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PRIORITY CONTRIBUTION

Foxes are now widespread in Tasmania: DNA detection defines the distribution of this rare but invasive carnivore

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Summary

1. Invasive vertebrate species are a world-wide threat to biodiversity and agricultural production. The presence of foxes, one of the most damaging invasive vertebrates introduced to Australia, has now been confirmed in the island state of Tasmania, placing at risk many species of native vertebrates and substantial agricultural industry.

2. Effective eradication of such a rare but elusive carnivore requires robust strategies informed by novel but systematic detection.

3. We combine DNA detection approaches for trace samples with systematic stratified and opportunistic surveys of carnivore scats to estimate the current distribution of foxes in Tasmania. We use that DNA evidence and other hard evidence provided by carcasses and other material to build a predictive model of fox habitat suitability for all of Tasmania.

4. We demonstrate that this destructive species is widespread in northern and eastern Tasmania but has not yet reached the limits of its range. The widespread nature of this distribution reveals that targeting fox activity hotspots only for eradication is unlikely to be successful and that a strategic and statewide approach is required. Our habitat suitability model can provide a basis for prioritizing areas for fox management.

5. *Synthesis and applications*. Our approach highlights the importance of early and pre-emptive surveys of recently established, and therefore rare, invasive species and the necessity of providing a sound and defensible approach to determining the distribution of the invasive species. This approach provides a template for the systematic detection of rare cryptic carnivores.

Key-words: eradication, extinction, habitat suitability modelling, mitochondrial DNA, scats, species distribution, strategic survey

Introduction

Invasive vertebrate species are a world-wide threat to biodiversity and agricultural production (Pimentel *et al*. 2001; May 2010). Their effects manifest through direct predation, competition and damage to food crops and indirect interactions such as costs of control and disease and parasite transmission (Bomford & Hart 2002). Invasive species are particularly likely to cause major disruption to isolated ecological communities, particularly islands, where endemic species have evolved in the absence of strong competition or predation (Reaser *et al*. 2007).

Invasions into new and vulnerable ecosystems by species that are likely to be detrimental must be subject to prompt and rigorous action. The most appropriate strategy is prevention followed by detection and eradication (McNeeley *et al*. 2001; Wittenberg & Cock 2001). The least favoured and most costly option is to accept establishment of the species and to then undertake sustained control. Designing a successful invasive species eradication program requires among other things a science base,

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exposure of all individuals to eradication techniques, no risk of reinvasion, methods that can detect the last survivors, and a monitoring phase to ensure that eradication has been achieved (McNeeley *et al.* 2001; Wittenberg & Cock 2001). The last two points are especially problematic for recently introduced species which are usually sparsely distributed, yet potentially widespread and therefore difficult to detect. When these species are cryptic, and the probability of an actual sighting is low, conventional surveillance systems are unlikely to be sufficient for eradication. In addition, the dispersal and habitat usage of a predator in a new environment and at low densities is usually unknown and likely to be quite different to those exhibited by the same species in areas in which it is naturalized and at high densities. In such circumstances, identifying the distribution and likely habitat usage are the key to defining the eradication problem and to the employment of the strategies necessary to prosecute the eradication.

Australia has a poor record of failing to acknowledge the threat posed by invasive vertebrates (Olsen 1998). At least 80 species of non-indigenous vertebrates have now established wild populations in Australia, and more than 30 of these species have become pests (Bomford & Hart 2002). The devastation inflicted by invasive vertebrates on Australia's indigenous wildlife has been massive, and despite general support for wildlife protection, the threat is unabated (Saunders *et al.* 2010a). A key indicator is Australia's unenviable record of half the known mammalian extinctions world-wide in the past 200 years (Short & Smith 1994). While habitat destruction, degradation and fragmentation have been important players (McKenzie *et al.* 2007), predation by foxes *Vulpes vulpes* has been central to the high Australian extinction rate (Kinnear, Sumner & Onus 2002; Saunders, Gentle & Dickman 2010b) and they are recognized by the Australian Government as a Key Threatening Process under the *Environment Protection and Biodiversity Conservation* Act 1999.

In contrast to this devastation on the Australian mainland, the native fauna of Tasmania has remained relatively unchanged by European settlement, with the thylacine being the only known extinction in historical times. Tasmania has remained fox free, and this appears to be a key factor in the lack of extinction among Tasmanian fauna. The establishment of foxes in Tasmania would therefore place at risk 78 species of native terrestrial vertebrates (Saunders *et al.* 2006).

