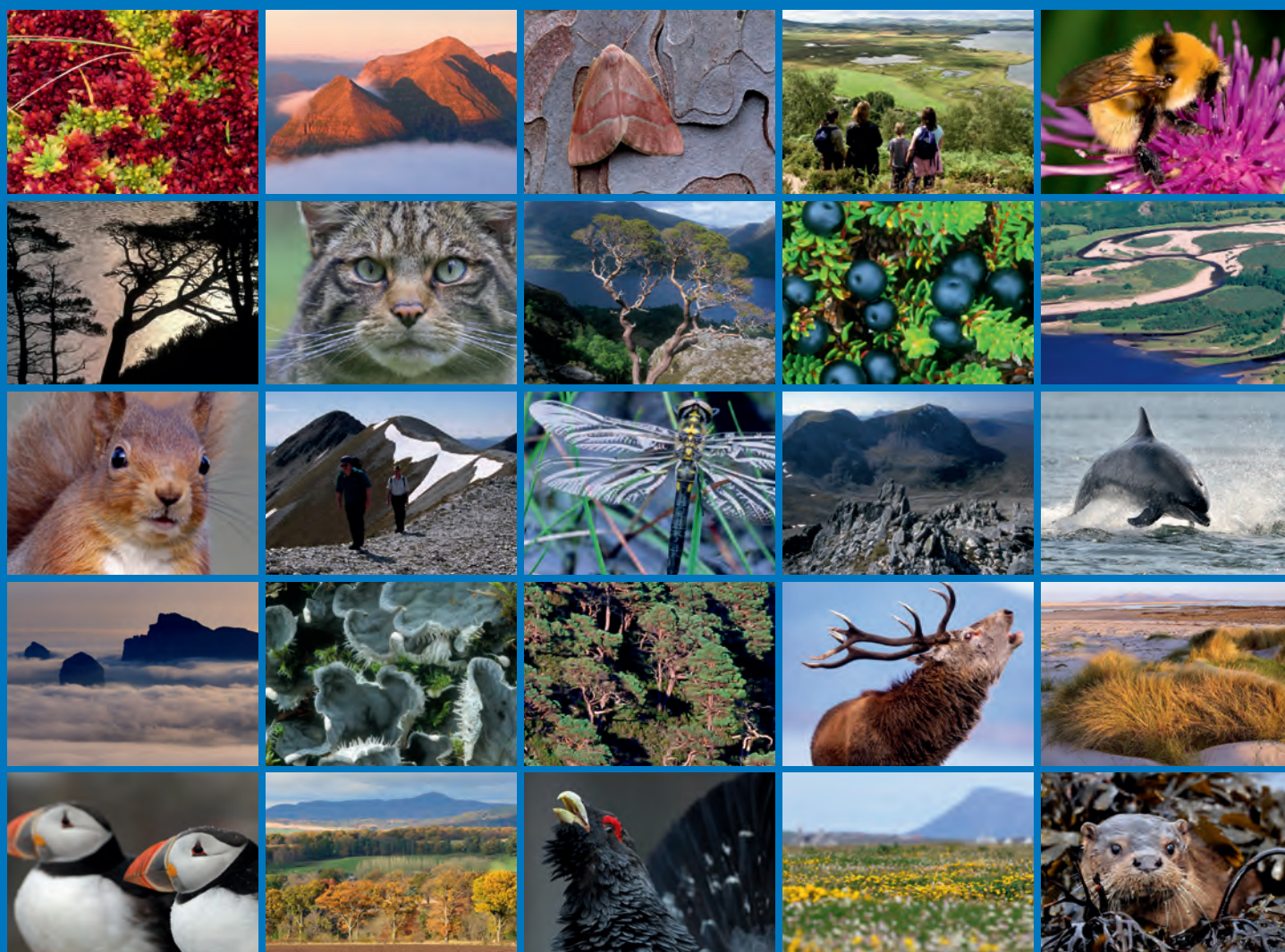


Expansion zone survey of pine marten (*Martes martes*) distribution in Scotland





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COMMISSIONED REPORT

Commissioned Report No. 520

Expansion zone survey of pine marten (*Martes martes*) distribution in Scotland

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COMMISSIONED REPORT

Summary

Expansion zone survey of pine marten (*Martes martes*) distribution in Scotland

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Background

This report arises from a partnership project involving the Vincent Wildlife Trust and Scottish Natural Heritage. The aim of this field-based survey was to gather new information on the changing distribution of the pine marten in 2012. Given the anecdotal evidence of the species' continuing re-colonisation of Scotland following its historical decline, this survey concentrated upon an 'Expansion Zone' beyond the pine marten's known range revealed by surveys in the 1980s (Velander 1983) and 1990s (Balharry *et al.* 1996).

The Expansion Zone targeted for survey in 2012 lies predominantly to the south and east of the pine marten's mainly Highland range identified in the 1990s by Balharry *et al.* (1996), and covers parts of eastern Caithness, Moray, Aberdeenshire, Angus, mid and southern Argyll, Perthshire, Stirlingshire and the Trossachs, Fife and Dumfries and Galloway. The main survey method involved experienced teams searching for pine marten scats (faeces) along 1km transects during May to August; molecular techniques were used to extract DNA from scats to confirm their species of origin. Collation of recent records of pine martens within the Expansion Zone significantly reduced the area covered by the field survey.

Main findings

- Verified recent pine marten records were gathered from 61 hectads within the Expansion Zone; these records were provided by a variety of recording organisations, land managers, researchers and naturalists.
- The presence of pine martens was confirmed via DNA from scats in 54 (25%) of the 220 hectads surveyed during the field survey.
- The survey was hampered by the unusually high proportion (48%) of scats from which identifiable DNA could not be extracted (the exceptionally heavy rainfall over much of Scotland in summer 2012 is one likely explanation); this affected 41 hectads (19%) in which experienced surveyors believed pine marten scats were collected but DNA confirmation was lacking. In most cases these 'probable positive' hectads lay adjacent to a 'DNA positive' hectad.
- In 108 hectads (49%) no possible pine marten scats were found. These are likely to include a significant proportion (>20%) of 'false negatives' arising because the survey's relatively low sampling frequency failed to detect the sparse presence of pine martens towards the fringes of the species' range, as observed in a previous survey in 1983.
- Pine martens have spread north into Sutherland and Caithness. There has been considerable spread eastwards from the Great Glen into Moray, Deeside and elsewhere

in Aberdeenshire. The range expansion has continued through Perthshire, Tayside and the Trossachs and martens have re-colonised much of Stirlingshire and limited parts of western Angus and Fife. Martens have spread south through Argyll onto the Kintyre and Cowal Peninsulas and are now present in some hectads in the Central Belt. The expansion in the Galloway Forest has been limited compared with that in the core marten range; only restricted spread has occurred from Glen Trool Forest, where martens were reintroduced in the early 1980s. Records collected in addition to the field survey indicate that martens have continued their natural colonisation of Skye and are now present on Mull due to translocation.

- Although the pine marten has become more widespread in Scotland since the last survey, in many areas field signs of the species were sparse; this may indicate incomplete re-establishment or limitations arising from the methodology.

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1. INTRODUCTION

1.1 Background

The pine marten *Martes martes* is one of six members of the weasel family native to Scotland. Its exceptional climbing ability is an adaptation to life in its preferred woodland habitat, where it consumes a wide variety of food from beetles and fruit to rodents, birds and carrion. Because of its recent scarcity, the pine marten is fully protected under UK and Scottish wildlife legislation.

Anthropogenic pressures have caused substantial historical changes in pine marten distribution and abundance in Scotland. It is suggested that, once stable woodland cover was fully established across the country in the Mesolithic, the pine marten was then one of the most numerous and widespread carnivores (Maroo & Yalden, 2000). In the Neolithic, clearance of woodland by humans for agriculture signalled the start of a hugely influential loss of pine marten habitat. Being a woodland-adapted mammal, such clearance was probably the major driver of the pine marten's decline initially, exacerbated latterly by culling associated with trapping for fur and predator control by gamekeepers (Langley & Yalden, 1977). At its distributional nadir in the early 1900s, when woodland cover was also at or close to a minimum of <5% of the land area, the pine marten in Scotland was largely confined to parts of the sparsely populated north-west Highlands. Here, in the near-absence of its preferred woodland habitat across Scotland, this rocky, mountainous landscape provided the alternative three-dimensional habitat features that enabled the pine marten to survive (Webster, 2001). It is suggested that this 'retreat to the Highlands' was more a consequence of active habitat selection at a time of low woodland cover, rather than a response to human persecution at the time (Birks & Messenger, 2010).

In the early 1900s the north-west Highlands of Scotland, one of three main British refugia identified by Langley and Yalden (1977), represented the pine marten's largest mainland stronghold; an estimated 1,500 animals survived here compared with 400 in northern England and 100 in Wales (Birks & Messenger, 2010). Subsequently, reforestation and reductions in culling pressure led to a slow recovery and range expansion by the pine marten in Scotland through the 20th century; full legal protection for the species in 1988 is likely to have assisted this process latterly.

While evidence of pine martens in England and Wales persisted in a few core areas through the 20th century and into the new millennium, the animal has remained rare here and there has been no convincing evidence of natural recovery south of the Scottish border (Strachan *et al.*, 1996; Birks & Messenger, 2010). Furthermore, recent genetic analyses indicate that the original relict genetic stock of pine martens once found in England and Wales has probably been lost, with wild-living individuals apparently represented mainly by animals translocated from Scotland, or the descendants thereof (Jordan *et al.*, 2012). Thus, today Scotland holds the only truly relict pine marten population in Britain that is recovering naturally from a severe historical decline.

1.2 Survey aims

The aim of the Expansion Zone Survey was to provide reliable information on current patterns of pine marten distribution beyond the species' range previously recorded in surveys undertaken in Scotland during the 20th century. This included comparisons with the findings of these previous surveys, reviewed below.

1.3 Review of previous surveys

1.3.1 Lockie (1964)

The early stages of the pine marten's recovery in Scotland were reported by Lockie (1964). In about 1926, with the marten's highly restricted main Scottish distribution at that time extending from north Sutherland to Moidart, there was the first reported increase in marten numbers on estates in north-west Sutherland, with a modest south-eastward range expansion apparent from the 1930s and beyond. By 1946 pine martens were recorded at various locations on the north side of Loch Ness, and Lockie observed that here the waters of the loch and river system in the Caledonian Canal represented a partial barrier to further south-eastward range expansion. By the early 1960s, however, pine martens were clearly established at sites south of the Caledonian Canal. Lockie attributed this modest range expansion to reductions in trapping pressure during and following the First World War of 1914-18, rather than to any increase in woodland cover. It was apparent that trapping remained a major cause of marten mortality, leading one or two individuals to urge restraint in the interests of conservation. Lockie (1964) felt certain that reafforestation had not contributed to the marten's initial recovery, because no such landscape change had affected north-west Sutherland at the time.

Following Lockie's (1964) suggestion of a possible relationship between the number of scats (faeces) found on paths and the abundance of martens at Beinn Eighe National Nature Reserve, studies in Scotland have been influential in the evolution of a standard survey method for detecting pine martens. The 'scat survey' method, involving standardised searches of selected transects for distinctive pine marten scats, was developed and tested in Scotland through status assessments at various scales (reviewed by Birks *et al.*, 2004). However, concerns have been raised about the untested assumptions upon which this method is based and the interpretation of survey findings (Birks *et al.*, 2004). Notably, concerns about surveyors' ability to identify marten scats in the field led to a study in Galloway Forest in 1999-2000 (where pine martens were reintroduced in the early 1980s; Shaw & Livingstone, 1994) in which surveyors' judgements were tested by DNA typing of all 'marten' scats collected (Davison *et al.*, 2002). Experienced marten surveyors were found to misidentify up to 29% of scats, with those of the red fox *Vulpes vulpes* most frequently misidentified as being from pine martens. Thereafter, DNA typing of all scats became standard practice in scat-based marten surveys, with a distribution survey in Ireland being the first to apply this new level of rigour on a wide scale (O'Mahony *et al.* 2012). Davison *et al.*'s (2002) scat verification study revealed that the scat misidentification rate was very much higher in those areas, such as England and Wales, where pine martens are very scarce and surveyors typically struggle to find field signs of the species.

1.3.2 Velandar (1983)

Velandar (1983) undertook the first wide-scale distribution survey of pine martens in Britain over an 18 month period in 1980-1982. Her main method involved searching for marten scats along survey routes of 0.7 to 2.0km on selected tracks and paths through suitable marten habitat (95% of surveys were based on routes of ≥ 1 km). Additional methods involved interviews with local naturalists and appeals for records of dead martens. In Scotland, this survey focused on 273 hectads (10km x 10km squares of the Ordnance Survey national grid) within or close to the pine marten's core range in the north-west Highlands, where a total of 155 hectads were positive on the basis of scats (77 hectads), interviews (57 hectads) and dead martens (21 hectads). This survey confirmed that Britain's pine marten population was still mainly confined to the Scottish Highlands, with range expansion apparent but also local extinctions occurring. Velandar made reference to game-keeping activities being the major cause of known pine marten mortality in Scotland at the time of her survey.

It is important to note that Velander's (1983) survey was not based on the verification of scat identity by modern DNA techniques. Nevertheless, the fact that Velander used two other corroborative sources of evidence to confirm marten presence in target hectads is likely to reduce the risk of errors arising from scat misidentification. In her survey report, Velander (1983) considers a range of factors that might influence the abundance and 'detectability' of pine marten scats on survey transects, such as surveyor skill, weather, transect condition and seasonal variations in marten home range use. She further suggested that some transects may lie in gaps between marten home ranges, so no scats may be found despite the presence of martens in the locality. In a later study of pine marten ecology in Inverness-shire, Velander (1986) reported that pine marten scat densities on forest transects showed seasonal variations of >100-fold.

In comparison with recent recommendations, the density of sites surveyed for scats by Velander (1983) in Scotland was relatively low, with a mean of one transect per hectad (Cresswell *et al.*, 2012 suggest a minimum of five 1-2km transects per hectad, albeit in connection with development-related status assessments rather than wide-scale surveys). Notably, Velander's survey revealed the significant risk of falsely inferring pine marten absence on the basis of low density scat surveys alone: she found that 33 hectads in Scotland yielded no scats on the transects surveyed, but were otherwise positive for martens on the basis of carcasses or reliable sightings. Many of these 'false negatives' were in hectads peripheral to the marten's main population in the north-west Highlands, and Velander suggests that this was due to problems associated with finding field evidence of martens where they were present in low numbers while they were establishing populations in new areas. This caveat is clearly relevant to the current Expansion Zone Survey in Scotland, where a similar transect density was used at the periphery of the marten's range, and so a comparable risk of false negatives may arise.

