



Myotismart Ltd

FLEET BASIN PINE MARTEN PROJECT

Final Report

**A report to:
Forestry Commission Scotland
and Peoples' Trust for Endangered Species**

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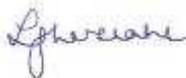
© Swift Ecology Ltd
Fartown Barn
Pensax
Abberley
Worcs
WR6 6AE

Tel 01299 890261

Email swifteco@swiftecology.co.uk

Website www.swiftecology.co.uk

QUALITY CONTROL

DATE	VERSION	NAME	SIGNATURE
21.12.16	Draft prepared	Johnny Birks MCIEEM Principal Ecologist/Director	
22.12.16	Checked by	Lisa Kerslake CEcol FCIEEM Principal Ecologist/Director	
22.12.16	Reviewed and issued	Johnny Birks MCIEEM Principal Ecologist/Director	

The information which we have prepared and provided is true, and has been prepared and provided in accordance with the Chartered Institute of Ecology and Environmental Management's Code of Professional Conduct. We confirm that the opinions expressed are our true and professional *bona fide* opinions.

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SUMMARY

- This report describes fieldwork on pine martens in the Fleet Basin area of Galloway Forest Park between September 2014 and May 2016.
- Non-invasive collection of scat and hair samples – and subsequent genotyping - were used to produce a population estimate for pine martens in the Fleet Basin in autumn 2014.
- 52 hair samples and 114 scats from autumn 2014 were analysed by the Waterford Institute of Technology to determine species, gender and individual genotype. 89% of samples were confirmed as pine marten; genotype success rates differed between hair samples (43%) and scats (23%).
- On the basis of genotypes identified, a minimum of 15 pine martens (seven males and eight females) was recorded as present in the Fleet Basin during autumn 2014, although up to one third of these are likely to have been dependent juveniles still occupying their natal ranges. The Capture Recapture programme *Capwire* produced a population estimate of 18 individuals.
- The 50 '*Galloway Lite*' pine marten den boxes installed in September 2014 were inspected in May 2015 and January and May 2016 for evidence of use by pine martens; a cumulative total of 31 boxes (62%) showed evidence of occupancy but no breeding was confirmed; this is likely to have been a consequence of low prey availability or habitat factors.
- Patterns of scat abundance, activity at hair tubes and den box occupancy since September 2014 suggest some marked variations in pine marten activity across the Fleet Basin. These are likely to be associated with variations in forest structure linked to the harvesting cycle, but this relationship was not investigated during this study.
- This study has successfully established baselines for future monitoring of the pine marten population in the Fleet Basin. Systematic non-invasive sampling of scats and hairs can be repeated in future years in order to derive genotypes for population estimates; and patterns of scat and hair sample abundance can be combined with data on *Galloway Lite* den box occupancy to provide information on variations in pine marten activity across the Fleet Basin.

1 INTRODUCTION

1.1 *Background*

This is the final report on the Fleet Basin Pine Marten Project. It describes bouts of fieldwork between September 2014 and May 2016 – involving collection of scat and hair samples and the installation and checking of den boxes - and subsequent genotyping of scat and hair samples in order to identify individuals present within the study area. This information was used to produce a pine marten population estimate for the Fleet Basin. Simple patterns of variation in pine marten activity across the Fleet Basin were derived from variations in the abundance of scats, hair samples and instances of den box occupancy.

The aim of the project was to establish a non-invasive monitoring programme for pine martens in The Fleet Basin Red Squirrel Stronghold (part of Galloway Forest Park in south-west Scotland) in order to inform red squirrel conservation efforts. This project is partly inspired by recent research in Ireland by Dr Emma Sheehy, which suggests that, under certain circumstances, pine martens contribute to red squirrel conservation efforts by suppressing grey squirrel populations. Currently red squirrels in The Fleet Basin are threatened by the encroachment of grey squirrels carrying squirrel pox virus.

The main objectives of the project were as follows:

- To establish a baseline inventory of pine martens present in The Fleet Basin in 2014;
- To identify broad patterns of pine marten distribution and activity within The Fleet Basin in 2014;
- To establish a long-term monitoring programme focusing on pine marten distribution and abundance in The Fleet Basin;
- To facilitate further research through provision of information and material of relevance to red squirrel conservation in The Fleet Basin (e.g. marten breeding success and faecal samples for dietary analysis).

This is a collaborative project, involving contributions from Forestry Commission Scotland (FCS), the Molecular Ecology Group at Waterford Institute of Technology (WIT) and two ecological consultancies involved in pine marten conservation, surveys and monitoring: Myotismart and Swift Ecology. The project is supported by funding from FCS and the Peoples' Trust for Endangered Species (PTES).

1.2 *Purpose of this Report*

This final report summarises and expands upon a series of interim reports produced to inform our funders, partners and collaborators about progress with the project. In order to keep the interim reports brief and readable we avoided detailed descriptions of methodology and full analysis and evaluation of results; these are now presented in this final report.

1.3 Non-Invasive Genetic Sampling

Non-destructive genetic sampling became viable with the discovery of the polymerase chain reaction (PCR), which allows amplification of DNA from small quantities of both fresh and dry tissues (Tarberlet & Luikart, 1999). Subsequent advancements revealed that DNA could be extracted from shed hair or feathers, shed skin, faeces or saliva. These methods allow for genetic sampling of wild animals without animals needing to be disturbed, captured or even observed. Genetic tagging is advantageous over physical tagging as it reduces the stress and mortality involved in capturing animals, reduces bias resulting from variable responses to traps and can increase the number of observations and thus improve population estimates (Miller *et al.*, 2005). The use of non-invasive methods to collect samples for genetic analysis is now widely applied and is used to gather information on distribution, population composition and identification of individuals (Zielinski *et al.*, 2006).

In recent years, non-invasive sampling of faeces, hair and urine has been employed to study populations of a variety of elusive carnivore species. These non-invasive methods have also been employed in several population studies of *Martes* species. This typically involves snaring of hair, which is a desirable survey method as capture of the animal is not required, the snaring device does not restrain or harm the animal and the cost of materials is low (Foran *et al.*, 1997). Individual animals can be identified by genotyping hair samples, which allows population size and density to be calculated (Mowat & Paetkau, 2002).

Hair snares were first trialled for American martens *Martes americana* to identify species, sex and individuals from hair samples (Foran *et al.*, 1997). Hair snares comprise a wooden, triangular or square squeeze tube in which patches of glue traps are fixed, along with bait. The snare is attached to a tree trunk at chest height and when an animal enters to take the bait, a small sample of hairs is left on the glue patch. Subsequently, this design has been used to estimate abundance and density of American martens in British Columbia (Mowat & Paetkau, 2002), and to assess population estimates of reintroduced populations of American martens and fishers *Pekania pennanti* in Michigan (Williams *et al.*, 2009). Hair snares have also been incorporated into track plates fixed to the ground, which has been successful in collecting hairs from American martens and fishers in California (Zielinski *et al.*, 2006).

The traditional hair snare design was modified by Mullins *et al.* (2010) to collect samples from pine martens in Co. Waterford, south-east Ireland. This design comprises a lightweight polyvinyl chloride tube with a patch of glue-based mouse trap which is baited and fixed to trees (for full description of the design see Methods or Mullins *et al.* (2010)). The hair tube proved successful in collecting hair from pine martens, with a 90.5% success rate, and provided population estimates following the identification of individuals through genotyping (Mullins *et al.*, 2010). The hair tube method has subsequently been employed to estimate pine marten population abundance and density in the Mourne Mountains, Northern Ireland (O'Mahony *et al.*, 2014) and at sites in the Cairngorms National Park, Scotland (Kubasiewicz, 2014).

1.4 Personnel

The project was led by Johnny Birks (JB) of Swift Ecology and John Martin (JM) of Myotismart, with the valuable administrative and fieldwork support of Shirley Martin (SM) also of Myotismart. Catherine O'Reilly and Peter Turner of the Waterford Institute of Technology (WIT) were crucial members of the team: they arranged the genotyping of all non-invasive samples collected and they undertook analysis and interpretation of the results. Lizzie Croose of the Vincent Wildlife Trust (VWT) made a substantial contribution to the project through her involvement in fieldwork and data analysis for the 2014 Fleet Basin population estimate; this was the subject of her MSc thesis at Edinburgh University (Croose, 2015), and the text and figures in this report draw heavily upon her work. Subsequently Lizzie has provided much helpful input and mapping. Dooley and Zella provided valuable canine assistance during scat collection operations.

During bouts of fieldwork the team was joined by many naturalists and researchers interested in learning about the project; the following people were involved: David Tosh, Faith Billington, Chas Mooney, Trina Barrett, Stephen Parker, Emma Sheehy, Jane Sedgeley-Strachan, Pete Garson, Peter Lurz and Martin Bagot; we were also pleased to involve local members of FCS staff, notably Andrew Jarrott, Martin Webber and Gareth Ventress.

1.5 Acknowledgements

We are very grateful to Nida Al-Fulaij of PTES and Andrew Jarrott and Kenny Kortland of FCS for their support and encouragement and for arranging funding for the project via their respective organisations. Martin Webber and Gareth Ventress of FCS provided valuable logistical support and information on harvesting patterns, as well as good company in the field. We are pleased to acknowledge the assistance during fieldwork of the naturalists and researchers listed in section 1.4 above.

We thank Kirsty Park of the University of Stirling for kindly lending hair tubes for the population estimate during autumn 2014. And we are grateful to Laura Kubasiewicz and Declan O'Mahony for their generous advice on abundance and population density modelling methods.

We are especially grateful to Peter Garson and Eliska Robson for kindly accommodating JM and Zella during the final stages of the autumn 2014 hair tube element of the project. We warmly thank Alan Vinnie and his staff at *Galloway Lodge* for providing frequent shelter and sustenance at the Clatteringshaws Visitor Centre; for hosting a successful public pine marten event with excellent refreshments in September 2015; and we thank Martin Webber and FCS for organising and subsidising the event.

2 METHODS

2.1 The Study Area

The study area was based on a block of multi-purpose forest known as the Fleet Basin, at the south-eastern edge of Galloway Forest Park in south-west Scotland (55°02 N, 4°16 W) (see Figure 1). The region has a mild climate with average temperatures ranging from 1.3 °C in the winter to 19.5 °C in the summer (Met Office, 2013) and average annual rainfall of around 1600 mm (Forest Enterprise, 2012). The Fleet Basin is a commercial conifer plantation covering approximately 100 km² and is dominated by Sitka spruce *Picea sitchensis* and lodgepole pine *Pinus contorta*, with minor coverage of larch *Larix* spp., Scots pine *Pinus sylvestris*, Norway spruce *Picea abies* and Douglas fir *Pseudotsuga menziesii*, and very little broadleaf (Forest Enterprise, 2012). The dominant tree age structure is pole stage and mature and old forest stage crops with a smaller proportion at establishment or thicket stage (Forest Enterprise, 2012). Evidence of disease caused by *Phytophthora ramorum* was confirmed on larch in the forest in 2011 (Forestry Commission Scotland, 2013) and consequently, sanitation felling of larch was carried out in the Fleet Basin during the study period, in order to contain outbreaks and spread of the pathogen. The Fleet Basin is designated as a Red Squirrel Stronghold, whereby management is driven by the requirements of red squirrel *Sciurus vulgaris* conservation.



Figure 1. The location of the Fleet Basin in south-west Scotland.

2.2 Fieldwork Timeline

The Fleet Basin Project was based on five bouts of fieldwork, commencing with the initial phase in autumn 2014 (from which a pine marten population estimate was derived) and ending with the final check of the Galloway Lite den boxes in early summer 2016. The timings, main activities and personnel involved on these five bouts are summarized in Table 1 below.

