

Non-invasively determined multi-site variation in pine marten *Martes martes* density, a recovering carnivore in Europe

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Abstract Pine martens (*Martes martes*) are subject to national and international conservation legislation throughout most of their European range. Yet population density and abundance estimates, which are a key component of effective conservation management, are lacking in many countries. In this study, a large-scale non-invasive survey was undertaken in 14 forested study sites throughout Ireland to assess variation in pine marten density and abundance. Pine marten hair samples were collected in each study site and analysed using genetic techniques to determine individual identity data. Density and abundance estimates were obtained using spatially explicit capture-recapture models and CAPWIRE. Across all study sites, a total of 93 individual pine marten were identified and captured 217 times. Estimated pine marten density varied from 0 to 2.60 individuals per km² of forested habitat, with all but a single site having estimated densities of ≤ 1 pine marten per km² of forest habitat. Mean population abundance estimates across all study sites ranged from 0 to 27 individuals. Spatially explicit capture-recapture models on combined data across all 14 study sites provided a mean density estimate of 0.64 (95% CI 0.49–0.81). Combining this with data on the current distribution and estimated area of forest habitat occupied by the species in Ireland, the total pine marten population abundance of pine marten in Ireland was estimated at 3043

(95% CI 2330–3852) individuals. This research has conducted the largest scale investigation of pine marten density and abundance in any part of its global distribution and provided an improved basis for future population assessment and monitoring of this species.

Keywords *Martes martes* · Density · Abundance · Spatially explicit capture-recapture · Conservation management · Habitats Directive

Introduction

Multi-scale assessment of wildlife population density and abundance is a critical management concern for species that are of conservation interest throughout the world. A wide variety of methods exist to estimate wildlife density ranging from field-based surveys of animal sign to the revolutionary application of non-invasive techniques, and involving analyses of relatively simple indices per unit area to complicated Bayesian statistics (Burnham et al. 1980; Wilson and Delahay 2001; Mowat and Paetkau 2002; Rowcliffe et al. 2008; Efford et al. 2009; Royle et al. 2009; Gopalaswamy et al. 2012). Increasingly, it is acknowledged that standardisation in survey designs, analytical frameworks and data reporting is of critical importance at the global scale (Fukuda et al. 2013; Gula and Theuerkauf 2013; Jansen et al. 2014; Burton et al. 2015). Standardisation provides for more reliable inference across studies in terms of biological and ecological parameters of interest at various spatial scales and more rigour in terms of the management of species. For example, the requirement for such standardisation has recently been highlighted in relation to the endangered northern black rhino *Ceratotherium cottoni* where non-standardised data collection and analysis procedures in home range estimators may have misled population

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management for this species (Plotz et al. 2016). Standardised methodologies have been implemented or are advocated for camera trapping (Jansen et al. 2014), invasive species monitoring (Jarnevich et al. 2015), wildlife road traffic accident reporting (Collinson et al. 2014), wildlife disease monitoring (Guberti et al. 2014) and calls for standardisation in data collection and analyses are likely to become an increasing demand into the future.

The European pine marten (*Martes martes*) is a widely distributed species, and throughout its range is of interest either in terms of conservation or population management. Despite its wide ranging distribution, economic and social importance, nonetheless there have been relatively few studies on the species population density or abundance, even though reporting on such parameters is a key element of assessing species status in EU member states under the terms of the Habitats Directive (Council Directive 92/43/EEC on the conservation of natural habitats and of wild fauna and flora). Estimates or indices of pine marten density have been obtained using snow tracking (Kurki et al. 1998), hunting statistics (Helldin 2000), scats (Baines et al. 2013), live trapping (Lynch et al. 2006; O'Mahony 2014), territory mapping (Zalewski and Jedrzejewski 2006), camera trapping (Manzo et al. 2012) and other non-invasive survey techniques (Mullins et al. 2010; O'Meara et al. 2014; O'Mahony et al. 2015; Balestrieri et al. 2016a; Croose et al. 2016). Methods of estimating pine marten density, or surrogates thereof, in these studies have ranged from relatively simple indices per transect length, minimum number alive divided by estimated study area size, CAPWIRE estimates, random encounter models and spatially explicit capture-recapture analyses. The inherent differences in study design, scale, methodological approaches, temporal duration and lack of inclusion of formal statistical analysis or put simply a lack of standardisation in methods across studies elicit uncertainties concerning the reliability of comparing results, which may have important consequences in terms of the management of pine marten locally, nationally and internationally. Zalewski and Jedrzejewski (2006) have applied a standardised formula to derive density estimates from 29 localities where density indices had been previously calculated by snow tracking and found mean density estimates that varied from 0.1 to 8.9 marten per 10 km² and maximum densities of 0.3 to 17.5 per 10 km². That study considered only a few sites from Western Europe where snow tracking data were sparse and the comparability of these estimates with the majority of current studies that are largely derived from other forms of non-invasive research is debatable.

The divergence in methods and analyses used to estimate pine marten density is exemplified by data from several recent studies in Ireland, a country which may now represent the most studied in terms of pine marten density and abundance estimation. Over the past 10 years in Ireland, pine marten densities have been determined using live trapping and simple

area versus minimum number alive calculations (Lynch et al. 2006; O'Mahony 2014), with molecular genotyping of individuals from non-invasively collected hairs within an estimated study area size (Mullins et al. 2010; Sheehy et al. 2014), and using spatially explicit capture-recapture and CAPWIRE estimates of non-invasively collected hair samples (O'Mahony et al. 2015). These studies were carried out in sites that ranged in size from 80 to 3350 ha, had temporal study periods of 2 to 14 months and only a single study conducted formal capture-mark-recapture statistical analyses. This is not a criticism of each individual study, each of which had inherent objectives, study designs, analyses and interpretations that fully justified their outcomes. However, the differences in methods and analyses across studies may confound the reliability of comparisons in terms of knowledge of the variation in pine marten density that may exist within Ireland, which is an important parameter for conservation management of this species.

In this study, a large-scale standardised non-invasive genetic survey was undertaken across multiple sites in Ireland to investigate pine marten density. The objectives were to determine site specific pine marten density and abundance estimates, investigate what level of variation in pine marten density existed across the range of the species in Ireland and provide a national population estimate of pine marten to inform Habitats Directive reporting requirements for this species.