There have been several attempted introductions of foxes into Tasmania over the past 100 years, but evidence of an established population has been lacking (Statham & Mooney 1991). Recently however, probably between 1998 and 2001, an unknown number of foxes were introduced into Tasmania (Saunders *et al.* 2006). Although the deliberate or accidental nature of this introduction remains unsubstantiated (Saunders *et al.* 2006), the discovery of four fox carcasses including males, females and immature individuals (Fig. 1) has confirmed the presence of foxes and suggests breeding populations in the central north coast and midland areas. Commensurate with the arrival of foxes, the top mammalian carnivore in Tasmania, the

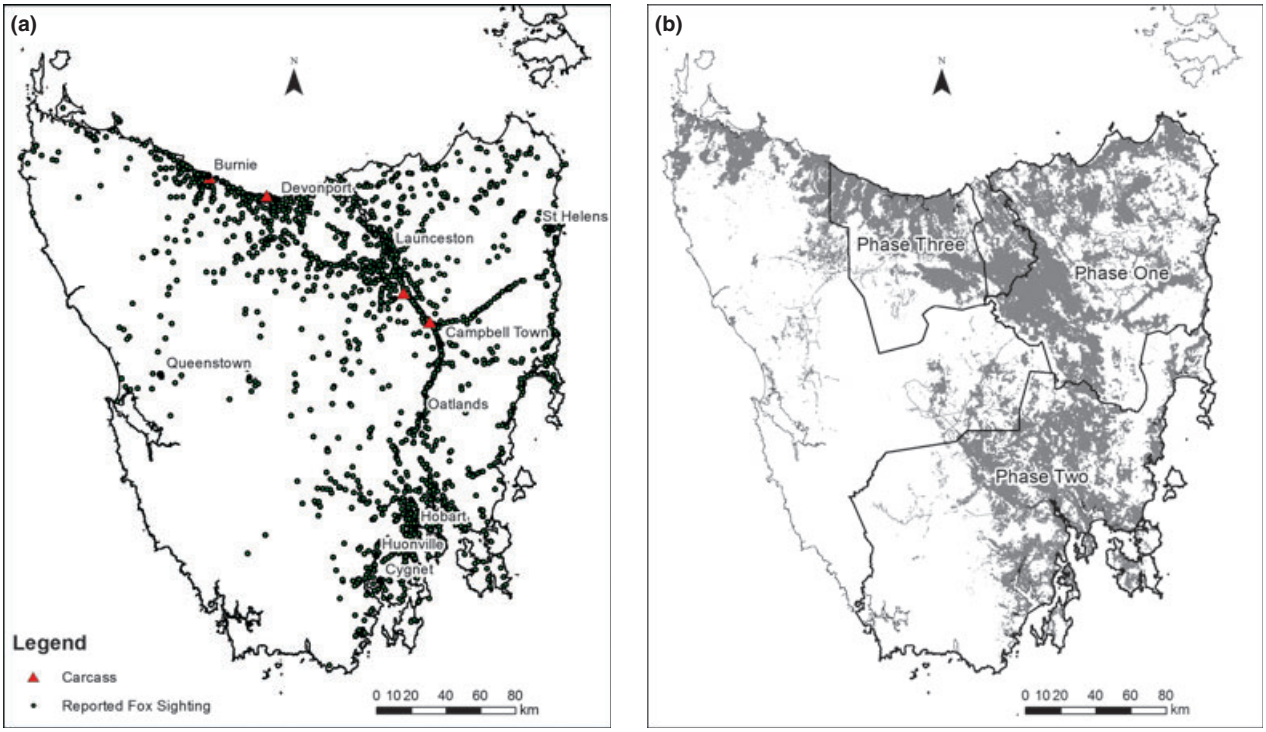

Fig. 1. (a) Reported public fox sightings and carcasses in Tasmania, 2002–2009. Data from the Tasmanian Department of Primary Industry Parks Water and the Environment. (b) Location of survey units containing at least 6% priority one habitat (grey shading) including the areas encompassed by the three survey phases.

Tasmanian devil *Sarcophilus harrisii*, contracted a new cancerous disease that is causing high mortality and a rapid and widespread collapse in devil numbers (Hawkins *et al*. 2006). These two unfortunate and coincident occurrences could affect profoundly Tasmania's fauna, with the widespread demise of devils reducing any role they may play in preventing fox establishment through competition and predation.

Over 2000 unconfirmed fox sightings have been reported by the public since 2002 (Fig. 1), suggesting that the fox may already be widespread on the island. However, given the likely high incidence of erroneous reporting, and the concentration of sightings around major roads and towns, it is likely that the sightings data provide a biased and inaccurate indication of fox distribution. This lack of unequivocal longer-term evidence has led to public scepticism about the threat posed by foxes and significant political pressure to reduce funding to monitor and control the invasion (Saunders *et al*. 2006). Moreover, the Fox Eradication Program, implemented by the Tasmanian Department of Primary Industry Parks Water and the Environment (DPIPWE), has been directed towards tactical baiting with the poison 1080 (sodium monofluoroacetate). These baits are set by hand, buried and then retrieved in order to minimize non-target deaths and to allay community concerns about toxins. This expensive and time-consuming approach makes accurate information about the current and likely (predicted) distribution of foxes critical.

The low detectability of foxes, even at normal densities, requires five to nine repeat spot-light visits to establish a reliable count (Field *et al*. 2005), and this count can also vary substantially from sample to sample through observer bias, weather and seasonal conditions, differences in sightability, and accessibility. Although the probability of detection by spotlighting is unknown, it is likely to be very low in Tasmania given the presumed low density of the animals (Saunders *et al*. 2006). It is therefore unsurprising that spot-light surveys conducted in Tasmania between 2002 and 2006 yielded no confirmed fox sightings (Mooney pers comm.).