Although the original aim was for four bouts of fieldwork, an unscheduled extra visit was made by JM and SM in January 2016 in order to reduce the risk of deterioration of sticky hair collection devices and any associated hair samples over winter.

Table 1. The timings, main activities and personnel involved in the five bouts of fieldwork in the Fleet Basin between September 2014 and May 2016.

Dates	Activity	Personnel
Sept/Oct 2014	Installation of hair tubes and establishment of scat collection transects; subsequent servicing (e.g. re-baiting of hair tubes) and collection of non-invasive samples for genotyping; installation of 50 Galloway Lite den boxes	JB, JM, SM, Lizzie Croose, Pete Garson, Chas Mooney
May 2015	Checking of Galloway Lite den boxes	JB, JM, SM, Faith Billington, Pete Garson, Pete Turner, Peter Lurz, Stephen Parker
September 2015	Scat collection, including a repeat of the transects established in September 2014; installation of sticky hair patches at Galloway Lite den boxes; raising of half of the GL boxes to greater elevation; public pine marten event	JB, JM, SM, Trina Barrett, Chas Mooney, Pete Garson and David Tosh
January 2016	Visits to all Galloway Lite den boxes for retrieval of sticky hair patches	JM and SM
May 2016	Checks of all Galloway Lite den boxes	JB, JM, SM, Lizzie Croose, Emma Sheehy, Jane Sedgeley-Strachan, Pete Garson and Martin Bagot

2.3 Deployment and Servicing of Hair Tubes

Hair traps developed at Waterford Institute of Technology (WIT) have been used by Peter Turner, Catherine O'Reilly and their colleagues to collect hair samples and develop microsatellite-based genotyping for pine martens in Ireland (Mullins *et al.* 2010). This method has also been used by the same team in a long-running study to monitor the pine marten population in Portlaw woods, Co. Waterford since 2009. The team has pioneered the genotyping of marten hairs from tubes in order to

distinguish individual martens and construct maps and Gantt charts of marten occupancy.

In the Fleet Basin we used the same design of tubes as our Irish colleagues and, in designing our study, we drew upon the recent experience of Laura Kubasiewicz who first deployed this approach in Scottish forests as part of her PhD studies in the north-east of the country. Recognising that pine martens in Galloway Forest occur at a considerably lower population density than those in Ireland, and because the Fleet Basin study area extends over some 10,000 ha of forested land, we opted for a relatively low density of one hair tube per kilometre square.

Hair tubes, comprising lightweight polyvinyl chloride tubes measuring 250 mm in length and 118 mm in width, were used to gather hair samples (for full details, see Mullins *et al.*, 2010). 100 tubes were installed by two teams of two people working independently over 2.5 days (7-9 September 2014), with approximately 1 km spacing between each tube where practicable (see Figure 2). Grid squares that had less than 50% forest cover and those without vehicle tracks for easy access were excluded. Hair tubes were installed within 10 m of the forest track and were fixed vertically to the trunk of a tree using wire, approximately 1 m off the ground. Two patches of 'mouse glue' (Pest Control Supermarket, <http://www.pestcontrolsupermarket.com>) cut to 1 cm x 1.4 cm rectangles and stuck with double-sided sticky tape to blocks of 4 mm thick Correx Board measuring 2 cm x 2.5 cm were fitted to the base of the tube to catch hair when an animal entered. To aid relocation, each tube site was identified by coloured marker tape on the edge of the forest stand; GPS locations (10-figure grid references) were also recorded for each tube.

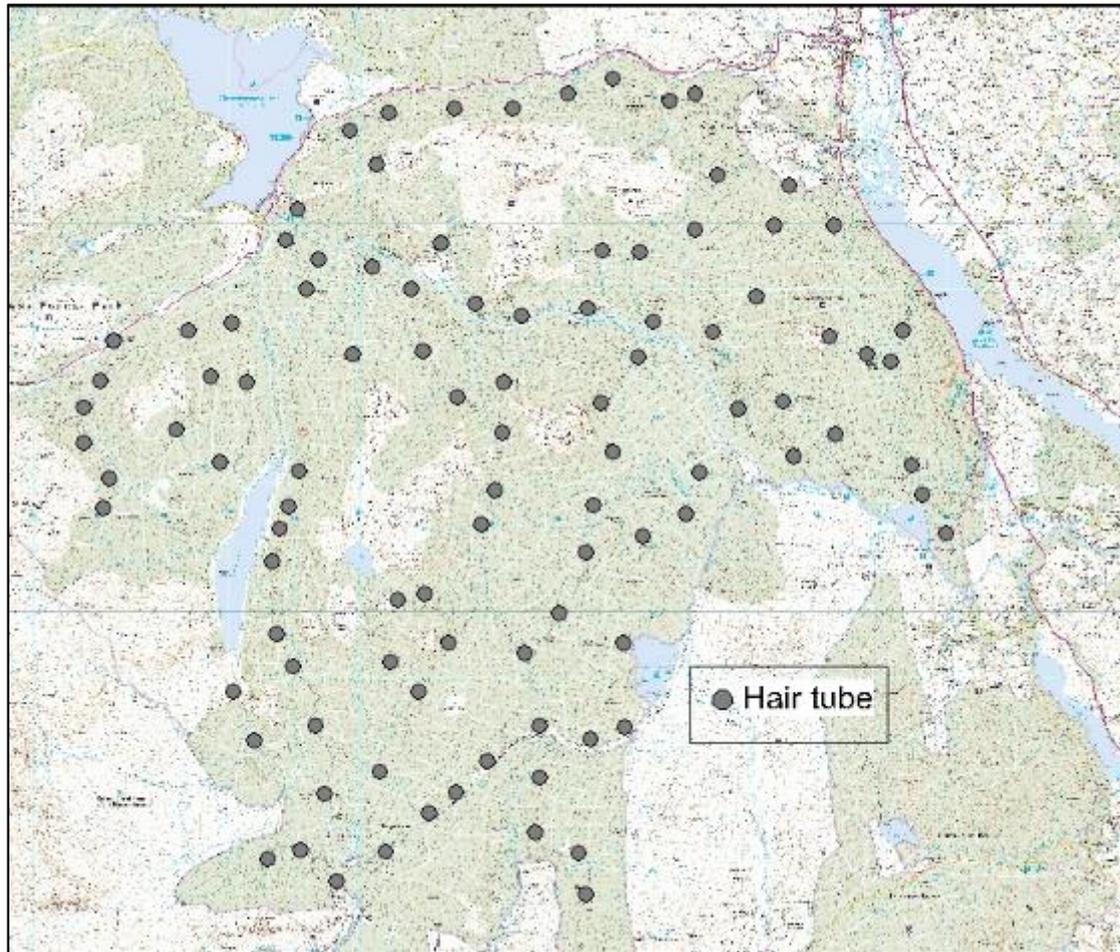


Figure 2. The distribution of the 100 pine marten hair tubes installed and monitored in The Fleet Basin during September and October 2014 (Based upon Ordnance Survey material with the permission of the Controller of HMSO © Crown Copyright (2013) Licence no. 100017908).

Tubes were baited with chicken meat attached to the inside of the tube lid with wire or string. Four sampling sessions were conducted, with tubes sampled on visits between 5-9 days apart over a total of 32 days; the mean period between all visits to install and service tubes was 6.4 days. During each sampling session, any glue patches that had collected a hair sample were removed and placed in a plastic sample pot, labelled with a unique sample number and subsequently frozen at -4°C within 12 hours of collection. Fresh glue patches were fitted and fresh bait was installed in the tube on each occasion when patches were removed. Peanut butter was smeared on the tree after the first check as an added attractant for pine martens. All hair tubes were removed on 7/8 October 2014.



Plate 1. Servicing a hair tube (left); close-up of tube to show sticky patch inside base (right).

2.4 Scat Collection

2.4.1 Scat transects

Where their populations are well-established pine martens deposit some of their scats on tracks and paths through suitable habitat as a means of communication and territory defence. Consequently, in forests with established networks of roads and tracks, non-invasive survey and monitoring information on pine martens can be gathered via the detection and identification of scats according to standard protocols. We adopted the standard protocol for pine marten scat surveys based upon searching 1 km transects and collecting all fresh scats for genotyping (see Croose *et al.*, 2013 and 2014). Two surveyors (JM and JB) used trained dogs to assist in the detection of pine marten scats.

Each transect was searched for pine marten scats once during September 2014, and the exercise was repeated in September 2015. A count was made of all probable pine marten scats detected on each transect; and all fresh marten scats were collected, individually bagged and stored deep-frozen for subsequent genotyping. 10-figure grid references were recorded for all scats collected. A count was also made of fox scats detected on transects.

In order to achieve proper spacing of transects, we divided the Fleet Basin into 21 zones of equal size (approximately 5 km²) and selected a single 1 km transect for survey within each zone. Precise transect locations were selected by surveyors working on the ground so as to ensure that all main forest habitats were represented. All transects were based upon lengths of forest road (see Figure 3

below). 10-figure grid references were recorded for the start and end of each transect to ensure that surveys could be repeated in future years.



Plate 2. Examples of scat transects in the Fleet Basin.

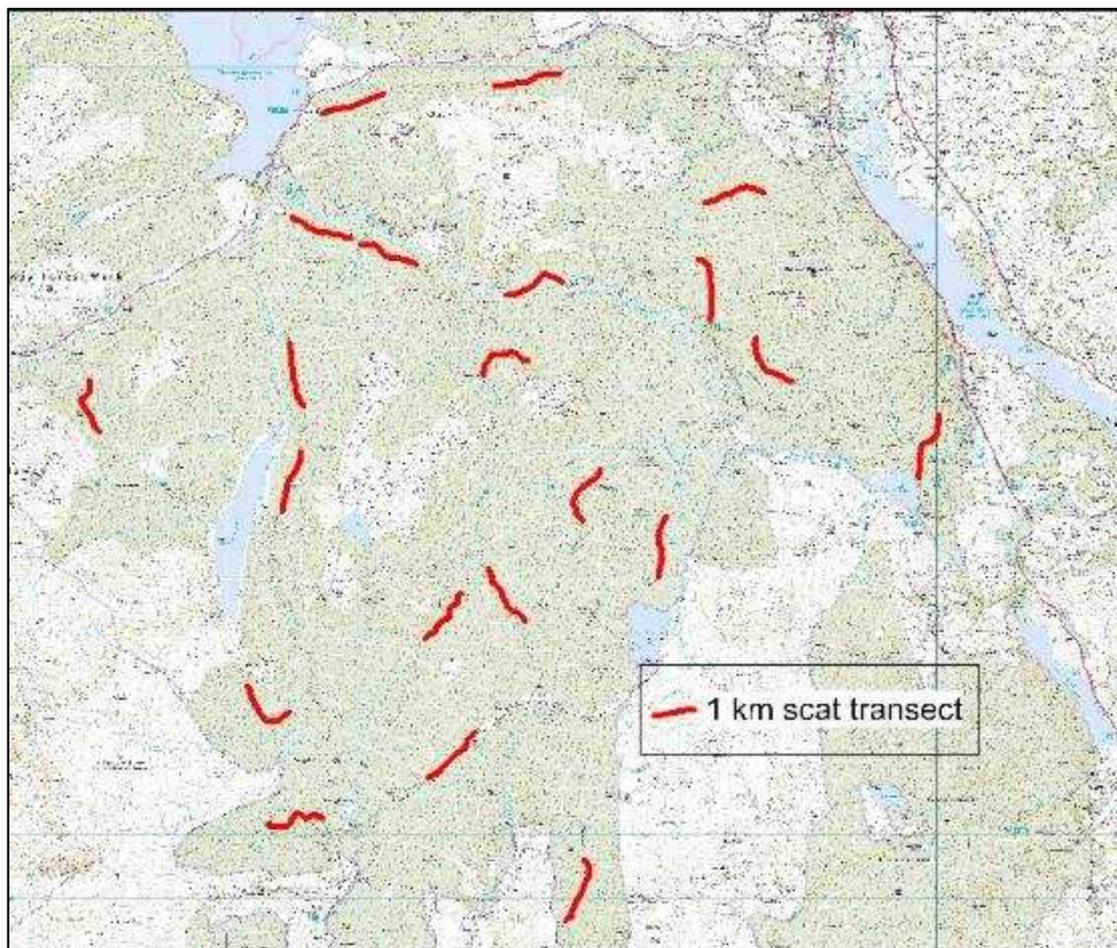


Figure 3. The location of 21 1 km transects searched for pine marten scats in The Fleet Basin during September 2014 and 2015 (Based upon Ordnance Survey material with the permission of the Controller of HMSO © Crown Copyright (2013) Licence no. 100017908).

