

Provided by the author(s) and NUI Galway in accordance with publisher policies. Please cite the published version when available.

Title	The role of the pine marten in Irish squirrel population dynamics
Author(s)	Sheehy, Emma
Publication Date	2013-09-19
Item record	http://hdl.handle.net/10379/3949

Downloaded 2018-11-14T10:29:44Z

Some rights reserved. For more information, please see the item record link above.





The role of the pine marten in Irish squirrel population dynamics

Thesis submitted for the degree of Ph.D. to the National University of Ireland, Galway by:

Emma Sheehy B.Sc. (Hons) H.Dip. (GIS)

Supervisor:

Dr. Colin Lawton

School of Natural Sciences

National University of Ireland, Galway

Discipline of Zoology

September 2013

Table of Co	ontents Page
Abstract	V
Acknowledgements	vii
Declaration	іх
List of Figures	х
List of Tables	xii

1 General Introduction

1.1	Sciurus vulgaris	1
1.2	Sciurus carolinensis	2
1.3	The replacement of red squirrels by grey squirrels	3
1.4	Grey squirrel control	4
1.5	Martes martes	5
1.6	History of the pine marten in Ireland	7
1.7	Squirrel distribution in Ireland	7
1.8	Marten predation on squirrels	8
1.9	Project aims	10

2	Squirrel and pine marten distribution surveys

2.1	Introduction		11
2.2	Aims		14
2.3	Methods		14
	2.3.1	Sightings surveys	14
	2.3.2	Hairtube surveys	15
	2.3.3	Laboratory analysis	18
	2.3.4	Data analysis	18
2.4	Results		19
	2.4.1	Sightings surveys	19
	2.4.2	Hairtube surveys	23
	2.4.3	Changes in distribution	26

2.5	Discussion		26
	2.5.1	Distribution in the midlands	26
	2.5.2	Distribution in the east	28
	2.5.3	Distribution in the buffer zone	28
2.6	Conclusion		29

3	Squirrel	population	studies
---	----------	------------	---------

3.1	Introduction		30
3.2	Aims		31
3.3	Methods		32
	3.3.1	Site selection and study sites	32
	3.3.2	Trapping and handling squirrels	33
	3.3.3	Demography	35
	3.3.4	Body size and fitness	35
	3.3.5	Reproduction	36
	3.3.6	Habitat quality	37
3.4	Results		38
	3.4.1	Squirrel density	38
	3.4.2	Sex ratio	40
	3.4.3	Breeding and Recruitment	40
	3.4.4	Body size	41
	3.4.5	Fitness	41
	3.4.6	Habitat quality	46
3.5	Discussion		47
	3.5.1	Competitive release	48
	3.5.2	Density and fitness	48
	3.5.3	Squirrel and pine marten distribution	50
3.6	Conclusion		52

4	A non-invasive approach to determining pine marten abundance			
	and predation			
4.1	Introduction		53	
4.2	Aims		55	
4.3	Methods		56	
	4.3.1	Field methods	56	
	4.3.1.1	Abundance study	56	
	4.3.1.2	Dietary study	59	
	4.3.2	Laboratory methods	61	
	4.3.2.1	Abundance study	61	
	4.3.2.2	Dietary study	63	
4.4	Results		65	
	4.4.1	Abundance study	65	
	4.4.1.1	Assessing genotyping errors	69	
	4.4.1.2	Genetic variability	69	
	4.4.2	Dietary study	71	
	4.4.2.1	Molecular and macro analyses	72	
4.5	Discussion		76	
	4.5.1	Pine marten abundance	76	
	4.5.2	Non-invasive techniques	78	
	4.5.3	Scat density	81	
	4.5.4	Dietary analysis	82	
4.6	Conclusion		84	
E	Grovenuirral	consitivity to produtor adour		
5	Siey squirier	sensitivity to predator odour		

5.1	Introduction		86
5.2	Aims		92
5.3	Methods		92
	5.3.1	Field methods	92
	5.3.2	Statistical analyses	94
5.4	Results		95

5.5	Discussion	99
5.6	Conclusion	102

6 General Discussion

6.1	Population crashes in formerly invasive species	103
6.2	Failure of the grey squirrel to establish in the west of Ireland	105
6.3	Range retraction of the grey squirrel	106
6.4	Why has the grey squirrel population declined?	107
6.5	Implications for Ireland, Britain and Europe	111