1.3.3 Balharry *et al.* (1996)

This was the second wide-scale survey in Scotland based upon systematic searches for pine marten scats. It aimed to map the apparently expanding area of marten distribution to the south and east of that identified by Velander (1983). Additional aims were to establish thresholds of marten scat density that could be used to define areas supporting resident breeding marten populations; to use this information to distinguish between areas supporting resident/breeding populations and those supporting dispersing/pre-established martens; and to compare the new distribution with that of Velander (1983) in order to estimate the rate of re-colonisation.

The survey was based upon 'search areas' of variable size defined and spaced on the basis of the distribution of woodland habitat at approximately 20km intervals within suitable habitat beyond the limits of marten distribution as defined by Velander (1983). Within each search area four 1km transects along woodland paths or tracks were searched for marten scats; fox scats were also collected. Care was taken to collect only those scats that were obviously from martens, using the morphology and unique smell of marten scats as a guide. As in Velander's (1983) survey, DNA typing to confirm scat identity was not available during this survey.

A minimum density of marten scats (accumulated over at least 8 weeks) associated with a known breeding population was derived from Balharry's (1993) radio-tracking study in Wester Ross. Mean scat density on 95 1km transects searched between February and September in Wester Ross varied between 2.88 (September) and 6.22 (February). This was compared with scat density data from seven other areas in Scotland known to support breeding pine martens in the 1990s (mean scat abundance 2.75-9.0 scats per 1km transect). Based on the 95% confidence level that, where martens were established (i.e. breeding), at least 7 scats would be found if 4km of track was searched (or at least 4 scats if only 3km

was searched), this threshold was used to separate the survey area into three groups: Group 1 – scat densities indicating established populations; Group 2 - lower scat densities indicating dispersing or transient martens; and Group 3 – no marten scats found.

The broad conclusions of the Balharry *et al.* (1996) survey were as follows: *“marten have now re-colonised some of the northern and western parts of Grampian, Tayside, Central and Strathclyde regions, and are thus no longer confined to the Highlands. To the south, marten have become established along the side of Loch Awe, in Glen Dochart and possibly Strathyre. In central Scotland the spread eastwards extends to the glens of Loch Tay, Tummel and Rannoch. However, the spread into the north-east has not been so extensive, and the distribution is similar to that found in 1982.”*

Considering the pine marten population established in south-west Scotland via a reintroduction in the early 1980s (Shaw and Livingstone, 1994), the Balharry *et al.* (1996) report concluded that a small but viable population was present, with all recent breeding records occurring within a 12km radius of Glen Trol Forest. On the basis of scat counts it was considered unlikely that martens were established outside Glen Trol Forest.

Balharry *et al.* (1996) drew attention to the greater difficulty martens had apparently encountered in re-colonising north-east Scotland compared with areas further south. They specifically identified the area east and south-east of Inverness where *“marten appear to be having difficulty getting established”* as indicated both by the limited distance covered since 1982 and by the number of survey areas supporting transient martens only as opposed to established populations, as indicated by scat counts. The authors speculate that these differences may have been an artefact of methodological flaws; or genuine results that reflected either differences in habitat suitability or the greater intensity of predator control in the north-east of Scotland compared with other areas more readily re-colonised by pine martens.

Balharry *et al.* (1996) used the figure of 250 ha of woodland per breeding pair of pine martens (from Balharry, 1993) to calculate pine marten population estimates for both the Velandar (1983) and Balharry *et al.* (1996) surveys. This produced estimates of 1,200 martens for 1982 and 2,600 in 1994, suggesting that the population had approximately doubled in the 12 years between the two surveys. Considering the greater extent of woodland available in Scotland beyond the limits of the pine marten's established range in 1994, Balharry *et al.* (1996) estimated that the population was less than one third of its potential size at that time. The authors concluded with a warning that *“if the current re-colonisation of Scotland by marten is to be encouraged, then it may become necessary to examine the issue of predator control in areas on the periphery of the current marten distribution.”*

2. METHODS

2.1 Definition of Expansion Zone Survey area

There is abundant anecdotal evidence demonstrating that the pine marten's distribution in Scotland expanded in the second half of the 20th century and into the 21st century. Anecdotal records indicate that the species' range had spread considerably from the Highland stronghold identified in the previous surveys. The targeted Expansion Zone Survey area was broadly defined as those hectads (10km x 10km squares of the national Ordnance Survey grid) located beyond the previously recorded pine marten range identified in the last systematic pine marten distribution survey (Balharry *et al.*, 1996) (see Figure 1). The targeted area was intended to extend slightly beyond the periphery of existing marten records. This area is predominantly south and east of the known 1990s pine marten Highland range, and covers parts of eastern Caithness, Moray, Aberdeenshire, Angus, mid and southern Argyll, Perthshire, Stirlingshire and the Trossachs, Fife and much of Dumfries and Galloway (approximately 324 hectads, see Figure 2 and Table 1). An extensive area in Dumfries and Galloway was targeted in view of the expected expansion of the population that was reintroduced in the early 1980s (Shaw & Livingstone, 1994). The rest of southern Scotland, south of the Central Belt, was excluded from the survey as available information suggested that there was unlikely to be an established marten population in this area.

The survey did not include those hectads that were negative during the 1994 distribution survey (i.e. those in Sutherland, Ross-shire, mid and north Argyll and eastern Perthshire). As there is abundant anecdotal evidence that pine martens are now present to the south and east of these areas, it is assumed that previously negative hectads within the 'core' marten range can be deemed positive.

Although there is recent evidence of pine marten establishing populations on Skye (R. Cottis, pers. comm.) and Mull, these areas were not included in the expansion zone survey, due to resource constraints.

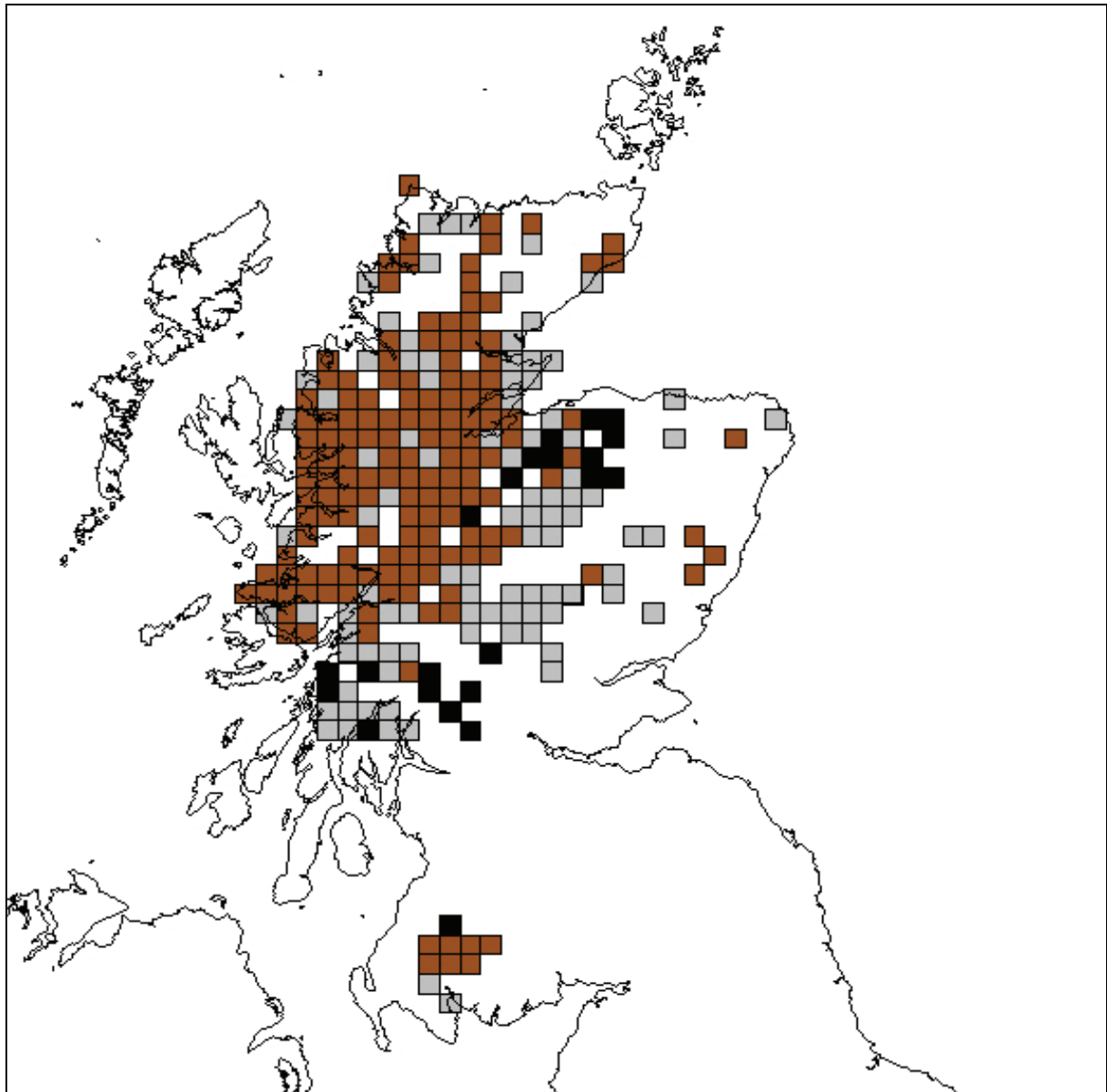


Figure 1. Hectads positive for pine marten during distribution surveys in 1980-1982 (Velandar, 1983) (hectads shaded brown) and in 1994 by field survey (hectads shaded black) and questionnaire (hectads shaded grey) (Balharry et al., 1996). Based upon Ordnance Survey material with the permission of the Controller of HMSO © Crown Copyright (2013) Licence no. 100017908.

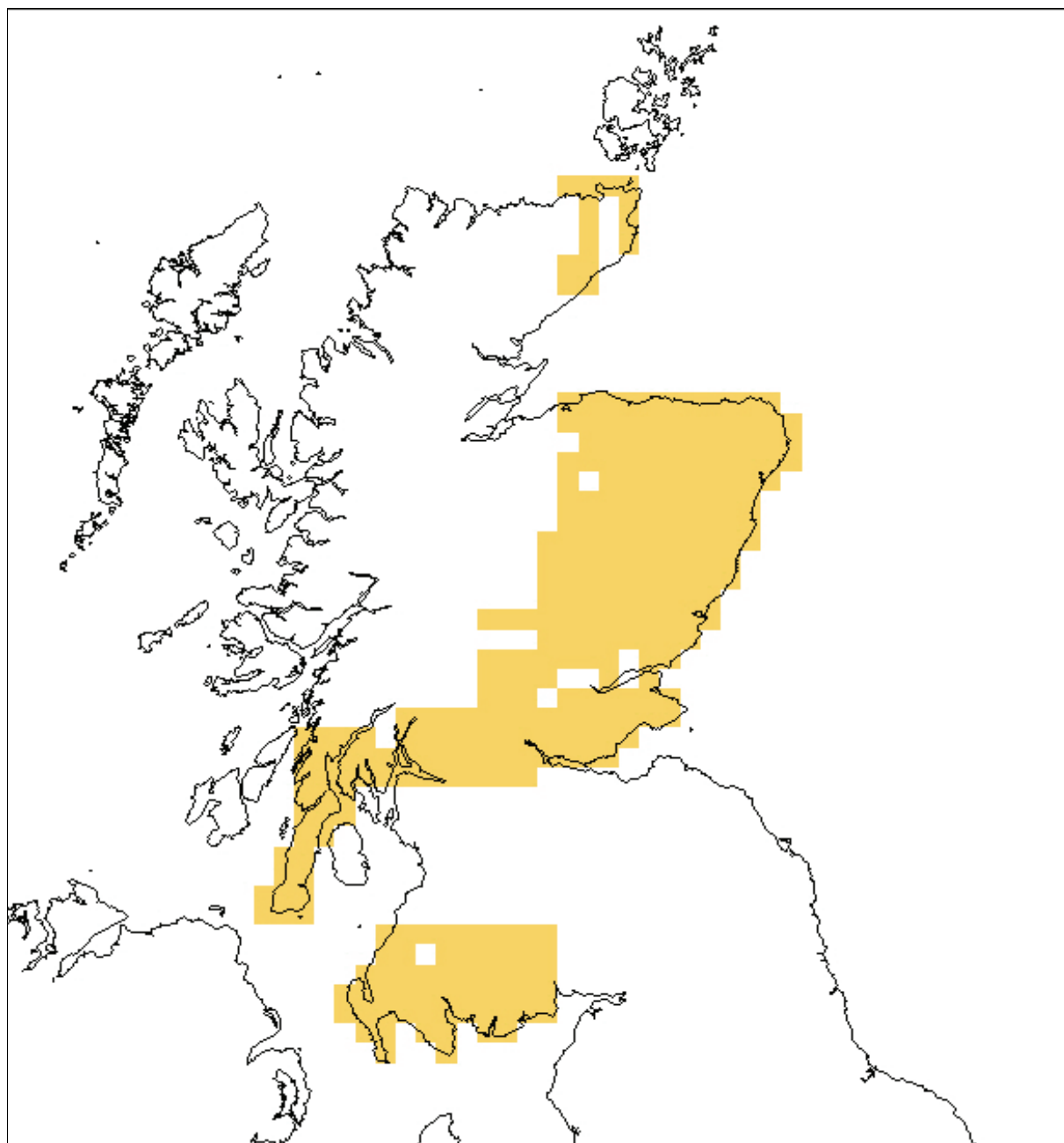


Figure 2. The Expansion Zone Survey area. Based upon Ordnance Survey material with the permission of the Controller of HMSO © Crown Copyright (2013) Licence no. 100017908.

2.2 Collection of recent records

In order to define the Expansion Zone Survey area, verified records of pine martens collected post-1999 were gathered from a variety of organisations and conservation projects. These records consisted of DNA-typed scats and hairs, photographs, road kills and sightings, collected from Local Biological Record Centres, Forestry Commission Scotland, the Scottish Beaver Trial, universities, researchers and naturalists. Sightings used were deemed verified if they had been validated by Local Biological Record Centres and/or were from experienced, reliable observers. In addition, pine marten records were extracted from the recently published Atlas of Highland Land Mammals (Scott, 2011). These confirmed the continuing presence of pine martens in the Highlands and indicated some recent expansion into Sutherland and Caithness and south-east of the Great Glen.

These pre-existing records were used to identify hectads that were already confirmed as positive for pine marten and were therefore not included in the field survey in order to optimise resource efficiency.