2.4.2 Ad hoc collection of scats

Throughout our fieldwork between 2014 and 2016 we collected fresh marten scats on an *ad hoc* basis from many locations within the Fleet Basin in order to maximise the number and distribution of samples for genotyping. In September 2014 many of these scats were collected by Lizzie Croose who conducted additional searches in parts of the Fleet basin beyond the 21 systematic transects shown in Figure 3 above. Several scats were collected from close to hair tubes during servicing visits and subsequently close to den boxes during checks.

2.5 Genotyping scat and hair samples

Genomic DNA was isolated from the hair samples using the ZR Genomic DNA™-Tissue MicroPrep (D3041) kit (ZYMO Research, CA, USA) using the protocol for hair DNA extraction (D3040). For DNA isolation from scats approximately 0.2 g of scat material was transferred to 1 ml S.T.A.R.® buffer (Stool Transport and Recovery buffer, Roche) in a 2 ml microfuge tube. The tube was vortexed for 15 seconds and then left to stand for at least 60 minutes. 200 µl of the supernatant was transferred to a microcentrifuge tube for DNA isolation. DNA was isolated using the ZR Genomic DNA™-Tissue MicroPrep (D3041) kit (ZYMO Research, CA, USA).

Real-time quantitative qPCR assays for species and sex identification were carried out as described in Mullins *et al.* (2010). Two PCR replicates were carried out for molecular sexing (Lynch and Brown 2006). Females were identified through the amplification of ZFX only, while a signal from both ZFX and ZFY probes indicated male DNA was amplified. The ZFX allele therefore acted as an internal amplification control for the assay. Samples were selected for genotyping based on the results of the qPCR sex-typing assay with samples with a ZFX C_T value < 34 selected for genotyping.

Microsatellite analysis to identify individual pine martens was carried out using 8 microsatellite markers. These were: Gg7; Ma2; Mel105; Mvi1341; Mvis075 (see Mullins *et al.* 2010) and Mar21; Mar53 and Mar08 (Natali *et al.* 2010). Each sample was analysed in duplicate and only samples giving identical results in the replicates were scored. PCR reactions were carried out in 3 multiplex reactions. Multiplex 1 contained Gg7 and Mvi1341; Multiplex 2 contained Mar21, Mar53 and Ma2; and Multiplex 3 contained Mel105, Mar08 and Mvis075. Each PCR reaction contained 5 µl GoTaq Hotstart master mix (Promega Inc.), 1 µl multiplex primer mix (200 nM each primer) and 4 µl DNA extract.

The PCR protocol was 95°C initial denaturation for 5 min, followed by 40 cycles of 94 °C for 30 s, 58 °C for 1 min. and 72 °C for 30 s, with a final extension time of 30 min. at 72 °C. Fragment analysis was carried out on an ABI PRISM 310 genetic analyser under standard run conditions with 4 % polyacrylamide. Alleles were scored against a GS500 LIZ™ size standard using GeneMapper software version 3.7 (Applied Biosystems). Genotype data were analysed for probability of identity (PI and PIsibs), observed (H_o) and expected (H_e) heterozygosity, allele frequencies and relatedness using GENALEX version 6 (Peakall and Smouse 2006).

2.6 Pine Marten Population Size and Density Estimates

A population abundance estimate was derived for autumn 2014 using the capture-recapture programme Capwire (Miller *et al.*, 2005). Capwire is appropriate for estimating population size in non-invasive genetic studies as it allows for sampling with replacement and takes account of multiple observations of an individual within a sampling session. Capwire also takes account of the capture heterogeneity within the sampled population by applying the Two Innate Rates Model to assign a 'capture type' to individuals (a high or low probability of being captured) (Miller *et al.*, 2005). Capwire has been shown to provide accurate population estimates when analysing small populations with substantial capture heterogeneity (Miller *et al.*, 2005). All captures of individual pine martens, identified through genotyping, were grouped into a single sampling session scheme for analysis. The maximum population size was set as 100 and 95% confidence intervals were used to calculate a population estimate with 1,000 parametric bootstrap replicates. The Likelihood Ratio Test (LRT) was implemented to choose the most suitable of two models for analysis: the Even Capture Model (ECM), which assumes that there is no capture heterogeneity and that each individual has an equal chance of being captured; or the Two Innate Rates Model (TIRM), which assigns a high or low capture probability to individuals.

To estimate the population density, an effective trapping area was estimated, with the inclusion of a buffer strip, to account for 'edge effects' caused by movement of animals in and out of the area (Otis *et al.*, 1978; Royle *et al.*, 2013). For the study area, a convex hull was delineated by the locations of the outermost hair tubes and scat transects using MapInfo Professional (v12.0). The maximum distance moved was calculated for individual pine martens that were detected at a minimum of two locations ($n=9$) and a straight line was drawn between the two detection locations that were the furthest apart using MapInfo. The mean maximum distance moved for all of these individuals was calculated to provide an estimator of home range diameter (mean=2.42 km) and the width of half mean maximum distance moved (1,021 m) was applied to the study area convex hull to create an effective trapping area (Royle *et al.*, 2013). Lochs and significant areas of open ground, which mostly comprised moorland, were excluded from the effective trapping area as pine martens strongly avoid this type of habitat (Balharry, 1993; Caryl *et al.*, 2012). The minimum area of open ground that was excluded was 1.37 km². Population density was calculated by dividing the population size by the effective trapping area (Otis *et al.*, 1978). Two post-breeding population density estimates were produced: one based on the minimum population size as derived from individual genotypes; and one based on the population estimate determined by Capwire. A third density estimate was derived based on the number of adult pine martens estimated to be in the study area; this was estimated based on information on the relatedness of individual pine martens, which allowed presumed juveniles to be identified and therefore excluded from the estimate.

Fisher's Exact Test was used to test for statistical significance in the genotyping success rate for hair samples.

2.7 Galloway Lite Den Boxes

A new lightweight design of pine marten den box, the *Galloway Lite*, was installed at 50 evenly-spaced locations across the Fleet Basin (this resulted in a density of approximately one box per 2 km² of forested habitat). These boxes, designed by JM, are an alternative to the successful VWT den box that, because of its large size and weight, has to be installed on a substantial tree. The advantage of the smaller, lighter *Galloway Lite* box is that it can be installed on a relatively young tree so, in commercial plantations, there is less constraint upon the selection of sites for installation of boxes.



Plate 3. The modular 'Galloway Lite' den box (left); box installation in a spruce stand (right).

2.8 Constraints

2.8.1 Weather

Some of the project's fieldwork elements were prone to influence by the direct or indirect effects of weather, which may in turn have influenced the success of genotyping hair and scat samples collected.

Autumn 2014

The weather in Galloway during mid-September 2014 was characterised by unusually mild, dry and calm conditions thanks to an established area of high pressure, so for human surveyors the conditions for detecting scats were very good during the first two weeks of fieldwork when most scats were collected. Fresh scats were generally in good condition in the absence of rain and high humidity; however, these conditions were less than ideal for the scat detection dogs Dooley and Zella, which need some breeze and humidity in order to detect the scent of fresh marten scats.

From late September 2014 onwards the weather became unstable with abundant rain and some strong winds. Generally the weather was not felt to be a significant constraint upon this phase of the project.

May 2015

The weather in Galloway was cool and mainly dry in May 2015 so was not a significant constraint upon this phase of the project.

September 2015

The weather in Galloway during late September 2015 was characterised by low pressure systems tracking eastward and bringing rain showers (some heavy and/or prolonged) and sunny spells in generally mild conditions. Although the rain pattern is likely to have affected the condition of some marten scats and the prospects of extracting good quality DNA, generally the weather was not felt to be a significant constraint upon this phase of the project.

May 2016

The weather in Galloway during mid-May 2016 was mainly calm, warm and dry, so this was not viewed as a constraint.

2.4.2 Sanitation felling

Many parts of the Fleet Basin were affected during the study period by routine harvesting, and by sanitation felling and removal of larch *Larix* sp. stands in order to tackle the serious outbreak of the plant pathogen *Phytophthora ramorum*. Most of the *Galloway Lite* boxes were not directly affected by this work, although inevitably there were some instances where boxes had to be relocated because of harvesting activity. Because of the extent of felling of larch, on top of routine harvesting, the proportion of the Fleet Basin represented by recently felled land in 2016 was greater than normal. The likely impacts of extensive clear-felling just before and during the study period on the Fleet Basin Pine Marten Project are difficult to estimate. The following impacts cannot be ruled out:

- Disturbance effects upon individual pine martens as a result of extensive removal of tree cover, leading to changes in the animals' patterns of foraging, breeding, scating and resting behaviour (e.g. avoidance of recently felled areas);
- Adverse impacts upon the viability of some individual pine marten home ranges affected by extensive clear-fells, to the extent that the Fleet Basin might support a reduced number of pine marten home ranges until habitat quality improves;
- Accidental impacts upon monitoring equipment (hair tubes and *Galloway Lite* den boxes) installed as part of this project in stands harvested during or after fieldwork;
- Interference with the systematic recording of pine marten scats during this project arising from a reduction in the detectability of scats by human surveyors on tracks used heavily by timber lorries and other vehicles.

2.4.3 Other constraints

Pine marten activity in the Fleet Basin was also likely to be influenced by the field vole cycle: our monitoring of vole abundance indicates that there was a population peak in 2014/15, followed by a crash, so that in 2016 numbers were at a low point; this crash might have an adverse effect upon pine marten numbers and/or activity, including reproduction.

3 RESULTS

3.1 *Non-invasive hair and scat samples*

3.1.1 Hair samples

Servicing of the 100 hair tubes in September and October 2014 produced 52 hair samples for genotyping over the 32-day period covered. Table 2 below illustrates the increase in the number of tubes producing hairs over the four checks (from 4 to 25) and the cumulative increase in the number of hair samples arising from multiple marten visits to tubes (from 4 to 52) and 'positive' hair tubes (from 4 to 26).

Table 2. A summary of hair sample collection during the four visits to the 100 tubes installed in the Fleet Basin in September 2014.

Check no.	No. tubes with hair samples	Cumulative number of hair samples collected	Cumulative number of tubes producing hair samples
1	4	4	4
2	10	14	13
3	13	27	15
4	25	52	26

Of the 52 hair samples, 98% (n=51) were identified as pine marten by DNA analysis, of which 88% (n=46) were gender-typed (30 male samples and 16 female samples) (see Table 3). There were almost twice as many male detections as female detections in the hair tubes. Forty-three percent (n=22) of hair samples were genotyped (the remainder failed because of the inadequate quantities of DNA), identifying five individual pine martens: two males and three females. The number of times an individual pine marten was detected in a hair tube varied from two to nine (average = 4.4).



Plate 4. Sticky patches from a hair tube with samples of pine marten hair attached.

Figure 4 below illustrates the extent to which the 26 'positive' hair tubes were concentrated mainly in the south-central and eastern parts of the Fleet Basin study area, with many tubes in the far north and west recording no visits by pine martens.