Methods

Survey design

A random sampling design was used to sample the pine marten population in Ireland, which involved randomly selecting 14, 10 × 10 km study sites (hereafter 10 km) throughout the species known current range that had a minimum of 200 ha of forest habitat (Fig. 1a). Pine marten is considered a species with an association and dependence on forest cover (Balestrieri et al. 2016b; O'Mahony 2017) to establish home ranges and provide requirements such as dens, resting sites and foraging opportunities. Therefore, surveying forested habitat only was a necessary logistical consideration in this study. The species is, however, adaptable in terms of using non-wooded and fragmented forest landscapes (Pereboom et al. 2008; Mergely et al. 2011; Caryl et al. 2012; Moll et al. 2016). Where an initial randomly selected 10 km grid square was not possible to survey ($n = 2$) for reasons related to the unavailability of landowner permission and site access, a random selection of an adjacent 10 km that had at least 200 ha of woodland habitat was undertaken. Each study site was assessed and its total forest cover determined using GIS datasets in ArcMap 10.3 (ESRI Systems, USA).

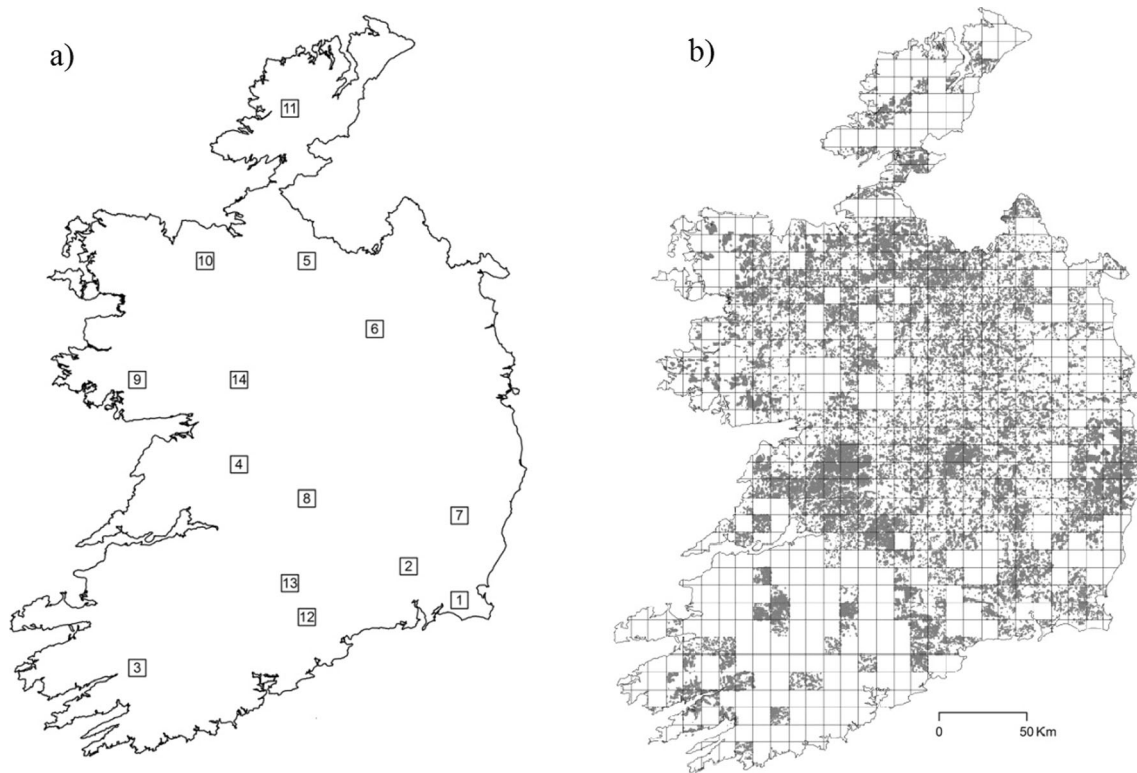


Fig. 1 **a** Locations of 14 randomly selected study sites in Ireland which were used to estimate pine marten density and abundance. **b** The distribution of forestry within current pine marten range in Ireland as

represented at the 10×10 km square level. Pine marten distribution data provided by the National Biodiversity Data Centre from records of pine marten between 2010 and 2015

Hair tube surveys

Hair tubes (see Mullins et al. 2010; O'Mahony et al. 2015) were used as the basis for sampling the pine marten population in each study site. Due to logistical constraints including the requirement to complete all of the surveys within a relatively short sampling timeframe (i.e. 5 months), the maximum number of hair tubes that could be surveyed at any single study site was 30. It was aimed to have an approximate tube density of 3 hair tubes per 100 ha of surveyed forest habitat, where practicable, a similar tube density to other studies that have used this methodology (O'Mahony et al. 2015). In each individual study site that had less than 1000 ha of forest cover, all accessible habitat was surveyed. Where more than 1000 ha of forest occurred in an individual study site, then a subsampling approach was adopted wherein each separate block or management unit of forest was provided with a unique identity, and forest blocks that were surveyed were randomly selected so that the approximately 30 tubes or an equivalent habitat area of approximately 1000 ha was surveyed.

Once forests that were to be surveyed were identified in each study site, experienced field surveyors were provided with GPS positions of potential hair tube deployment locations at approximate intervals of 600 m. Field surveyors then deployed tubes in study sites either at, or close to, the potential hair tube deployment locations provided. The individual field

surveyors made field-based decisions on tube locations based on site specific data such as forest management considerations, recent felling and unsuitable or inaccessible habitat. Each study site was surveyed for approximately 1 month, with three weekly sampling sessions per site. During the first week in any study site, field surveyors deployed baited hair tubes and recorded tube locations with a Garmin 62 handheld GPS unit. At 5–7-day intervals, field surveyors revisited each tube in each study site and ascertained whether or not hair samples were present. If hair samples were present then the sticky patches were removed from the tube and placed in a labelled sample tube for storage at -20 °C. New sticky patches were then placed in the rebaited hair tube. If tubes had not been visited, the bait was replaced but clean sticky patches were left in situ. In total, each tube in each study site was visited on three sampling occasions, with tubes removed during the last sampling session. Tubes that were removed were cleaned and reused in other study sites. All study sites were surveyed between January and June 2016.

Genetics

Hair samples were recovered from sticky patches using 1–2 drops of xylene to soften the glue and hairs were transferred to 1.5 ml microfuge tubes using forceps. Forceps were heated to red heat and cooled between samples to prevent

cross-contamination. Hair was digested in 1.5 ml microfuge tubes containing 90 µl HPLC grade water; 90 µl 2× digestion buffer (ZR Genomic DNA II Kit™ (ZYMO Research, CA, USA)); 10 µl 20 mg/ml proteinase and 10 µl 1 M-dithiothreitol (Sigma-Aldrich). Digestion was at 56 °C for 1–3 h using a shaking heating block. DNA was purified using ZR Genomic DNA II Kit™ (ZYMO Research, CA, USA) according to appropriate manufacturer's method. Final eluates were stored at –20 °C.

Real-time quantitative PCR (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 et al. 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.

