Density estimates and detection models inform stoat (*Mustela erminea*) eradication on Resolution Island, New Zealand

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Abstract Resolution Island (20,800 ha) in Fiordland, New Zealand, has long held great potential as a sanctuary for the protection and reintroduction of highly threatened bird species. In 2008, the New Zealand Department of Conservation initiated a programme to eradicate wild stoats (*Mustela erminea*) from Resolution Island. Following the establishment of a trapping network, but prior to the traps being set, hair-snagging devices were deployed on approximately one quarter of the island, in order to obtain an independent estimate of population density. Stoat hair samples were collected from devices approximately daily over a 10-day period. DNA was extracted from 117 hair samples, and resulting genotypes were analysed using the spatially explicit mark-recapture software DENSITY, which provided a population density estimate for the study area of 0.48 stoats km^{-2} (95% CL 0.31 – 0.74; CV 23%). Hair tubes underestimated the 'minimum number alive' population density calculated from the number of stoats subsequently captured in kill-traps (an estimate of 1.4 stoats km^{-2}) but provided precise information on detection parameters. They also gave an independent measure of initial trapping success with 21 out of 22 stoats detected in tubes being subsequently caught in traps. The above data in a Lincoln-Peterson index, with hair samples as the mark and trap samples as the recapture, gave a population estimate slightly above the actual number trapped. In a preliminary analysis, we modelled trap-capture data in a Bayesian framework and estimated that the probability of stoats persisting would be <1% after 10 consecutive checks with no captures. These models also yield a population slightly higher than the number of animals actually caught. We conclude that DOC150 traps were efficient at detecting stoats, but trapping stoats to extinction on Resolution Island will not be achieved in the near future and that initial trap spacing may have contributed to this.

Keywords: Bayesian modelling, detection parameters, Fiordland, genotyping, *Mustela erminea*, Resolution Island, restoration, stoat

INTRODUCTION

Stoats (*Mustela erminea*) are an invasive alien predator implicated in the historical and continued decline of many highly threatened bird species in New Zealand such as kiwi (*Apteryx* spp.), kaka (*Nestor meridionalis*), mohua (*Mohoua ochrocephala*), takahe (*Porphyrio hochstetteri*), and blue duck (*Hymenolaimus malacorhynchos*)(King and Murphy 2005). One way to effectively manage the threats posed by stoats is to eradicate them from offshore islands, thereby creating 'island sanctuaries'.

In 2002, following successful invasive mammal eradications on other New Zealand islands and around the world (Simberloff 2001; Veitch and Clout 2002; Howald *et al*. 2007), the New Zealand Department of Conservation (DOC) initiated a plan to eradicate stoats from Resolution Island (detailed in Edge *et al*. 2011). The island (ca 20,800 ha) is the largest of Fiordland's near-shore islands. The only introduced mammals on the island are stoats, mice (*Mus musculus*), and deer (*Cervus elaphus*). The eradication of stoats would create the largest island sanctuary in New Zealand for highly threatened bird species such as the kakapo and those with large home range requirements such as kiwi and kokako (*Callaeas cinerea wilsoni*) (McMurtrie *et al*. 2008).

The size and remote location of Resolution Island have made this attempt extremely challenging. Furthermore, at its narrowest point the island is only 520m offshore. Stoats are trapped on the adjacent mainland coast, but the narrow channel is well within their swimming capabilities (Taylor and Tilley 1984). Although design of the current operation involved scaling up from previous campaigns on smaller islands (Edge *et al*. 2011), it was not known how the capacity of stoats to reinvade might compromise the eradication attempt (Elliott *et al*. 2010).

The planned eradication of stoats from Resolution Island provided an important opportunity to apply learning from earlier eradication campaigns and to fit these and the current research into an adaptive management framework. Key questions revolved around the number of stoats on the island prior to control, and the number of stoats remaining following the initiation of control. Independent estimates of the initial population can be obtained by: 1) using microsatellite DNA analysis of hair to identify individuals (Foran *et al*. 1997) and analysing these data in a mark-recapture framework; and 2) using a Bayesian analysis of the kill-trapping data. Microsatellite DNA analysis can also be used to determine the genetic relatedness between island and mainland populations (McMurtrie *et al*. 2011), which is important for identifying the origin of animals that are caught during later phases of the eradication programme.