One hair tube was lost to harvesting operations towards the end of the study (between servicing visits 3 and 4).

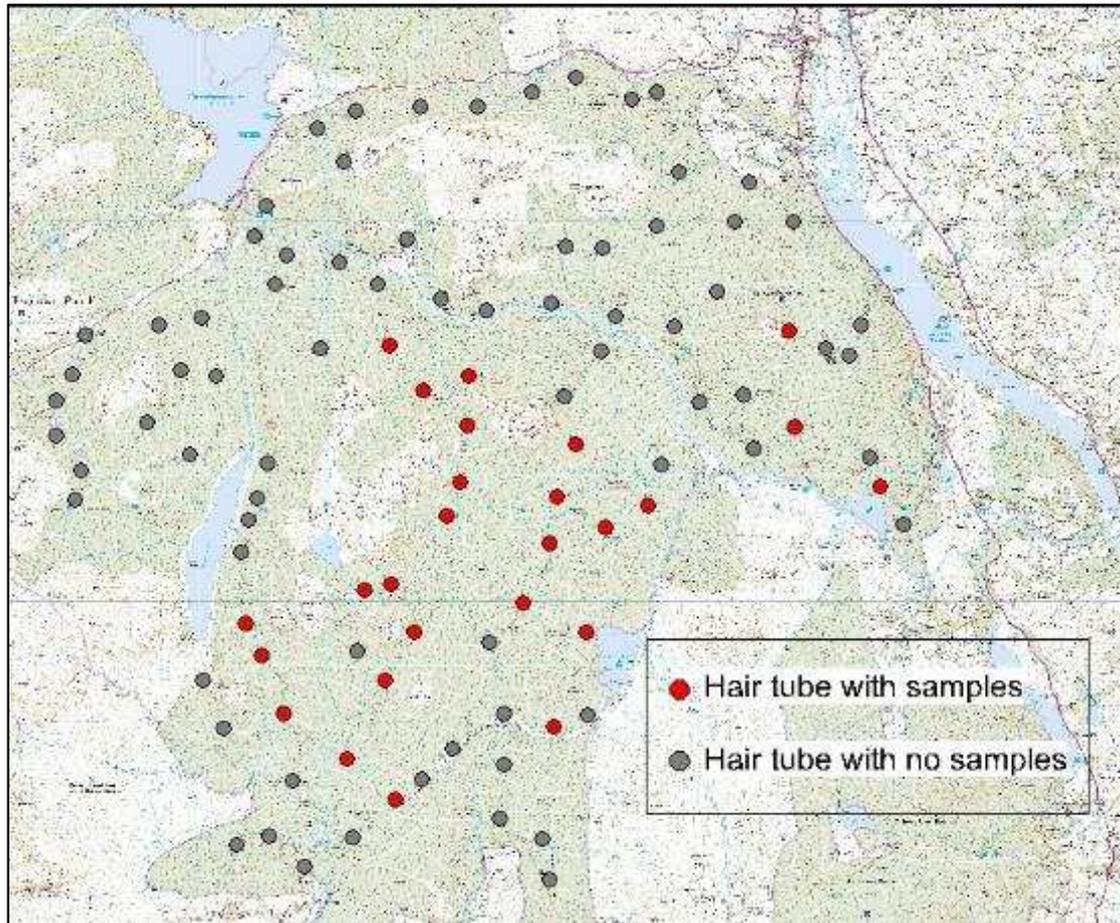


Figure 4. The distribution of positive and negative hair tubes in The Fleet Basin during September and October 2014 (Based upon Ordnance Survey material with the permission of the Controller of HMSO © Crown Copyright (2013) Licence no. 100017908).

3.1.2 Scats

September 2014

The 21 scat transects were completed successfully during mid-September 2014 in good weather conditions. 73 probable pine marten scats were detected on the 21 transects at a mean density of 3.5 scats per transect. However, scat density varied greatly across transects (see Figure 5 below), with eight negative transects (i.e. no scats found) and one transect producing 35 scats; this unusually productive transect was in an area believed to be occupied by a breeding female pine marten during summer 2014. 19 out of the 21 transects revealed five or fewer scats. 47 relatively fresh scats were collected for genotyping from 12 transects.

September 2015

Nineteen of the 21 scat transects were re-surveyed successfully during mid-September 2015 in reasonable weather conditions; two transects (nos. 11 and 19) could not be surveyed due to active resurfacing works; some other transects had been recently resurfaced but were nonetheless surveyable.

91 probable pine marten scats were detected on the 19 transects at a mean density of 4.8 scats per transect (this compares with 73 scats across 21 transects in September 2014, with mean scat density 3.5 per 1 km transect). However, as in 2014, scat density varied greatly across transects (see Figure 5 below), with two negative transects (i.e. no scats found) and three transects producing 14, 19 and 22 scats. These 'super-productive' transects appeared to be ones where the track contained a scat-marking site used frequently by one or more martens, sometimes with ten or more scats of various ages clustered at one location. 13 out of the 19 transects revealed five or fewer scats. 22 of the most recent/fresh scats were collected for genotyping from 12 transects (a further 22 scats were collected from elsewhere in the study area).

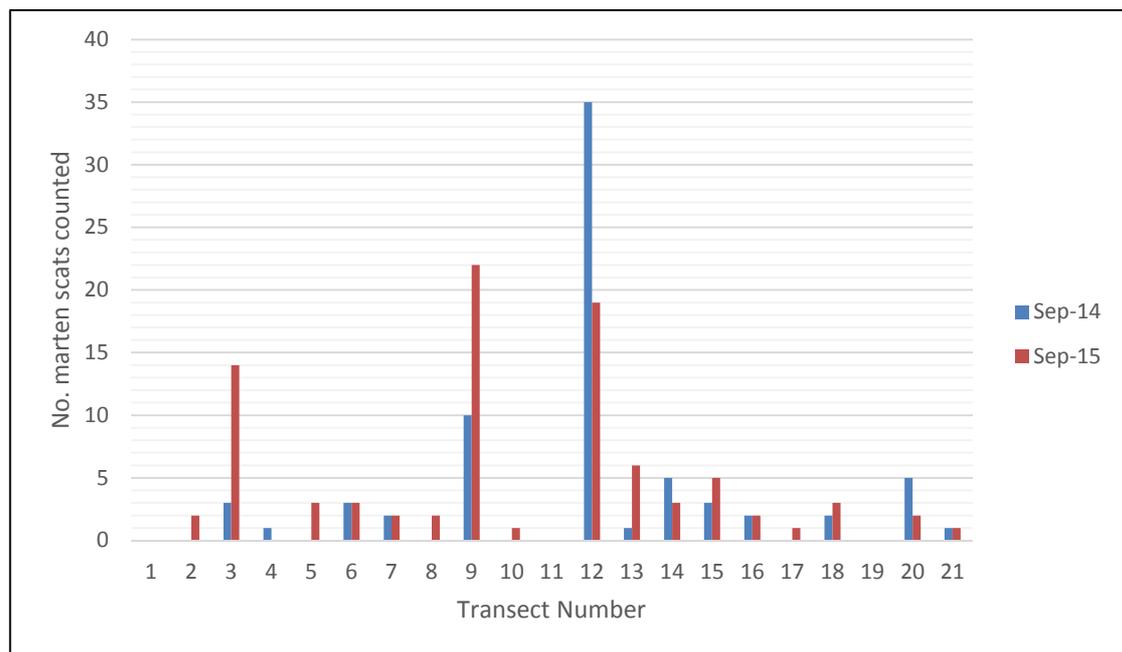


Figure 5. A comparison of the abundance of probable pine marten scats counted on the 21 1 km transects surveyed in The Fleet Basin during September 2014 and September 2015 (note that during September 2015 transects 11 and 19 could not be surveyed due to track resurfacing works).



Plate 5. Examples of fresh marten scats collected in the Fleet Basin during September 2014.

Ad hoc collection of scats from throughout the Fleet Basin resulted in a further 67 scats from 2014, and a further 44 scats from September 2015. Subsequently, the 44 scat samples collected from the Fleet Basin in September 2015 and the eight scat and ten hair samples collected in January 2016 were subjected to genetic analysis by Waterford Institute of Technology. Samples were generally too poor to provide individual genotypes, but most (85%) were sufficient to confirm species and some (35%) to identify gender.

The distribution of all 158 scats collected during 2014 and 2015 (both from transects and via *ad hoc* collection) is shown in Figure 6 below, together with the hair samples collected from hair tubes during 2014. A comparison of the map in Figure 6 with that in Figure 4 indicates that marten scats were detected at many sites where hair tubes were not visited by pine martens (so no hair samples were available).

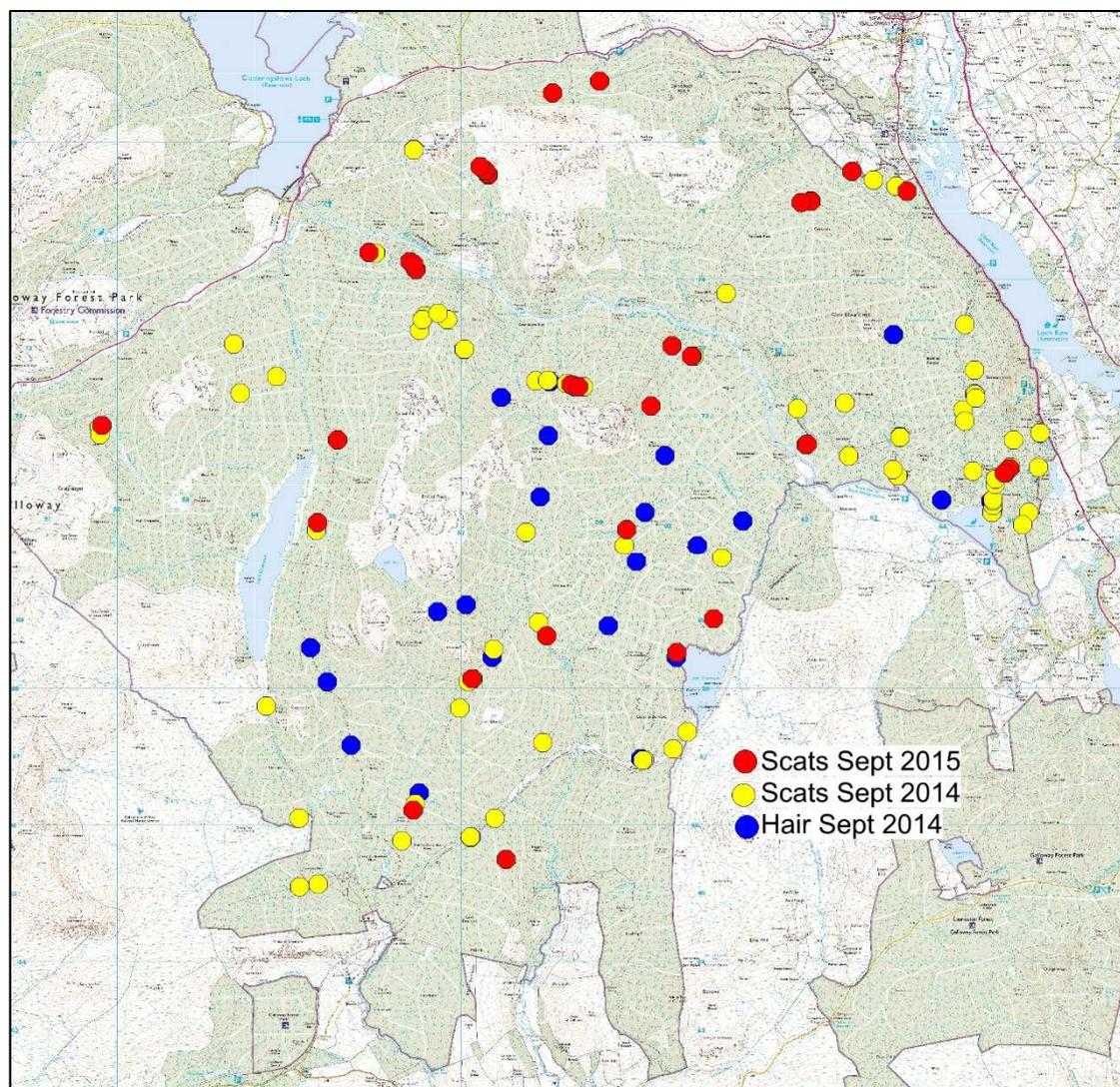


Figure 6. The locations of hair samples and scats collected in The Fleet Basin during September 2014 and 2015 (Based upon Ordnance Survey material with the permission of the Controller of HMSO © Crown Copyright (2013) Licence no. 100017908).