7 List of References

Abstract

There are two squirrel species found in Ireland. The native red squirrel, *Sciurus vulgaris*, and the invasive North American grey squirrel, *Sciurus carolinensis*. In Ireland, the UK and Italy the grey squirrel threatens the survival of the red squirrel as the effects of competition and disease from the grey squirrel almost inevitably lead to total replacement of the red squirrel population. In 2007, the results of a national squirrel survey suggested that the normally invasive grey squirrel population in the midlands of Ireland had gone into decline. Whilst occasionally grey squirrel introductions have failed elsewhere, such a decline in range has not been recorded anywhere else for this species subsequent to having established itself as an invasive population. In the 2007 report, the decline in grey squirrel range was anecdotally attributed to an increase in European pine marten, *Martes martes*, range and numbers. The pine marten population in Ireland reached a nadir in the early 20th century as a result of habitat loss and persecution, but the core population in the west and midlands of Ireland have recovered in recent decades.

The 2007 survey recommended the theory that the pine marten population may be involved in the decline of the invasive grey squirrel's range be investigated. This project serves to carry out that study by investigating the role, if any, of the European pine marten in red and grey squirrel population dynamics in Ireland.

The distribution and status of all three species were examined in both the midlands of Ireland and a control region in the east, where all three species are also present but the grey squirrel range had not been reported as having gone into decline. Detailed distribution surveys of the study and control regions were carried out by means of sightings and field surveys which confirmed that the grey squirrel is now rare in approximately 9,000km² of its former invasive range. The distribution survey also established that the red squirrel has successfully recolonised much of this area and a live-trapping program confirmed the red squirrel population in the midlands is now in competitive release.

v

The grey squirrel continues to thrive in other parts of the country however, including the control region in the east of the country. Results from a pine marten diet and density study suggest that the abundance of the native predator population may be a critical factor in the grey squirrel's success or failure as an invasive species. Non-invasive methods of studying this elusive carnivore were also investigated, and the genotyping of remotely plucked hair samples in the study and control regions revealed the pine marten to be considerably more abundant in the midlands than the east of Ireland. Real-time PCR techniques were utilised to determine the frequency of occurrence of squirrels and other small mammals in pine marten diet and the results were compared to a sub sample of scats which were subject to traditional macro analysis. Both techniques were found to yield similar results suggesting molecular techniques are a useful tool in studying pine marten diet. The first evidence of European pine marten predating upon the North American grey squirrel is reported, however a negative correlation between pine marten and grey squirrel presence at woodland level resulted in a small sample size of scats in areas that the grey squirrel was available as a prey item. This negative correlation in distribution also suggested that the non-lethal effects of an abundant predator population may be influencing the grey squirrel's ability to succeed as an invasive species in Ireland more so than direct predation itself. The possibility that pine marten scent marking may be influencing grey squirrel behaviour was also explored through a literature review and an experiment with grey squirrel sensitivity to predator odour.

Red squirrel distribution at landscape level was positively correlated with that of its natural predator, and the area which the red squirrel has recolonised falls entirely within the midlands pine marten population's range. The dietary study confirmed that the red squirrel is a low frequency prey item in the abundant pine marten population's diet. The true relationship between the pine marten and the grey squirrel has yet to be established however, and recommendations for future studies are provided.

vi

Acknowledgements

There are so many people that I would like to thank for their help and support during the course of this project. The first is my supervisor Colin Lawton. Thank you for giving me the opportunity to work with you on such a fascinating project. I will always be very grateful to you for giving me that opportunity, and for your guidance and support along the way.