Microsatellite analysis to identify individual pine marten was carried out using six microsatellite markers (Table 1). Microsatellite amplifications were performed in a total volume of 10 µl with 3 µl DNA extract, 5 µl GoTaq® Hot Start Green Master Mix (Promega) and primer concentrations as indicated in Table 1. Cycling parameters were as follows: a single cycle at 95 °C for 5 min, 40 cycles of 95 °C for 30 s, 57 °C for 90 s and 72 °C for 30 s with a final single hold at 60 °C for 30 min.

Samples were rapidly cooled to 4 °C. Samples were diluted 1:2 in molecular grade water. One microlitre of each sample was denatured in 15 µl Hi-Di formamide (Applied Biosystems) with 0.15 µl GS500LIZ™ size standard (Applied Biosystems) for 5 min at 95 °C, followed by rapid cooling to 4 °C. The samples were run on a ABI3500 genetic analyser (Applied Biosystems). The genotypes were scored using Peak Scanner v1.0 software (Applied Biosystems).

Statistics

Site-specific pine marten density estimation

For each study site, the unique individual identity data and capture histories for each individual pine marten derived from genetic analyses were used in capture-recapture statistical approaches. Spatially explicit capture-recapture analyses (secr), also referred to as spatial capture-recapture (scr), were used to determine study site specific, across site averaged density and national population density and abundance of pine marten in Ireland. Spatially explicit capture-recapture modelling is a recent advancement on traditional forms of capture-recapture analyses. To model population density, secr includes spatial information on an individual's capture and home range activity centre inferred from inputted spatial data, overcoming

Table 1 Details of microsatellite loci used in this study. These six loci were used in a single multiplex reaction. To facilitate the multiplex reaction, the forward primer for Ma2 and the reverse primer from Mar21 were redesigned. The fluorescent dyes used to label the forward primers were FAM, PET, ATTO565 and Yakima Yellow (YAK). The reverse primers (except for Mar53) were modified with a 5' sequence of GTTCTT to promote non-templated nucleotide addition (Brownstein et al. 1996)

Locus	Primer sequence 5'–3'	Source	Reference	Size range	Primer (µM)
Ma2	F: <u>YAK</u> -ccatgtactttctctatcttttagga	Eurofins	This study	131–137	0.25
	R: <u>GTTCTT</u> atcttgcatacaactaaaat	Eurofins	Davis and Strobeck 1998		
Mel1	F: <u>FAM</u> -CTGGGGAAAATGGC TAAACC	Eurofins	Bijlsma et al. 2000	110–118	0.06
	R: <u>GTTCTT</u> GTCTTTATAAATCT GAAAATTAGGAATTC	Eurofins	Mullins et al. 2010		
Gg7	F: <u>FAM</u> -GTTTTCAATTTTAG CCGTTCTG	Eurofins	Davis and Strobeck 1998	170–178	0.08
	R: <u>GTTCTT</u> GTTTATCTCCCTCT TCCTACCC	Eurofins	Davis and Strobeck 1998		
Mvi1341	F: <u>PET</u> -GTGGGAGACTGAGA TAGGTCA	Applied Biosystems	Vincent et al. 2003	168–178	0.09
	R: <u>GTTCTT</u> GGCA ACTTGAATGGACTAAGA	Eurofins	Vincent et al. 2003		
Mar-21	F: <u>ATTO565</u> -ACATGCATACCTCC CAGACC	Eurofins	Natali et al. 2010	208–218	0.06
	R: <u>GTTCTT</u> TCTTCCCATTCCAC TTTGTCTACT	Eurofins	This study		
Mar-53	F: <u>FAM</u> -TCTCCAGCATTAC CTTTACCC	Eurofins	Natali et al. 2010	242–248	0.08
	R: GAACAGCCAACCCCATACC	Eurofins	Natali et al. 2010		

issues associated with non-spatial estimation techniques such as edge effects (Efford 2004; Efford et al. 2004; Royle et al. 2009; Borchers and Fewster 2016). Spatially explicit capture-recapture techniques are increasingly being used in density estimation studies of species of conservation and management concern throughout the world (Karanth 1995; Royle et al. 2011; Gray and Prum 2012; Head et al. 2013; Rouco et al. 2013; Anile et al. 2014; Borchers et al. 2014; Stetz et al. 2014; Dumond et al. 2015; Morin et al. 2016; Sirén et al. 2016).

Using the genotyped data for each individual pine marten detected in each specific study site, capture histories were created for each sampling occasion ($n = 3$ per study site). For secr analysis, the detector type chosen was 'proximity' to allow for multiple individuals to be captured at the same location; a Poisson distribution of home range centres was specified; and probability density functions were modelled using half-normal detection functions, which are commonly used in secr analyses (Dumond et al. 2015). Half-normal functions assume that the probability of pine marten capture increases linearly with proximity of a hair tube to the home range of an individual animal, which is biologically reasonable. As the sample size of the number of individual pine marten and associated capture rates were generally low for each site (Table 2), it was advisable that relatively simple models of detection and spatial processes would be the most biologically meaningful (Royle et al. 2009; O'Brien and Kinnaird 2011).

Pine marten ecology, behaviour and spacing patterns can vary between males and females (Zalewski and Jedrzejewski 2006; O'Mahony 2014), and these ecological differences can

affect capture probabilities ($g\theta$) and spatial scales (σ). To account for this, a hybrid mixture model (hcov) was implemented in secr (Efford 2016). In addition to candidate models that accounted for variation in $g\theta$ and σ by sex, behavioural models that specified a behavioural response to capture b , and whether an animal had been previously detected at a specific site bk , and sampling time occasion effect models t were also implemented in effecting $g\theta$. A null model, where $g\theta$ and σ were assumed to be equal amongst individuals and sexes, was also specified. Overall model selection was based on the lowest Akaike information criterion value, corrected for small samples sizes (AICc). Where candidate models were closely related to each other, model averaging was undertaken.