3.1.3 A comparison of scats and hairs collected in 2014

In total, genotypes were identified for 31% (n=45) of hair and scat samples collectively from the autumn 2014 sampling exercise. A Fisher's Exact Test was conducted to compare the genotyping success rate for hair and scat samples. Genotyping success rate was significantly higher for hair samples (43% n=22) than scat samples (24%, n=23; $p=0.02$, Fisher's Exact Test).

Of the 15 individuals sampled, all individuals except one (93%, n=14) were detected in scats, whereas only 33% (n=5) of individuals were detected in hair samples (see Figure 8). Twenty-seven percent (n=4) of individuals were identified in both scat and hair samples. The number of times an individual was detected ranged from one to ten (average = 3), with the majority of individuals (73%, n=11) detected less than four times; notably, six individuals were detected on the basis of single scats only (see Figure 8). In total, there were 69 detections of males and 60 detections of female pine martens (see Figure 7).

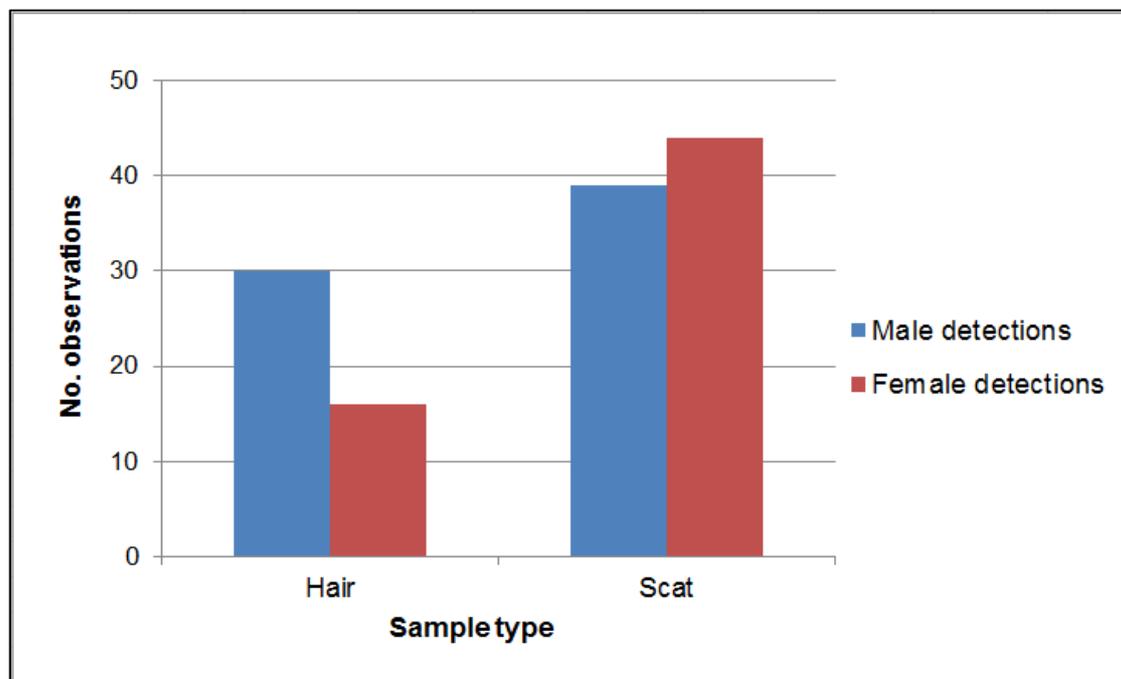


Figure 7. Detections of male and female pine martens in hair and scat samples.

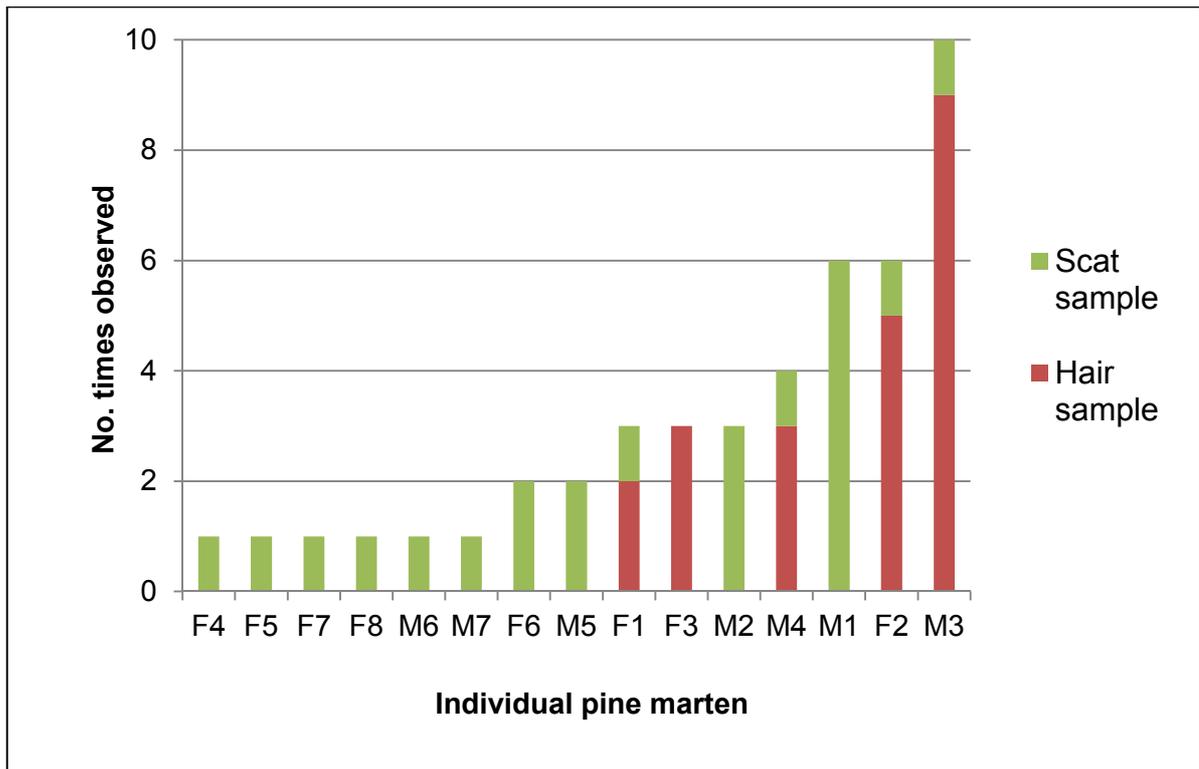


Figure 8. Detections of individual pine martens in scat and hair samples, in ascending order. Males are identified as M# and females as F#.

3.2 A 2014 Pine Marten Population Estimate

A total of 15 individual pine martens were identified through genotyping hair and scat samples collected in autumn 2014, comprising seven males and eight females; a sex ratio of 47% to 53%. This minimum population size estimate of 15 represents the pre-dispersal post-breeding population at its annual peak, because the kits born in the spring prior to this study would very likely still have been within their mother's territory before dispersing during the winter, thus temporarily inflating the population size. Therefore, the number of adult martens occupying fixed home ranges in the study area is likely to be lower than the total population size observed.

Table 3. The results of the DNA analysis of hair and scat samples from 2014. *The percentage is of the samples confirmed as pine marten.

	Samples collected	Samples identified as pine marten	Samples determined to gender*	No. male: female detections	Samples determined to genotype*	No. individual genotypes (males: females)
Hair	52	51 (98%)	46 (88%)	30:16	22 (43%)	5 (2:3)
Scat	114	96 (84%)	83 (86%)	39:44	23 (24%)	14 (7:7)
Total	166	147 (89%)	129 (88%)	69:60	45 (31%)	15 (7:8)

3.2.1 Capwire population abundance estimate

The Likelihood Ratio Test (LRT) was implemented in Capwire and selected the Two Innate Rates Model (TIRM) model to analyse the data, based on individual heterogeneity in the capture data ($p=0.002$). Capwire generated a population size estimate of 18 (95% CI 15 to 25) (see Table 4). The Capwire population estimate is similar to the minimum population size as derived from the number of individual genotypes ($n=15$). The average observation per individual pine marten was three.

Table 4. Population size estimates for pine martens in the Fleet Basin in 2014.

Sample size	Minimum population size	Capwire population size	Lower Confidence Interval	Upper Confidence Interval
45	15	18	15	25

3.2.2 Population density

Three population density estimates for pine martens in the Fleet Basin were determined (see Table 5). These estimates range from 0.13 to 0.15 pine martens per km² for the post-breeding population, representing the presence of pre-dispersal juveniles still within their mother's territory. The pre-breeding population density estimate for adults only was 0.08 pine martens per km², although it is possible that density could be lower than this if the number of adults present is fewer than the ten individuals presumed to be present. The density estimate based on the minimum population size is a conservative estimate based on raw genotype data only; and the estimate for adults only is based upon the exclusion of five individuals presumed to be pre-dispersal kits still within their mothers' territories.

Table 5. Pine marten population density estimates for the Fleet Basin in autumn 2014. Density is expressed as the number of pine martens per km².

Post-breeding density (minimum population size)	Post-breeding density (Capwire population size)	Pre-breeding density (adults only)
0.13	0.15	0.08