In this paper, we present results from research on Resolution Island which aimed to: 1) determine the initial population size and density using mark-recapture models based on genotyped hair samples (Lincoln Peterson index, Seber 1982; Program DENSITY, Efford *et al*. 2004) and a Bayesian model for the trap-capture data; 2) estimate spatial detection parameters (capture probability and home range width) of the stoat population prior to eradication using the same molecular data; and 3) provide an estimate of the search effort necessary to declare eradication success.

METHODS

Study area

Resolution Island in Fiordland, New Zealand (45° 41.4′S, 166° 41.5′E) reaches 1069 m (Fig. 1). The vegetation is a mix of southern beech (*Nothofagus menziesii* and *N. solandri* var. *cliffortioides*) and podocarp-broadleaf forest dominated by kamahi (*Weinmannia racemosa*) and rimu (*Dacrydium cupressinum*); manuka (*Leptospermum scoparium*) shrublands; tussock grasslands dominated by *Chionocloa acicularis*; and small areas of wetland, coastal scrub and fellfield vegetation (Ledgard and Rance 2008). The climate is cool temperate, with mean annual temperature of C. 10° C, and annual rainfall of C. 4000 mm spread evenly throughout the year (Bayliss *et al*. 1963).

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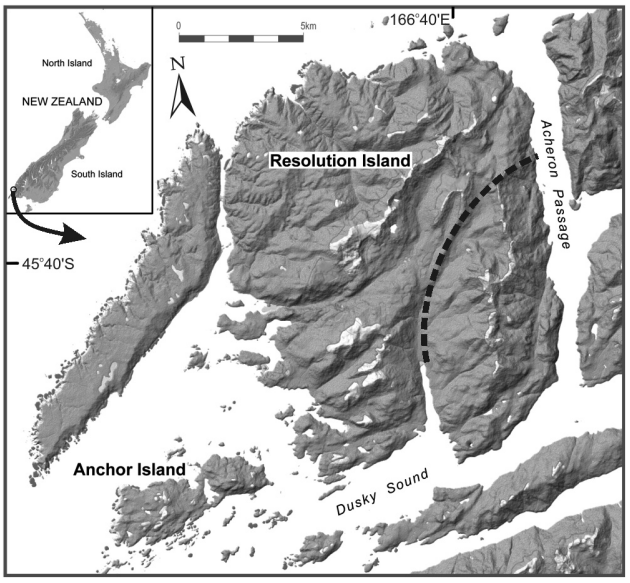

Fig. 1 Resolution Island in Fiordland, New Zealand. The study area used for non-invasive sampling of DNA from the stoat population prior to initial knock-down is south-east of the dotted line.

Pre-baiting and kill-trapping

The trapping regime for stoats on Resolution Island was similar to that for Secretary Island (McMurtrie *et al*. 2011), with a network of tracks covering the island and DOC150 traps spaced at C. 100 m intervals on each track. Each trap was placed inside a protective wooden tunnel (400 mm × 150 mm × 200 mm) and the goal was to have no point on the island more than 700 m from a trap. Track spacing was approximately equal across the whole island. Pre-baiting with eggs and meat was conducted twice, on 20 May and 24 June 2008 and kill-traps were set, checked and re-baited twice over two 3-day periods from 15–24 July and once more during 5–12 August 2008. This resulted in a total of three initial trapping sessions over 20 days during the "knockdown" phase.