A habitat mask that incorporated a map of surveyed forests and a specific buffer zone (Efford et al. 2004) were created in ArcMap 10.3. The buffer distance should be sufficiently large to ensure that all animals with a negligible probability of encounter are included (Royle and Converse 2014) to minimise bias in density estimation (Efford et al. 2004). In this study, a buffer zone distance of 2000 m was specified, equating to approximately four home range centres for pine marten in Ireland (O'Mahony 2014). Density was expressed as number of pine marten per km² of forest habitat. All density estimation was undertaken using package *secr* (Efford 2016) in R version 3.3.1 (R Core Development Team 2016), with some preliminary data exploration in Density 5.0 (Efford et al. 2004). In secr analyses, all study site data were simultaneously modelled with each candidate model. The most parsimonious model was then fitted to each individual study site unique

Table 2 Summary of pine marten capture data for each study site. Hair samples were defined as pine marten by DNA analysis and as individuals by unique genotypes at the initial six microsatellite loci

Site code	Tube no.	Hair samples	Pine marten hair		Sex type		Genotyped samples		Unique genotypes
			<i>n</i>	% of hair	<i>n</i>	% male	<i>n</i>	% marten	
1	24	11	11	100	10	40	10	91	6
2	30	34	34	100	32	56	27	79	9
3	29	23	19	83	17	82	18	95	8
4	30	21	21	100	20	55	20	95	10
5	30	16	16	100	16	81	16	100	5
6	23	11	11	100	11	45	11	100	6
7	30	0	0	na	na	na	0	na	0
8	31	20	20	100	20	85	20	100	11
9	30	16	16	100	16	63	15	94	6
10	30	25	25	100	23	70	22	88	8
11	25	2	2	100	2	100	2	10	1
12	26	4	3	75	2	100	2	67	2
13	30	26	24	92	18	67	16	67	7
14	22	38	38	100	38	37	38	100	14
	390	247	240	97	225	60	217	90	93

capture data, trap and habitat mask area configurations to derive site specific density estimates. Abundance estimates for each site were obtained using CAPWIRE (Miller et al. 2005) with the TIRM model implemented on combined capture data across each sampling session, in each study site.

A national population estimate for pine marten

To estimate a national mean density for pine marten, all study site data were simultaneously modelled with each candidate model and the best performing model was then applied to produce a mean density estimate across all study sites. CAPWIRE was also applied to the 14 randomly selected sites to obtain an overall abundance estimate for these study sites.

The mean pine marten population density was scaled to a national pine marten population abundance estimate for Ireland by incorporating data on the estimated forest habitat available within the current distribution of pine marten in Ireland (see Fig. 1b), determined from various Irish forestry and landscape GIS datasets. This approach is frequently used when data on regional or national population abundance estimates are required for species of interest (Erb and Sampson 2009; Frary et al. 2011; O'Mahony et al. 2012; Fechter and Storch 2014; Humm et al. 2015; Gervasi et al. 2016). It obviously has underlying assumptions that include current knowledge on pine marten distribution is correct, study sites are randomly selected, all available habitat in the species distribution is occupied by pine marten, GIS layers are accurate and habitat suitability is accounted for. Given pine martens established relationship with forested habitat, the basis for scaling up density estimates in terms of the availability of forest habitat in the current study was well founded.

Results

A total of 247 hair samples were collected from 390 hair tubes deployed throughout the 14 study sites (Table 2). The number of hair samples collected per study site varied from 0 to 38. DNA analysis verified that 97% of hair samples were pine marten and 90% of those yielded genotypes with the six microsatellite loci. All loci were polymorphic with 3–4 alleles per locus. The probability of identity (PI) using all six loci was $PI = 0.00042$ and $PI_{sibs} = 0.024$. No deviation from Hardy-Weinberg equilibrium was observed. In total, 93 unique individual pine marten were determined across all study sites, 53 male and 37 female (3 were undetermined). The number of individual pine marten detected per study site varied from 0 to 14 (Table 2). Across the 14 study sites, 61.53% of hair tubes yielded pine marten hair samples (240/390; Table 2). Across sampling sessions, the number of unique individual pine marten captured did not significantly

differ between sessions $\chi^2 = 0.19$, $df = 2$, $P > 0.90$ (session 1 = 29, session 2 = 32, session 3 = 32), and the levels of animal recaptures increased as sampling sessions progressed (session 1 = 37, session 2 = 51, session 3 = 69). Across all study sites and sampling sessions, on average, individual pine marten had a recapture rate of 2.33 (SE 0.19; range 1–10), with 50.53% of individuals detected once, and 49.47% of pine marten captured more than once. In a similar pattern as observed with pine marten capture rates, on average, pine marten was captured in 1.65 unique hair tubes (SE 0.103; range 1–5), which does not include reuse of the same tube. Detection of multiple pine marten individuals at the same hair tube location only occurred in 6.99% (10/143) of tubes, and in 35.71% (5/14) of study sites.

Based on secr analyses, the best performing models were hybrid mixture models that included sex and site-specific detection effects on pine marten capture probability and sex effects on spatial scale (Table 3). The estimated habitat mask area including all forested habitat within a 2000-m buffer of all surveyed sites was 30,100 ha (Table 4). Estimated pine marten density varied from 0 to 2.60 pine marten per km² of forested habitat across study sites (Table 4; Fig. 2). In all but a single site, pine marten density estimates were below 1 individual per km² of forest habitat. Confidence intervals associated with density estimates from study sites overlapped for most of the study sites (Fig. 2), with the exception of study site 14, which was significantly greater than other sites as judged by the lack of confidence interval overlap with other estimates. Abundance estimates of pine marten for each study site, based on using CAPWIRE, ranged from 6 to 29 individual pine marten across study sites (Table 4).

To estimate a national population abundance of pine marten, secr analyses were conducted on capture data from across all 14 study sites to estimate a mean pine marten density. This dataset consisted of 93 individual

Table 3 Akaike information criterion (AIC) model selection for spatially explicit capture-recapture analyses of pine marten density. Model specified capture probability at home range center ($g\theta$), and spatial scale (σ), modelled with variation in sex ($h2$), individual behaviour response to capture (b), whether an animal had been previously detected at a specific site (bk), sampling time occasion effect models (t), and null model (1). Number of parameters (npar), log-likelihood (LogL), AICc is AIC with a correction for finite sample sizes

Model	npar	LogL	AIC	AICc
g0~bk + h2, σ ~h2	7	-732.18	1478.36	1479.68
g0~b + h2, σ ~h2	7	-768.47	1550.94	1552.26
g0~t + h2, σ ~h2	8	-769.34	1554.67	1556.39
g0~h2, σ ~h2	6	-796.61	1605.23	1606.20
g0~1, σ ~1	4	-802.35	1612.69	1613.15

Table 4 Pine marten density and abundance estimates for each study site using secr based and CAPWIRE analyses of pine marten capture data across 14 study sites in Ireland. In site 7 as no pine marten were detected, density could not be calculated and was assumed to be zero, and no abundance estimates for sites 11 and 12, due to small sample size. Estimated habitat mask area refers to the surveyed area within which hair tubes were located in each study site, including a 2000-m buffer of suitable habitat. SE is standard error; LCL is lower 95% confidence interval and UCL is upper 95% confidence interval