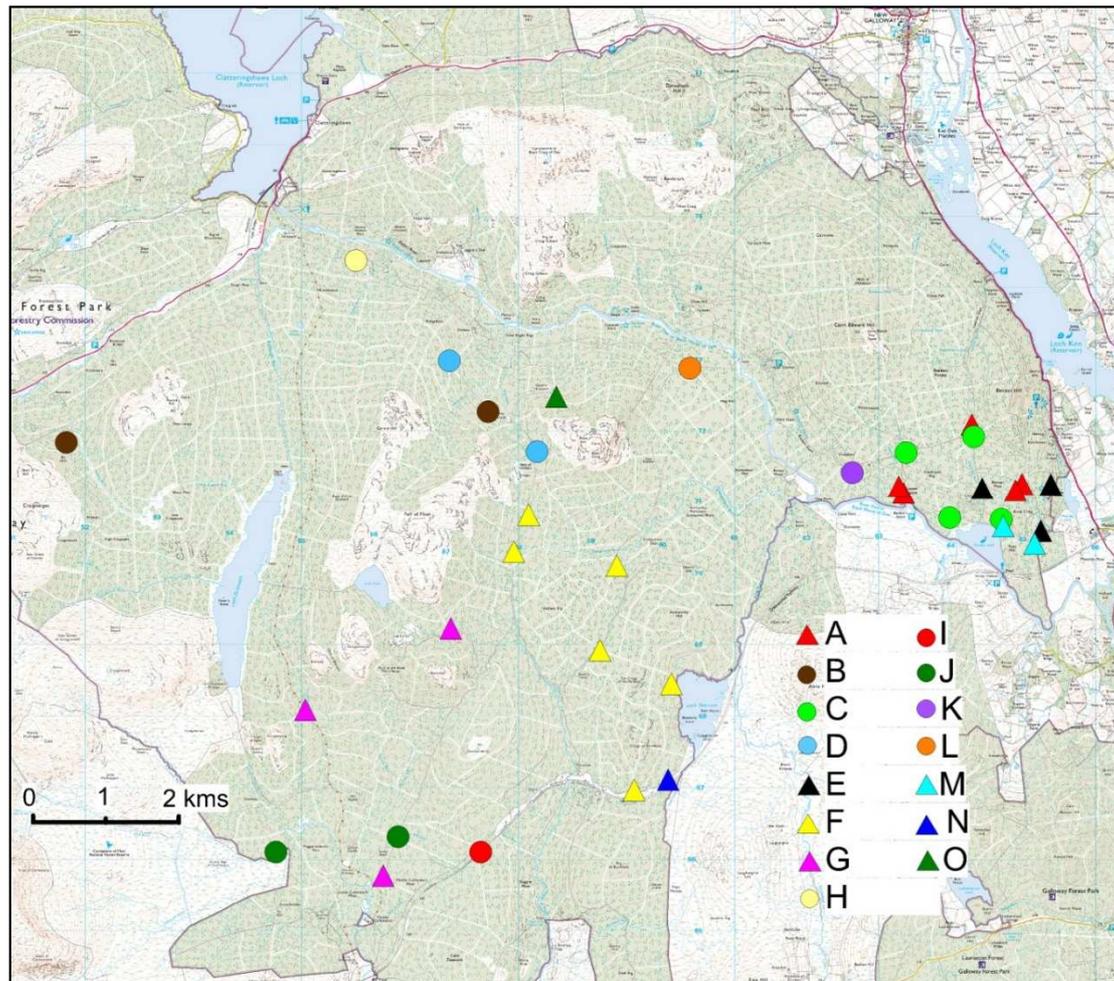


Figure 9. The distribution and gender of individual genotyped pine martens in the Fleet Basin in autumn 2014, based on non-invasively collected hair and scat samples. Males are represented by triangles and females are represented by circles. Each individual marten is assigned a unique letter. Two clusters of 3-5 individuals are apparent in the north/centre and far east, and these are believed to represent pre-dispersal marten families.

3.2.3 Population Composition

The genetic analysis allowed some potential relationships to be determined between some of the individuals identified in autumn 2014 as either parent-offspring or siblings (see Table 6). However, overall genetic diversity in the population was low, meaning that it was possible only to determine that individuals were either related as parent-offspring or siblings, but not possible to determine which. From this information, it is possible to infer the relationships between some of the individuals within the population, but this process is speculative so the relationship information presented below cannot be regarded as wholly reliable.

Table 6. Potential relationships between individual pine martens, as determined by genetic analysis. Each row represents two individuals which are related, either as parent-offspring or as siblings. Males are identified as M# and females as F#.

Individual 1	Sex	Individual 2	Sex
M1	♂	F2	♀
F2	♀	M2	♂
F1	♀	F6	♀
F4	♀	F7	♀
F5	♀	M5	♂
F3	♀	M7	♂
F8	♀	M7	♂
M6	♂	M7	♂

Two areas of probable breeding, where juveniles were apparently still within their mother's territory, can be presumed within the study area. Firstly, in the far east of the Fleet Basin, there were three related individuals: one female (F2) that is related to two males (M1 and M2) (see Figure 9). It is likely that the female is the mother of the two males, which were born in the spring of 2014. This assertion is reinforced by evidence that a female marten bred in a den box in this area in the spring prior to this study. However, the genetic analysis did not indicate that the two males (M1 and M2) were related, so this relationship is somewhat unclear. There is no evidence to suggest that the other two individuals in this area, a male (M5) and a female (F7), are related to these three individuals (F2, M1 and M2).

Secondly, in the centre of the study area, there was a male (M7) that is related to two females (F3 and F8) detected nearby and also related to another male (M6) towards the south of the study area (see Figure 9). It is possible that the male (M7) is the father of these three individuals or that he is the offspring of one of the females. It is not known whether the other female in this area (F1) is related to any of these individuals.

Given the identification of five possible juveniles in these two breeding areas, it is reasonable to assume that the total adult population could number 10 individuals or fewer. It is very likely that some of the other individuals detected are juveniles within their mother's territory, thus temporarily inflating the population. The estimate of 10 resident adult martens was used to calculate the population density estimate for adults only. All of the other individuals that were determined to be related (F1 and F6; F4 and F7; M5 and F5) are geographically separated with least cost distances (the shortest distance) between the related individuals varying from 5.6km to 8.5km. These relationships could comprise animals born in previous years and related either as siblings, or as parent-offspring, or could be kits born during the spring that are making pre-dispersal forays from their mother's territory, or have dispersed early (i.e. before or during September).

Where three or more locations were recorded for a genotyped individual, it was possible to illustrate its area of activity as a Minimum Convex Polygon (MCP) as shown for six animals in Figure 10. However, because this approach is based on very few samples it should not be taken as indicative of the true size and shape of the individuals' home ranges, which are very likely to be substantially larger than those shown in Figure 10.

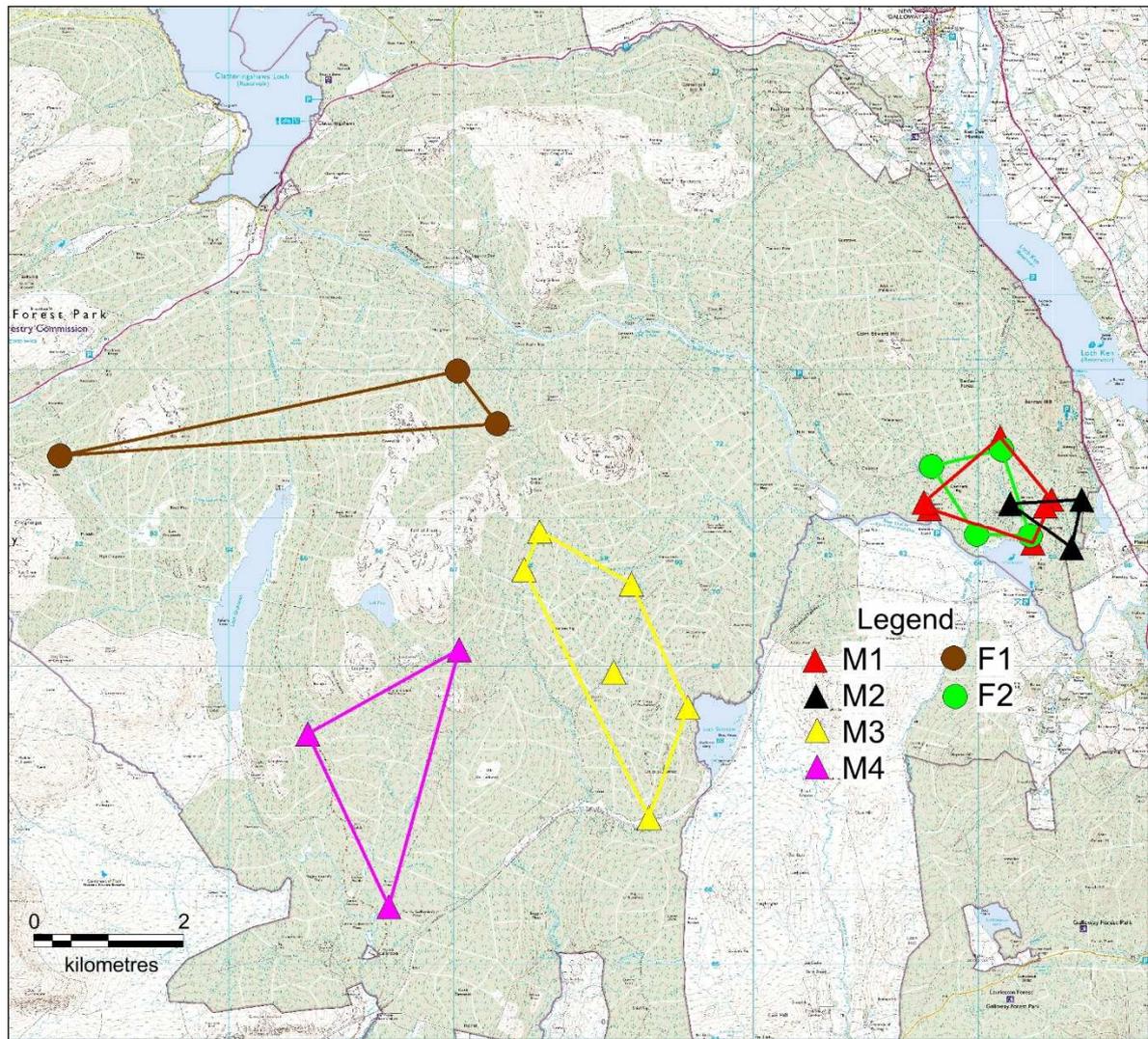


Figure 10. The minimum convex polygons (MCP) of individual pine martens that were detected at three or more locations in the Fleet Basin. Males are represented by triangles and females are represented by circles. Excludes individuals for which MCPs could not be calculated. © Crown Copyright and database right [2014]. All rights reserved. Ordnance Survey Licence number 100021242.

3.2.4 Non-genotyped samples

Further information on population composition and distribution can be inferred by mapping the locations of pine marten samples for which the gender of the animal was determined but the genotype was not (see Figure 11).