Thanks to my family, especially my parents Dermot and Nuala for the moral, emotional and financial support you have given me. I could not have done this without your help. Mum for all the dinners after Wicklow fieldwork, I think you know how much they helped keep my strength up! Thank you Dad for absolutely always being there, and at the ripe old age of 70 for being one of three generations of Sheehy men who so generously helped me in the woods. Thanks especially to my brothers and my nephew Sam, Michael and Mark for all the help in Tomnafinnoge. It made such a huge difference to me to have the physical help with the work, but also your company was so much appreciated. Thanks also to Sarah Murray and David Lawton who both volunteered so much of their time to help in the woods.

And to my squirrel sisters, Emily and Cat, thanks for everything! Mammal Ecology Group members past, Niamh, Peter, Conall, for your support and encouragement over the years, from when I joined you in Room 101 as an undergrad all those years ago, and since we've all moved on. Thanks to my molecular biology buddies Vijay, Carsten and especially Ishla and Kelly. And to James, Brian and Liam who adopted a stray Mammal Ecologist in her final year - thanks for the cups of tea, the biscuits and not to mention the Friday sessions!

Thanks to the Irish Research Council for providing me with funding and thanks to Eoin MacLoughlin for help with all things technical. Thanks to David-Hutton Bury, Donal Whelan, the Coillte forest managers, NPWS and the late Geoffrey Tottenham for allowing me to work in their woods.

vii

Thanks to Denise O'Meara, who I met at the first AIMS in 2009 and have collaborated with since. Thanks to Catherine O'Reilly for giving me the opportunity to learn something completely new in WIT, and to all in the Molecular Ecology Research Group who made a field ecologist feel very welcome. A special thanks to David O'Neill for your patience and help in the lab.

Thanks to my sister Ruth for designing the project logo and posters. Thanks to Alan Murphy for doing an amazing job with the website and for being so patient. Thanks to Anthony Smart for doing a great job with the pine marten scats, it was a real pleasure working with you. Thanks to all of the student volunteers who have helped over the years. Thanks to the hundreds of 'citizen scientists' who contributed to the study and a special thanks to Michael Carey and to Huw Denman for advice and support with the project from the very beginning, and all at the Vincent Wildlife Trust for your kind encouragement and good will. Thanks to Andrew Kendell and all at the European Squirrel Initiative for your generous grant towards the molecular work. Thanks to Colin Stafford-Johnson for providing me with more than my fair share of airtime on national television and radio. I'm very grateful to have had the opportunity to bring the story of my research to so many people in such a nice way.

Thanks to my sister Debbie for the long skype chats and to Noelle and Lara for getting me away from the books on a regular basis! A special thank you to Aoife Kelly, and to all of the girls in Dublin.

And finally, the biggest thanks of all has to go to the squirrels and the pine marten, for providing me with years of entertainment and hopefully many more to come.....

Declaration

This thesis has not been submitted in whole or in part to this or to any other university for any other degree and is, except where otherwise stated, the original work of the author.

Chapter four has been submitted in its current form to the European Journal of Wildlife Research. The author list is as follows: Sheehy E, O'Meara D, O'Reilly C, Smart A, Lawton C

Input of each author to chapter 4:

Author 1: Experimental design, all field and laboratory work and all analysis except for the following:

Author 2: Section 4.3.2.1 *Data Analysis* was carried out and written up by Denise O'Meara, Waterford Institute of Technology.

Author 4: Section 4.3.2.2 *Macro analysis* was carried out as part of an undergraduate project by Anthony Smart, National University of Ireland, Galway.

Authors 3 and 5: Catherine O'Reilly, Waterford Institute of Technology and Colin Lawton, National University of Ireland, Galway, had supervisory roles in the project.

Two anonymous reviewers provided helpful input on an earlier version of the manuscript.