Genotyping

Prior to the initial knockdown, and between pre-baiting sessions (4–13 June 2008), we obtained DNA from hair follicles (Foran *et al*. 1997) of stoats on C. 5900 ha (28% of the island; Fig. 1) using hair-snagging tubes (Duckworth *et al*. 2006). A total of 208 sections of PVC drainpipe (250mm length x 40 mm diameter) were placed 1–2 m from every second wooden trap box (i.e. every C. 200 m). Each tube contained two rubber bands stretched through slots at each end, pasted with a 50:50 mixture of trapper glue (Bell Laboratories, Wisconsin, USA) and toluene ultimAR (Mallinckrodt chemicals) and baited with a small piece of fresh rabbit meat, secured to the ground with a wire hook. Tubes were checked and re-baited when weather conditions permitted; on average three out of every four days to provide a 1-session closed population estimate with five occasions over a 10-day period. Tubes containing hair samples were replaced with a fresh tube. The hairs obtained were left attached to each rubber band, which was snipped off using forceps and scissors. Each length of rubber band with hairs attached was then wrapped in filter paper and samples sent to EcoGene™ (Auckland, NZ) for DNA extraction. Tissue samples (tail tips) were also collected from stoats captured in kill-traps and those samples that came from the study area were included in the Lincoln Peterson mark-recapture estimate.

In the laboratory, 50 mg of muscle tissue and caudal skin were removed from the tail tips and DNA was isolated using a Bio-Rad AquaPure Genomic Tissue Kit (Cat# 732-6343) following the manufacturer's protocol. DNA extraction from hair samples used a modified protocol following Walsh *et al*. (1991). Hair follicles were placed in an Eppendorf tube containing 100 µl of extraction buffer (5% chelex 10 mM Tris, 0.1 mM EDTA), followed by an addition of 1 µl Proteinase K (20 mg/ml) and 2.7 µl of 1 M DTT. Samples were incubated at 56°C for 2 h. A further 1 µl of Proteinase K was added and samples incubated an additional 2 h at 56°C, tapping occasionally. Samples were then boiled for 8 min, vortexed at high speed for 15 s and centrifuged (13,000 rpm) at room temperature for 3 min. Supernatants were transferred to new tubes with a wide-bore pipette tip, and stored at −20 °C.

Microsatellite amplification and genotyping across 16 variable microsatellite loci followed McMurtrie *et al*. (2011). Evidence for allelic drop-out, scoring error due to stutter, and presence and frequency of any null alleles were assessed with MICRO-CHECKER (Oosterhout *et al*. 2004). Genotyping was carried out using a step-wise protocol of exclusion that has been shown elsewhere to ensure rigorous and conservative determination of identity (Paetkau 2003; Weaver *et al.* 2005). We required a perfect match between the two amplifications in order to accept each genotype and to eliminate PCR errors resulting in either allelic drop-out or false alleles. Any samples that differed by one locus were checked for potential scoring or amplification errors (Paetkau 2003). If these differences were not able to be explained by errors in scoring/typing, samples were then subjected to a further round of PCR and scoring (Poole *et al*. 2001; Mowat and Paetkau 2002). Samples that were not able to be accurately genotyped for the majority of loci were rejected from the analysis.

We used the software package GIMLET (v. 1.3.3; Valiére 2002) to estimate P_{ID} and $P_{ID\text{-}sib}$ among full siblings as that provides an upper limit to the probability that pairs of individuals will share genotypes (Taberlet and Luikart 1999).

DATA ANALYSES

Stoat density on the south-eastern part of Resolution Island was estimated in two ways. First, by spatially-explicit capture-recapture in Program DENSITY (Maximum Likelihood method) (Efford *et al*. 2004) using the individual genotypes identified with DNA extracted from hair follicles. Estimating population densities (D) using DENSITY also enabled us to calculate two spatial detection parameters: 1) g0, which is per-night probability of capture at the centre of the home range, and 2) σ, which is the spatial scale over which the probability of capture declines with distance from the home range centre. The precision of the estimates of D, g0, and σ was measured using the coefficient of variation (CV); the standard deviation of an estimate divided by the estimate. Secondly, we used the total number of stoats caught from the three initial trapping sessions on the south-eastern part of the island to estimate the minimum density on the island. These data were also used to calculate initial population size (N) and the probability of capturing each stoat (θ) with the deployed traps as follows:

$$y \sim binomial(\Theta, N).$$

$$\Theta = 1 - \exp(-\rho * Effort)$$

where ρ is the rate parameter describing the relationship between number of sessions (Effort) and

detection probability, θ. In this analysis we did not attempt to incorporate heterogeneity of detection in males and females. We then used Bayes theorem and the relationship between trapping effort and detection probability to predict the probability of stoat persistence given no detection (Ramsey *et al*. 2009).

We also used the total number of stoats caught from the three initial trapping sessions across the whole island to estimate the minimum density of stoats on Resolution Island.

Areas were calculated using ARCGIS (ESRI, Redlands California, USA).

RESULTS

Genotyping

Of 191 hair samples and 112 tissue samples obtained, 117 hair samples and all tissue samples were successfully genotyped for all 16 loci. Where DNA genotyping was not possible, most were <5 hairs and DNA yield was subsequently low. For these samples, either PCR amplification was not possible for any loci, or it was infrequent and all loci could not be reliably genotyped. The P_{ID} within the population across all loci was 0.097% and the $P_{ID\text{-}sib}$ of 4.4×10^{-5} was well below the 1% threshold. No identical genotypes were obtained amongst the tissue samples. There was no evidence of allele dropout or scoring error due to stutter. One locus (Mer041) exhibited some evidence of a null allele; however, because it would not affect the ability to differentiate individuals, this allele was not removed from the analysis. Given these results, identical genotypes within different individuals from this population were extremely unlikely and it is reasonable to conclude that hair samples with identical genotypes are from the same individual.

Stoat captures in kill-traps and hair tubes

Two hundred and ninety stoats were caught in kill-traps during the knockdown phase of trapping (Table 1) giving an initial minimum population estimate of 1.4 stoats km^{-2} across the island. More females than males were caught in all trapping sessions. The overall ratio of female to male stoats was >3:1 and differed significantly from 1:1 (exact binomial test; P=0.002). Most stoats (75%) were caught in the first 3-day trapping session and were caught across the whole island, in all habitat types and altitudes. In the study area, 81 stoats were captured in traps and this also equated to 1.4 stoats km^{-2} (Table 1).

Table 1 Number of stoats caught during the 'knockdown phase' of trapping on Resolution Island. Traps were pre-baited twice, set and checked twice over two, 3-day cycles (July) then checked again 14 days later in August; and detected by hair tubes within the study area.

Trapping (whole is.)	Fem	Male	Unkn	Total	Density[1]
Session 1 (July)	157	61	1	219	
Session 2 (July)	35	4	0	39	
Session 3 (August)	32	0	0	32	
Total	224	65	1	290	1.39
Trapping (study area)	64	17	0	81	1.37
Hair tubes					
Unique individuals	13	8	1	22	
Recaptures	9	7	0	16	

[1]Density estimates were derived from trap catch data divided by the sampling area (whole island, or study area only).

Twenty-two individual stoats were identified from the 117 hair samples. Twenty one of the 22 individuals (95%) identified in the hair tubes were subsequently captured in kill-traps. The ratio of female:male stoats detected in tube trap samples was 1.6:1 and not significantly different from an equal ratio (P=0.286). The median number of detected tube entries per stoat was 2.5 (range 1–27).

Population density and detection probability

The capture-recapture data (Table 1) gave an initial estimate of population density D in the south-eastern part of the island of 0.48 stoats km^{-2} (95% CL 0.31 – 0.74; CV 23%). The sampled stoat population had a g0 estimate of 0.13 day^{-1} (95% CL 0.07 – 0.22; CV 31%), and σ was 397 m (95% CL 322-489; CV 11%). In other words, a stoat had a per-night probability of being captured in a tube at the centre of its home range of approximately 13%, and a home range radius of 486 m (half of 2.45×σ; Efford *et al*. 2004). The Lincoln-Peterson index gave an estimated population in the study area of 85 stoats, which is slightly higher than the number actually caught.