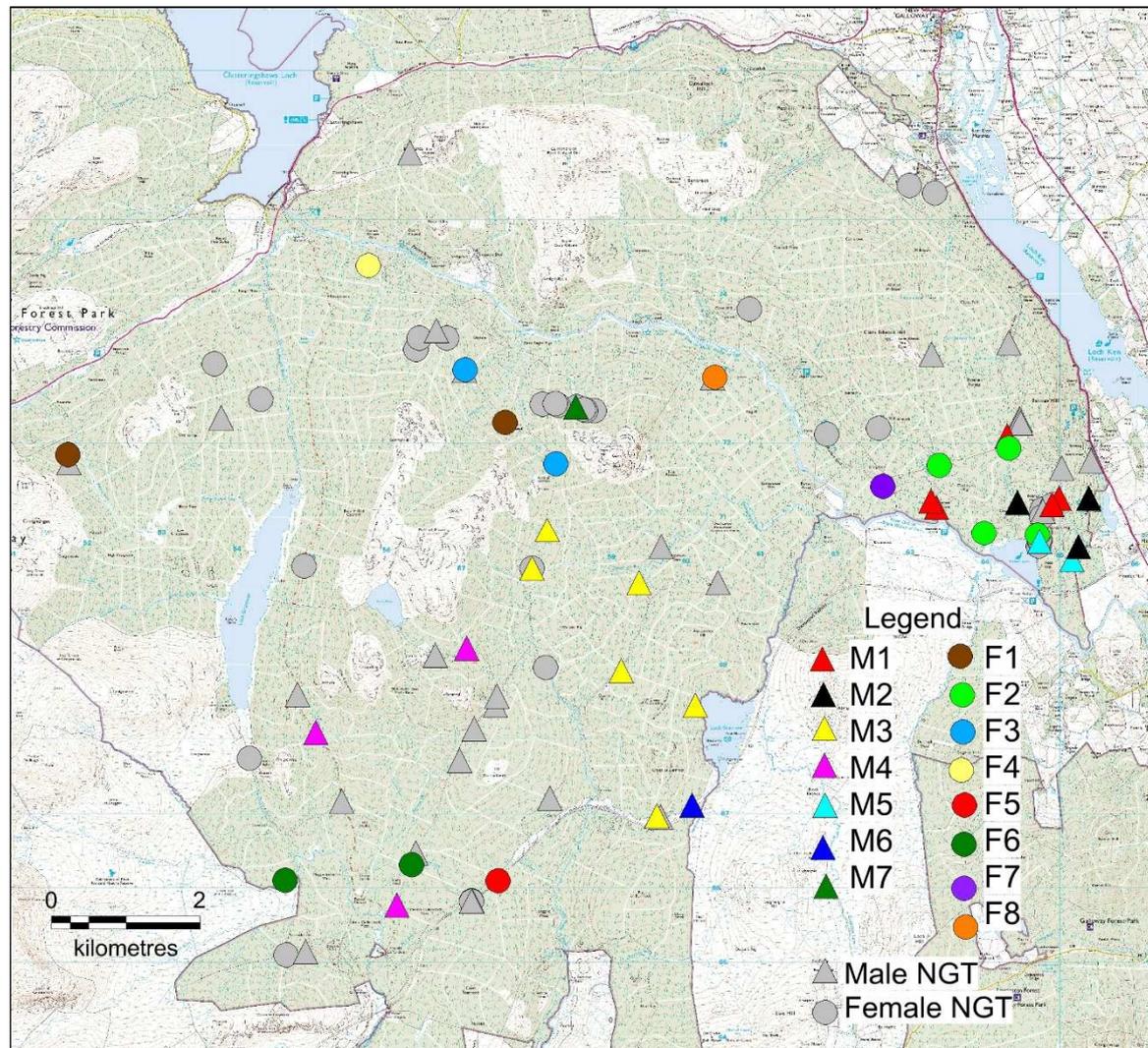


Figure 11. The distribution of individual pine martens and non-genotyped but gender-typed samples in the Fleet Basin. Males are represented by triangles and females are represented by circles. NGT denotes non-genotyped samples. © Crown Copyright and database right [2014]. All rights reserved. Ordnance Survey Licence number 100021242.

Although it is possible that most of the non-genotyped samples represent previously genotyped individuals, particularly when found in close proximity to genotyped samples, we cannot be certain of this. When non-genotyped samples were found at greater distances from genotyped samples, it is likely that they represent an unidentified individual. For example, there was a non-genotyped male sample in the far west that is 4.9 km from the nearest other male sample (M4) and another non-genotyped male sample in the north-west of the study area that is approximately 4.1 km from the nearest other male sample (M7); these could both represent previously unidentified individuals. There were also two non-genotyped female samples in the far north-east, 3.6 km from the nearest female sample (F2), which possibly represent a previously unidentified female.

3.3 Patterns of Pine Marten Activity in 2014

Geographical variations in the abundance of pine marten scats and hair samples collected during intensive fieldwork in autumn 2014 provide a crude indication of variations in pine marten activity across the Fleet Basin. These are shown as the combined total of pine marten samples recorded in each monad in Figure 12. However, there is an important caveat to bear in mind when interpreting any patterns revealed: survey effort across all 103 monads sampled in the Fleet Basin was not quite even, because not every monad was subject to the same intensity of scat searching; for example, some monads did not include sections of the scat transects shown in Figure 3. Nevertheless, the variations in pine marten activity shown in Figure 12 are based upon very broad abundance categories, which would tend to override minor elements of sampling bias.

Of the 103 monads subject to searches for pine marten hairs and scats during autumn 2014, pine marten activity was recorded in 63 (61%). Of these 'marten active' monads, only a low level of activity (a single scat or hair sample) was recorded in 43 (68%) monads, with relatively moderate activity (2-5 samples) recorded in 17 monads (27%) and high activity (>5 samples) recorded in just three monads (5%).

The distribution of these activity categories across the 103 monads, shown in Figure 12, suggests a marked clustering of pine marten activity in the far east of the study area, where a block of six contiguous monads were classed as 'moderate' or 'high'; this coincides with the area where a marten family was believed to be present in autumn 2014, as indicated in Figure 9. Further west there are many contiguous monads with 'low' or 'moderate' activity and a single monad with 'high' activity south of the River Fleet; this monad also coincides with an area believed to be occupied by a marten family in autumn 2014 (see Figure 9). In the far north and north-west there are substantial blocks of contiguous monads in which no marten activity was recorded.

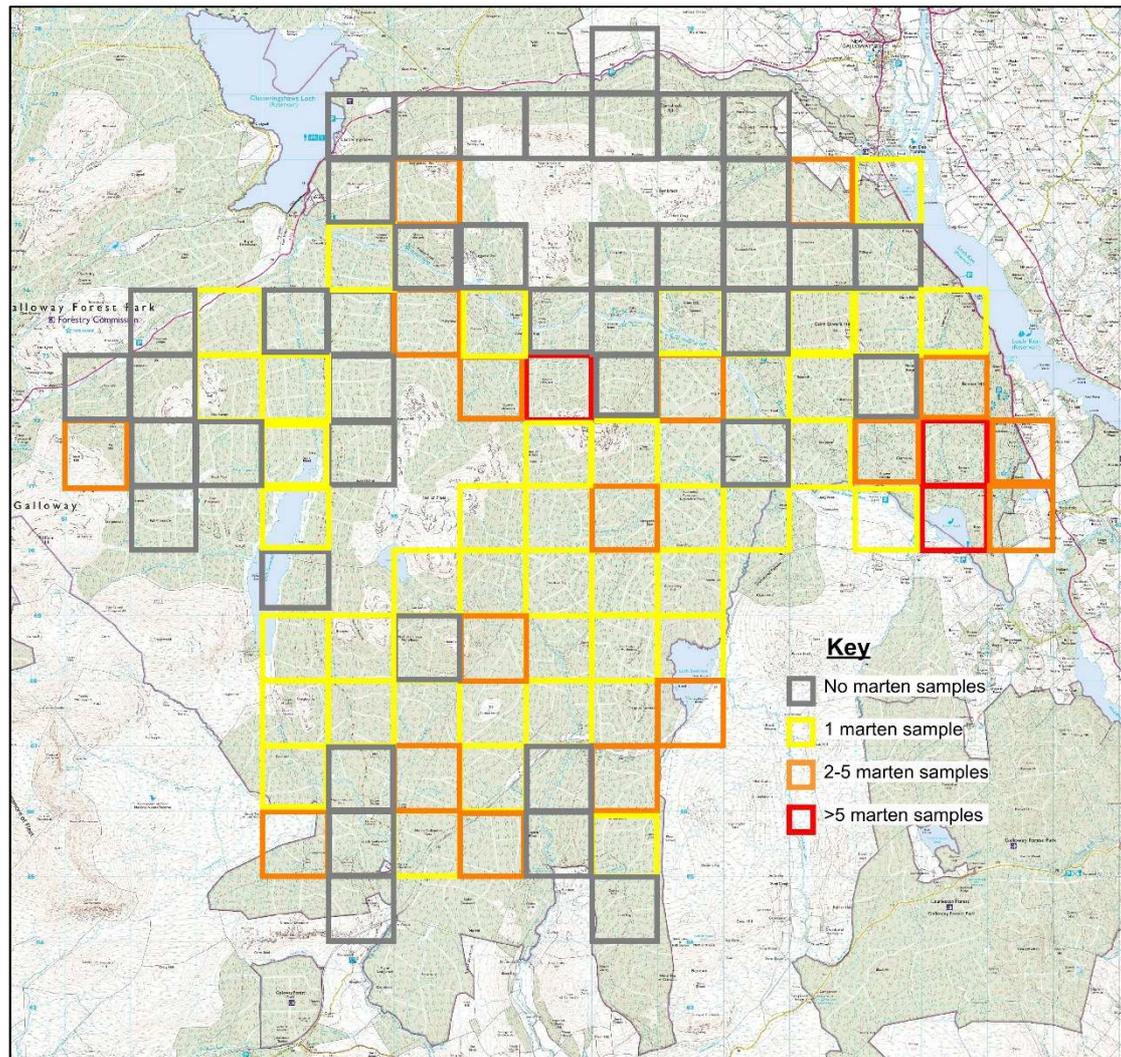


Figure 12. Spatial variations in the abundance of pine marten scat and hair samples collected in the Fleet Basin during autumn 2014. The 103 grid squares are monads (1 x 1 km²) of the Ordnance Survey. © Crown Copyright and database right [2014]. All rights reserved. Ordnance Survey Licence number 100021242.

3.4 Galloway Lite den Boxes

3.4.1 Patterns of use by pine martens

The *Galloway Lite* den boxes installed in September 2014 were fully checked for evidence of use in May and September 2015 and in May 2016 (the check in January 2016 was not a full check because it focused primarily on removing and replacing hair-sampling devices). Patterns of box occupancy are summarized in Table 7 and Figure 13 below. The low level of use of the boxes in May 2015 is probably a consequence of the relatively short time (seven months) having elapsed since their installation.

Table 7. Patterns of use by pine martens of the Galloway Lite den boxes installed in the Fleet Basin in September 2014. Note that, although on some checks fewer than the full 50 boxes could be checked because of harvesting or theft, all percentages are based on the nominal total of 50 boxes.

Check date	No. boxes used	Cumulative no. boxes used
May 2015	6 (12%)	6 (12%)
September 2015	22 (44%)	22 (44%)
May 2016	22 (44%)	31 (62%)

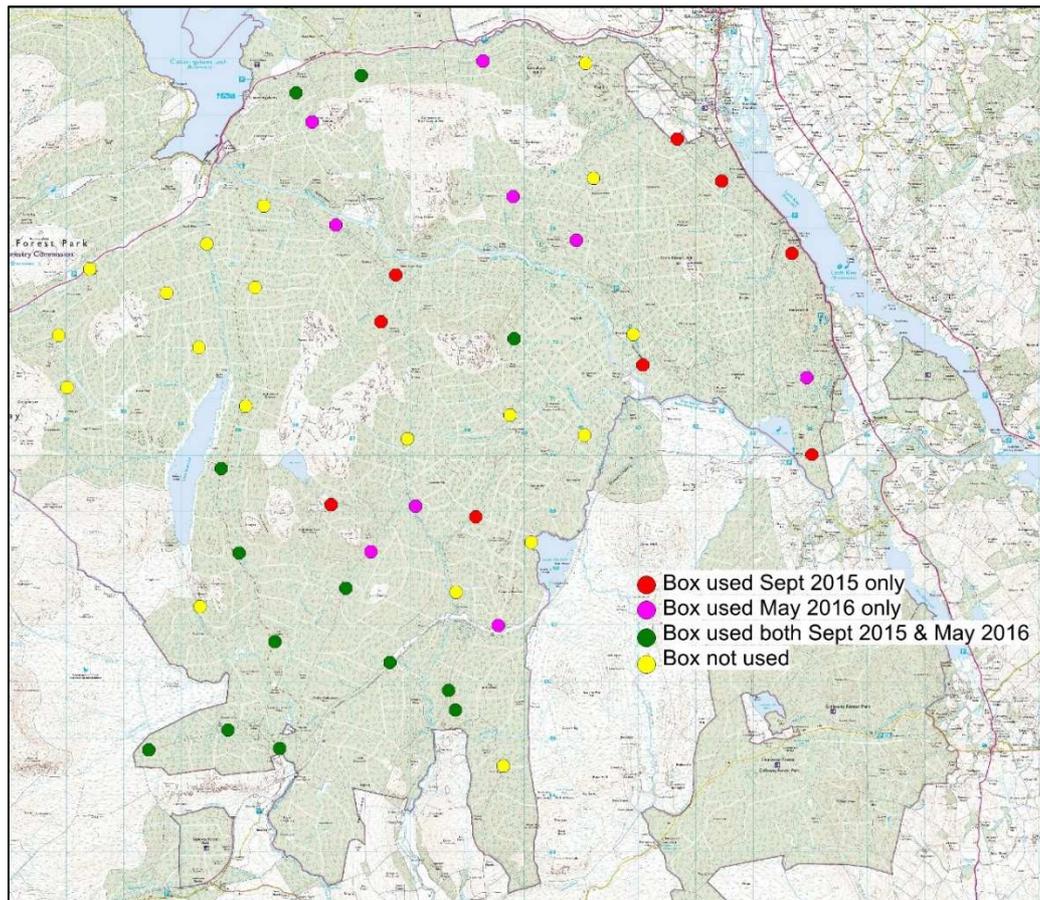


Figure 13. The distribution of the Galloway Lite pine marten den boxes installed in The Fleet Basin during September 2014, showing patterns of box occupancy from September 2015 to May 2016 (Based upon Ordnance Survey material with the permission of the Controller of HMSO © Crown Copyright (2013) Licence no. 100017908).