An alternative approach and the method that we selected was the detection of fox faeces (scats). Canid scats can persist in the field for weeks or even months (Kohn *et al*. 1999), and several species, including foxes, tend to leave them in prominent places such as along tracks (MacDonald 1980). Fox scats have been used previously for broad-scale survey (Baker, Harris & Webbon 2002; Webbon, Baker & Harris 2004), and good scat persistence times make feasible the detection of foxes at low densities. Another advantage of scat surveys is that relatively untrained personnel can collect robust presence or absence data with precise locational information. However, definitive morphological diagnosis of scats can be exceedingly difficult (Reynolds & Short 2003), a problem exacerbated in Tasmania where six major mammalian predators (foxes, cats, dogs, devils, spotted tail quolls and eastern quolls) can produce scats of similar size and shape. To ensure reliable scat identification, we developed a mitochondrial DNA-based test for foxes and tested it for robustness using scats exposed to field conditions (Berry *et al*. 2007). Our testing demonstrated that fox DNA could be reliably detected in scats for at least 3 months after deposition, while preliminary analysis revealed two scats collected in Tasmania to contain fox DNA (Berry *et al*. 2007).

The confirmed presence of foxes makes determination of their distribution essential in order for a full and systematic eradication program to be developed and implemented. Here, we report on the development and conduct of a large-scale DNA-based analysis of the presence and absence of foxes in eastern Tasmania. We use those data to model the likely distribution of foxes relative to their likely habitat. Our analysis provides the first systematic appraisal of the nature of the fox problem in Tasmania and provides the basis for an achievable approach to one of the most challenging eradications yet attempted.

Materials and methods

SURVEY STRATEGY AND DESIGN

A majority of fox occurrence evidence in Tasmania was collected as part of a strategic scat survey or by field investigations (where fox scats were found as the result of investigations of fox sightings), both conducted by the Tasmanian Fox Eradication Branch. The state of Tasmania was divided into contiguous 9-km^2 (3 × 3 km) cells giving a total of 7772 in the state. The strategic survey involved searching one of every three units that contained at least 6% by area of habitat considered as highly suitable (priority 1) to foxes, as judged by fox ecologists. Priority 1 habitat included habitat variables belonging to two main groups, Agricultural and Urban environments and Native Grasslands (Fig. 1b). Biophysical variables describing vegetation type, annual rainfall, elevation, aspect and slope were derived for each survey unit from spatial layers held by DPIPWE. The area of each vegetation class was derived for each survey unit and used in the analyses. The mean, standard deviation and minimum and maximum were also derived for annual rainfall, elevation, slope and aspect for each unit.

The strategic scat survey comprised three phases, each of which covered a single geographic region on the eastern and northern Tasmanian mainland and contained between 200 and 300 9-km^2 units. Urban areas were not considered in the survey as these areas are heavily populated, making sightings, and the gathering of hard evidence of presence more likely than in less densely populated regions, reducing the need for formal survey. The surveys were conducted in autumn (March to June) in each of 2008 (Phase 1), 2009 (Phase 2) and 2010 (Phase 3). Autumn was selected because it represents a time of relative fox population stability following juvenile dispersal and preceding mating (Saunders *et al*. 1995) and because pasture is usually short at that time of year increasing scat detectability. Each unit selected for survey was subjected to searching for a period of ten person-hours, focusing search effort on landscape features such as water bodies, vehicle and walking tracks, hedgerows and fence lines. All

carnivore scats found were collected, except those from devil latrines where only 10 smaller scats were collected. Each scat collected was placed in a paper bag using disposable implements and the GPS position recorded. All scats were air-dried at room temperature in their collection bag before DNA analysis.

DNA APPROACHES

Extraction and PCR analysis

All DNA extractions and PCR set-up were structured to minimize the risk of laboratory contamination of samples. The focus of these measures was the spatial separation of PCR set-up, DNA extraction and post-PCR analysis, and the one-way movement of samples and laboratory technicians from PCR set-up areas to DNA extraction stations to the analytical laboratory. All PCR reagents were handled in a DNA-free area with dedicated pipettes and equipment. DNA templates were subsequently added to PCR mastermix aliquots in the DNA area of the laboratory. Biohazard safety cabinets and other equipment dedicated to the analysis of trace DNA samples were used for all DNA extraction work and other standard trace DNA measures, such as the inclusion of negative controls at all stages of preparation, were incorporated as routine.

We used the multiplex PCR fox-diagnostic test developed by Berry *et al.* (2007) for identifying fox DNA present in carnivore scats. That test, which targets mitochondrial DNA, consists of a PCR multiplex that contains i) primers that amplify a 134-bp PCR product from cytochrome B gene in the presence of fox DNA only and ii) universal primers for the 12S ribosomal gene that amplify a PCR product in the presence of any of the candidate mammalian carnivores. DNA was extracted from each sample using the Chelex method described in Berry *et al.* (2007) with the following modifications: 350 μL of supernatant was added to 350 μL of chelex 100 solution (10% in water; BioRad) along with 0·15 mg of proteinase K. Following chelex extraction, 100 μL of each sample was cleaned using the IntronBio PCRquick-spin PCR Product Purification Kit (iNtRON Biotechnology, Seongnam, Korea) according to the manufacturer's instructions and adding 100 μL of isopropanol to the binding buffer/sample mix before addition to the spin column. Elution occurred in 30 μL of elution buffer. The PCR conditions described by Berry *et al.* (2007) were used here; however, bovine serum albumin was excluded from the reaction and the concentration of DNA used as template were not quantified, merely 2 μL of 1 : 10 diluted template was used. At least two no-emplate PCR negatives (2 μL PCR water added instead of DNA) and one positive control (2 μL of known fox DNA template at 25 ng μL^{-1}) were included in every PCR. PCR cycling conditions also followed Berry *et al.* (2007), except that annealing temperature was increased to 60 °C and the number of PCR cycles was increased to 35.