Modelling the abundance of stoats using the kill-trapping data gave an estimate of N=94 stoats on the south-

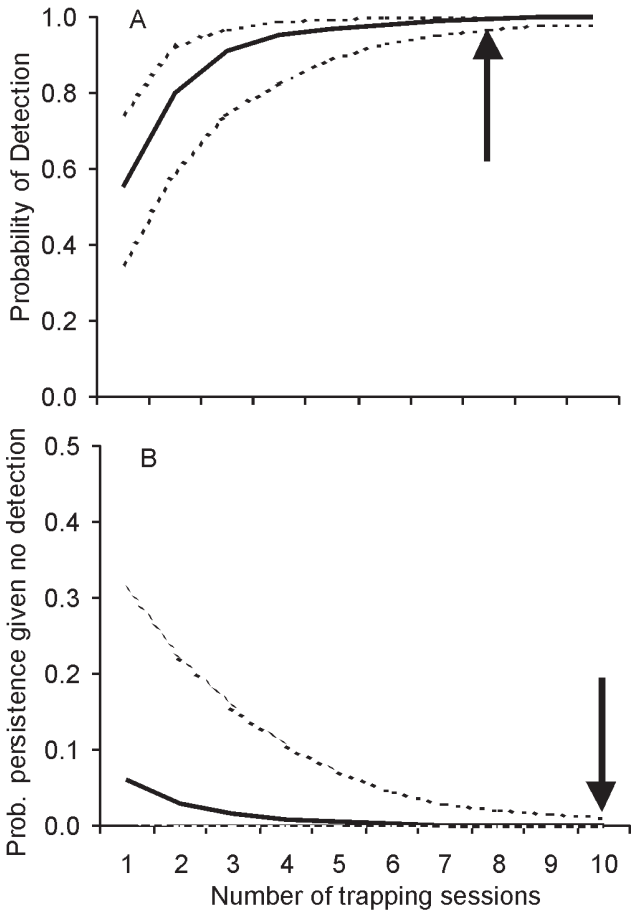

Fig. 2 Modelled probabilities of (A) detection and (B) persistence of stoats on Resolution Island out to 10 trapping sessions, using information obtained from stoats captured during the initial knockdown period (three trapping sessions). Dotted lines represent 95% credible intervals. Arrows indicate (A) a lower credible interval of 0.972, i.e. a probability of 0.028 that stoats present on the island would not be detected after 8 trapping sessions, and (B) an upper credible interval of 0.008, i.e. a 0.8% chance that stoats would remain on the island without being detected.

eastern part of the island (95% Credible Interval 72–140). This translated to a density of 1.6 stoats km^{-2}. The estimated probability of detection in traps increased very quickly with the number of sessions and was projected to have a lower 95% credible interval (conservative estimate) of 0.972 after eight trapping sessions (Fig. 2). Using these results to predict the probability of persistence in the confirmation phase (when stoats are no longer being detected in traps), we found that after ten trapping sessions with no stoat detections, the conservative upper 95% credible interval would be 0.008; a 0.8% chance that stoats remain on the island without being detected (Fig. 2).

DISCUSSION

Knowing the initial population size and detection probability of an invasive species is highly informative for eradication efforts. Furthermore, independent estimates using different methods are rarely obtained, so having multiple measures of these parameters increases confidence in the estimates. We were able to compare empirical estimates using non-invasive genetic sampling with data obtained from stoats captured during the initial knockdown phase of the eradication attempt on Resolution Island.

