science of the Total Environment* **643**, 134-144. doi:10.1016/j.scitotenv.2018.06.207

Lohr, M. T. and Davis, R. A. (2018). Anticoagulant rodenticide use, non-target impacts and regulation: A case study from Australia. *Science of the Total Environment* **634**, 1372-1384. doi:10.1016/j.scitotenv.2018.04.069

Marquez, A., Khalil, R. A., Fourel, I., Ovarbury, T., Pinot, A., Rosine, A., et al. (2019). Resistance to anticoagulant rodenticides in Martinique could lead to inefficient rodent control in a context of endemic leptospirosis. *Scientific Reports* **9**, 13491. doi:10.1038/s41598-019-49661-5

Maser, C. and Brodie, E. D. (1966). A study of owl pellet contents from Linn, Benton and Polk counties, Oregon. *The Murrelet* **47**, 9-14. doi:10.2307/3536232

McColl-Gausden, E. F., Weeks, A. R., Coleman, R. A., Robinson, K. L., Song, S., Raadik, T. A., et al. (2021). Multispecies models reveal that eDNA metabarcoding is more sensitive than backpack electrofishing for conducting fish surveys in freshwater streams. *Molecular Ecology* **30**, 3111-3126. doi:10.1111/mec.15644

IN REVIEW

538 Menning, D. M., Uher-Koch, B. D., Flamme, M. J., Simmons, T., Schmutz, J. A. and Talbot, S.
539 L. (2023). eDNA Metabarcoding analyses of diet in Yellow-billed Loons of Northern Alaska.
540 *Waterbirds* **45**, 159-166. doi:10.1675/063.045.0206

541 Miller-Brown, A. (2022). eDNA and dissection analysis of owl pellets: A method validation
542 study of Benamane et al. (2019). Honours Thesis, Ohio Dominican University.

543 Nakayama, S. M., Morita, A., Ikenaka, Y., Mizukawa, H. and Ishizuka, M. (2019). A review:
544 poisoning by anticoagulant rodenticides in non-target animals globally. *Journal of Veterinary*
545 *Medical Science* **81**, 298-313. doi:10.1292/jvms.17-0717

546 Nance, A., Mitchell, W., Clarke, R., Wilson, M., Brown, S., Macgregor, N., et al. (2020).
547 Norfolk Island Robin (*Petroica multicolor*). In 'The Action Plan for Australian Birds 2020. Vol.
548 1.' (Eds S. Garnett and G. Baker.) pp. 741-744. (CSIRO Publishing: Melbourne.) doi:
549 10.1071/9781486311910.

550 Nance, A., Wilson, M., Cook, C. and Clarke, R. H. (2023a). Monitoring for the presence of
551 invasive arboreal rodents: an assessment of monitoring tools across forest strata. *Biological*
552 *Invasions*, In review. doi:10.21203/rs.3.rs-2444671/v1

553 Nance, A. H., Mitchell, W. F., Dawlings, F., Cook, C. and Clarke, R. (2023b). Rodent predation
554 and specialised avian habitat requirements drive extinction risk for endemic island songbirds in
555 the south-west Pacific. *Emu-Austral Ornithology*, 1-15. doi:10.1080/01584197.2023.2228350

IN REVIEW

556 Noh, A. A. M., Ahmad, A. H. and Salim, H. (2023). Efficacy of cholecalciferol rodenticide to
557 control Wood Rat, *Rattus tiomanicus* and its secondary poisoning impact towards Barn Owl,
558 *Tyto javanica javanica*. *Scientific Reports* **13**, 2854. doi:10.1038/s41598-023-29499-8

559 Olsen, J. (2012). 'Australian High Country Owls.' (CSIRO Publishing: Melbourne)

560 Olsen, P. (1997). Recovery Plan for the Norfolk Island Boobook Owl, *Ninox novaeseelandiae*
561 *undulata*. Australian National University, Canberra.