Polymerase chain reaction products were visualized on a 2% TBE gel (1% high-resolution Ultrapure Agarose 1000 [Invitrogen, Grand Island, NY, USA] + 1% general purpose Agarose I [Amresco, Solon, OH, USA]) with 0·02 μL mL^{-1} SYBR Safe DNA gel stain (Invitrogen) run at 110 volts for 25 min. PCR products (5 μL of each) were run in separate wells, with 2 μL of positive control PCR and 2 μL of size standard (Hyperladder II; Bioline, Alexandria, NSW, Australia) run in one well each per row for comparison. For samples that failed to provide an amplified product, the PCR was repeated using undiluted template DNA. Any samples from which fox-indicative cytochrome b (CytB) bands were identified were subsequently investigated (either at 1 : 10 diluted template or else undiluted as appropriate) as follows. The multiplex PCR was repeated for each sample to confirm the result, while three additional PCRs using just the CytB VvF and CytB VvR primers were performed to produce 30 μL of PCR product containing a single band ready for sequencing. To sequence, 25 μL of PCR product was made up to 100 μL with Bioline PCR-grade water and cleaned using the IntronBio PCRquick-spin PCR Product Purification Kit according to above methods.

Samples were sequenced using Beckman Coulter GenomeLab DTCS Quick Start Kit (Beckman Coulter Inc., Indianapolis, IN, USA) at half the manufacturer's recommended volume using 0·5 μM primers and 4–5 μL of CytB PCR product as template DNA. The reaction was cycled through (96 °C for 20 s, 53 °C for 20 s, 60 °C for 2 min) × 40. Samples were cleaned and ethanol precipitated according to the manufacturer's recommendations as outlined in the Beckman Coulter GenomeLab DTCS Quick Start Kit instruction sheet. Immediately prior to analysis, each sample was resuspended in 30 μL formamide. Capillary electrophoresis was performed using the Beckman Coulter CEQ 8000 Genetic Analysis System, and the results were analysed using the Beckman Coulter CEQ 8000 software version 8.0.52. Sequence quality and base calls were checked manually, and sequences were aligned relative to known sequences using SEQUENCHER version 4.9 (Gene Codes). Sequence identities were confirmed by BLASTn analysis (Altschul *et al.* 1997).

FOX HABITAT SUITABILITY MODELLING

Locations of physical evidence of foxes (scats, carcasses, skulls) were used to build a model of fox habitat suitability using a maximum-likelihood method developed for presence-only data (Royle *et al.* 2012) with the 'maxlike' R package (Royle *et al.* 2012). Unlike other popular methods for modelling presence-only data (e.g. maxent - Phillips, Anderson & Schapire 2006), the maximum-likelihood method (hereafter 'maxlike') has been shown to directly estimate the probability of species occurrence (occupancy probability). Occurrence probability is a natural parameter for modelling species distributions and is also a natural summary of habitat suitability (Royle & Dorazio 2008), which is a relevant interpretation when projecting occurrence probability across a landscape using bioclimatic predictors or when the species in question has yet to colonize all available suitable habitat (Gormley *et al.* 2011). The assumptions underlying the maxlike method are that a random sample of presence locations are obtained from the area of interest and that species detection across the area is constant (Royle *et al.* 2012). Fox presence locations ($n = 65$) defined by 'hard' evidence, such as carcasses, skulls or DNA-confirmed scats, were used to build the model. The state of Tasmania was divided up into contiguous 1-km^2 cells (66 456 total cells) with the 65 locations of unique fox evidence assigned to an individual cell. Typically with presence-only data, 'pseudo-absence' data are often used in place of true absence data. These pseudo-absence data (locations where the species has not been observed) are usually randomly sampled from the area of interest (background data). The formulation of the maxlike method specifies the entire set of n locations (cells) in the area of interest as the random quantity and the likelihood are conditioned on the probability of observing a cell x given the species has been observed

($y = 1$) (Royle *et al*. 2012). Hence, the 'data' used in the modelling comprised the entire set of 1-km^2 cells in Tasmania ($n = 66\,456$).

Bioclimatic and biophysical covariates

For each cell, bioclimatic and biophysical variables describing vegetation type, annual rainfall, elevation, aspect and slope were derived from spatial layers held by DPIPWE (Appendix S1, Supporting information). The proportional area of each vegetation class was derived for each cell and used in the analyses. For annual rainfall, elevation, slope and aspect, the mean, standard deviation, minimum and maximum were also derived for each cell. These variables were standardized before analysis. Correlations between each variable were calculated and where any two variables had an absolute correlation coefficient >0·7, one of the variables was removed from further analysis leaving a total of 154 potential variables from which to model fox occurrence.