ix

List of Figures Pag		
2.1	Red and grey squirrel distribution in Ireland in 2000 as per	12
	O'Teangana	
2.2	Pine marten distribution in Ireland in the 1980's according to	12
	O'Sullivan and in 2006 according to O'Mahony (from O'Mahony et	
	al. 2012)	
2.3	Map of the midlands, east and buffer zone regions and locations of	16
	hairtube survey sites	
2.4	Map showing position of squirrel and pine marten hairtubes during	17
	survey of Charleville Forest	
2.5	Percentage of sightings for red squirrel, grey squirrel and pine	20
	marten throughout the study regions	
2.6	Scatter plots displaying correlations in the number of red squirrel	22
	and pine marten sightings per hectad in each study area	
2.7	Map showing locations and densities of red squirrel, grey squirrel	24
	and pine marten sightings reported between 2010 and 2012	
2.8	Portion of hairtubes used by each species during hairtube surveys	25
3.1	Map showing position of squirrel traps in Charleville	34
3.2	Map showing position of squirrel traps in Tomnafinnoge	34
3.3	Red and grey squirrel density in Charleville using MNP estimates	39
3.4	Grey squirrel density in Tomnafinnoge using MNP estimate	39
3.5	Linear regression of body weight and shin bone length of adult red	44
	squirrels in Charleville	
3.6	Linear regression of body weight and shin bone length of adult grey	45
	squirrels in Tomnafinnoge	
3.7	Annual seed count data for large seeded trees in Charleville and	47
	Tomnafinnoge	
4.1	Map of the study area and pine marten study sites in Ireland within	58
	the fragmented forested landscape	

4.2	The number of successful hair trapping events and the portion of	66
	hair samples successfully genotyped from each study site	
4.3	Linear regression between the %FO of mammalian prey items	73
	detected using molecular and macro analyses	
4.4	Overall results for molecular and macro analysis of mammalian	74
	prey items in pine marten diet	
4.5	Comparison of results from both molecular and macro techniques	75
4.6	Percentage relative biomass (%BPI) of mammalian prey items as	76
	determined by macro analysis	
5.1	The natural ranges of the European pine marten and the red	87
	squirrel	
5.2	The natural ranges of the fisher, the American marten and the grey	88
	squirrel	
5.3	Map of squirrel traps in Tomnafinnoge as divided into grids for	93
	predator odour experiment	
5.4	Box plot showing the range of capture rate values for predator	97
	odour treated and non-predator odour treated grids	
5.5	Variation in grey squirrel capture rate per day during the predator	98
	odour treatment	

List of Tables		
3.1	Sex ratios, reproductive activity and recruitment values for squirrel	43
	populations in Charleville and Tomnafinnoge	
3.2	Fitness values for adult squirrels in Charleville and Tomnafinnoge	46
3.3	Fitness values and density ranges for the red squirrel population in	49
	Charleville compared to other Irish red squirrel populations	
4.1	List of scat survey sites indicating which species of squirrel was	60
	present, number of scats collected and the number of squirrels	
	detected in pine marten diet	
4.2	List of microsatellite loci and primers used in pine marten	62
	genotyping	
4.3	The total number of pine marten identified at each site, mean	67
	number of captures per animal and the number of adults resident	
	at each site.	
4.4	The site each pine marten was recorded at, the number of times	68
	genotyped and number of months recorded for each individual	
4.5	Descriptive statistics for microsatellite analysis of pine marten	70
	population	
4.6	Squirrels as detected as prey items in molecular and macro analysis	72
	of pine marten scats	
5.1	The number of traps available and the number of traps used during	96
	each trapping session per odour treatment	

1 General Introduction

Squirrels are rodents belonging to the family *Sciuridae*, of which there are approximately 289 species worldwide, in 51 genera (Thornington and Hoffmann 2005) including tree squirrels, gliding squirrels, chipmunks, marmots, ground squirrels and prairie dogs. There are 28 species of tree squirrels in the genus *Sciurus*, most of which are found in Central and North America. There are three species naturally occurring in Eurasia; the Persian squirrel, *Sciurus anomalus*, which is restricted to the middle-east, the Japanese squirrel, *Sciurus vulgaris*, which is widespread throughout the Palearctic. The red squirrel's range reaches from Ireland in the west to the island of Hokkaido, Japan (Lurz *et al.* 2005) in the east.