562 Olsen, P. D. (1996). Re-establishment of an Endangered subspecies: the Norfolk Island Boobook
563 Owl *Ninox novaeseelandiae undulata*. *Bird Conservation International* **6**, 63-80.
564 doi:10.1017/S0959270900001313

565 Park, B., Scott, A., Wilson, A., Haynes, B. and Breckenridge, A. (1984). Plasma disposition of
566 vitamin K1 in relation to anticoagulant poisoning. *British Journal of Clinical Pharmacology* **18**,
567 655-662. doi:10.1111/j.1365-2125.1984.tb02526.x

568 Penck, M. and Queale, L. F. (2002). The diet of Southern Boobooks in South Australia. *Emu*
569 **102**, 355-360. doi:10.1071/MU00075

570 Peters, D. H., Schumacher, K., Schumacher, R. J. and Baigent, D. W. (2014). Goodnature
571 automatic traps for vertebrate pest control: field trials using new kill traps targeting animal pests
572 in New Zealand. *Proceedings of the Vertebrate Pest Conference* **26**, 404-410.
573 doi:10.5070/V426110618

574 Porter, R. D. and Wiemeyer, S. N. (1969). Dieldrin and DDT: effects on Sparrow Hawk
575 eggshells and reproduction. *Science* **165**, 199-200. doi:10.1126/science.165.3889.199

IN REVIEW

576 Price, C. J. and Banks, P. B. (2012). Exploiting olfactory learning in alien rats to protect birds'
577 eggs. *Proceedings of the National Acadademy of Sciences* **109**, 19304-19309.
578 doi:10.1073/pnas.1210981109

579 Prowse, T. A., Cassey, P., Ross, J. V., Pfitzner, C., Wittmann, T. A. and Thomas, P. (2017).
580 Dodging silver bullets: Good CRISPR gene-drive design is critical for eradicating exotic
581 vertebrates. *Proceedings of the Royal Society B: Biological Sciences* **284**, 20170799.
582 doi:10.1098/rspb.2017.0799

583 Quasim, S., MacDonald, A. J. and Sarre, S. D. (2018). Towards more efficient large-scale DNA-
584 based detection of terrestrial mammal predators from scats. *Mammal Research* **63**, 387-393.
585 doi:10.1007/s13364-018-0369-x

586 R Core Team. (2023). R: A language and environment for statistical computing (4.2.2). *R*
587 *Foundation for Statistical Computing*. https://www.r-project.org/

588 Riaz, T., Shehzad, W., Viari, A., Pompanon, F., Taberlet, P. and Coissac, E. (2011). ecoPrimers:
589 inference of new DNA barcode markers from whole genome sequence analysis. *Nucleic Acids*
590 *Research* **39**, e145-e145. doi:10.1093/nar/gkr732

591 Roemer, G. W. and Wayne, R. K. (2003). Conservation in conflict: the tale of two Endangered
592 species. *Conservation Biology* **17**, 1251-1260. doi:10.1046/j.1523-1739.2003.02202.x

593 Rognes, T., Flouri, T., Nichols, B., Quince, C. and Mahé, F. (2016). VSEARCH: A versatile
594 open source tool for metagenomics. *PeerJ* **4**, e2584. doi:10.7717/peerj.2584

IN REVIEW

595 Russell, J. C., Meyer, J.-Y., Holmes, N. D. and Pagad, S. (2017). Invasive alien species on
596 islands: impacts, distribution, interactions and management. *Environmental Conservation* **44**,
597 359-370. doi:10.1017/S0376892917000297

598 Salo, P., Korpimäki, E., Banks, P. B., Nordström, M. and Dickman, C. R. (2007). Alien
599 predators are more dangerous than native predators to prey populations. *Proceedings of the*
600 *Royal Society B: Biological Sciences* **274**, 1237-1243. doi:10.1098/rspb.2006.0444

601 Smith, C. R. and Richmond, M. E. (1972). Factors influencing pellet egestion and gastric pH in
602 the barn owl. *The Wilson Bulletin* **84**, 179-186.