2.3 Scat survey methodology

2.3.1 Hectad exclusion

Thirty-one hectads within the Expansion Zone Survey area had insufficient pine marten habitat for the survey methodology to be deployed, thus these hectads were excluded from the field survey. Hectads excluded included those in which woodland was scarce or absent, or those dominated by urban, suburban, mountainous and coastal land types. In hectads where woodland was present but no tracks were shown on the map, surveyors 'ground-truthed' the woodland to establish whether any tracks were present for the field survey.

2.3.2 Transect selection

The survey methodology was based on The Vincent Wildlife Trust's baseline water vole survey of Britain (Strachan & Jefferies, 1993), which was subsequently promoted with minor modifications by Macdonald *et al.* (1998). In relation to monitoring pine martens, Macdonald *et al.* (1998) recommend 'standardised 1km transect searches for field signs along tracks, paths and field boundaries.' This transect approach has recently been applied in a survey of pine marten distribution in Ireland (O'Mahony *et al.*, 2012.).

The density of transects selected for surveys is usually a compromise between survey objectives and resourcing constraints. New guidance on presence/absence surveys for pine martens in relation to Environmental Impact Assessment and development proposals recommends a minimum of five transects per hectad (Cresswell *et al.*, 2012). However, this relatively high density is costly and labour-intensive unjustifiable in wide-scale distribution surveys where many hectads need to be surveyed; for example, a recent wide-scale pine marten survey in Ireland was based upon three transects per hectad (O'Mahony *et al.*, 2012). In the current survey, one transect was selected in each hectad, in line with the approach adopted by Velander (1983) and in order to fit within resourcing constraints; it is recognised that this approach might lead to an increased risk of 'false negative' hectads (see section 2.5 below).

2.3.2.1 Targeting of suitable woodland

Transect selection operated at two levels: targeting of suitable woodland blocks for survey and selection of transect routes within targeted woodland blocks. Within each hectad the most extensive woodland block was targeted for transect selection; in hectads with low and/or fragmented woodland cover, transects were located in the largest available woodland.

2.3.2.2 Selection of transect routes

Transects were preferentially routed along forest tracks or footpaths in extensive woodland with little vehicle use and reasonable access for surveyors (i.e. close proximity to a public road) (see Plate 1). Each transect was separated from transects in adjacent hectads by at least 5km where practicable, in order to reduce the chances of collecting scats from the same animal in adjacent hectads.

Transects were mapped onto Ordnance Survey maps using MapInfo Professional 11.5. Each transect was allocated a unique identifier number (UID) matched to a 10 figure National Grid Reference which corresponded to the starting point of the 1km transect.

Plate 1. Examples of transects used for scat surveying



Plate 1a. Transect in Angus (© E. Croose)



Plate 1b. Transect in the Trossachs (© H. Macmillan)

2.3.3 Scat survey approach

Scat surveys were undertaken in 2012 between May and August, as previous fieldwork has indicated that pine marten scats are likely to be most abundant in the summer months (e.g. Velander, 1986; Cresswell *et al.*, 2012) and the weather is typically more favourable for fieldwork.

Experienced surveyors walked each transect for 1km, measured using a GPS unit, and searched for and collected possible pine marten scats. The number of scats collected per transect varied in relation to the number of possible marten scats found and their likelihood of yielding DNA in the judgement of surveyors. For example, where several possible marten scats of various ages and states of deterioration were found, only the freshest were collected; conversely, where only one or two scats were found, all were collected. Furthermore, in areas where possible marten field signs were very scarce an inclusive approach to scat collection was adopted, with surveyors collecting any scats that could not confidently be ruled out as coming from other species. This inclusive approach was intended to maximise the likelihood of encountering marten scats; an inevitable consequence was that, compared with sites where marten scats were frequently encountered, it was more likely to result in collection of some scats from other species.

Seven teams, comprising a total of twelve surveyors, were used in the Expansion Zone Survey; all were either staff of the VWT and/or experienced volunteers (most of whom were professional conservationists or ecologists) and contractors with extensive experience of marten scat surveys in Britain and Europe. Surveyors worked individually or in pairs; each transect was searched for scats on the outward and return journeys. An average of five transects per team were surveyed per day. Two surveyors used scat detection dogs to assist in finding marten scats on transects (see section 2.3.3.3 below).

2.3.3.1 Scat collection

Scats were lifted from the transect surface by using clean wooden 'lolly' sticks (small hard wooden sticks, measuring 11cm long by 4cm wide), which were used once only to avoid contamination of DNA between scats. Each scat was placed into a separate plastic zip-locked sample bag with a unique identification number. This scat collection technique followed that used by O'Mahony *et al.* (2012) in their marten survey of Ireland where genetic verification was also involved.

Each scat collected was assigned a unique identification number which corresponded to the number on the sample bag. For each scat, a 10 figure grid reference, habitat code and description of the scat were recorded on a survey sheet. For each transect, regardless of whether marten scats were found, surveyors also recorded other key variables, including woodland habitat type and structure, weather and the number of fox scats seen.

After collection, all possible marten scats were frozen in order to preserve DNA and subsequently posted to Waterford Institute of Technology for genetic analysis.

2.3.3.2 Transect alteration

When a survey team arrived at a pre-selected transect and found it to be either unsuitable for the field survey (e.g. the track was overgrown or heavily-used by vehicles) or inaccessible (e.g. due to harvesting operations or private access restrictions), another transect was selected within the same hectad, where available. In hectads where woodland was sparse and no other suitable transects were present, the hectad was not surveyed.

2.3.3.3 Use of scat detection dogs

The practice of involving scat detection dogs in wildlife surveys to maximise detection success is well-established (Wasser *et al.*, 2004). By harnessing the olfactory searching ability, high play drive and reward-based motivation of certain breeds, dogs can be trained to detect and respond to the scent left by a wide range of elusive species. In this survey, two contract surveyors (J. Martin and J. Birks), operating independently as solo surveyors, were each accompanied by a dog that had been trained to detect the scent of pine marten scats; one was a two year-old Labrador and the other a four year-old Labrador-lurcher cross. Training in advance of the 2012 survey involved the handlers creating an association within the dogs between the scent of pine marten scats and verbal praise combined with a 'fun' reward (usually a game with a favourite toy). The scats used during training were fresh samples of known origin collected from the lids of pine marten den boxes in Galloway Forest and frozen to preserve their scent prior to thawing for training purposes. It should be noted that the abilities of the two dogs in detecting marten scats were not tested or compared before the survey.

The dogs searched each survey transect in close proximity (<20m) to the human surveyors, who inspected each scat where the dog showed an interest. Because of the association with marten scat scent established in training, the dogs' behaviour on encountering such scent on a survey transect was distinctive when compared with their reaction to other scents: typically the dogs lingered longer when smelling marten scats and tended to look at their owners in anticipation of a reward. All scats producing such a response were collected as detailed above. In addition to observing their dogs, the human surveyors searched for scats along the same transects and collected any that appeared to be from pine martens.

2.3.4 The Galloway Forest Control Study

In the first phase of the survey in May 2012, surveyors undertook a control study to assess the effects of surveying more than one transect in selected hectads in Galloway Forest, where a population of pine martens is known to have established following a reintroduction in the early 1980s (Shaw & Livingstone, 1994). This study was intended to indicate the likelihood of false negatives arising where only one 1km transect per hectad was surveyed. Only those hectads in which recent evidence of pine martens was confirmed were targeted for the study. Surveyors completed three to five 1km transects within each of five hectads and collected possible pine marten scats for DNA analysis as detailed below.

2.4 DNA analysis

DNA was extracted from scats as described previously (O'Reilly *et al.*, 2008). The presence of pine marten and fox DNA was determined using the pine marten and fox specific quantitative real time polymerase chain reaction (qPCR) assay as described previously in O'Reilly *et al.* (2008) and Mullins *et al.* (2010). The qPCR assay was also used to quantify the DNA to identify samples suitable for DNA sequencing. A 315 bp fragment of the mitochondrial control region was PCR amplified using primers LMS3 (5' TCC CTA AGA CTC AAG GAA GA 3') (Statham, 2005) and PM-Rev (5' GGC CCG GAG CGA GAA G 3') (O'Reilly *et al.* 2008). The 10 µl PCR reaction contained 5 µl GoTaq® Hot Start Green Master mix (Promega Corporation, 2800 Woods Hollow Road, Madison, WI 53711, USA, Cat. No. M5123), 200 nM each primer and 2-10 ng DNA. The thermal cycling protocol was 94°C initial denaturation for 2 minutes, followed by 50 cycles of 94°C for 20 seconds, 57°C for 30 seconds and 72°C for 30 seconds with a final extension for 10 minutes at 72°C. PCR products were purified for sequencing using a DNA Clean and Concentrate-5™ (Zymo Research Corporation Cat. No. D4004). The PCR products were sequenced on both strands using the Applied Biosystems BigDye® v3.1 cycle sequencing kit (Life Technologies Corporation, 5791 Van Allen Way, PO Box 6482, Carlsbad, California 92008, USA) used according to the manufacturer's instructions. The sequencing reaction was analysed using

the ABI 310 Genetic Analyser. DNA sequences were analysed using the Lasergene software package (DNASTAR Inc.3801 Regent Street, Madison, WI 53705 USA).

2.5 Caveats associated with the scat survey method

In spite of the new rigour applied to pine marten scat surveys through routine DNA-typing of scats (Davison *et al.*, 2002, O'Mahony *et al.*, 2012), there are other methodological limitations that must be taken into account when interpreting survey results. These have been reviewed by Birks *et al.* (2004) via an analysis of several pine marten surveys, some of which were carried out in Scotland. Of special relevance to the present survey, in which transect density is low at one per hectad, is the significant risk of 'false negative' hectads, as indicated by the findings of Velander's (1983) survey in northern Scotland. The present survey targeted those parts of Scotland into which the pine marten population is believed, either to have expanded recently (post-1994), or is still re-colonising. It therefore follows that in some areas martens may be present at very low density, so the abundance of scats on transects is likely to be correspondingly low with an attendant likelihood of false negatives. This limitation is most likely to be encountered at low sampling intensities.

Beyond the use of scat surveys for determining the presence of pine martens, it is tempting also to infer some link between the abundance of scats and numbers of martens present at a locality, as suggested by Lockie (1964). However, this is unwise because some variables quite unrelated to marten abundance may influence the number of scats detected on a survey transect, such as the physical characteristics of the transect route, the nature and extent of vegetation cover, and differential scat decay rates (Laing *et al.*, 2003, Sanchez *et al.*, 2004). One conclusion arising from the review by Birks *et al.* (2004) is that '*the field relationship between scat abundance on transects and marten numbers has not been established.*'

3. RESULTS

3.1 Survey area

Two hundred and thirty-four hectads were targeted for the field survey (see Figure 3). Of these, 220 hectads were surveyed successfully. Eleven hectads were not surveyed due to access restrictions or tracks being unsuitable for surveying (i.e. overgrown or non-existent). No alternative transects were available in these hectads so these were excluded from the field survey. A further three hectads did not have identifiable tracks in suitable habitat shown on the maps used for transect selection. These hectads were visited by surveyors and 'ground-truthed' to assess whether any suitable woodland tracks were present. In all three cases, no suitable tracks for surveying were present so the hectad was not surveyed. The majority of hectads where suitable transects and habitat were not present were at the periphery of the Expansion Zone area (e.g. in coastal areas).

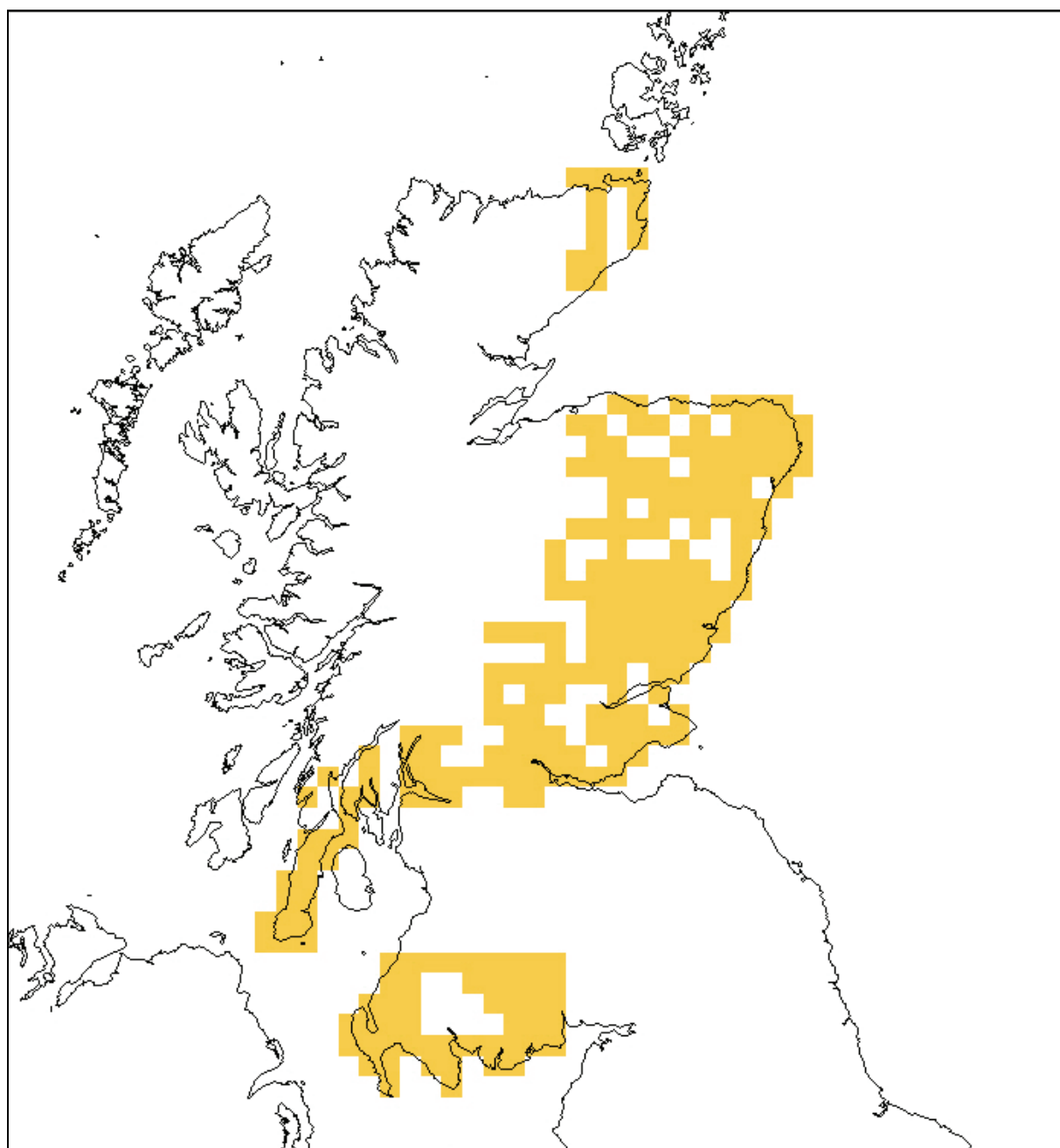


Figure 3. Hectads targeted for field survey. Based upon Ordnance Survey material with the permission of the Controller of HMSO © Crown Copyright (2013) Licence no. 100017908.