1.1 Sciurus vulgaris

The natural habitat for the red squirrel throughout much of the Palearctic is boreal coniferous forest, consisting mainly of larch (*Larix* spp.), pine (*Pinus* spp.) and spruce (*Picea* spp.). In central and southern Europe the red squirrel is also found in mixed and broadleaved woodlands. Tree seeds and fruits are the most important food items, followed by tree shoots, buds, flowers, berries, fungi, bark and lichens. The majority of the red squirrel's active time is spent foraging, most of which takes place in the canopy (Kenward and Tonkin 1986), however in autumn, if tree seeds are abundant, a considerable amount of time is spent caching food (under the surface of soil or in leaf litter) for later consumption. Red squirrels typically exist at densities of 0.3 to 1.5 squirrels.ha⁻¹ with lowest densities found in habitat dominated by sitka spruce, *Picea sitchensis*, and highest in mixed deciduous and mixed coniferous habitat (Gurnell 1983; Lurz *et al.* 1995; Wauters and Lens 1995; Lurz *et al.* 1998). Densities do not typically exceed 1.5 squirrels.ha⁻¹ irrespective of habitat (Gurnell 1987).

The red squirrel is a natural coloniser of Ireland, having arrived before the end of the last Ice Age. However, while the current population is considered to be native, it was widely believed that by the 17th and 18th centuries, the red squirrel was extremely rare, if not extinct, in Ireland (O'Teangana *et al.* 2000) as a result of hunting and loss of habitat through deforestation. In the 19th century a number of reintroductions from Britain took place (Barrington 1880) and a recent study using molecular techniques to determine the origin of the Irish population suggests that whilst there may be some individuals in the south east and south west of the country that are remnant native Irish haplotypes, the current population are largely descendent from more recent British populations than the original natural colonisers (Finnegan *et al.* 2008).

1.2 *Sciurus carolinensis*

The North American grey squirrel, *Sciurus carolinensis*, known in its native range as the Eastern gray squirrel, is found from Texas to Canada and east to the Atlantic coast. It has been introduced to Britain, Ireland, Italy, Australia, South Africa and parts of western North America (Thornington and Hoffmann 2005). Most introductions took place in the late nineteenth and early twentieth centuries. Australian introductions were not successful, and the grey squirrel became extinct in Australia by the 1980's (Seebeck 1984). In the case of the European introductions, the grey squirrel has flourished at the expense of the red squirrel populations, replacing its native congener in much of Britain and Wales, and in those parts of Ireland and Italy where the population has established successfully. In 2004 they were classified as one of the world's most invasive alien species (Lowe *et al.* 2004) due to damage caused by the bark stripping of trees in their invasive range, and the detrimental impact on the Eurasian red squirrel populations as a result of competition and disease.

Grey squirrels are native to the extensive hardwood forests of eastern North America and reach their highest densities in habitats composed of large-seeded tree species that produce winter-storable foods such as oak (*Quercus*), hickory

(*Carya*) and walnut (*Juglans*) and the majority of the grey squirrel's active time is spent foraging on the ground (Kenward and Tonkin 1986). Grey squirrels typically live at densities of 2 to 16 squirrels.ha⁻¹ (Gurnell 1987) in broadleaved habitat but have been found to live at lower densities in coniferous habitat. However, even in lower density, coniferous habitat populations, the grey squirrel reaches higher densities than those of the red squirrel (Kenward *et al.* 1998; Wauters *et al.* 2000; Gurnell *et al.* 2004).

1.3 The replacement of red squirrels by grey squirrels

The process of competitive replacement and its causal factors have been the subject of much research to date (e.g. Kenward and Holm 1993; Kenward et al. 1998; Wauters et al. 2000; Wauters et al. 2002a; Gurnell et al. 2004). The natural habitat for the red squirrel throughout its range is predominantly coniferous and mixed broadleaf woodlands. The grey squirrel is a native of the mixed hardwood forests of North America, however within the same habitat, the two species show very similar preferences - not sufficiently different to avoid the competitive exclusion of one by the other (McKinnon, 1978). Whilst the red squirrel has evolved without any significant interspecific competition, the grey squirrel has evolved within the context of strong interspecific competition from the American red squirrel (Tamiasciurus sp), and the fox squirrel (Sciurus niger), and has therefore benefited selectively from such pressures. Beneficial traits include higher recruitment rates, higher density populations, larger body mass with greater fat reserves and a more varied diet (MacKinnon 1978). An average time for local replacement, once the grey squirrel arrives in a previously "red-only" area, has been calculated to be 15 years (Pepper and Patterson 1998), however the local extinction of a red squirrel population can occur up to twenty times faster when disease is a contributing factor (Rushton et al. 2006).