603 Smithers, C. N. (1998). A species list and bibliography of the insects recorded from Norfolk
604 Island. *Technical Reports of the Australian Museum* **13**, 1-55. doi:10.3853/j.1031-
605 8062.13.1998.1282

606 Soulé, M. E., Estes, J. A., Berger, J. and Del Rio, C. M. (2003). Ecological effectiveness:
607 conservation goals for interactive species. *Conservation Biology* **17**, 1238-1250.
608 doi:10.1046/j.1523-1739.2003.01599.x

609 Sousa, L. L., Silva, S. M. and Xavier, R. (2019). DNA metabarcoding in diet studies: Unveiling
610 ecological aspects in aquatic and terrestrial ecosystems. *Environmental DNA* **1**, 199-214.
611 doi:10.1002/edn3.27

612 Sperring, V., Brown, S., Macgregor, N., Olsen, P., Clarke, R., Wilson, M., et al. (2021). Norfolk
613 Island Morepork *Ninox novaeseelandiae undulata*. In 'The Action Plan for Australian Birds

IN REVIEW

2020. Vol. 1.' (Eds S. Garnett and G. Baker.) pp. 360-363. (CSIRO Publishing: Melbourne.) doi: 10.1071/9781486311910.

St Clair, J. J. (2011). The impacts of invasive rodents on island invertebrates. *Biological Conservation* **144**, 68-81. doi:10.1016/j.biocon.2010.10.006

Stephenson, B. M. (1998). The ecology and breeding biology of morepork, *Ninox novaeseelandiae,* and their risk from secondary poisoning, in New Zealand. Masters Thesis, Massey University, New Zealand.

Stephenson, B. M., Minot, E. O. and Armstrong, D. P. (1999). Fate of moreporks (*Ninox novaeseelandiae*) during a pest control operation on Mokoia Island, Lake Rotorua, North Island, New Zealand. *New Zealand Journal of Ecology* **23**, 233-240.

Threatened Species Scientific Committee (2016). Conservation Advice *Ninox novaeseelandiae undulata* Norfolk Island Boobook Owl. Department of the Environment, Canberra.

Trost, S., Olsen, J., Rose, A. and Debus, S. J. (2008). Winter diet of Southern Boobooks *Ninox novaeseelandiae* in Canberra 1997–2005. *Corella* **32**, 66-70.

Van den Brink, N. W., Elliott, J. E., Shore, R. F. and Rattner, B. A. (2018). 'Anticoagulant Rodenticides and Wildlife.' (Springer: Switzerland.)

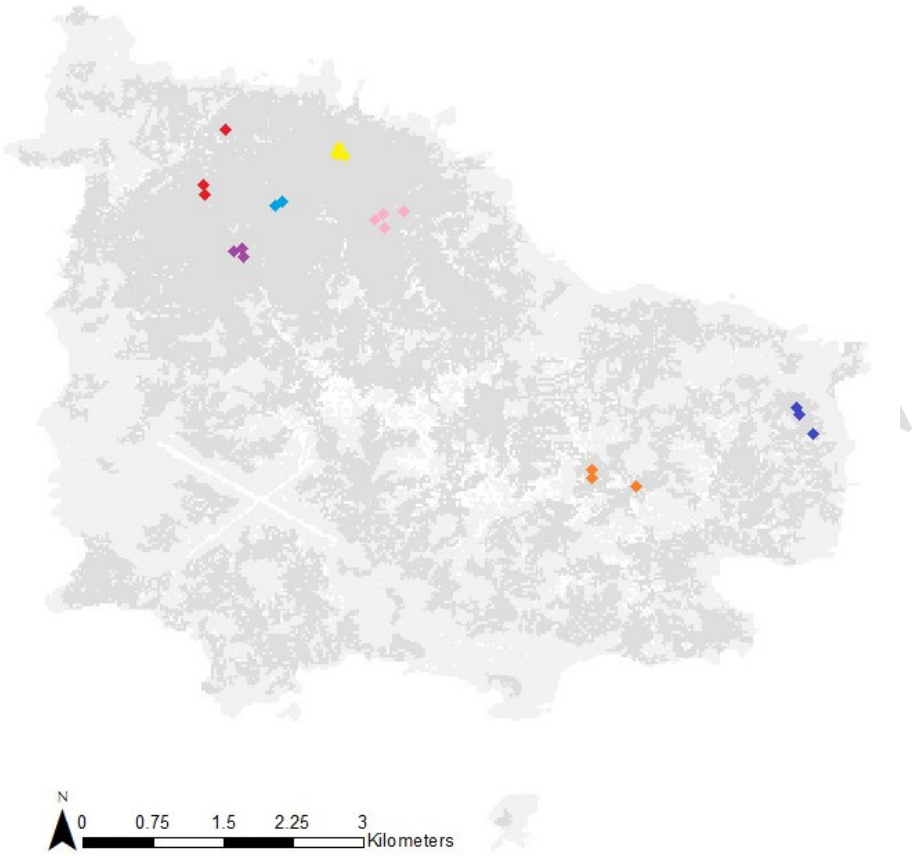
Wheeler, R., Priddel, D., O'Dwyer, T., Carlile, N., Portelli, D. and Wilkinson, I. (2019). Evaluating the susceptibility of invasive Black Rats (*Rattus rattus*) and House Mice (*Mus musculus*) to brodifacoum as a prelude to rodent eradication on Lord Howe Island. *Biological Invasions* **21**, 833-845. doi:10.1007/s10530-018-1863-4