For the purpose of analysis, the entire Expansion Zone is subdivided into the sub-zones identified in Table 1.

Table 1. The distribution of hectads proposed, targeted and actually surveyed according to sub-zones and OS 100km grid squares.

Survey sub-zone	OS 100km grid squares	*Estimated number of hectads initially proposed for survey	**Number of hectads targeted for field survey	***Number of hectads actually surveyed	Survey teams involved
Caithness	ND	30	12	8	C
North-east (Aberdeenshire, Moray, Angus)	NJ, NK, NO	138	108	101	A, B, D, F
Centre (Perthshire, Stirling, the Trossachs, Fife, the Central Belt, mid and south Argyll)	NN, NT, NS, NR	106	61	60	A, B, C, D
South-west (Dumfries & Galloway)	NX	50	53	51	A, B, E, F, G
Total		324	234	220	

** Identified in the initial proposal from SNH. ** Targeted after the exclusion of hectads that were positive on the basis of recent pine marten records and those unsuitable for field survey due to habitat constraints identified during the desk selection. Also includes some hectads that were added due to survey area being larger than anticipated. *** Excludes hectads that were not surveyable due to access restrictions and/or habitat constraints encountered on the ground.*

3.2 Field survey

3.2.1 Scat collection and DNA analysis

254 scats were collected during the field survey. Of these, 52% yielded DNA in the laboratory (n=131): 38% (n=97) were pine marten (see Plate 2); 13% (n=33) were fox; and 0.4% (n=1) were mink. 48% (n=123) did not yield sufficient DNA to be determined in the laboratory (see Table 2).

Table 2. Scat results as determined by DNA analysis.

Species (determined by DNA analysis)	Number of scats	Percentage of scats
Pine marten	97	38%
Fox	33	13%
Mink	1	0.4%
Not determined	123	48%
Total	254	

There are several reasons why 48% of scats collected could not be determined to species level in the laboratory. Firstly, the DNA contained in scats may have been lost or degraded by exposure to the elements, particularly the heavy rain that was experienced at times during the field survey. Secondly, scats may have been from a species other than pine marten, fox or mink and therefore not identified through sequencing, though this is believed to be an unlikely explanation in view of the high level of surveyor experience. Thirdly, some technical failure in the DNA extraction and sequencing procedure cannot be ruled out. This is discussed further in section 4.3.3.



Plate 2. A pine marten scat in the field (© E. Croose)

3.2.2 Surveyor accuracy

If one excludes all non-determined scats collected, overall surveyor accuracy at identifying pine marten scats in the field was 74%.

The relative contributions, in terms of hectads surveyed and marten scats counted, collected and confirmed by DNA, varied between teams according to both the duration and locations of their involvement in the survey (see Table 3). For example, Table 1 shows that Teams E and G, which were involved only for a limited period in the south-west of Scotland where evidence of martens was very scarce (see Table 5), covered the fewest hectads and had the lowest success rate in terms of confirmed marten scats. Conversely, Team A was involved throughout the survey in three of the four sub-zones and so covered the most hectads and achieved a relatively high success in terms of scats collected and confirmed as pine marten on DNA evidence. Team F (including a scat detection dog) was involved only in south-west Scotland and peripheral areas of possible marten distribution in north-east Scotland, so this may explain the relatively low success rate in terms of marten scats counted, collected and confirmed.

Table 3. Variations in scat identification accuracy across the survey teams.

Survey Team	No. hectads surveyed	Total probable marten scats counted	□No. probable marten scats counted per 1km transect	Total probable marten scats collected	□No. probable marten scats collected per 1km transect	No. (and %) of marten scats confirmed by DNA analysis	□No. marten scats confirmed per 1km transect	No. (and %) of fox scats collected	No. (and %) of scats from other species collected	No. scats not determined (i.e. no DNA extracted)	***Surveyor success rate (% collected marten scats confirmed by DNA)
A	64	82	1.28	74	1.16	28 (38%)	0.44	3 (4%)	1 (1%)	42 (57%)	88%
*B	54	107	1.98	69	1.28	33 (48%)	0.61	6 (9%)	0 (0%)	30 (43%)	85%
C	25	46	1.84	44	1.76	16 (36%)	0.64	5 (11%)	0 (0%)	23 (52%)	76%
D	20	57	2.85	43	2.15	16 (37%)	0.8	2 (5%)	0 (0%)	25 (58%)	89%
**E	12	10	0.83	9	0.75	1 (11%)	0.08	8 (89%)	0 (0%)	0 (0%)	11%
*F	35	16	0.46	8	0.23	3 (38%)	0.09	3 (38%)	0 (0%)	2 (25%)	50%
**G	10	10	1	7	0.7	0	0 (0%)	6 (86%)	0 (0%)	1 (14%)	0%
Total	220	328		254		97 (38%)		33 (13%)	0 (0.4%)	123 (48%)	

Percentages rounded to nearest whole number. *Use of scat detection dog. **Surveyors were only involved in surveys in Dumfries & Galloway where marten scats were very scarce ***When non-determined scats are excluded from the calculation. □Note that the calculation of these figures includes many transects on which no marten scats were counted or collected. Data on scat counts excluding these negative transects are presented by vice-county in Table 5.

3.2.3 *The occurrence of positive hectads*

Pine marten scats, confirmed by DNA analysis, were found in 54 hectads (25% of hectads surveyed); these were classed as 'DNA positive' hectads (see Figure 4).

An additional 45 hectads were classed as 'probable positive' hectads on account of the unverified nature of the evidence recorded. These hectads largely comprised those in which surveyors collected scats that they believed to be from pine martens, but that failed to yield any DNA in the laboratory (n=41). Based on the experience of the surveyors involved, it is likely that many of these scats were from pine martens, although this might vary across teams/surveyors, use or not of a scat detection dog, and the pine marten population density in each given area, but the DNA was too degraded to allow the species to be confirmed by laboratory analysis. Probable positive hectads also included four hectads in which surveyors met local people who reported seeing or knowing of pine martens within the hectad.

This approach, including hectads positive on the basis of surveyor opinion, enables comparisons to be drawn with previous surveys in which DNA verification was not available. Although the aim of the current survey was to establish pine marten distribution on the basis of the presence of scats yielding pine marten DNA, the relatively poor success rate in yielding DNA from scats to determine species (see section 4.3.3) limited the data available. Considering surveyor judgement in identifying scats by sight may introduce a subjective element to the results but this was considered to be justified to maximise all the information available.

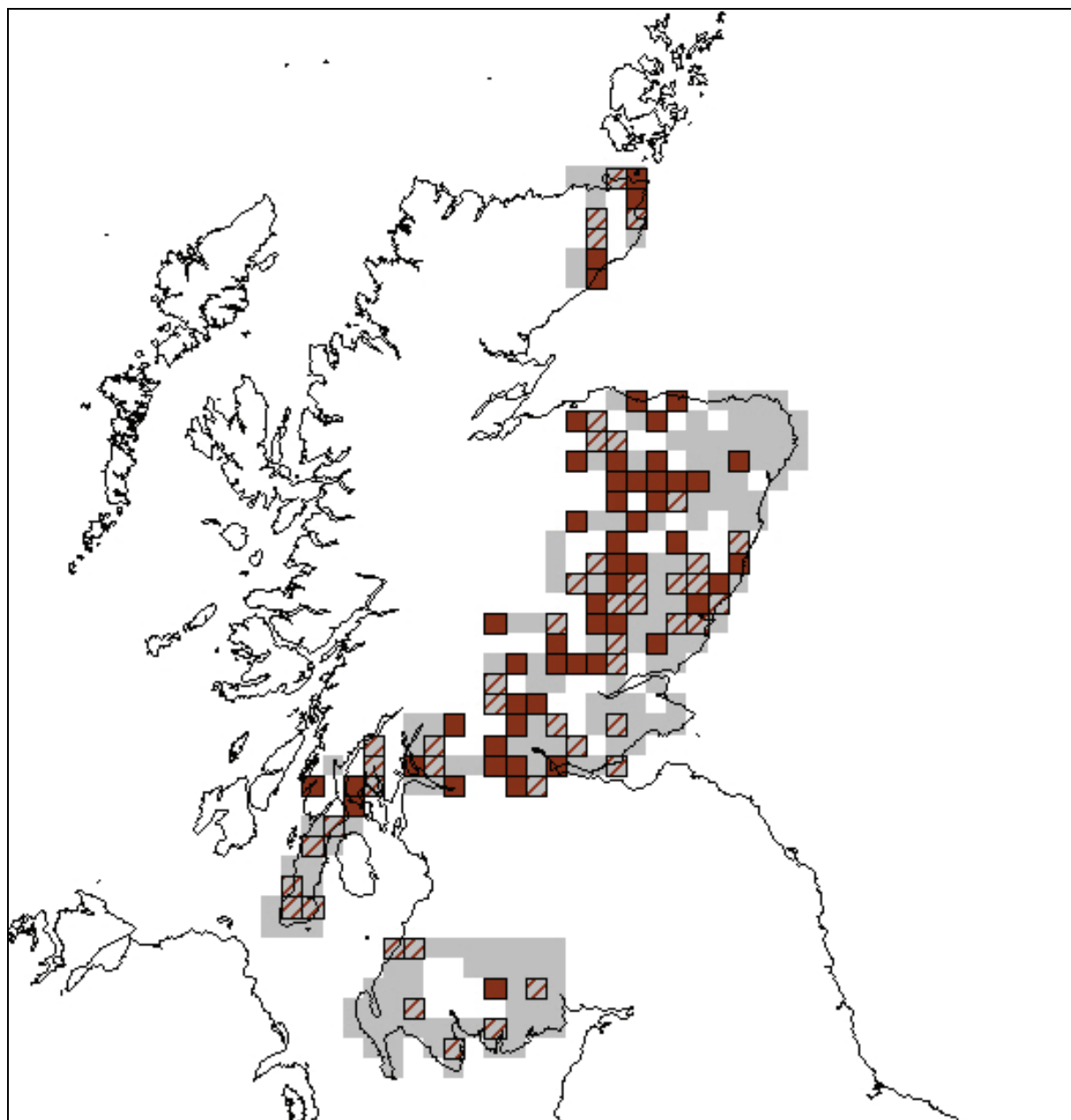


Figure 4. Field survey results summary by hectads: DNA positive (brown solid shading); probable positive (brown hatched shading) and negative (grey solid shading). Based upon Ordnance Survey material with the permission of the Controller of HMSO © Crown Copyright (2013) Licence no. 100017908.

3.2.4 Patterns of scat abundance on survey transects

The number of possible marten scats counted on 1km transects varied between one and 15 (see Figures 5 and 6); 108 transects produced no possible pine marten scats.

Table 4 indicates that DNA positive transects were characterised by a greater abundance of scats when compared with probable positive transects.

Table 4. The frequency distribution of transects on which different numbers of scats were found and proportions positive for pine marten from DNA analysis.

Scats per transect	No. of transects	No. of transects positive for pine marten on basis of DNA analysis	% of transects positive for pine marten on basis of DNA analysis
0	108	N/A	N/A
1	43	13	30
2	23	10	43
3	15	9	60
4	13	8	62
5	6	3	50
6	3	2	67
7	1	1	100
8	3	3	100
10	2	2	100
14	2	2	100
15	1	1	100

Figure 5 shows that 54% (51 out of 95) of all positive hectads - those based on both DNA-positive (n=54) and non-determined scats (n=41), as discussed in section 3.2.3 - comprised transects with counts of fewer than three marten scats. These low scat density transects comprised 43% of hectads confirmed on the basis of DNA-confirmed scats and 68% of those positive on the basis of probable (non-determined) marten scats.

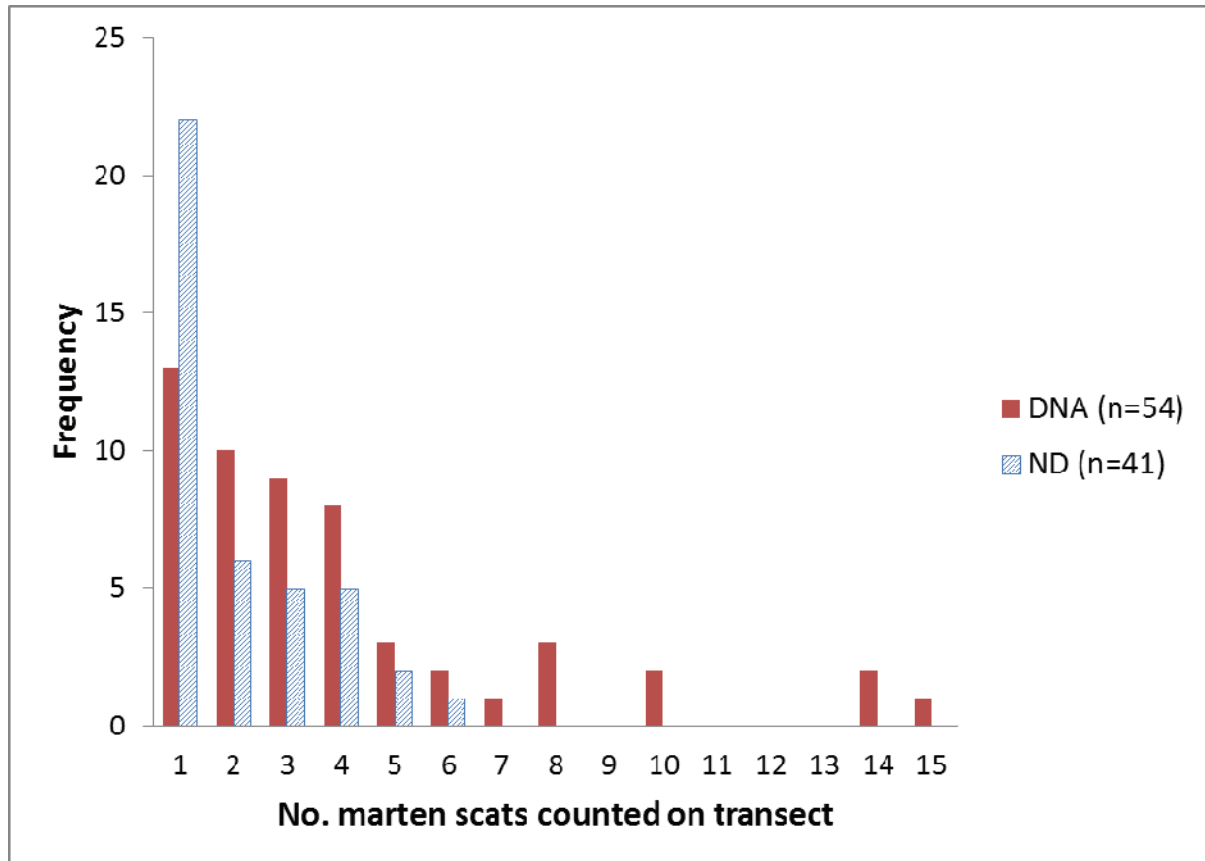


Figure 5. A comparison of the frequency of occurrence of possible marten scats on DNA positive and non-determined (ND) transects.