The distribution of boxes showing signs of occupancy by martens is shown in Figure 13, with some geographical differences apparent in the pattern of use. It is clear that the most frequently used boxes (those used on both September 2015 and May 2016) are concentrated mainly in the south-western sector of the Fleet Basin. It is likely that patterns of box use were influenced, in part, by timber harvesting operations, which in some cases left boxes relatively isolated or exposed in areas that had previously supported closed canopy stands when the boxes were originally installed.

No breeding was confirmed in any of the *Galloway Lite* den boxes. However, in May 2016, the field vole population was at a low point in its population cycle and no breeding by pine martens was confirmed at any other locations in the Fleet Basin, including the wooden VWT den boxes in which breeding had been recorded in previous years.

The January 2016 check of the *Galloway Lite* den boxes (mainly to remove and replace sticky hair patches) confirmed occupancy of two of the boxes by pine martens (see Plate 6 below), indicating that the boxes are used as over-winter resting sites by non-breeding pine martens.



Plate 6. *Galloway Lite* den boxes occupied by pine martens in January 2016, an aggressive male (left) and a placid female (right).

3.4.2 Hair collection from den boxes

The sticky patches attached to all *Galloway Lite* den boxes in September 2015 and January 2016 had largely failed to gather adequate samples of marten hair at boxes used recently by martens. This was probably a consequence of the patches losing their stickiness after prolonged exposure to humid conditions; also at some occupied boxes the patches had been removed from their original positions and were found on the ground beneath the boxes (we presume that this was done by the marten occupying the box).

3.4.3 The influence of box height on occupancy

In September 2015 half of the 50 *Galloway Lite* boxes had been raised to heights of 4-5 m above ground, so the May 2016 visit was the first opportunity to detect any

differences in occupancy patterns between these higher boxes and those remaining at the original height of 2-3 m above ground.

Table 8. Patterns of marten occupancy of Galloway Lites in May 2016 in relation to box height above ground level.

Box height	No. boxes with signs of marten occupancy	No. boxes with no signs of marten occupancy	Total
Low (2-3 m)	13	11	24
High (4-5 m)	9	15	24
Total	22	26	48

Although a slightly higher proportion of 'Low' boxes had signs of recent occupancy (54% as opposed to 37.5%), this difference was not statistically significant ($\chi^2 = 1.342$, $df = 3$, $P > 0.05$).

4 DISCUSSION

4.1 A Pine Marten Population Estimate

The 2014 population estimate of 15-18 pine martens (including pre-dispersal juveniles) in the Fleet Basin provides a baseline against which to measure future responses to external influences, such as changes in forest structure, habitat quality and prey availability related to harvesting operations, as well as to habitat enhancement measures such as provision of artificial den boxes: for example, the 2014 population estimate effectively pre-dates the likely beneficial effect of installing 50 *Galloway Lite* den boxes in the Fleet Basin.

The pine marten population density estimates for the Fleet Basin of 0.08 pine martens per km² for a pre-breeding population to 0.13-0.15 pine martens per km² for a post-breeding population are within the range of densities observed for pine martens elsewhere in Scotland, but are towards the lower limit (see Table 9).

*Table 9. A comparison of pine marten population densities reported from studies in Scotland. For method, NIS= non-invasive sampling and RT= radio-tracking. *Density specified for adults only.*

Study site	Method	Population density (martens per km ²)	Reference
Scotland			
Minnoch, Galloway Forest	RT	0.030-0.094*	Bright & Smithson (1997)
Mar Lodge, Cairngorms National Park	NIS	0.07	Kubasiewicz (2014)
Fleet Basin, Galloway Forest	NIS	0.08*-0.15	This study
Glen Trool, Galloway Forest	RT	0.119-0.221*	Bright & Smithson (1997)
Inshriach, Cairngorms National Park	NIS	0.18	Kubasiewicz (2014)
Abernethy, Cairngorms National Park	NIS	0.38	Kubasiewicz (2014)
Strathglass, Inverness-shire	RT	0.42	Balharry (1993)
Novar, Ross-shire	RT	0.58*	Halliwell (1997)

4.2 Non-invasive Methods as a Population Assessment Tool

The proportion of hairs and scats confirmed as pine marten by DNA analysis (98% and 84%, respectively) was relatively high, as was the proportion of pine marten samples subsequently determined to gender (88% for hairs, 86% for scats). The genotyping success rate for hair samples in this study (43%) was considerably lower than that achieved in other non-invasive studies of pine marten populations: 54% in

O'Mahony *et al.* (2014); 66% in Sheehy *et al.* (2013); 72% in Kubasiewicz (2014); and 92% in Mullins *et al.* (2010). This could account for the proportionately small number of individuals (n=5) detected in the hair tubes compared with in scats; it is probable that additional individuals used the hair tubes but the hair samples left were not of sufficient quality to yield a genotype. The quality and degradation rate of DNA is influenced by time and climatic variables; notably, the length of time that samples are in the field, as well as temperature and dew point, significantly influence DNA amplification success rates (Murphy *et al.*, 2007). It appears unlikely that the DNA in the hair samples degraded significantly prior to collection, as the time between sampling sessions was five to nine days. In the study by Sheehy *et al.* (2013), hair traps were sampled once per month; thus, samples were left in situ for up to a month prior to collection, yet a higher genotyping success rate (66%) was achieved than in this study. Genotyping success rates are known to increase with the number of hairs and hair follicles in a sample (Mowat & Paetkau, 2002; O'Mahony *et al.*, 2014; Kubasiewicz, 2014) and are significantly greater with samples with more than 10 hairs (O'Mahony *et al.*, 2014). Some of the hair samples gathered in this study had flying insects stuck to the glue patch, which resulted in marten hair not sticking to the patch and made removing the hairs and extracting DNA problematic. Insects are likely to have been attracted to the hair tubes due to the presence of the meat bait, although this has not been such an issue in other studies in which meat has been used (P. Turner, pers. comm.). In addition, some of the hair samples comprised very few hairs. In these cases it is likely that the pine marten did not spend much time in the tube removing the bait; this may have occurred on occasions when the bait was not secured sufficiently in the tube and thus was removed easily when the marten encountered it. To ensure collection of a higher number of hairs, it is essential that the bait is secured sufficiently well so the martens have to spend as much time as possible removing it from the tube.

The genotyping success rate for scats in this study (24%) was comparable to that from other studies. It is typically more difficult to determine genotypes from faecal material than from hair samples due to the poorer quality of the DNA in the former. The relatively good genotyping success rate for scats during this study may be due to the long period of dry weather experienced during the scat collection in autumn 2014. Success rates for amplifying DNA from faeces are highest when climatic conditions are either dry or very cold and the samples are as fresh as possible (Murphy *et al.*, 2007); and for carnivores, PCR amplification rates decline significantly when scats are exposed to wet weather (Farrell *et al.*, 2000). In a recent study using molecular analysis of pine marten and stone marten scats, 24% of scats identified as suitable for further screening were determined to genotype (J. Power, pers. comm.). Other studies of pine marten populations have not incorporated genotyping of scats due to low genotyping success rates, which declined with the amount of time the scats were exposed to rainfall (Kubasiewicz, 2014).

Despite the lower genotyping success rate, the use of data derived from scats as well as hairs in this study contributed to a considerably more robust population estimate than would have been possible had only hair tubes been used (hair samples identified only one third of the 15 animals confirmed as present on the basis of

genotypes from both scats and hairs). The clear benefit of this diverse sampling approach in this study was the basis for a paper titled '*Sample diversity adds value to non-invasive assessment of a pine marten (*Martes martes*) population in Galloway Forest, south-west Scotland*' produced by Lizzie Croose for publication in the journal *Mammal Research* (Croose, 2016). Nevertheless, the distribution of some of the non-genotyped pine marten samples in this study raised questions about the existence of otherwise undetected individuals in the Fleet Basin, and suggested that the higher Capwire population estimate of 18 might have been more accurate than the lower one.

4.3 Patterns of Pine Marten Activity in the Fleet Basin

The simple quantification of hair and scat samples collected in each monad provides a basis for identifying changes in geographical patterns of pine marten activity in future years. A similar approach, involving counts of scats along fixed transects, has been used recently by the Vincent Wildlife Trust to assess levels of pine marten activity in Scottish forests before and after the live-trapping and removal of small numbers of animals for translocation to Wales as part of the Pine Marten Recovery Project (J. Macpherson, pers. comm.).

The Fleet Basin data from 2014 suggest that pine marten activity is not distributed evenly over the forested land in the Fleet Basin, but is concentrated in certain sectors; in contrast other sectors revealed very low levels of activity, or no activity at all. This project did not set out to identify the factors that explain such patterns, although there is considerable interest in improving understanding of such effects in order to inform forest design plans and other measures to conserve biodiversity in commercial forests (Gareth Ventress pers. comm.). It is understood from Scottish radio-tracking studies that, in commercial forests, variations in forest structure exert a powerful influence upon patterns of pine marten activity, with a clear preference shown for mature, closed-canopy woodland over younger stands and open, clear-fell areas, which tend to be avoided by martens (e.g. Bright & Smithson, 1997). However, a recent study has demonstrated that martens in Scotland do make some use of more open habitats, such as tussock grassland with high vole populations (Caryl, 2012).

Interpretation of variations in patterns of pine marten activity in future could be informed by characterising each monad in terms of forest structure. The robustness of this approach could be improved by ensuring, as far as possible, that sampling effort to record patterns of pine marten activity is spread evenly across all monads in the Fleet Basin.

4.4 The Galloway Lite Den Box Design

The 62% cumulative occupancy rate of the *Galloway Lite* den boxes confirms that the boxes provide suitable conditions for resting martens. This high level of occupancy just 18 months after installation also supports the view that, in commercial forests dominated by biologically young trees, elevated den sites for pine martens are scarce or absent and might be a limiting factor affecting populations (Brainerd, 1990; Birks

et al. 2005). Therefore, the *Galloway Lite* den box represents a significant habitat enhancement measure for pine martens in modern forests. Its lightweight design means that it can be installed easily on a wide age range of trees, thereby avoiding the geographical constraints associated with installing heavier box designs; this design also carries fewer risks to health and safety than heavier boxes during installation and relocation. Finally, evidence from this study suggests that boxes installed on trees at heights of 2-3 m are no less likely to be used by pine martens than higher boxes installed at 4-5 m, so there is no need to install boxes at great heights in order to guarantee occupancy.

The absence of any evidence of breeding by pine martens in the Fleet Basin during 2016 means that the *Galloway Lite* den box design has still to be confirmed as suitable for use as a pine marten natal den site. We anticipate that such opportunities will arise in the near future in response to recovery in the local field vole population.

Because of their even distribution and relatively high density (one box per 2 km²), and their frequent use by martens, geographical variation in the use of boxes is likely to be a useful additional indicator of patterns of marten activity within the Fleet Basin. Efforts to use the boxes as a means to gather non-invasive hair samples from pine martens were not successful during this study; further work to develop an effective, weather-proof hair-snagging method will continue.

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