Site	Estimated habitat mask (ha)	Density	SE	95% LCL	95%UCL	CAPWIRE abundance (95% CL)
1	1462	0.75	0.21	0.44	1.29	12 (6–30)
2	1908	0.97	0.24	0.60	1.56	9 (9–9)
3	2607	0.60	0.15	0.37	0.99	12 (8–21)
4	4269	0.52	0.13	0.32	0.83	19 (10–32)
5	2399	0.46	0.15	0.24	0.86	6 (5–10)
6	1435	0.89	0.27	0.50	1.59	7 (6–10)
7	1652	0.00	–	–	–	–
8	1792	0.93	0.17	0.64	1.32	27 (11–44)
9	1832	0.54	0.14	0.32	0.88	6 (6–6)
10	2218	0.72	0.18	0.44	1.16	8 (8–8)
11	2099	0.12	0.09	0.03	0.45	–
12	2108	0.25	0.14	0.09	0.68	–
13	3211	0.50	0.15	0.28	0.87	11 (7–18)
14	1141	2.60	0.56	1.71	3.95	19 (14–26)
Total	30,106					148 (119–165)

pine marten captured 217 times. The best performing model specifying sex and site-specific detection effects on g_0 and sex effects on σ was applied to the dataset, providing a mean density estimate across all study sites of 0.64 (95% CI 0.49–0.81) pine marten per km² of forest habitat. In CAPWIRE, the estimated total pine marten

population abundance across the 14 randomly selected study sites was 148 (95% CI 116–165). It was estimated that 475,565 ha of forest habitat existed within pine marten range. Combining this data with the mean density estimate, the current pine marten population in Ireland was estimated at 3043 (95% CI 2330–3852) individuals.

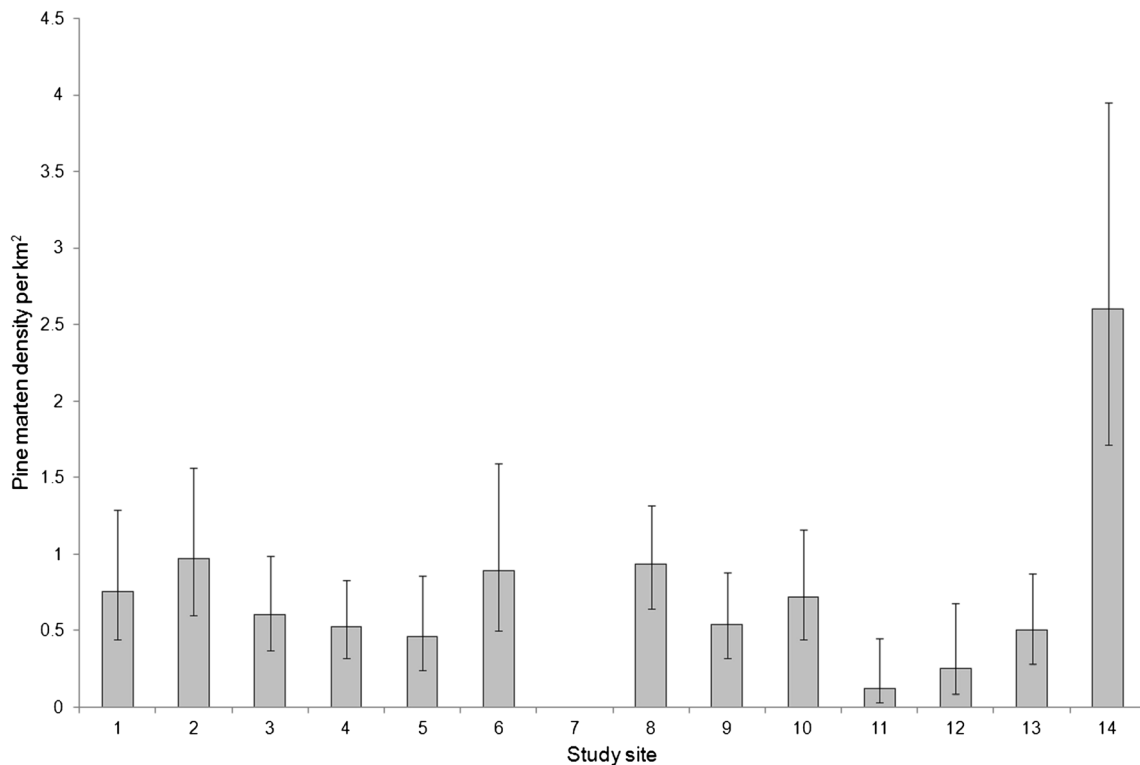


Fig. 2 Variation in pine marten density across random study sites in Ireland. 95% CI for each site are indicated by error bars. No pine marten density estimates were available in site 7 as no pine marten

were detected. Densities were estimated with the most parsimonious hybrid mixture models using spatially explicit capture-recapture models

Discussion

This is the first study that has explicitly aimed to determine variation in pine marten density across multiple sites throughout Ireland and produce a national population abundance estimate for the species. To our knowledge, this also represents the largest scale population density assessment of this species in any country or region within the species range. This information is critical in terms of the conservation and management of protected and important species such as pine marten and provides the basis for part fulfilment of national and international conservation obligations with reference to this species. The current density estimates are within the ranges reported for pine marten in Ireland (Lynch et al. 2006; Mullins et al. 2010; Sheehy et al. 2014; O'Mahony et al. 2015) and for the species across its range (Zalewski and Jedrzejewski 2006; Manzo et al. 2012; Balestrieri et al. 2016a; Croose et al. 2016), although comparisons between studies should be treated cautiously due to differing objectives, methods and analyses. The combined dataset that consisted of 93 individual pine marten, captured 217 times, a recapture rate of 2.33 compares well with studies involving capture-recapture estimation for related species such as the American marten *M. americana* and fisher *Pekania pennanti* (Mowat and Paetkau 2002; Sweitzer et al. 2015) and provided reliable estimation of pine marten density across the study sites surveyed.

It was clear that in the study sites sampled, the variation in estimated pine marten density was largely non-significant as judged by the overlap in associated confidence intervals. The sample size of individual pine marten and the number of captures were low in most of the sites surveyed, with only 21.42% of sites (3/14) having detected 10 or more individual pine marten. As each study site was surveyed for 1 month and included a minimum of several hundred, if not thousands, of hectares of forest habitat, and that the non-invasive technique used in the study has proven effective in Ireland (Lynch et al. 2006; Mullins et al. 2010; Sheehy et al. 2014; O'Mahony et al. 2015), this is clearly indicative of generally low numbers of pine marten throughout the majority of study sites considered in the current study. Traditionally, in capture-recapture studies, sample sizes of individuals of less than 20 may be too small for reliable density estimation (Otis et al. 1978; White 1982). However, spatially explicit capture-recapture techniques can produce more reliable density estimates with small sample sizes of individuals (Borchers and Efford 2008; Gardner et al. 2010; O'Brien and Kinnaird 2011; Sollmann et al. 2011).