IN REVIEW

634 Whittaker, R. J. and Fernández-Palacios, J. M. (2007). 'Island Biogeography: Ecology,
635 Evolution, and Conservation.' (Oxford University Press: Oxford.)

636 Zeale, M. R., Butlin, R. K., Barker, G. L., Lees, D. C. and Jones, G. (2011). Taxon-specific PCR
637 for DNA barcoding arthropod prey in bat faeces. *Molecular Ecology Resources* **11**, 236-244.
638 doi:10.1111/j.1755-0998.2010.02920.x
639

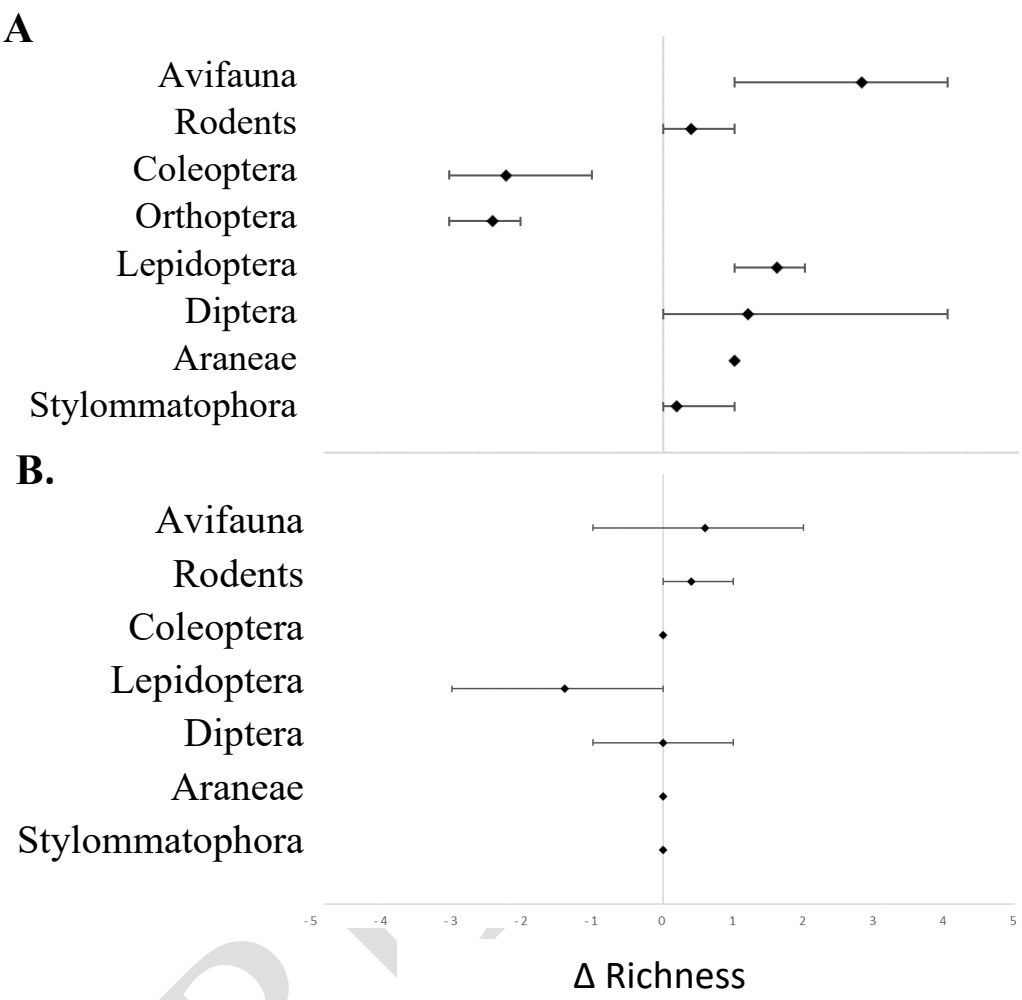
IN REVIEW



640

641 Figure 1. Roost sites for each Norfolk Island Morepork sampled across Norfolk Island during the
642 austral spring of 2020 and austral autumn of 2021. Each colour represents a different owl. The
643 owls marked in pink and purple were sampled only in spring. The owl marked in light blue was
644 sampled only in autumn.
645

IN RE



646

647 Figure 2. Average Δ richness for each taxonomic group comparing (A) visual and eDNA
648 analysis and (B) eDNA analysis of scats and pellets collected from Norfolk Island Moreporks. In
649 Figure 1A, negative values represent greater taxa richness detected with visual analysis and
650 positive values represent greater richness with eDNA. In Figure 1B, negative values represent
651 greater taxa richness identified in scats and positive values represent greater richness in pellets.
652 Minimum and maximum Δ richness per owl are shown with horizontal bars.

653