Owing to the large number of potential covariates available for modelling fox occurrence, we used a bidirectional stepwise procedure for model building using AIC as the criterion for adding or deleting variables to the model. Starting with an intercept, each covariate was added to the model in turn with the covariate that resulted in the largest reduction in AIC retained. This step was then repeated with the remaining variables retaining the next best variable in terms of the model with the lowest AIC value. For each of these forward selection steps, only variables that resulted in a reduction in the model AIC of at least two were considered for inclusion in the model. Once a variable was added to the model following the first forward selection step, we conducted an elimination step by deleting each covariate in turn, excluding the one just added. Any covariate removed that resulted in a change in the model AIC of ≤ 1 was discarded from the model. The low number of presence locations made it necessary to increase the maximum number of iterations in the optimization procedure to 1000 as well as to use the 'BFGS' algorithm in order to obtain convergence of the numerical minimization of the log-likelihood.

Following the construction of the final model from the above, we predicted the occurrence probability values (ψ) for the whole of Tasmania by calculating

$$\text{logit}(\psi_x) = \beta_0 + \beta_i Z_{ix}$$

where $\text{logit}(\psi_x)$ is the log odds of ψ at location x, β_0 is the intercept parameter, β_i are the parameter estimates for each of the i covariates, and Z_{ix} are the values for each of the i covariates at location x. We calculated a 95% confidence interval for ψ_x by first calculating the standard error of $\text{logit}(\psi)$ using the delta method and then back-transforming the endpoints of the interval.

Model performance was assessed by determining how well the model discriminated between suitable and unsuitable habitat over a range of thresholds. For any threshold of habitat suitability, presence locations are either correctly classified as being in suitable habitat ('true positives') or misclassified as being in unsuitable habitat ('false negatives'). To assess performance of the final model as selected by the bidirectional stepwise procedure outlined above, we plotted a receiver operating characteristic (ROC) curve, which compares the model sensitivity (true positives) against the complement of the specificity (false positives) over the entire range of thresholds (Fielding & Bell 1997). For presence-only modelling, there are no absence data and hence, false positives cannot be estimated. However, we adopted the interpretation of Phillips, Anderson & Schapire (2006) by considering the classification problem as distinguishing presence from random, rather than presence from absence. Hence, the area under the ROC curve (AUC) represents the probability that a randomly chosen presence site will be ranked as more suitable than a randomly chosen background site. A model that performs no better than random will have an AUC of 0·5, whereas a model with perfect discrimination would have an AUC of 1. However, for presence-only data, the maximum AUC that can be obtained is actually less than 1 (Phillips, Anderson & Schapire 2006). We divided up the presence data into 'training' and 'testing' data by randomly selecting 25% of the presence records to make up the 'testing' data with the remaining 75% used as the 'training' data and calculated AUC values using the testing data. In addition to AUC, we also calculated the threshold that maximized the sum of the sensitivity and specificity (using fractional predicted area) of the testing data. We used this threshold to delineate the region of Tasmania into 'suitable' and 'unsuitable' habitat. We repeated these calculations on 30 random training and testing subsets of the presence locations (random subsets validation) and aggregated the results.

A problem with the use of AUC as a measure of predictive performance is that the AUC values vary depending on the spatial extent of the background used for inference (Lobo, Jiménez-Valverde & Real 2008). It has been shown that model evaluation statistics, such as AUC, tend to be inflated when absence or background locations are increasingly further away from presence locations (Lobo, Jiménez-Valverde & Real 2008). This phenomenon has been dubbed 'spatial sorting bias' (SSB) (Hijmans 2012). We examined the extent that SSB affected the calculation of AUC for our model and conducted the analysis recommended by Hijmans (2012) to remove it. We again divided up the presence data into 'training' and 'testing' data as described previously and calculated the mean distance between testing-presence locations to the nearest training-presence locations (Dp) and the mean distance of testing-absence locations to the nearest training-presence locations (Da) with the ratio (Dp/Da) used as an estimate of SSB. SSB values close to zero indicate a high degree of spatial sorting bias with a value of SSB = 1 indicating no spatial sorting bias (Hijmans 2012). Hijmans (2012) showed that spatial sorting bias can be removed using pairwise distance sampling to subsample the testing data. Each testing-presence location is paired with the testing-absence location that has the most similar distance to its nearest training-presence location. If the relative difference between the two distances was greater than 33%, the presence location was not used (see Hijmans 2012 for further details). The model evaluation statistic AUC was then re-calculated following the application of pairwise distance sampling. We again repeated the above analysis for 30 random training and testing subsets of the presence locations. We used the functions ssb() and pwdSample() in the R package dismo (Hijmans 2012) under R ver. 2.15.1 (R Development Core Team 2005) to undertake these analyses.

Results

HARD EVIDENCE OF FOX DISTRIBUTION

Deoxyribonucleic acid was extracted from over 9500 scats and other trace samples collected as part of the strategic survey of eastern Tasmania and tactical investigations (Appendix 1). Overall, we successfully amplified PCR

product from 7658 scats (79%) of those samples using our fox DNA test of which 186 exhibited bands that required sequencing, confirming 56 scats as containing fox DNA. A further 47 scats were found to contain fox-like sequence, but could not be conclusively identified as fox (usually through incomplete or poor-quality sequence). These samples were not included in our analysis as positive for fox. The non-fox-amplified product was usually DNA from rabbits or hares, most likely prey DNA contained within the scats.