Where small numbers of individuals exist within a population, limiting the complexity of implemented secr-based candidate models is advisable (Royle et al. 2009; O'Brien and Kinnaird 2011) and was the approach undertaken in this study. Similarly, CAPWIRE was originally designed specifically for small populations (Miller et al. 2005). High pine marten densities (i.e. >2 per km²) were rare in this study, having occurred

in a single study site only. Potential reasons for this high density are speculative and may include that the largest wood in the site had varied mixed coniferous/deciduous forest type and structure, had been established for hundreds of years as part of an early demense, and that the site was within core pine marten population range in Ireland (O'Mahony et al. 2012). Such densities have been reported in the literature for pine marten previously from Ireland and Italy (Lynch et al. 2006; Mullins et al. 2010; Sheehy et al. 2014; Balestrieri et al. 2016a; this study). Other studies such as Zalewski and Jedrzejewski (2006) and Manzo et al. (2012) also suggested what were termed relatively high-density pine marten population estimates of 0.34 per km² and 0.36 to 0.76 per km², respectively, which were within the range of the majority of estimates in the current study. This highlights an interesting paradigm with reference to what researchers and managers refer to as 'high'-density wildlife populations across a species range. Clearly for pine marten as a population throughout its range, high, or relatively high population density estimates cannot exist at ranges from 0.34 to >2.60 individuals per km² in specific sites, as derived from the aforementioned studies.

It is suggested that the general lack of studies on density estimation on this species and the different methods used in calculating densities is a barrier to identifying where true high-density populations may exist across the species range. At biogeographic spatial scales, primary drivers of variation in pine marten density include winter severity and seasonal variation in the productivity of ecosystems, with higher densities of pine marten in areas with milder winters and lower seasonality (Zalewski and Jedrzejewski 2006). The climate of Ireland is heavily influenced by its small size and oceanic influence, and can best be described as mild and wet, with a lack of temperature extremes and relatively little snow cover. Mean annual temperatures range from 9 to 10 °C, average annual rainfall is 1230 mm and prolonged snow cover is rare, with lowlands experiencing few to none days of snow, and the uplands having up to 20–30 days in any typical year. According to the analysis of Zalewski and Jedrzejewski (2006), Ireland fits with predictions of a country that has the potential to experience high-density pine marten populations. The estimate of 2.60 per km² at one site in the current study is amongst the highest yet reported for the species, although the mean figure of 0.64 calculated from this study and the estimates from the majority of study sites were not unlike other countries surveyed.

The national pine marten abundance estimate in the current study [3043 individuals (95% CI 2330–3852) individuals] in Ireland is, as far as we are aware, the first in the EU to be based on a large-scale pine marten population survey. In other jurisdictions that have been subject to Habitats Directive conservation assessments, national abundance estimates for pine marten have been largely achieved using limited data sources and expert opinion (see <http://bd.eionet.europa.eu>). For

instance in the UK, the pine marten population has been assessed as approximately 3800 individuals, with the majority of that population existing within Scotland. The current study may provide a framework for more robust estimates of this species density and abundance in other jurisdictions. However, it is suggested that pilot studies are carried out in specific suites prior to any large-scale deployment of this technique to ensure that hair tubes are an effective survey method (see Bartolommei et al. 2012). This study also suggests that future research that focuses on pine marten density estimation in Ireland, and perhaps in other countries with highly fragmented forest habitat, should consider adopting a landscape scale approach to study area delineation such that it is likely to be sufficient to contain at least 15–20 individual pine marten. Based on evidence from the current study on mean pine marten density, a minimum potential study area size of 1500 to 3000 ha of forest habitat could be a useful indicative area. That is of course unless a priori rationale exists for suspecting high-density pine marten populations in smaller sites.

Pine marten populations consist of territory holding resident adult individuals, subadults and juveniles that have a requirement to establish their own territories. Territories can be established by dispersing to unoccupied suitable habitats or acquiring parts of existing territories of unrelated individuals (Zalewski 2012). In the current study, it was not possible to determine the proportion of resident pine marten included in density estimates. However, this study occurred during January to June, when any subadults/juveniles detected in hair tubes would have been 6 to 12 months of age. Given the majority of hair tubes detected unique single individual pine marten, and not multiple individuals at the same tube, it is suggested that the majority of animals detected in this study were potentially resident territory holding individuals. Higher proportions of subadult to adult pine martens may exist in more fragmented habitat and they may occupy smaller home ranges (Mergey et al. 2011), but such habitats are likely to have significant fitness consequences in terms of survival and reproduction, and they probably represent suboptimal habitat for the species. Research into the ecology of pine marten populations that exist in highly fragmented habitats in Ireland would be beneficial in terms of providing input into future density and abundance estimates for the species.

A clear finding from the current study is that, despite recent signs of recovery in the pine marten's range in Ireland, pine marten population abundance is relatively low. However, this needs to be considered in terms of the potential carrying capacity of the landscape for the species in Ireland, which will ultimately be influenced by forest habitat availability. There is a duality in the dependence on forest habitat for pine marten in that not only is forest habitat a key resource for the species, it can also be viewed as a key limiting resource for the population in poorly forested (9% landcover) island nations such as

Ireland, in that pine martens are unlikely to have established and sustainable populations in habitats that are largely devoid of some level of forest cover. This duality must be acknowledged when considering management of the species in Ireland according to current knowledge. The density and abundance results of the current study should be seen in the context of Ireland having a limited habitat resource for pine marten, which taken together with the species socio-spatial organisation and solitary behaviour indicates that the ultimate potential carrying capacity of the pine marten population in the Irish landscape may inherently be limited. This study also emphasises the benefits of standardising methods of density estimation for pine marten across its range and undertaking appropriate statistical analyses to estimate density. Improving the ability of managers and researchers to accurately determine and compare population densities will lead to improved understanding and management of pine marten populations nationally and internationally.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

- Anile S, Ragni B, Randi E, Mattucci F, Rovero F (2014) Wildcat population density on the Etna volcano, Italy: a comparison of density estimation methods. *J Zoology* 293:252–261
- Baines D, Aebischer N, Macleod A, Woods J (2013) Pine marten *Martes martes* and red fox *Vulpes vulpes* sign indices in Scottish forests: population change and reliability of field identification of scats. *Wildl Biol* 19:490–495
- Balestrieri A, Ruiz-González A, Vergara M, Capelli E, Tirozzi P, Alfino S, Minuti G, Prigioni C, Saino N (2016a) Pine marten density in