Table 1. Dietary items consumed by Norfolk Island Moreporks as shown by eDNA analysis. Numbers represent the total number of pellets or scats that contained a particular prey item. Scat samples are shown in bold. Detections from scat and pellet samples collected on the same day are included. Total count represents the total number of detections of each species, with samples from the same or consecutive day excluded. Paired owls are identified with 'a/b.'

		Morepork individual					Total
Taxa	Common name	1a/b	2	3	4	5a/b	count
Psittacidae sp.	Green Parrot	1	-	-	-	-	1
Columba livia	Rock Dove	1	-	-	-	-	1
Gallus gallus	Feral Chicken	-	-	-	-	1	1
Passer domesticus	House Sparrow	-	1	-	-	-	1
Rhipidura fuliginosa	Grey Fantail	-	-	1,**1**	-	-	1
Gerygone sp.	Norfolk Gerygone	-	1,**2**	-	-	-	2
Stumidae sp.	Common Starling	-	-	1	-	-	1
Zosterops sp.		1,**1**	-	3,**2**	**1**	**1**	6
Rattus rattus	Black Rat	-	-	-	-	1	1
Rattus sp.		1,**3**	-	1	1,**2**	2,**1**	9
Mus musculus	House Mouse	-	1	1	-	-	2
Coleoptera sp.		-	-	1,**1**	-	-	2
Araneae sp.		**1**	1,**1**	4,**1**	1,**1**	1,**1**	9
Lepidoptera		1					1
Agrotis ipsilon	Black Cutworm	-	-	-	-	**1**	1
Athetis thoracica		-	-	-	**1**	-	1
Mocis frugalis	Sugarcane Looper	-	**1**	-	-	-	1
Leucania stenographa	Sugarcane Armyworm	**1**	-	2,**2**	-	-	4
Mythimna convecta	Australian Armyworm	-	-	-	-	**1**	1
Mythimna separata	Northern Armyworm	-	-	-	-	**1**	1
Spodoptera sp.		-	**1**	**1**	**1**	-	1
Calliphora stygia	Brown Blowfly	**1**	-	-	-	-	1
Culex sp.		**2**	-	-	-	-	2
Diptera sp.		1	-	-	-	**1**	1
Helina sp.		1	-	-	-	-	1
Chironomus sp.		-	1	-	-	-	1
Stylommatophora sp.		2,1	-	-	-	-	3
Number of samples		4,7	2,2	5,2	2,3	2,3	