Figures 6a-d show the geographical distribution of the scat count information, by sub-zone, summarised in Figure 5. The maps in Figure 6 indicate that, although the pine marten apparently has a widespread distribution within the Expansion Zone, there were marked variations in the number of marten scats counted per transect within the same geographical area. Notably, there are cases where relatively high scat counts (indicative of established marten populations according to Balharry *et al.* (1996)) are in hectads close to those with very low scat counts (i.e. zero or one marten scat per 1km). This extreme patchiness in the abundance of scats is not merely a feature of the periphery of the marten's range, as there are several hectads with one or zero scats closer to the marten's core range; for example, in Moray and west Aberdeenshire.

Figure 6. Number of scats counted on transects in the four sub-zones.

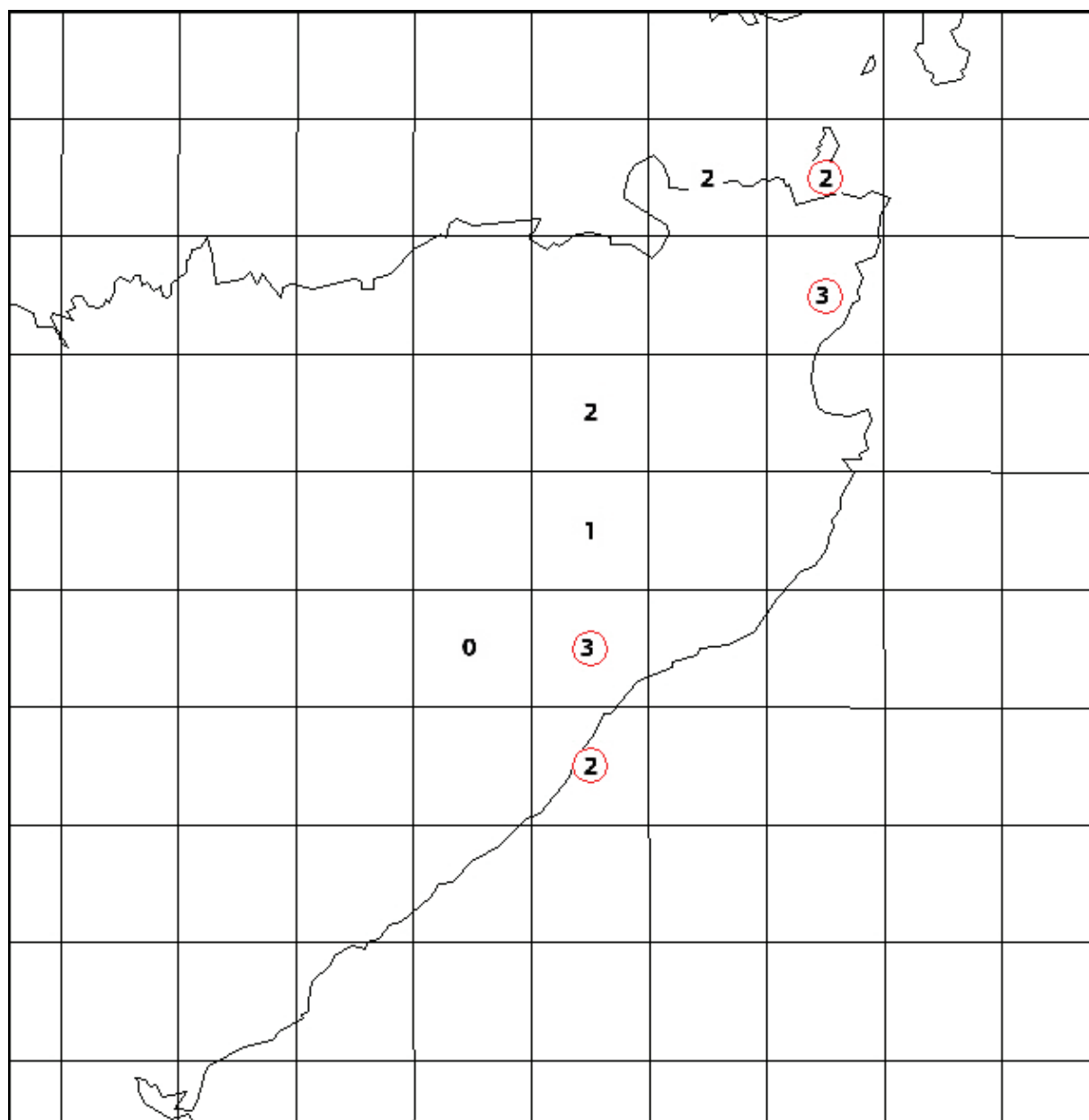
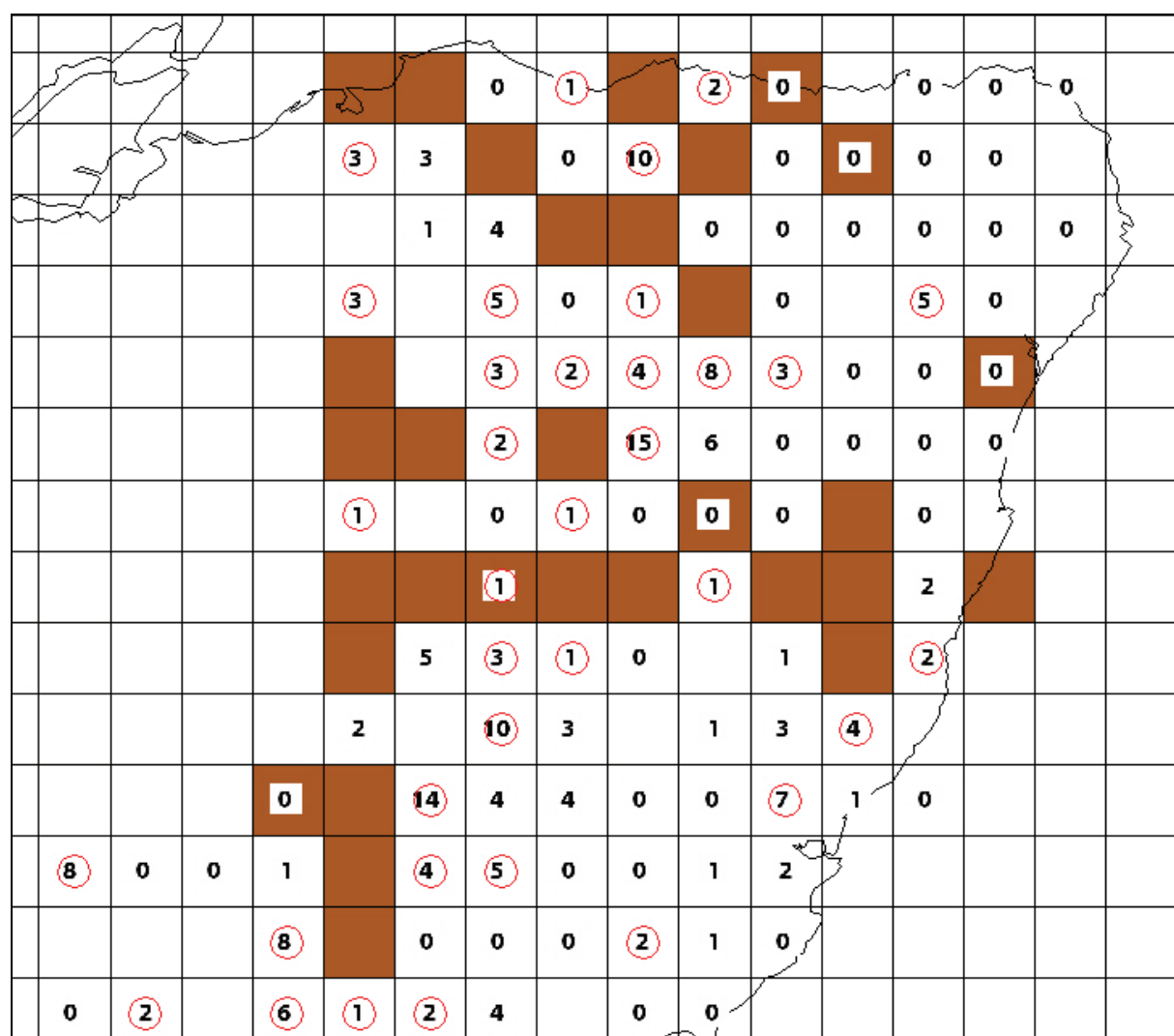


Figure 6a. Number of scats counted on transects in the Caithness sub-zone. Transects on which pine marten scats, confirmed by DNA analysis, were collected are ringed in red. Based upon Ordnance Survey material with the permission of the Controller of HMSO © Crown Copyright (2013) Licence no. 100017908.



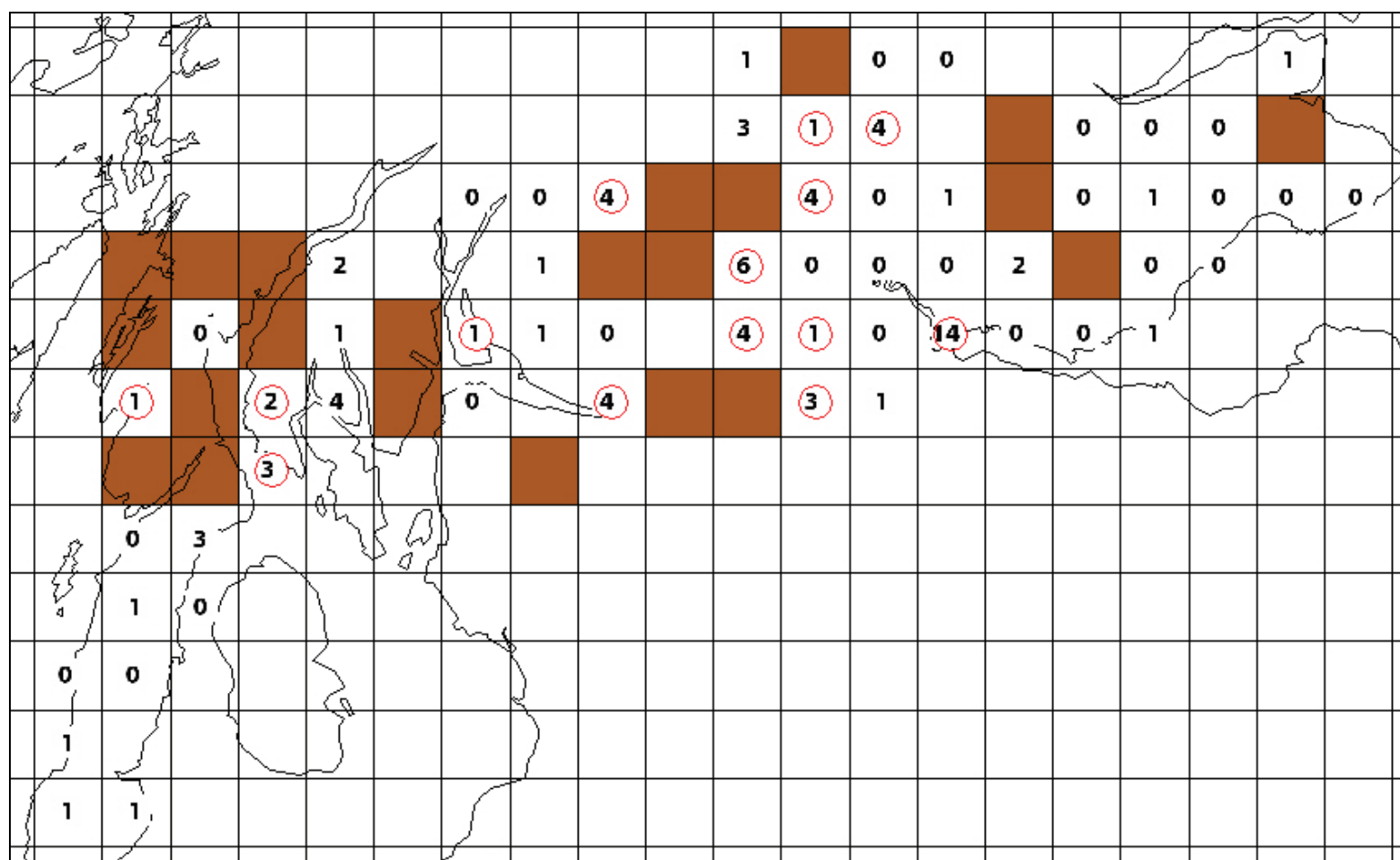


Figure 6c. Number of scats counted on transects in the centre sub-zone. Transects on which pine marten scats, confirmed by DNA analysis, were collected are ringed in red. Hectads with pine marten records collected outside of the field survey are shaded brown. Based upon Ordnance Survey material with the permission of the Controller of HMSO © Crown Copyright (2013) Licence no. 100017908.