- lowland riparian woods: a test of the random encounter model based on genetic data. *Mamm Biol* 81:439–446
- Balestrieri A, Bogliani G, Boano G, Ruiz-González A, Saino N, Costa S, Milanese P (2016b) Modelling the distribution of forest-dependent species in human-dominated landscapes: patterns for the pine marten in intensively cultivated lowlands. *PLoS One* 11:e0158203
- Bartolommei P, Manzo E, Cozzolino R (2012) Evaluation of three indirect methods for surveying European pine marten in a forested area of central Italy. *Hystrix* 23:91–94
- Bijlsma R, Van De Vliet M, Pertoldi C, Van Apeldoorn R, Van de Zande L (2000) Microsatellite primers from the Eurasian badger, *Meles meles*. *Mol Ecol* 9:2215–2216
- Borchers DL, Efford M (2008) Spatially explicit maximum likelihood methods for capture–recapture studies. *Biometrics* 64:377–385
- Borchers D, Fewster R (2016) Spatial capture–recapture models. *Stat Sci* 31:219–232
- Borchers D, Distiller G, Foster R, Harmsen B, Milazzo L (2014) Continuous-time spatially explicit capture–recapture models, with an application to a jaguar camera-trap survey. *Methods Ecol Evol* 5:656–665
- Brownstein MJ, Carpten JD, Smith JR (1996) Modulation of non-templated nucleotide addition by Taq DNA polymerase: primer modifications that facilitate genotyping. *BioTechniques* 20:1004–1006
- Burnham KP, Anderson DR, Laake JL (1980) Estimation of density from line transect sampling of biological populations. *Wildl Monog* 72:3–202
- Burton AC, Neilson E, Moreira D, Ladle A, Steenweg R, Fisher JT, Bayne E, Boutin S (2015) REVIEW: wildlife camera trapping: a review and recommendations for linking surveys to ecological processes. *J Appl Ecol* 52:675–685
- Caryl FM, Quine CP, Park KJ (2012) Martens in the matrix: the importance of nonforested habitats for forest carnivores in fragmented landscapes. *J Mammal* 93:464–474
- Collinson WJ, Parker DM, Bernard RTF, Reilly BK, Davies-Mostert HT (2014) Wildlife road traffic accidents: a standardized protocol for counting flattened fauna. *Ecol Evol* 4:3060–3071
- Croose E, Birks JD, O'Reilly C, Turner P, Martin J, MacLeod ET (2016) Sample diversity adds value to non-invasive genetic assessment of a pine marten (*Martes martes*) population in Galloway Forest, south-west Scotland. *Mamm Res* 61:131–139
- Davis C, Strobeck C (1998) Isolation, variability, and cross-species amplification of polymorphic microsatellite loci in the family Mustelidae. *Mol Ecol* 7:1776–1778
- Dumond M, Boulanger J, Paetkau D (2015) The estimation of grizzly bear density through hair-snagging techniques above the tree line. *Wildl Soc Bull* 39:390–402
- Efford MG (2004) Density estimation in live-trapping studies. *Oikos* 106: 598–610
- Efford M (2016) secr 2.10-spatially explicit capture–recapture in R.
- Efford MG, Dawson DK, Robbins CS (2004) DENSITY: software for analysing capture–recapture data from passive detector arrays. *Anim Biodivers Conserv* 27:217–228
- Efford MG, Borchers DL, Byrom AE (2009) Density estimation by spatially explicit capture–recapture: likelihood-based methods. Modeling demographic processes in marked populations. Springer, In, pp 255–269
- Erb J, Sampson B (2009) Distribution and abundance of wolves in Minnesota. Minnesota Department of Natural Resources. Grand Rapids, Minnesota
- Fechter D, Storch I (2014) How many wolves (*Canis lupus*) fit into Germany? The role of assumptions in predictive rule-based habitat models for habitat generalists. *PLoS One* 9:e101798
- Frary VJ, Duchamp J, Maehr DS, Larkin JL (2011) Density and distribution of a colonizing front of the American black bear *Ursus americanus*. *Wildl Biol* 17:404–416
- Fukuda Y, Saalfeld K, Webb G, Manolis C, Risk R (2013) Standardised method of spotlight surveys for crocodiles in the tidal rivers of the northern territory, Australia. *North Territ Nat* 24:14–32
- Gardner B, Reppucci J, Lucherini M, Royle JA (2010) Spatially explicit inference for open populations: estimating demographic parameters from camera-trap studies. *Ecology* 91:3376–3383
- Gervasi V, Brøseth H, Gimenez O, Nilsen EB, Odden J, Flagstad Ø, Linnell JDC (2016) Sharing data improves monitoring of trans-boundary populations: the case of wolverines in central Scandinavia. *Wildl Biol* 22:95–106
- Gopalaswamy AM, Royle JA, Hines JE, Singh P, Jathanna D, Kumar N, Karanth KU (2012) Program SPACECAP: software for estimating animal density using spatially explicit capture–recapture models. *Methods Ecol Evol* 3:1067–1072
- Gray TNE, Prum S (2012) Leopard density in post-conflict landscape, Cambodia: evidence from spatially explicit capture–recapture. *J Wildl Manag* 76:163–169
- Guberti V, Stancampiano L, Ferrari N (2014) Surveillance, monitoring and surveys of wildlife diseases: a public health and conservation approach. *Hystrix* 25:3–8
- Gula R, Theuerkauf J (2013) The need for standardization in wildlife science: home range estimators as an example. *Eur J Wildl Res* 59:713–718
- Head JS, Boesch C, Robbins MM, Rabanal LI, Makaga L, Kuhl HS (2013) Effective sociodemographic population assessment of elusive species in ecology and conservation management. *Ecol Evol* 3:2903–2916
- Helldin JO (2000) Population trends and harvest management of pine marten *Martes martes* in Scandinavia. *Wildl Biol* 6:111–120
- Humm J, McCown JW, Scheick BK, Clark JD (2015) Annual report to the Florida fish and wildlife conservation commission on contract R112219563 with the University of Tennessee.
- Jansen PA, Ahumada J, Fegraus E, O'Brien T (2014) TEAM: a standardised camera-trap survey to monitor terrestrial vertebrate communities in tropical forests. Wildlife Research and Management CSIRO Publishing, Melbourne, Australia, Camera Trapping
- Jamevich CS, Simpson A, Graham JJ, Newman GJ, Barger CT (2015) Running a network on a shoestring: the global invasive species information network. *Manage Biol Invas* 6:137–146
- Karanth KU (1995) Estimating tiger *Panthera tigris* populations from camera-trap data using capture–recapture models. *Biol Conserv* 71: 333–338
- Kurki S, Nikula A, Helle P, Linden H (1998) Abundances of red fox and pine marten in relation to the composition of boreal forest landscapes. *J Anim Ecol* 67:874–886
- Lynch AB, Brown MJF, Rochford JM (2006) Fur snagging as a method of evaluating the presence and abundance of a small carnivore, the pine marten (*Martes martes*). *J Zool* 270:330–339
- Manzo E, Bartolommei P, Rowcliffe JM, Cozzolino R (2012) Estimation of population density of European pine marten in central Italy using camera trapping. *Acta Theriol* 57:165–172
- Mergey M, Helder R, Roeder J-J (2011) Effect of forest fragmentation on space-use patterns in the European pine marten (*Martes martes*). *J Mammal* 92:328–335
- Miller CR, Joyce P, Waits LP (2005) A new method for estimating the size of small populations from genetic mark–recapture data. *Mol Ecol* 14:1991–2005
- Moll RJ, Kilshaw K, Montgomery RA, Abade L, Campbell RD, Harrington LA, Millsbaugh JJ, Birks JDS, Macdonald DW (2016) Clarifying habitat niche width using broad-scale, hierarchical occupancy models: a case study with a recovering mesocarnivore. *J Zool* 300:177–185
- Morin DJ, Kelly MJ, Waits LP (2016) Monitoring coyote population dynamics with fecal DNA and spatial capture–recapture. *J Wildl Manag* 80:824–836