Non-invasive hair tubes identified about 25% of the stoats that were subsequently captured in kill-traps during the initial knock-down phase of the eradication attempt. Hair tubes were thus less effective detection devices relative to kill-traps once differences in their deployment are considered. Our low density estimates from hair tube sampling may have three origins. First, stoats may have been neophobic to hair tubes. Trap boxes had been in situ for several months prior to knock-down and had received two rounds of pre-baiting, so stoats may have become more used to their presence relative to the newly-placed hair tubes. Consequently, we could have sampled a smaller subset of the population. Second, in retrospect the period for hair tube sampling was insufficient to give precise density estimates. An interval of 2–3 weeks may have been more appropriate in order to make a direct comparison with the kill-trap data. Finally, the proportion of clean genotypes obtained from samples was only 60%, so the remaining samples, if resolved, would have increased the DNA-derived density estimates.

Our data suggest that kill-traps efficiently detected stoats at the moderately low density of 1.4 stoats km^{-2} measured on Resolution Island (see King and Murphy (2005) for other NZ stoat density estimates). We were also able to provide an informal, independent assessment of trapping success during the knockdown phase and conclude that it was >90%. This is particularly important for the current management of invasive mammals on Secretary and Resolution Islands, where traps are used in perpetuity to increase the chance of resident stoats being trapped and to prevent incursions from the mainland (Edge *et al*. 2011). Those naive stoats that do occasionally swim to the island (McMurtrie *et al*. 2011; Elliott *et al*. 2010) are likely to encounter an effective kill trap soon after arriving. However, it seems that some stoats survived the initial kill trapping and might represent trap-shy or narrow-ranging individuals. Female stoats usually retain between six and 13 blastocysts inside the uterus for up to a year (King and Murphy 2005), so survivors of a trapping programme will strongly contribute to the continuation of a stoat population in an area.

We were able to provide reasonably precise estimates of g0 and σ, which usefully tested the trap spacing on the island (McMurtrie *et al*. 2008). Our estimates of the spatial detection parameters are similar to other published studies (e.g., Smith *et al*. 2008; Efford *et al*. 2009), and gave an estimate of home range radius for stoats (c. 486m) similar to but slightly less than many of those derived by radio-tracking (King and Murphy 2005). So the initial goal of having a maximum of 700 m from any point on the island to the nearest kill-trap (McMurtrie *et al*. 2008) now seems to have over-estimated resident stoat home range sizes.

Catch-effort modelling of the data obtained from kill-trapping gave a less biased measure of the initial stoat population density prior to the knock-down, and was also useful for obtaining an independent estimate of the probability of detection for the current trap array. We could then predict how many trapping sessions would be required before being confident that eradication of stoats from Resolution Island had been achieved (assuming no in-situ breeding and no further incursions from the mainland). This knowledge is of little use at present, as stoats still inhabit the island (P. McMurtrie pers. comm., Feb 2011). A more useful analysis would be to model *in situ* breeding and likely immigration rates, which we are currently undertaking. The proposed Bayesian modelling approach will ultimately incorporate both the kill-trap and the genetic-mark-recapture data to provide improved estimates of the initial population size. The improved model will also incorporate the sex ratio bias, population growth rate, and the ongoing probability of immigration from the mainland. Improved modelling should also account for the possibility of decreasing detection probabilities as the population is reduced to near zero. Further, we have now established a genetic database of stoats from the island prior to the eradication, which can be used in the future to infer whether captured individuals are survivors or recent arrivals.

The attempted eradication of stoats from Resolution Island represents a large, complex and ambitious project. A key component of the planning and implementation of the eradication programme was to learn as much as possible about stoat behaviour and trappability on the island in order to adapt the operational aspects of the programme through time (Edge *et al*. 2011). We provide evidence that the kill-trap devices chosen, strong emphasis on pre-baiting to avoid neophobia, and ongoing use of the control tool (kill-traps) as a surveillance device were sound operational decisions for the eradication of stoats on Resolution Island. However, an increased density of kill-traps may be required if eradication is to be achieved. The DNA sequencing techniques we developed represent an important advance, but further research that reduces the problems of mixed samples would be beneficial. Finally, to ensure a successful programme, future work is needed to better understand detection probabilities at the very low population densities of stoats on Resolution Island and to combine multiple sources of uncertain, imprecise or sparse information.

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