The distribution of the hard evidence is widespread ranging from the coastal north-west near the town of Burnie through to the far north-east corner and as far south as Cygnet. Many of these sites overlap with areas that show high numbers of sighting reports (unpublished data). Although the majority of the positive samples were collected in 2008 (26) and 2009 (18), a number of the scats and other hard evidence of foxes were collected over a longer period dating back to 2001. Thus, the distribution contains a temporal element. Overall, the distribution of hard evidence shows a broad but patchy distribution that encompasses much of what was originally considered by expert knowledge to be habitat 'highly suitable' to foxes.

FOX HABITAT SUITABILITY MODELLING

The final model as selected by the bidirectional stepwise procedure contained nine covariate values (Table 1). The proportion of the deviance explained by this model was 0·20. Although all variables contributed to reductions in model AIC by at least 2, some variables were estimated with fairly low precision. The variables estimated to be the most important for predicting fox occurrence included agricultural land (FAG), urban environments (FUR, FUM) and *Eucalyptus amygdalina* inland forest and woodland on Cainozoic deposits (DAZ). Other covariates were strongly negatively related to fox occurrence. The predicted habitat suitability for foxes in Tasmania and the corresponding 95% confidence intervals are given in Fig. 2.

The model evaluation statistic AUC calculated from the average of 30 random training and testing partitions of the presence data was equal to 0·93 (SE = 0·008), indicating fairly high predictive performance (Fig. 3a). The threshold that maximized the sum of the sensitivity and specificity (predicted area) was equal to 0·022 (SE = 0·004), suggesting that *c*. 29% of Tasmania represented suitable fox habitat (Fig. 4). However, estimates of average SSB were equal to 0·13 (SE = 0·006), indicating a high degree of spatial sorting bias, and hence, the estimate of AUC was likely to be positively biased. Following subsampling of the presence locations using pairwise distance sampling, the estimate of SSB was 0·93 (SE = 0·049), indicating that spatial sorting bias had been greatly reduced. The corresponding AUC calculated after the subsampling was equal to 0·71 (SE = 0·021), much lower than the uncorrected AUC value (Fig. 3b) but greater than the 0·7 suggested by Hijmans (2012) as representing a 'good' model.

Discussion

DISTRIBUTION OF FOXES

The history of Australia since European settlement is one of well-meant, but often destructive, introductions of exotic vertebrates. Although Tasmania has seen its share of attempted fox introductions (Saunders *et al.* 2006), until now it has been spared this destructive influence. The study presented here makes it clear that one or more introductions of foxes to Tasmania have occurred and that as a consequence, the isolation of Tasmania from this pest can no longer be assumed. Our combination of DNA detection technology, broad-scale systematic survey, ad

Table 1. Parameter estimates for the final maximum-likelihood model ('maxlike') fitted to the 65 locations of confirmed fox presence using a bidirectional stepwise model selection procedure

Vegcode	Title	Estimate	SE	*Z*	*P*
(Intercept)		−6·05	0·747	−8·103	<0·001
FAG	Agricultural land	1·77	0·593	2·990	0·003
FUR	Urban areas	218·3	109·87	1·987	0·047
MEAN_PREC	Mean annual precipitation (mm)	−2·68	0·754	−3·550	<0·001
DAZ	*Eucalyptus amygdalina* inland forest and woodland on Cainozoic deposits	14·7	9·00	1·632	0·10
FUM	Extra-urban miscellaneous	17·2	8·04	2·139	0·033
DTO	*Eucalyptus tenuiramis* forest and woodland on sediments	−276·2	1177·1	−0·235	0·81
DAS	*Eucalyptus amygdalina* forest and woodland on sandstone	−267·6	158·8	−1·685	0·092
NAL	*Allocasuarina littoralis* forest	109·5	98·5	1·112	0·27
DAM	*Eucalyptus amygdalina* forest and woodland on mudstone	−319·7	196·5	−1·627	0·10

SE, standard error; *Z*, Wald statistic; *P*, probability.