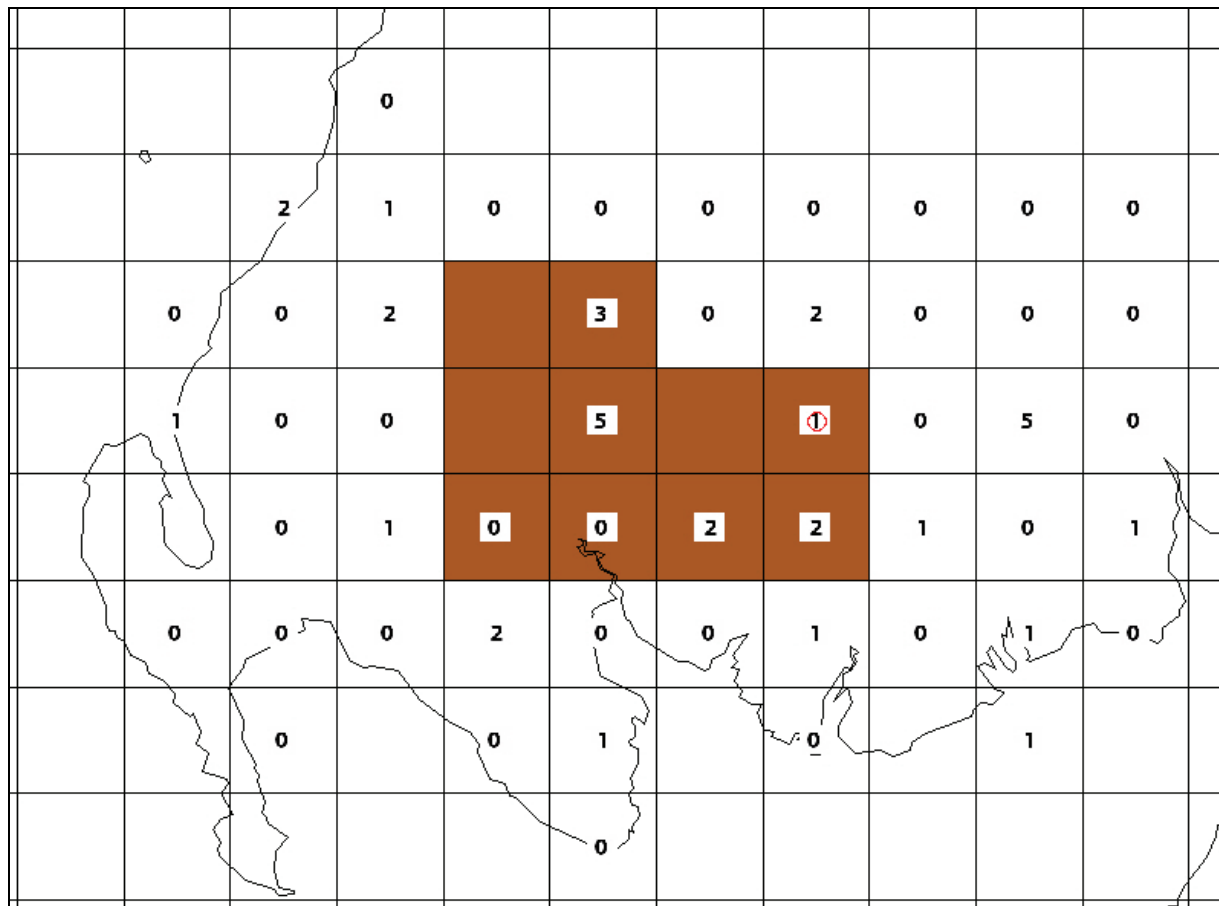


Figure 6d. Number of scats counted on transects in the south-west sub-zone. Transects on which pine marten scats, confirmed by DNA analysis, were collected are ringed in red. Hectads with pine marten records collected outside of the field survey are shaded brown. Based upon Ordnance Survey material with the permission of the Controller of HMSO © Crown Copyright (2013) Licence no. 100017908.

3.1.5 Use of scat detection dogs

The benefits of using dogs to assist in detecting marten scats on transects can be assessed in two ways:

1. by recording the number of scats (later confirmed as from pine martens) found by dogs that would not have been found by their human handlers on a transect by transect basis (e.g. where dogs found scats off the transect in vegetation, or found scats on the track which were small and/or hidden and therefore easily missed by human surveyors); and
2. by comparing overall numbers of scats found and the proportion that were confirmed as marten scats between teams working with and without dogs.

The first of these was not recorded consistently by the two surveyors working with a dog, although subjectively, the dogs quite frequently detected scats that would have been missed by a human surveyor, especially on those transects where the substrate rendered the scats difficult to detect by sight, such as during the Galloway Control Study. Here, on one transect along a newly constructed forest road of dark crushed stone (on which scats were difficult to

detect visually), the dog found four marten scats that were missed by the human surveyor (who found only one) (see Plate 3).

The wider comparison of scat numbers counted and correctly identified by teams working with scat detection dogs (Teams B and F), and those without, is hampered by the variations in numbers and locations of hectads surveyed, as explained in section 3.2.1. Team F was mainly involved in searching hectads where probable marten scats were scarce or absent (see Table 3), so its success rate cannot reasonably be compared with most other teams. Nevertheless, despite finding and collecting fewer probable marten scats per transect than any other team, Team F achieved a higher marten scat identification rate and a lower fox scat misidentification rate than either Teams E or G, which surveyed in similar circumstances. Team B was involved in surveys across the full range of marten scat abundance, and achieved a relatively high success rate in terms of marten scats correctly identified (see Table 3).



Plate 3. Scat detection dog with a marten scat on stone between dog and leash (© J. Birks)

3.1.6 Haplotype of scats collected

All determined pine marten scats were sequenced in the laboratory to establish haplotype. 20% of pine marten scats (n=19) did not have sufficient DNA for the haplotype to be determined. All of the remaining 80% (n=78) of pine marten scats that yielded sufficient DNA for species identification were haplotype *a*. Haplotype *a* is currently the dominant haplotype for pine marten in Scotland (Jordan *et al.*, 2012).

3.3 Other records

Verified pine marten records, collected mainly in advance of the field survey (see Section 2.2) accounted for 61 positive hectads within the Expansion Zone (see Figure 7 and Table 5). Note that records were collected from an additional nine hectads on Skye and four hectads on Mull, which were not part of the Expansion Zone Survey.

3.4 Pine marten distribution in the Expansion Zone

The proportions of hectads positive and negative for pine martens by vice county and sub-zone is summarised in Table 5. In this report, in keeping with other recording exercises, we have used the system of Watsonian vice-counties (VCs).

A simple comparison of the proportion of positive hectads across the four sub-zones reveals a striking contrast between south-west Scotland (28%) and the remainder (54-59%). Notably, most of the positive hectads in south-west Scotland were based on records from Forestry Commission Scotland and the Dumfries and Galloway Environmental Records Centre; only one hectad was positive on the basis of a DNA confirmed scat found in the field survey. Positive hectads in this sub-zone were concentrated in Kirkcudbrightshire VC, where pine martens were reintroduced in the early 1980s in the Galloway Forest. There were few positive hectads in VCs Wigtownshire and Ayrshire and no positive hectads in Dumfriesshire.

In Caithness, marten presence was confirmed in both the south-west and the north-east of the vice-county, although the distribution was rather patchy. Records here came solely from the field survey; no records were contributed from other sources.

In the north-east sub-zone, the greatest concentration of positive hectads was in VCs Moray, Kincardineshire, Banffshire and South Aberdeenshire, where both field survey and other evidence contributed equally to the records. Considerably fewer positive hectads were found in North Aberdeenshire and especially towards the east coast, where woodland was reduced and fragmented. Marten distribution was patchy in Angus, where several hectads were probable positives (scats were non-determined following DNA analysis).

In the central sub-zone, the greatest concentrations of positive hectads were found in VCs Main Argyll, Stirlingshire, East Perth and West Perth (where both field survey and non-field survey records contributed almost equally to these hectads), and Dunbartonshire and Mid Perth (where contribution from non-field survey records was limited). Comparatively fewer positive hectads were found in Kintyre, Renfrewshire and Fife towards the southern limits of the marten's range, with DNA confirmed scats contributing to only two positive hectads in Kintyre and none in Renfrewshire or Fife.

Table 5. Number of positive and negative hectads for pine marten by vice county.

Vice-county (grouped by survey sub-zone)	Hectads included in expansion zone survey	Positive hectads (DNA typed scats found during field survey)	Probable positive hectads (non-determined scats)	Positive hectads (non field survey)	*Total number hectads positive for pine marten	% positive hectads (rounded to nearest whole number)	Total number hectads negative for pine marten	% negative hectads (rounded to nearest whole number)
Caithness sub-zone								
Caithness	14	4	4	0	8	57	6	43
Sub-zone Total	14	4	4	0	8	57	6	43
North-east sub-zone								
Moray	14	3	3	5	11	79	3	21
Kincardineshire	12	2	3	4	9	75	3	25
Banffshire	16	5	0	5	10	63	6	38
South Aberdeenshire	28	6	2	9	17	61	11	39
Angus	25	5	6	0	11	44	14	56
North Aberdeenshire	29	6	0	3	9	31	20	69
Sub-zone Total	124	27	14	26	67	54	57	46
Centre sub-zone								
Main Argyll	10	1	3	5	9	90	1	10
Stirlingshire	14	5	1	4	10	71	4	29
East Perth	14	3	3	4	10	71	4	29
West Perth	8	2	1	2	5	63	3	38
Dunbartonshire	7	2	2	0	4	57	3	43
Mid Perth	21	7	3	2	12	57	9	43
Kintyre	23	2	5	5	12	52	11	48
Renfrewshire	2	0	0	1	1	50	1	50
Fife	18	0	3	3	6	33	12	67
Sub-zone Total	117	22	21	26	69	59	48	41

South-west sub-zone								
Kirkcudbrightshire	27	1	2	8	11	41	16	59
Ayrshire	7	0	2	0	2	29	5	71
Wigtownshire	19	0	2	1	3	16	16	84
Dumfriesshire	5	0	0	0	0	0	5	100
Sub-zone Total	58	1	6	9	16	28	42	72
Overall Totals	313	54	45	**61	160	51	153	49

** Includes probable positive hectads. ** This total does not include records for Skye and Mull, which are shown in Figure 7.*

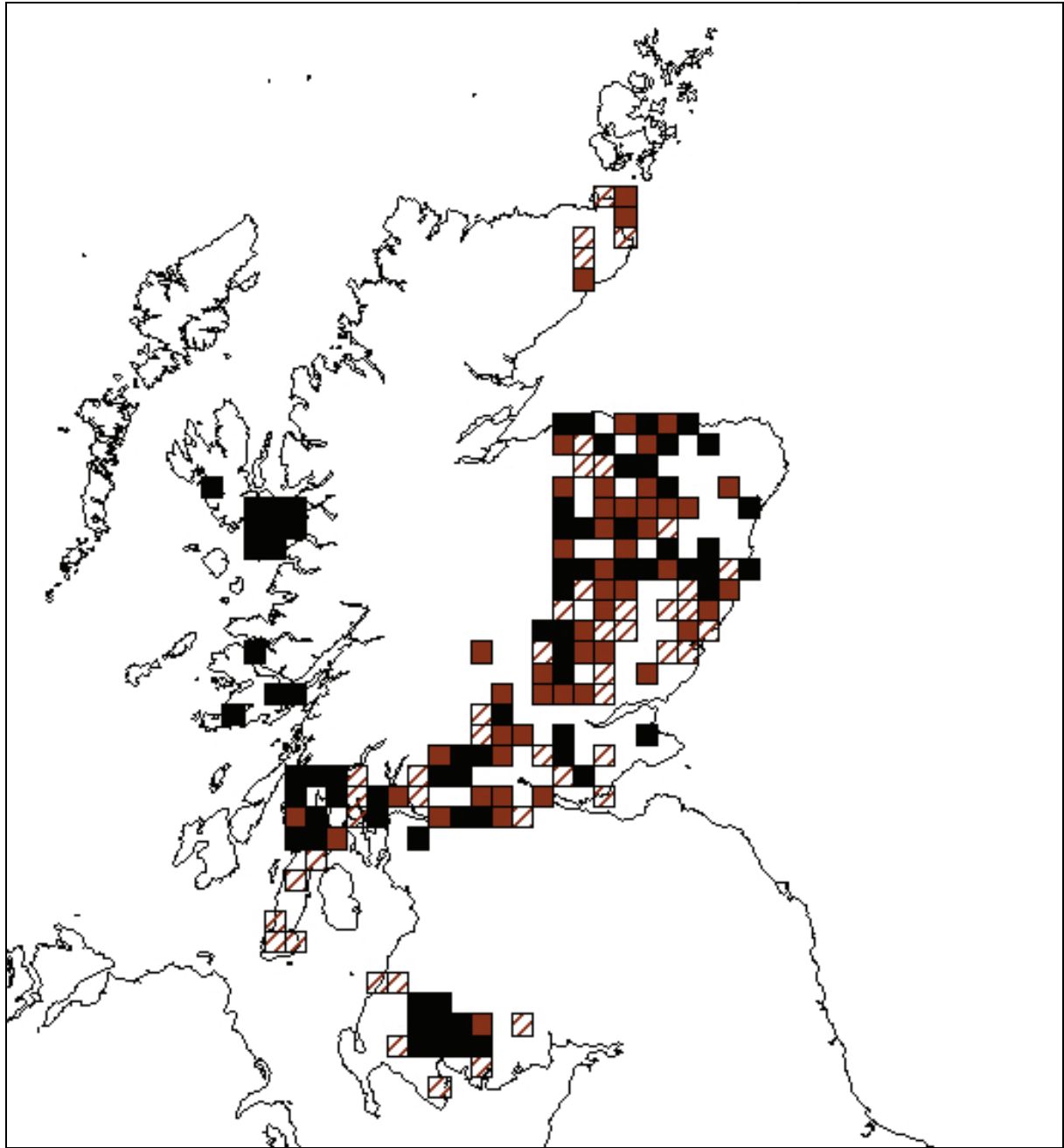


Figure 7. Hectads positive for pine marten: DNA positive from field survey (brown solid shading); probable positive from field survey (brown hatched shading); and pine marten records collected outside of the field survey (black solid shading). Note that records for Skye and Mull are included here, even though these islands were not part of the Expansion Zone Survey. Based upon Ordnance Survey material with the permission of the Controller of HMSO © Crown Copyright (2013) Licence no. 100017908.

The distribution of pine martens in Scotland as revealed by the current survey, the 1994 distribution survey (Balharry *et al.*, 1996) and the 1980-1982 distribution survey (Velandar, 1983) is shown in Figure 8. Although the pine marten's range appears fragmented and discontinuous in places, this is likely to reflect the sampling process and the associated occurrence of 'false negatives' and it is probable that the true range is more continuous than shown here. It is also worth noting that although the north-west of Scotland, where pine

martens were recorded in previous surveys, was not included in the current survey, there is abundant anecdotal evidence to suggest that pine martens are still present in these areas today.