IN REVIEW

Table 2. Abundance of each prey taxa identified for individual Norfolk Island Morepork using visual analysis of pellets in the austral autumn (above) and spring (below). Paired owls are identified with 'a/b.'

		Morepork individual						
Taxonomic group	Taxa ID	1a/b	2	3	4	5a/b	6	7
Coleoptera	*Pimelopus spp.*	2	1	1	1	0		
	Onthophagus spp.	0	1	0	0	0		
	Cerambycid sp.	2	3	3	2	4		
Lepidoptera	L1	0	1	3	2	3		
Orthoptera	*Insulascirtus spp.*	6	3	2	2	3		
	O1	5	2	5	1	3		
	O2	1	0	1	0	0		
Vertebrate	Rodent	0	2	0	0	1		
	Unidentified vertebrate	3	1	1	0	1		
Number of samples		7	4	6	2	5	-	-
Coleoptera	*Pimelopus spp.*		0	0	1	5	7	5
	Onthophagus spp.		1	8	1	6	2	3
	Cerambycid sp.		4	17	4	22	13	7
Lepidoptera	L1		1	11	2	13	2	0
Orthoptera	*Insulascirtus spp.*		2	6	3	13	13	12
	O1		0	1	1	6	4	10
	O2		0	0	0	0	0	0
Vertebrate	Songbird		0	0	0	1	0	0
	Rodent		0	2	1	4	1	0
	Unidentified vertebrate		3	6	3	7	11	3
Number of samples		-	6	21	5	25	15	14

IN REVIEW

Image S1. Typical Norfolk Island Morepork pellet collected on cotton sheet.

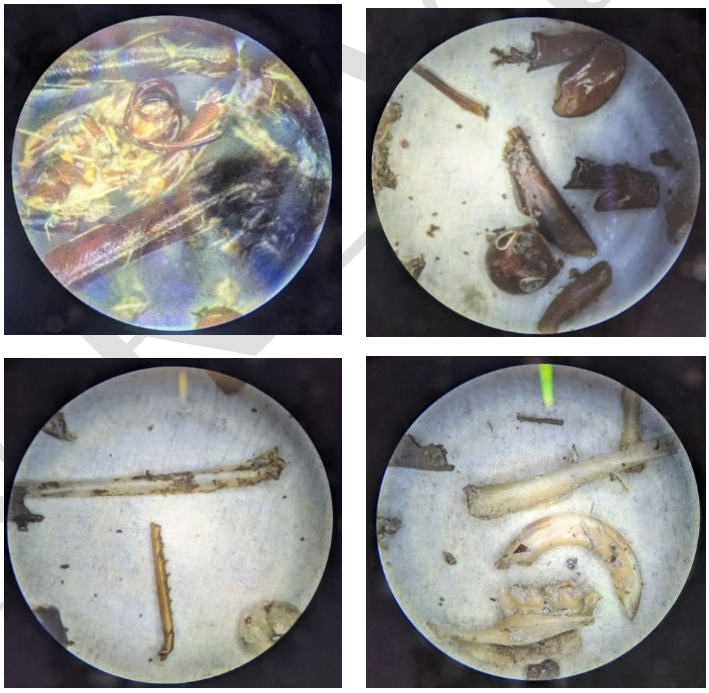


Image S2. Example images of a) Lepidoptera, b) Coleoptera, c) Orthoptera and d) Rodentia. Images represent the typical visual screening process for Norfolk Island Morepork pellets with other taxa also visible in most images.

IN REVIEW