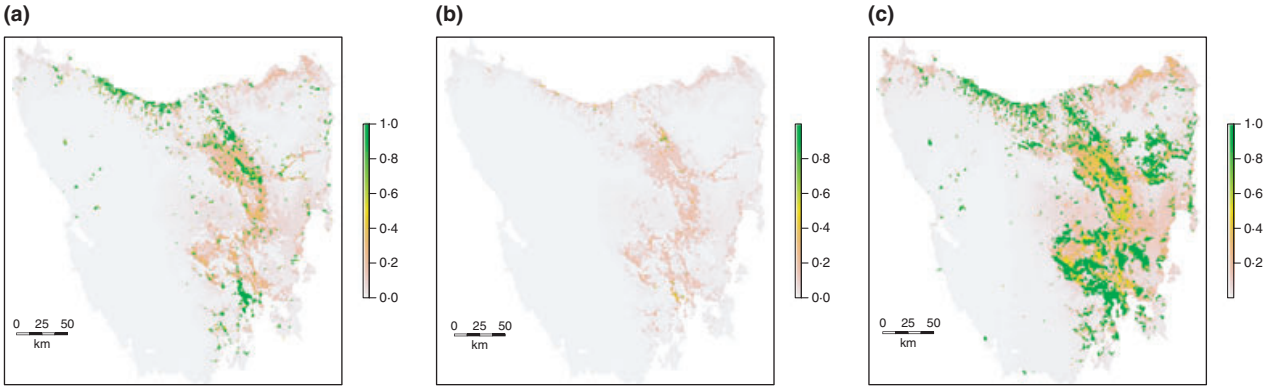

Fig. 2. Predicted values of fox occupancy probability (habitat suitability) for Tasmania using the maximum-likelihood method 'maxlike' from the final model fitted to 65 locations of confirmed fox presence (a) and the lower (b) and upper (c) 95% confidence limits of the predictions.

hoc on-ground investigations and habitat modelling approaches provides a clear view of this hitherto intractable problem of international conservation significance. We have shown that foxes are widespread, although possibly in disjunct centres of activity, but appear not to have yet expanded into their full potential range and conclude that if eradication is to be prosecuted effectively, then control action needs to be swift and broad-scale.

Our data confirm that foxes are present in the central north, midlands and in isolated areas of the north-east and south-east of Tasmania. Although widespread, the data may represent a distribution that is fragmented into eight clusters near the locales of Burnie, Devonport, Longford, the Midlands and Gladstone, as well as isolated sites near Hobart, Cygnet (south of Hobart), and Seymour on the central east coast. To some extent, this fragmented distribution is consistent with what is believed to be the recent history of the introduction of foxes to Tasmania. Two litters of 11 fox cubs were reported to have been deliberately introduced into Tasmania in 1999 and released in three areas: Longford/Cressy, an unknown site on the east coast (possibly St Helens) and Oatlands (Saunders *et al.* 2006). In addition, a fox was seen leaving a ship in the docks at Burnie in 1998. Burnie and Devonport are the two most important ports for cargo and passengers from mainland Australia and are therefore the most likely points of accidental introduction for foxes and therefore establishment of fox populations. Our data are consistent with these scenarios suggesting as they do, a concentration of hard evidence for foxes in northern Tasmania centred on these two ports, and in the midlands where two of the three deliberate release sites are believed to have occurred. Presence on the north-eastern coast (St Helens) is consistent with a release site in this area.

The absolute number of foxes represented by our findings is unclear. DNA profiles have been obtained from 18 sources (scats, carcasses, skull) (MacDonald, Berry and Sarre unpublished data) with many scats not providing sufficient quality DNA to genotype at microsatellite loci. The genotypes so far obtained have been shown to represent individual genotypes, suggesting a minimum of 18 individuals present in Tasmania over the past 4 years. These include both males (sry - male-specific gene present) and females (sry absent). These data also suggest that the likelihood that two scats identified as fox will be from the same individual is low. This is most likely a function of large home ranges, high rates of scat degradation and low detection probability causing a low density of a specific individual's scats in any given area. The life expectancy of a fox scat in Tasmania is unknown, but it is likely to vary

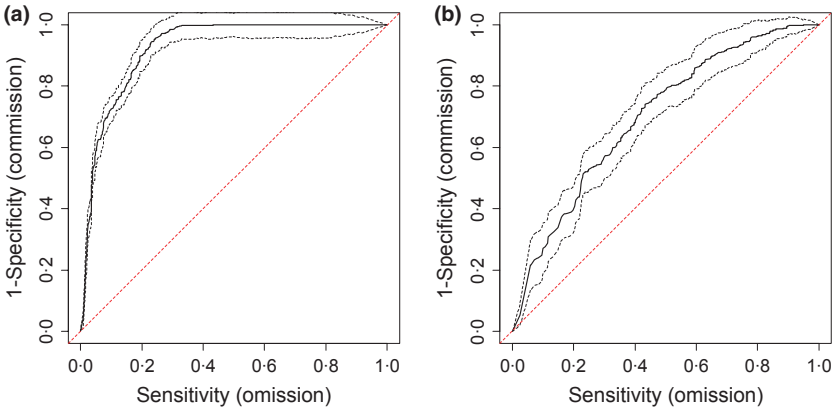

Fig. 3. Receiver operating characteristic (ROC) curve for varying thresholds of habitat suitability from (a) 30 random training and testing partitions of the original presence locations and (b) 30 random training and testing partitions of the presence locations following pairwise distance sampling to remove spatial sorting bias (see text for details).

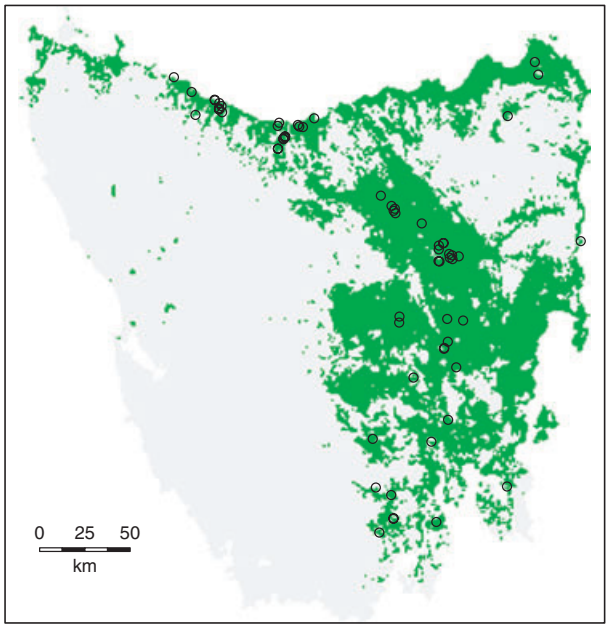

Fig. 4. Predictions of 'suitable' fox habitat using a threshold of predicted fox occupancy that maximized the sum of the sensitivity and specificity (using fractional predicted area) of the test locations (see text for details). Black circles are the fox presence data.

substantially with season, geography and among habitat type.