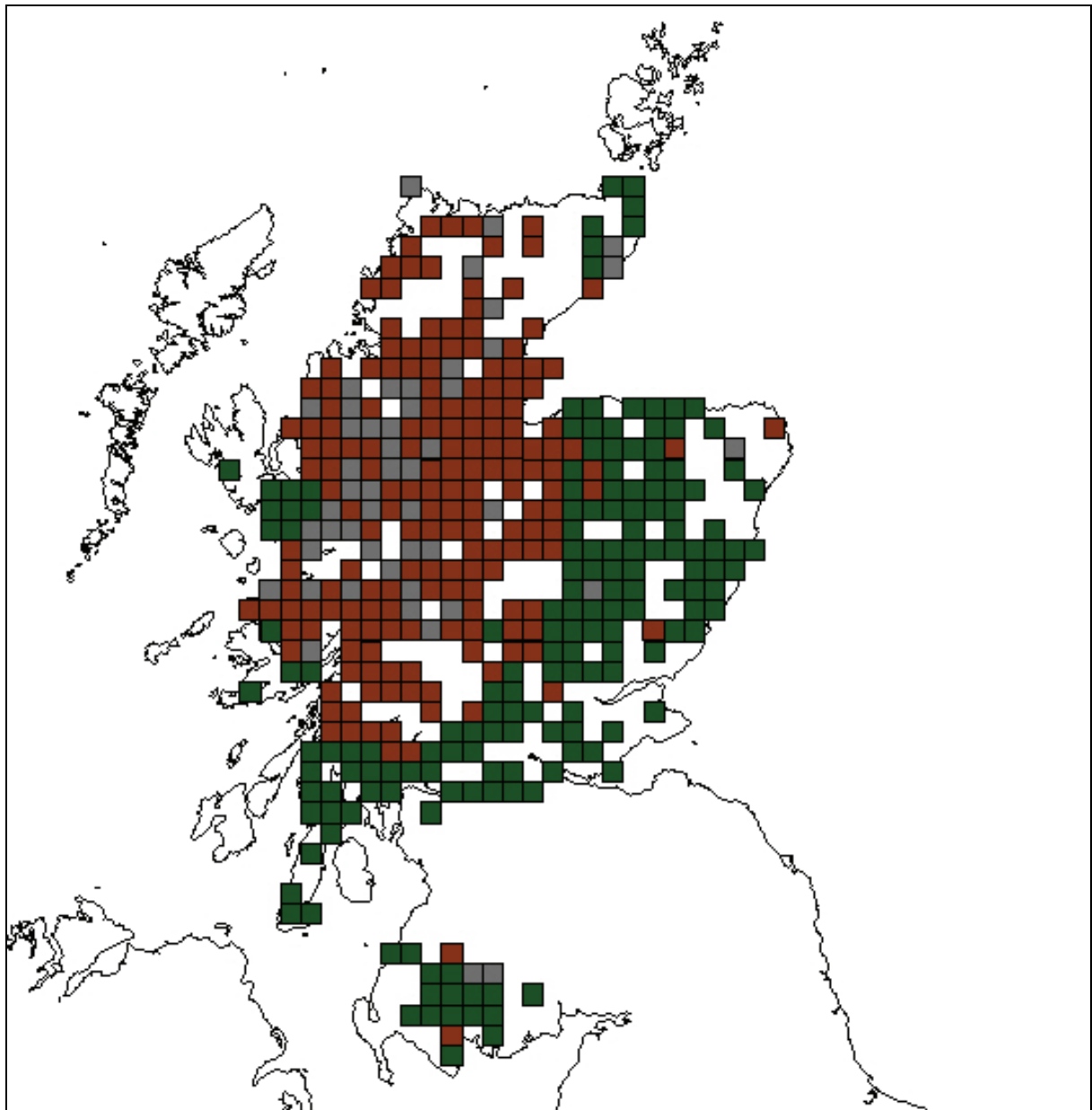


Figure 8. Overall distribution of pine martens in Scotland, comprising records collected from 1980 to 2012. This map includes records collected during the 1980-1982 distribution survey (Velandar, 1983) (hectads shaded grey), the 1994 distribution survey (Balharry et al., 1996) (hectads shaded brown) and records (both DNA positive and probable positive records from the field survey and non-field survey records) collected during the current Expansion Zone Survey (hectads shaded green). Based upon Ordnance Survey material with the permission of the Controller of HMSO © Crown Copyright (2013) Licence no. 100017908.

3.5 The Galloway Forest Control Study

This pilot exercise in May 2012 was undertaken in five hectads in Galloway Forest in which pine martens were known to be established on the basis of recent reliable records. Five separate teams each surveyed between three and five transects within one or more hectads, as summarised in Table 6. Most teams surveyed single hectads, but one team covered two and two separate teams surveyed different parts of the same hectad (NX38) – hence the use of the term ‘team/hectad’ below. All five hectads surveyed in this pilot were positive on the basis of pine marten DNA extracted from scats. Twenty-three of the 45 scats collected during this pilot were submitted for DNA analysis to confirm species: 13 (57%) were from pine marten, three (13%) from fox and seven (30%) were non-determined.

In seeking to understand the limitations of the main survey method based on a single 1km transect per hectad, it is useful to consider the results of this more intensive pilot on a team/hectad basis. Three teams surveyed hectads in which one or two transects yielded no scats, despite marten scats being found on other transects in the same hectad (e.g. 10 scats collected from one transect in NX67, where two other transects produced none). Of those transects that did yield scats (coincidentally all team/hectads produced three such transects), the number from which pine marten DNA was confirmed ranged from one to three in each team/hectad (mean 1.83). Only one team (B* in Table 6 below), which included a scat detection dog, achieved a 100% success rate in terms of pine marten DNA confirmed on every transect from which scats were collected.

Overall, in the areas of known (breeding) pine marten presence, the proportion of all transects surveyed per team/hectad that produced DNA-confirmed pine marten presence, ranged from 25-67%. This adds weight to the argument for completing more than one 1km transect per hectad in order to confirm pine marten presence, especially where the rate of DNA non-determination is unaccountably high.

Table 6. A summary of the Galloway Forest Control Study results.

Team/hectad	Survey team	Number of 1km transects surveyed	Number of transects on which scats were collected (and total number of scats)	Number of transects yielding marten DNA from scats	% of surveyed transects positive on the basis of pine marten DNA from scats	% of scat-yielding transects positive on the basis of pine marten DNA from scats
NX38	A	4	3 (5)	1	25	33
NX57	B*	5	3 (7)	3	60	100
NX67	F*	5	3 (16)	2	40	67
NX27	G	3	3 (5)	2	67	67
NX38	E	3	3 (8)	2	67	67
NX28	E	3	3 (4)	1	33	33
Total		23	18 (45)	11	48	61

**Use of scat detection dog.*

4. DISCUSSION

4.1 Overview of Distributional Change

Further to the findings of the Velandar (1983) and Balharry *et al.* (1996) surveys, pine marten range expansion has continued southwards and eastwards from the Highlands. Martens are now present throughout Moray, Deeside and much of the Cairngorms; and the isolated populations previously recorded in these areas by Velandar (1983) and Balharry *et al.* (1996) appear to have become contiguous. Martens have continued to re-colonise southern, western and south-eastern Aberdeenshire, joining up with isolated records towards the east coast, detailed in Velandar (1983); but expansion towards the north and east of this local authority area has been limited. Further south, the expansion through the former Central and Strathclyde local authority regions, as described by Balharry *et al.* (1996), has continued and martens are now present in limited parts of Angus and Fife, although their distribution in these areas is rather patchy.

Expansion has arguably been most extensive throughout Stirlingshire and the Trossachs, with martens now present in the Central Belt in some places. Further west, martens have spread south from Loch Awe into southern Argyll and into much of the Kintyre and the Cowal Peninsulas. In the Balharry *et al.* (1996) survey, it was concluded that the spread of martens to the north-east of the Highland local authority area had not been so extensive and suggested that the Flow Country may act as a barrier to further expansion. However, this survey has confirmed that martens are widespread in Sutherland and Caithness, albeit with a patchy distribution in some areas. In Galloway, martens have spread south and east from Glen Trool Forest where the species was reintroduced in 1980-1981 (Shaw & Livingstone, 1994), but this expansion has been limited (see section 4.1.1). Since most of the west of Scotland was not included in this survey, it is not possible to discuss distributional change in this area; however, pine martens have continued their post-1995 colonisation of Skye and are now present on Mull through translocation.

4.1.1 Expansion of the pine marten population in south-west Scotland

Following the release at two sites in Galloway Forest in 1980 and 1981 of 12 animals trapped in western Inverness-shire (Shaw & Livingstone, 1994), the pine marten population centred on Glen Trool in the south-west of Scotland is clearly now established and breeding; but the findings of subsequent surveys suggest that it has not expanded as widely as might have been expected.

In the 1994 distribution survey only one hectad in south-west Scotland had a sufficient scat density to suggest that an established population was present, though four other hectads had lower scat densities representing dispersing martens (Balharry *et al.*, 1996). On this evidence the authors concluded that '*there does not appear to have been much colonisation away from the original release site*'; and '*it is unlikely that martens are established out with Glentool Forest*'.

Further research on the Galloway Forest pine marten population in 1995-96 (15 years after the original release) by Bright & Smithson (1997) examined the pattern of population expansion in relation to release site locations and wider habitat availability. Methods involved extensive scat surveys, radio-tracking, habitat assessment and population modelling. This work indicated that although sightings of individual martens were reported from up to 70km away and scats were found up to 20km away from Glen Trool, the established breeding population was still confined to an 11km radius around Glen Trool, with a maximum estimated population of 36 adults. The authors considered that philopatry played a part in explaining this pattern, with individuals choosing to settle in relatively poor upland spruce

habitat around Glen Trool rather than expand into higher quality habitat available further afield in south-west Scotland. Population modelling by Bright & Smithson (1997) adds weight to the view of Shaw & Livingstone (1994) that one of the releases, into upland forest near Clatteringshaws Loch, may have failed to establish a population; so instead of 12 animals the original founder population probably comprised just six individuals released at Caldons in Glen Trool.

The current Expansion Zone Survey confirmed the presence of pine martens in 10 adjacent hectads centred on Glen Trool (with six further peripheral hectads producing probable marten scats). Most of the 10 hectads were positive on the basis of recent sighting records gathered by the Forestry Commission, rather than via the main scat survey. This represents a very modest range expansion over 30 years, especially considering the substantial extent of wooded habitat available to pine martens in the south-west of Scotland. It is possible that expansion of the pine marten population in this area could be limited by anthropogenic factors or genetic constraints arising from founder effects from the very small number of individuals that were reintroduced.

4.1.2 Factors contributing to pine marten recovery

There are at least two important factors that are likely to have been the driving forces behind the pine marten range expansion found in this survey. Firstly, the legal protection afforded the species under the Wildlife and Countryside Act (1981) has undoubtedly reduced persecution pressure on pine martens and allowed populations to expand. Prior to this protection, direct hunting and persecution led to the extirpation of pine martens across much of their former range in Scotland (Lockie, 1964). Increases in pine marten distribution have occurred following a reduction in persecution pressure in other parts of their European range (O'Mahony *et al.*, 2012; Ozolins & Pilats, 1995).

Secondly, there has been a large increase in forestry cover in Scotland during the last century. In 1900, during the nadir of the pine marten in Britain, only 5% of Scotland was covered in forest (Scottish Natural Heritage, 2010). By 1980, during the time of the 1980-1982 pine marten distribution survey (Velandar, 1983), forestry cover had increased to 12% (Forestry Industry Council of Great Britain, 1998) and by 2007, it had further increased to 17% of land area (Scottish Natural Heritage, 2010). This afforestation and expansion of woodland cover has increased the availability of suitable habitat for martens, hence parts of the pine marten's former range that were previously unsuitable (i.e. non-wooded) are now suitable for re-colonisation (Caryl, 2008). However, it is important to note that martens have been recorded on a number of occasions in non-woodland habitats, such as upland habitats in the Cairngorms (K. Kilshaw, pers. comm.), peatland blanket bog in the Flow Country, Sutherland (Bavin, 2010) and coastal habitats in Wester Ross (R. Raynor, pers. comm.). In other parts of their European range, pine martens have also frequently been documented in landscapes where woodland is not the dominant habitat type, such as agricultural landscapes (Balestrieri *et al.*, 2009), suggesting that they can adapt to fragmented habitat (Mergey *et al.*, 2011; Mergey *et al.*, 2012) and are capable of persisting in areas where woodland is sparse or even absent. Indeed, recent research in Scotland has demonstrated that a level of woodland fragmentation coincides with an initial increase in marten population densities (Caryl *et al.*, 2012).

4.2 Prospects for future expansion

It seems probable that martens will continue to spread southwards and eastwards from Aberdeenshire, Perthshire and Stirlingshire into Angus and Fife. Similarly, animals from

Moray and Aberdeenshire would be expected to spread into unoccupied parts of north-east Aberdeenshire.

It is unknown how well populations will re-colonise southern Scotland; any re-colonisation would be dependent on martens dispersing through the Central Belt and/or from Galloway Forest (see section 4.1.1 above and 4.2.1 below).

The vision of the Scottish Forestry Strategy to increase woodland cover to around 25% of Scotland's land area by the second half of the 21st century (Forestry Commission Scotland, 2006) may further help the pine marten to expand its range.

4.2.1 Constraints to further pine marten range expansion

The key barrier to pine marten range expansion southwards from the core Highland population is the industrialised Central Belt region. Balharry *et al.* (1996) also proposed that this presents a barrier to marten re-colonisation. It is possible that the extensive road networks and urbanisation in this area may present a significant obstacle for dispersing martens, hindering the re-colonisation of the species in southern Scotland. This situation is akin to the recovery of the polecat *Mustela putorius* in England, whereby large conurbations such as Liverpool and Manchester may present a barrier to northward dispersal from the population in the English Midlands (Birks, 2008). Pine marten records collected from both the field survey and other sources illustrate that martens are now present on the northern fringe of the Central Belt, including one DNA-confirmed pine marten scat found south of the M80 in Cumbernauld. Notably, there is a record of a pine marten in Clyde Muirshiel Regional Park (some 20km south-west of Glasgow) in 2002, which presumably moved southwards through the Central Belt. The Central Scotland Green Network may be beneficial to pine martens by providing an integrated habitat network throughout the area. Woodland creation and enhancement of other habitats, such as riparian corridors, provide effective dispersal corridors for pine martens. Given the limited expansion, particularly northwards and eastwards, of the pine marten population in Galloway Forest (see section 4.1.1) it is likely to be some time before martens are widespread in southern Scotland, with the lack of woodland potentially restricting future establishment of martens in some areas. Pine marten range expansion may also be limited in lowland habitats (for example, in Angus and Fife), where much of the habitat is agricultural and woodland habitat is frequently small and fragmented. Due to these habitat limitations, pine martens may remain present at low densities with a patchy distribution in these areas. Similarly, woodland cover is isolated and fragmented in VC North Aberdeenshire, particularly towards the east coast (see Plate 4), which may limit range expansion in this area. It has previously been suggested that gamekeeping pressures may also act as a limiting factor in the re-colonisation of martens in the North East (Balharry *et al.*, 1996). Nevertheless, as discussed in section 4.1.2, pine martens have been recorded persisting in fragmented habitats (Mergey *et al.*, 2011; Mergey *et al.*, 2012; Caryl *et al.*, 2012), thus woodland fragmentation may not be as limiting as previously believed.