Squirrel pox virus (SQPV), which is carried by grey squirrels, usually asymptomatically, but lethal to red squirrels, has played a major contributing role in the replacement of the red squirrel in Britain (Rushton *et al.* 2006). It is not known

what impact it has had on the Irish red squirrel population historically, as it was not detected on the island until 2011 (McInnes *et al.* 2012). SQPV has not been detected in the Italian population to date.

The survival of the red squirrel is also threatened in areas of Europe beyond the current grey squirrel range. Bertolino *et al.* (2008) predict that due to the availability of suitable habitat corridors, if the existing grey squirrel population in Italy is not contained successfully, it will continue to spread to France and Switzerland within a matter of decades and eventually potentially to much of Eurasia.

1.4 Grey squirrel control

To date, there has been no successful method developed in the long-term control (nor indeed the eradication) of grey squirrel populations. In order for control to be successful at a local level, short-term tactics must be employed intensively and on an ongoing basis (Gurnell and Pepper 1993). Methods include trapping, shooting (including drey-poking) and the use of poison baits such as Warfarin, although there are restrictions in the use of these methods if red squirrels are present. The motivation for controlling a grey squirrel population may be classified as either (a) the conservation of a red squirrel population or (b) reduction of financial losses incurred by the forestry industry.

In Ireland, grey squirrel control for the purpose of red squirrel conservation is not carried out, however there is some small scale control carried out by foresters, and the National and Regional Association of Game Councils (NARGC) who, for example, culled approximately 1,600 grey squirrels per annum in the Republic of Ireland in 2011 and 2012 as part of predator control programs. Lawton and Rochford (2007) found that grey squirrel control for the purpose of reducing damage to trees may be effective if carried out intensively during spring however a recovery in numbers was found to take place within ten weeks of intensive culling programs. "Bark-stripping" by grey squirrels involves the removal of not only the outer bark but also

the vascular tissue of the tree, which causes a disruption in the flow of nutrients, leading to reduced growth or even death of the upper part of the tree. Damaged trees are also left exposed to disease or fungal attack (Kenward 1983). The cost of damage to forestry from bark-stripping is currently estimated at €0.85 million *per annum* in Ireland (Kelly *et al.* 2013) and a further €8 million *per annum* in the UK.

Rushton *et al.* (2002) estimated that the cost of trapping grey squirrels in sufficient numbers to ensure the persistence of the red squirrel population in the long term in Thetford Forest, England would be in excess of £300,000 (€345,000) per annum. Indeed intensive efforts to control grey squirrel range and numbers have taken place in the UK, in particular with the motivation of red squirrel conservation. However, with the exception of a few, small, intensively managed red squirrel populations the red squirrel is now almost entirely absent from England and Wales, although it is still relatively widespread in Scotland (Rushton *et al.* 2006).

1.5 Martes martes

The European pine marten, *Martes martes,* is a member of the family *Mustelidae* within the order *Carnivora*. The mustelid family includes the marten (*Martes spp*), otter, wolverine, badger, mink and weasel. Included within the genus *Martes* are the fisher, *Martes pennanti*, American marten, *Martes americana*, Japanese marten, *Martes melampus*, sable, *Martes zibellina*, and the stone marten, *Martes foina*. The European pine marten has a circumboreal distribution and is widespread throughout much of north and western Europe but rare or absent in much of its historic southern European range (Yalden 1999). It is the only member of the genus *Martes* in Ireland and is considered to be native, however it is possible that the pine marten was introduced by man, for its fur, several thousand years ago. The first fossil records appear in the Bronze Age (McCormick 1999) 4500 to 2800 years B.P. Molecular studies indicate the Irish pine marten population is more closely related to its Iberian counterparts than the current population in Britain (Davison *et al.* 2001; Jordan *et al.* 2012; Ruiz-González *et al.* 2013a), however it is not clear whether this is a result of artificial or natural colonisation processes.