- Mowat G, Paetkau D (2002) Estimating marten *Martes americana* population size using hair capture and genetic tagging. *Wildl Biol* 8: 201–209
- Mullins J, Statham MJ, Roche T, Turner PD, O'Reilly C (2010) Remotely plucked hair genotyping: a reliable and non-invasive method for censusing pine marten (*Martes martes*, L. 1758) populations. *Eur J Wildl Res* 56:443–453
- Natali C, Banchi E, Ciofi C, Manzo E, Bartolommei P, Cozzolino R (2010) Characterization of 13 polymorphic microsatellite loci in the European pine marten *Martes martes*. *Conserv Genet Res* 2: 397–399
- O'Brien TG, Kinnaird MF (2011) Density estimation of sympatric carnivores using spatially explicit capture–recapture methods and standard trapping grid. *Ecol Appl* 21:2908–2916
- O'Mahony DT (2014) Socio-spatial ecology of pine marten (*Martes martes*) in conifer forests, Ireland. *Acta Theriol* 59:251–256
- O'Mahony DT (2017) A species distribution model for pine marten (*Martes martes*) in the least forested region of Europe. *Mammal Res* 62:195–200
- O'Mahony D, O'Reilly C, Turner P (2012) Pine marten (*Martes martes*) distribution and abundance in Ireland: a cross-jurisdictional analysis using non-invasive genetic survey techniques. *Mammal Biol* 77: 351–357
- O'Mahony DT, Turner P, O'Reilly C (2015) Pine marten (*Martes martes*) abundance in an insular mountainous region using non-invasive techniques. *Eur J Wildl Res* 61:103–110
- O'Meara DB, Sheehy E, Turner PD, O'Mahony D, Harrington AP, Denman H, Lawton C, MacPherson J, O'Reilly C (2014) Non-invasive multi-species monitoring: real-time PCR detection of small mammal and squirrel prey DNA in pine marten (*Martes martes*) scats. *Acta Theriol* 59:111–117
- Otis DL, Burnham KP, White GC, Anderson DR (1978) Statistical inference from capture data on closed animal populations. *Wildlife Monogr* 62:3–135
- Pereboom V, Mergey M, Villerette N, Helder R, Gerard J-F, Lodé T (2008) Movement patterns, habitat selection, and corridor use of a typical woodland-dweller species, the European pine marten (*Martes martes*), in fragmented landscape. *Can J Zool* 86:983–991
- Plotz RD, Grecian WJ, Kerley GIH, Linklater WL (2016) Standardising home range studies for improved management of the critically endangered black rhinoceros. *PLoS One* 11:e0150571
- R Core Development Team (2016) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org/>
- Rouco C, Norbury GL, Smith J, Byrom AE, Pech RP (2013) Population density estimates of brushtail possums (*Trichosurus vulpecula*) in dry grassland in New Zealand. *New Zeal J Ecol* 37:12–17
- Rowcliffe JM, Field J, Turvey ST, Carbone C (2008) Estimating animal density using camera traps without the need for individual recognition. *J Appl Ecol* 45:1228–1236
- Royle JA, Converse SJ (2014) Hierarchical spatial capture–recapture models: modelling population density in stratified populations. *Methods Ecol Evol* 5:37–43
- Royle JA, Nichols JD, Karanth KU, Gopalaswamy AM (2009) A hierarchical model for estimating density in camera-trap studies. *J Appl Ecol* 46:118–127
- Royle JA, Magoun AJ, Gardner B, Valkenburg P, Lowell RE (2011) Density estimation in a wolverine population using spatial capture–recapture models. *J Wildl Manag* 75:604–611
- Sheehy E, O'Meara DB, O'Reilly C, Smart A, Lawton C (2014) A non-invasive approach to determining pine marten abundance and predation. *Eur J Wildl Res* 60:223–236
- Sirén AP, Pekins PJ, Abdu PL, Ducey MJ (2016) Identification and density estimation of American martens (*Martes americana*) using a novel camera-trap method. *Diversity* 8:3
- Sollmann R, Furtado MM, Gardner B, Hofer H, Jácomo AT, Tôrres NM, Silveira L (2011) Improving density estimates for elusive carnivores: accounting for sex-specific detection and movements using spatial capture–recapture models for jaguars in central Brazil. *Biol Conserv* 144:1017–1024
- Stetz JB, Kendall KC, Macleod AC (2014) Black bear density in Glacier National Park, Montana. *Wildl Soc Bull* 38:60–70
- Sweitzer RA, Popescu VD, Barrett RH, Purcell KL, Thompson CM (2015) Reproduction, abundance, and population growth for a fisher (*Pekania pennanti*) population in the Sierra National Forest, California. *J Mammal* 96:772–790
- Vincent I, Farid A, Otieno C (2003) Variability of thirteen microsatellite markers in American mink (*Mustela vison*). *Can J Anim Sci* 83: 597–599
- White GC (1982). Capture-recapture and removal methods for sampling closed populations. Los Alamos National Laboratory.
- Wilson GJ, Delahay RJ (2001) A review of methods to estimate the abundance of terrestrial carnivores using field signs and observation. *Wildl Res* 28:151–164
- Zalewski A (2012) Alternative strategies in the acquisition of home ranges by male pine martens in a high-density population. *Acta Theriol* 57:371–375
- Zalewski A, Jedrzejewski W (2006) Spatial organisation and dynamics of the pine marten *Martes martes* population in Bialowieza Forest (E Poland) compared with other European woodlands. *Ecography* 29: 31–43