Plate 4. Fragmented woodland in north-east Aberdeenshire (© E. Croose)

There are several threats that could affect or stall pine marten recovery. Firstly, pine martens are vulnerable to predator control undertaken for other species and significant mortality may result from the non-target effects of some forms of pest control. Conflict with humans, arising from predation of poultry and other livestock and the use of inhabited buildings for denning, may increase conflict with humans (see sections 4.5 and 4.6.1). Secondly, marten populations may be affected by woodland loss and fragmentation as a result of development.

4.3 Methodological constraints

4.3.1 The influence of limitations inherent in the field survey

The method chosen for the field survey in this assessment of pine marten distribution carries significant risks of failure at two stages in the process. The results of the Galloway Forest Control Study give some indication of the relative scale of these, though caution should be used when extrapolating these to the wider Scottish survey because there may be differences in performance between the marten population in south-west Scotland and those further north (see section 4.1.1).

4.3.2 False negatives due to the absence of detectable scats

The low density of transects selected for the field survey carries the risk of failure to detect pine marten presence in a hectad where the species is present; this risk arises where the single pre-selected 1km transect fails to coincide with detectable field signs of pine martens. In Velander's (1983) survey, which adopted a similar sampling approach (except that DNA confirmation of scat identity was not available), false negatives (whereby no marten scats were found, yet other evidence such as a carcass confirmed marten presence) accounted for a minimum 21% of all positive hectads. In the current survey, the control study in the Galloway Forest revealed that in three out of five hectads (comprising all of those where >3

transects were surveyed), one or two transects produced no possible marten scats. It is reasonable to conclude that such false negatives are most likely to occur where scat densities are low, such as in peripheral areas where an establishing pine marten population is sparse and/or fragmented – a situation likely to arise widely in the pine marten's expansion zone targeted by this survey. This effect may explain the occurrence of some negative hectads close to the fringes of the pine marten's current range.

4.3.3 *False negatives due to the failure to extract DNA from scats*

False negatives may also arise where scats from pine martens are collected during field surveys but these fail to produce pine marten DNA during laboratory analysis. In the Galloway Forest control study (based on hectads where pine martens were known to be established) 52% of all transects, and 39% of transects from which scats were collected for DNA typing, failed to reveal pine marten DNA. Note that in this control study not all scats collected were DNA-typed. In the main survey the proportion of non-determined scats was unusually high at 48%, perhaps due to the impact upon scats of rain encountered during the survey period. Despite repeated attempts to extract DNA from scats; this compares very poorly with a 2011 pine marten survey in Argyll in which 1% of 157 scats were non-determined (VWT, unpublished data) and a predator survey in which 25% of 414 scats were non-determined (Baines *et al.*, 2011).

A consequence of the high non-determined rate in the current survey was that experienced surveyors believed they had found fresh pine marten scats in many hectads but this was not confirmed subsequently by DNA typing. This affected 41 hectads, representing 19% of all hectads surveyed. This inclusion of probable positive hectads facilitates comparison with previous surveys in which DNA verification was not available.

4.3.4 *Is there a relationship between scat numbers and marten abundance?*

The number of scats deposited by pine martens on a 1km survey transect is influenced by seasonal patterns of marten activity and patterns of home range use and social or territorial behaviour (e.g. Velandar, 1986, review by Birks *et al.*, 2004). As a consequence, scat density may vary markedly between transects at various scales (as observed in the current survey), including those located within the same woodland. For example, in a recent, long-term study in Portlaw Wood, County Waterford, Ireland, where a stable and relatively dense population of eight pine martens occupies a 330ha site, a scat transect located on a territory boundary consistently produced large numbers of scats, while a nearby transect located within an occupied marten home range produced none (P. Turner, pers. comm.). In Inshriach Forest in Strathspey, where pine martens are well-established, scat abundance on a survey of 15 1km transects during autumn 2012 ranged between zero to five (EC personal data). It follows that the number of scats deposited by martens on survey transects is not a reliable proxy for marten abundance.

Beyond the confounding influence of patterns of pine marten activity, the abundance of detectable pine marten scats on a 1km transect is influenced by a number of other 'non-marten' variables, and this further undermines attempts to use scat density to derive estimates of marten abundance. For example, the 'standing crop' of detectable marten scats is inevitably influenced by patterns of recent rainfall and vehicle traffic on transects, both of which may limit the longevity of scats where impacts are high. In the current survey heavy recent rain was considered to be a likely influence on scat abundance. The content of marten scats may also differentially influence their longevity, with those composed of mammal fur lasting longer as detectable entities than those composed of insect remains, which are more prone to fragmentation. Another factor is the visibility of scats, which may be

enhanced or curtailed by such local effects as the texture and colour of the transect surface, patterns of leaf fall and the extent and nature of vegetation growing on the transect; even predation by slugs has been reported as an influence on marten scat abundance (Birks *et al.*, 2004).

Differences in the visual acuity and experience of surveyors may also influence the number of marten scats counted per transect. In the current survey, where seven teams of experienced surveyors were involved, detection levels were broadly similar across teams, except among those that concentrated heavily in areas where evidence of martens was very scarce, such as south-west Scotland. Also, the positive influence of a scat detection dog was apparent in one team, where the animal found several scats that were missed by the human surveyor and both the number of scats found per transect and the proportion of DNA positive scats were higher than in any other team.

4.3.5 Methodological recommendations for future surveys

The Galloway Forest pilot study has demonstrated that pine marten occupancy may be underestimated where only a single 1km transect is completed per hectad, leading to the risk of 'false negatives.' This risk can be reduced in future surveys by completing a minimum of three transects per hectad in suitable habitat. Scat surveys remain the most time-efficient and cost-effective survey method for detecting pine marten presence during systematic distribution surveys.

The relationship between numbers of scats detected and pine marten occupancy/abundance may not be a simple one and this deserves further study.

4.4 DNA verification of scats

DNA typing of scats is standard practice in scat-based pine marten surveys, as marten scats are easily misidentified as scats from other species, principally fox *V. vulpes* (Davison *et al.*, 2002). Unfortunately, the proportion of scats that successfully yielded DNA to determine species level during this survey was very low (52%) compared with other studies (see section 3.2). If one excludes all non-determined scats collected, overall surveyor accuracy at identifying pine marten scats in the field was 74%, thus 26% of scats were misidentified. This result is comparable to the study conducted in Galloway Forest in 1999-2000 where experienced surveyors were found to misidentify 18% of believed 'marten' scats collected, resulting in an average surveyor accuracy rate of 82% (Davison *et al.*, 2002). Similarly, in a pine marten survey conducted in Argyll in 2011, 66% of scats determined to species level by DNA were pine marten and 34% were fox (VWT, unpublished data). These accuracy rates are also comparable with a study by Baines *et al.* (2011), in which 77% of scats subjected to DNA analysis were correctly identified as pine marten by surveyors. The mean of the surveyor accuracy rates across these four studies is 75%.

This indicates that experienced surveyors are capable of correctly identifying a large proportion of pine marten scats, but a level of misidentification is always to be expected. Many scats found in the field can be identified as pine marten relatively easily due to morphology and smell, so the term 'classic' or 'typical' pine marten scat can probably still be employed in many cases. However, DNA analysis is still recommended as standard practice to ensure confirmation of scat identification and robustness of survey results.

4.5 Issues arising from pine marten recovery

Of the six native carnivores to have suffered complete or near-complete anthropogenic extinctions since the Mesolithic (also involving the brown bear *Ursus arctos*, wolf *Canis lupus*, lynx *Lynx lynx*, wildcat *Felis sylvestris* and polecat *M. putorius*), the pine marten is the first to make a substantial recovery in Scotland to the extent that it is widespread once again. An identical process involving a different carnivore is under way in southern Britain, where the polecat *M. putorius* has also been recovering its range from a western stronghold (Birks, 2008).

The natural recovery of a native mammal after many decades of rarity and restricted range is a welcome example of biodiversity restoration. However, the recovery of carnivore populations presents challenges (Breitenmoser, 1998), especially when a species returns after an absence of many years, as is the case in Scotland. Following the mammalian extinctions described above, with similar losses among birds of prey, the human population of Scotland has become accustomed over generations to living in a predator-poor environment in which certain patterns of activity are viable because some predators are scarce or absent. Similarly, wild prey populations become 'predator-naive', developing patterns of behaviour and reproductive output that are unsustainable in the presence of their natural predators. In this context, if native predators are encouraged to recover as a consequence of policy and legislative changes, a period of adjustment is required during which both human and wildlife populations adapt to a more natural predatory environment. As the first recovering carnivore, therefore, the pine marten in Scotland can be regarded as a pioneer species from which important lessons about managing that adjustment process may be learnt.

4.6 Recommendations for future work

4.6.1 Conflict resolution

As the pine marten re-colonises parts of Scotland with higher human populations than those in its historical Highland core range, the species is increasingly likely to come into conflict with human interests. The pine marten's omnivorous feeding habits, nocturnality and exceptional climbing ability combine to create patterns of behaviour that are a potential nuisance for householders, gardeners and keepers of game and poultry. Although this issue was not explored during the current Expansion Zone Survey, it is clear that some public perceptions of the pine marten in Scotland are already influenced by personal experience of its 'nuisance value' and by assumptions about its adverse impact upon some prey species. Currently, the main issues of concern in Scotland are as follows: the pine marten's use of the roofs of inhabited buildings for breeding, with associated disturbance to human occupants; predation of poultry, game and other small livestock where traditional exclusion and deterrent measures are inadequate; consumption of soft fruit in gardens; and perceived threats to vulnerable prey populations arising from pine marten predation. There is a need to develop, test and promote cost-effective, sustainable solutions to such conflicts as the pine marten re-colonises areas from which it has been absent for many decades. The Scottish public should be made aware that there are non-lethal choices available to them when measures are required to resolve problems created by pine martens. For example, incorporating an electrified overhang and sheet materials to game and poultry pens has been proved to be an effective method for deterring pine martens from accessing them (Balharry & Macdonald, 1996). When combined with the greater appreciation and tolerance of predators characteristic of a more enlightened age, such measures are an important component of the reintegration of recovering native carnivores in both a practical and cultural sense (Treves & Karanth, 2003).

Where populations of prey such as red squirrel *Scuirus vulgaris*, goldeneye *Bucephala clangula* and capercaillie *Tetrao urogallus* are perceived as threatened by the pine marten's recovery, it is important to acknowledge that the pine marten is a natural predator of such species, with which it has coexisted in Scotland for several thousand years. There is a case for an education exercise to affirm that the pine marten is a valuable component of woodland ecosystems.

4.6.2 Assess pine marten distribution in areas where its status is currently unknown

There is a need to undertake distribution surveys in areas where the current distribution and status of pine martens is unknown. There is a growing body of evidence that pine martens are present in parts of southern Scotland, yet detailed information on their distribution and status in this area is currently lacking. Pine martens have now been reported from parts of the Scottish borders including the vicinities of Peebles, Hawick, Denholm, Jedburgh, the Forest of Ae and parts of East and Mid Lothian. A distribution survey should encompass parts of southern Scotland that have not previously been surveyed, comprising areas south of the Central Belt but excluding much of Dumfries and Galloway, which was covered in this Expansion Zone Survey. The proposed survey area should include the vice counties of Roxburghshire, Berwickshire, East Lothian, Mid Lothian, West Lothian, Renfrewshire, Lanarkshire, Peebleshire, Selkirkshire and parts of Dumfriesshire and Ayrshire. It is important to establish the presence and distribution of pine martens in these areas, as populations here could provide an important mechanism for the re-colonisation of the species in southern Scotland and the north of England, where martens are currently very scarce. This is particularly pertinent given the lack of expansion from the marten population in Galloway Forest.

It is also evident that there are gaps in our distribution knowledge in parts of the Central Highlands: northern Argyll, the west coast and western Perthshire (i.e. the area between the hectads positive during the 1994 distribution survey and this Expansion Zone Survey). These areas should be surveyed to confirm, rather than assume, presence of pine martens.

4.6.3 The south-west population

The causes of the apparently poor performance of the reintroduced pine marten population in south-west Scotland should be determined. In particular, research should address the hypothesis that the population is genetically constrained by founder effects arising from the very small number of individuals released in the early 1980s. Relevant information could be gathered via a combination of non-invasive tissue sampling and monitoring of reproductive output (both facilitated by the martens' frequent use of den boxes in the Galloway Forest), together with further scat-based distribution surveys to monitor future range expansion. It is possible that anthropogenic factors could also be limiting the expansion of the population.

4.6.4 Targeted surveys of relict haplotype *i* populations

Recent research has demonstrated that a relict pine marten population of a rare genetic type (haplotype *i*) may be present in western Scotland (Jordan *et al.*, 2012). Haplotype *i* was the dominant haplotype of the historical pine marten population in England and Wales but appears to have become extinct during the mid-20th century and has not been detected in extant populations in these areas since 1924 and 1950 retrospectively. The dominant haplotype of the pine marten in Scotland is haplotype *a* (Davison *et al.*, 2001; Jordan *et al.*, 2012). However, in 2010, two haplotype *i* pine marten kits were found on the Isle of Eriska, off the west coast near Oban. This discovery suggests that this area may harbour a relict population of haplotype *i* individuals.

Relatively little is known of the distribution of haplotype *i* pine martens in Scotland yet haplotype *i* individuals here could potentially represent the logical source population for any future reintroduction or restocking attempt to England and Wales, where pine martens are very scarce (Birks & Messenger, 2010). As per International Union for the Conservation of Nature and Natural Resources (IUCN) guidelines for reintroductions, it is desirable that source animals come from wild populations, and that founder stock should be supplied from a population that is closely related genetically to the original native stock and shows similar ecological characteristics to the original sub-population (IUCN, 2012). Therefore, relict haplotype *i* populations in Scotland would be the most suitable source population for any future reintroduction or restocking programmes in southern Britain. To this end, survey work to determine the distribution and abundance of haplotype *i* pine martens in Scotland would be beneficial.

5. CONCLUSION

The pine marten is the first of those mammalian predators so nearly eradicated by humans in the late 19th century to make a substantial recovery in Scotland. Its slow 20th century re-colonisation of its former Scottish range has continued into the new millennium. Since the 1990s, the species has spread further beyond its core Highland stronghold and now occupies many lowland areas with higher human populations. However, in many areas covered in the 2012 Expansion Zone Survey signs of pine martens were sparse and patchy, suggesting that populations here may not yet be well-established. This is likely to be a temporary consequence of the pattern of re-colonisation, though it is not possible to rule out the adverse effects of habitat constraints and anthropogenic pressures that are more prevalent in the lowlands than in the pine marten's core Highland range. The sparse and patchy distribution recorded in some areas may also be a consequence of the scat survey methodology failing to detect pine marten populations occurring at a low density. Assumptions about the future spread of the pine marten must be tempered by uncertainty about its ability to establish populations in lowland areas with limited and fragmented woodland cover, or locations where predator control leads to increased mortality.

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