The pine marten is omnivorous and feeds upon a wide variety of food including small mammals, birds, reptiles and amphibians, invertebrates, fruit, nuts and carrion in various proportions throughout the seasons, however diet is usually dominated by just two or three food categories, depending on seasonal and regional food availability (Birks 2002). The pine marten is closely associated with forests and has traditionally been considered a forest specialist. Semi-retractile claws make it an agile tree climber, and indeed trees are used as refuge from terrestrial predators such as foxes, occasional hunting (e.g. birds, eggs and occasionally squirrels) and also as den sites, although most of their foraging takes place on the ground. Zalewski and Jedrzejewski (2006) estimated that 2 km² is the minimum area of forested habitat necessary to support an adult pine marten in mainland Europe's temperate forest zone. However, despite their close association with trees, pine marten are not strictly forest-dependent and have adapted to a variety of landscapes throughout their range, not least in Ireland and Britain where deforestation in the 19th century led to <5 % forest cover.

Pine martens are solitary mustelids, and social organisation is structured around intrasexually exclusive territories. Scent is an important means of communication and scent-marking, via abdominal scent glands and faecal deposits, serves in both territorial and reproductive communication. The pine marten is a slow breeding species, and females typically do not produce young until their third year. Mating takes place during July and August, after which implantation is delayed for approximately 6 months, with 1 to 3 kits born in March of the following year.

In a review of available literature on European pine marten densities, Zalewski and Jedrzejewski (2006) found that between 41° and 68°N densities declined exponentially with decreasing winter temperature and increasing seasonality, and suggested that both winter severity and availability of rodents are limiting factors on populations. The most recent population estimate for the island of Ireland is 3060 individuals (O'Mahony *et al.* 2012), although there is still relatively little known about pine marten population densities in Ireland which is the western-most part of their European range.

1.6 History of the pine marten in Ireland

Indeed, there was little known about the status or distribution of the pine marten in Ireland prior to a study in the 1980's (O'Sullivan 1983), as incidental sightings records, returns from taxidermists and trapping records comprise the majority of historical records for the species (O'Mahony et al. 2006). The pine marten has been hunted heavily for its fur throughout its range, and in Ireland predator control programs and habitat loss through deforestation, combined with hunting for fur, led to a major reduction in the range of the pine marten by the twentieth century (O'Sullivan 1983). O'Sullivan conducted the first comprehensive national survey for pine marten; scat transects were carried out within 428 hectads, covering much of the country. This survey found the pine marten population to be largely restricted to counties west of the river Shannon with one other population in the Slieve Bloom mountain range in counties Laois and Offaly. These core populations were found to have increased in recent decades, as a result of increased habitat availability through reforestation and importantly, protection by law under the Wildlife Act (1976). O'Mahony et al. (2006) resurveyed a random selection of hectads from the earlier study and found that although pine marten distribution had increased, it was still mainly concentrated around core populations in the west and midlands, along with some smaller populations in the south west and south east of the country.

1.7 Squirrel distribution in Ireland

The grey squirrel was introduced to Ireland from its non-native range in Britain in 1911 to one known location in the midlands (Castleforbes Estate, Co. Longford). Since then, the population has spread through much of the north, east and south of the country, at a mean rate of 1.94 km per year (O'Teangana *et al.* 2000). The most recent survey found grey squirrels present in 26 (of 32) counties (Carey *et al.* 2007), with the west and southwest representing the only parts of the country in which

the grey squirrel had not yet established itself. The survey also found that red squirrels were rare in areas that grey squirrels were longest established, and extinct in Meath and Westmeath. O'Teangana et al. (2000) noted for the first time however, that the red and grey squirrel populations in the northern counties of Cavan and Monaghan appeared "unsettled" and that the grey squirrel had been reported, anecdotally, to have declined locally since the 1980's, allowing for a red squirrel population resurgence. At this time, grey squirrels were present along the east banks of the River Shannon and considered to be ubiquitous in the midlands. Indeed the eastern side of the Shannon has historically represented the western most extent of the grey squirrel's range in Ireland (O'Teangana et al. 2000) and the river itself has traditionally been considered a barrier to its dispersal into the west of the country. In 2007, Carey et al. found a similar phenomenon to be occurring, namely grey squirrels declining and red squirrels reappearing after an absence of several decades, in the midland counties of Laois and Offaly. This apparent retraction of the grey squirrel's range coincided with the regionally resurgent pine marten population, and anecdotal evidence suggested the distribution patterns of the three species may be linked (Carey et al. 2007). If the grey squirrel population has in fact experienced a decline in Ireland, it is atypical for the species anywhere in its invasive range. This could prove highly significant both in terms of red squirrel conservation in Ireland, the UK and Europe and also in the reduction in damage to commercial forestry.