FOX HABITAT SUITABILITY

The predictions of habitat suitability developed here provide the best understanding yet of the regions and habitats most likely to be favoured by foxes as they expand their range into Tasmania. As such, these data should underpin eradication planning as well as the quantification of risks to native species. Decision support systems developed from these data will be crucial for determining the feasibility and costs of eradication and evaluating which alternative strategies such as containment or control are the most appropriate. Key variables as identified from the habitat modelling indicated that urban and fragmented agricultural environments are likely to be important determinants governing the distribution of foxes in Tasmania. These relationships are similar to the findings for foxes in mainland Australia as urban and agricultural landscapes provide a wide array of food, cover and den sites for foxes (Saunders *et al*. 1995).

The habitat model presented here using our chosen threshold predicts that approximately one-third of Tasmania is likely to be favoured by foxes. This threshold had a false-negative error rate of 4%. For invasive species, it is usually desirable to minimize false-negative errors as misclassifying suitable areas is likely to be more costly than misclassifying unsuitable areas (Gormley *et al*. 2011). Model evaluation indicated that, following correction for spatial autocorrelation of the presence locations, the model was still a useful predictor of habitat suitability, with a corrected AUC of 0·71. Hence, we believe our model can provide an important basis for prioritizing areas of Tasmania for fox management and the design of future monitoring programmes.

While the development of a habitat suitability model is an important step in quantifying the fox problem, it is important to realize that it is unlikely that we have a full picture of fox distribution in Tasmania. The relatively recent nature of the introduction means that fox distributions are likely to be dynamic and not at equilibrium in distribution or density. Thus, the picture represented by the distribution of positive scats and other hard evidence for fox is unlikely to provide a fully representative view of current fox distribution in Tasmania. We have the consummate predator released into a landscape that has abundant prey, few, if any, natural predators and very low numbers of conspecifics. The behaviour of foxes under such circumstances is unknown, but is likely to include home ranges and dispersal distances larger than those exhibited by foxes in established populations. Hence, accounting for dispersal and colonization processes (e.g. Václavík, Kupfer & Meentemeyer 2012) may be an important component in refining distribution models of an invading species such as the model presented here for foxes in Tasmania.

IMPLICATIONS FOR FOX ERADICATION IN TASMANIA

Our data demonstrate that DNA detection combined with systematic survey can provide the understanding necessary to detect a cryptic invasive carnivore that is rare, yet widespread. The development of DNA approaches enabled the broad-scale identification of the target species, foxes, from otherwise indistinguishable samples and allowed scat collection to become the preferred method of detection, with scats one of the few identifiable traces left in the landscape by foxes, and indeed most predators. Before this, there really was no way to collect presence data systematically. Public sightings are heavily biased towards areas of high human activity and provide little certainty in most circumstances as most putative fox sightings occur at night along major highways.

Our approach highlights the importance of early and pre-emptive surveys of recently established, and therefore rare, invasive species and the necessity of providing a sound and defensible approach to determining distribution. Our data highlight the need for rapid and multiple surveys for presence following incursion. The likely dynamic nature of the fox incursion emphasizes the need for a clear understanding of any distributional changes. The sheer size of Tasmania, the amount and broad distribution of habitat suitable for foxes and the initial absence of any suitable approach to sampling have prevented the rapid temporal assessment of fox distributions, yet it is this information that will determine where and when to

undertake control action and enable the assessment of the effectiveness of that action.

The enormous scale and breadth of the distribution of fox scats revealed by our analyses indicate that fox eradication processes will need to be implemented on a very wide scale if they are to succeed. Indeed, a recent external review of the Fox Eradication Program (Parkes & Anderson 2009) recommended a change from the tactical approach currently in place to that of a pre-emptive approach where baiting is rolled out across all suitable habitat on the island. We would go further than that. Our data suggest that foxes could be on the verge of becoming established irreversibly in Tasmania. Given their apparent widespread distribution, the moment may even already have passed for a feasible eradication although we do not suggest that now is the time to stop. Under the current planning and funding, a single baiting of all highly suitable fox habitat will take 5 years. We suggest that given the widespread fox distribution revealed here, such a timeframe will result in failure. Rather, we suggest that a massive upscaling of effort and perhaps more focused approach is going to be required to maximize the chances of a successful eradication. Otherwise, Australia stands on the precipice of a third major wave of mammalian extinctions – this time focused on the island of Tasmania.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Appendix S1. Bioclimatic and biophysical variables used in the fox habitat modelling.