1.8 Marten predation on squirrels

There is no evidence in the literature of the European or American marten species being a limiting force on tree squirrel populations (Gurnell 1987; Smith 2007). The European pine marten is a known predator of the Eurasian red squirrel. Indeed the range of the pine marten overlaps almost entirely with that of the western portion of the red squirrel's range and many studies on pine marten diet have been carried out in areas where the red squirrel is present as a prey item. The percentage frequency of occurrence in the diet is usually very low (Clevenger 1993; Balharry

1993; De Marinis and Masetti 1995; Gurnell and Lurz 1997; Caryl et al. 2012b; O'Meara et al. 2013). As their ranges largely overlap, the American marten also preys upon two sympatric tree squirrel species; the pine squirrel, Tamiasciurus hudsonicus, and the Douglas squirrel, Tamiasciurus douglasii. In contrast, little is known about marten predation on the North American grey squirrel. The natural range of the grey squirrel in North America only overlaps with that of the American marten (Martes americana) and fisher (Martes penanti) in a very small part of its north eastern and north western natural range limits. As a result of this limited range overlap, few studies have been carried out in areas of grey squirrel sympatry with either marten species. Where studies have been carried out in North America, the grey squirrel has appeared as a higher frequency prey item (16 and 17 % FO) in the diet of the American marten and the fisher. Furthermore, the introduced range of the grey squirrel has only coincided with the range of the European pine marten in very recent years in Ireland (and in Scotland), as the pine marten population has begun to recover. In both countries it has been suggested anecdotally that the range of the grey squirrel has retracted (Paterson and Skipper 2008; Carey et al. 2007) but to date there has been no research carried out to quantify the apparent range retraction of the grey squirrel. Nor has there been any evidence of the European pine marten preying on the North American grey squirrel anywhere in its invasive range.

1.9 Project Aims

The overall aim of this study is to determine the role, if any, of the European pine marten in red and grey squirrel population dynamics in Ireland. Populations of all three species in the midlands (Counties Laois and Offaly) and the east (County Wicklow) of Ireland will be examined in order to investigate the suggested changes in red and grey squirrel ranges first reported in Carey *et al.* (2007).

- To determine the current distribution and status of the red squirrel, the grey squirrel and the pine marten in the midlands region of Ireland (namely counties Laois and Offaly), where reports of a decline in the range of the grey squirrel and a red squirrel resurgence have been reported.
- 2. To determine whether correlations exist between the distribution of the three species
 - (a) at woodland level
 - (b) at landscape level
- To quantify red and grey squirrel population demographics in the midlands of Ireland whilst establishing the extent (if any) to which interspecific competition between red and grey squirrels is occurring.
- 4. To quantify pine marten abundance in the midlands of Ireland, and also in the east where they are considered to be less common.
- 5. To quantify the frequency of occurrence of both squirrel species in the diet of the pine marten.
- 6. To investigate whether "predator avoidance" is taking place in the grey squirrel population.

2. Squirrel and Pine Marten Distribution Surveys

2.1 Introduction

Previous Irish squirrel distribution surveys i.e. O'Teangana *et al.* (2000) (Figure 2.1) and Carey *et al.* (2007) were based largely upon sightings surveys where specialist groups such as foresters and ecologists were targeted for their knowledge on local squirrel distribution. They also included elements of "citizen science" where members of the public reported their animal sightings. Citizen science surveys are increasingly being used to monitor the spread and distribution of species (Cooper *et al.* 2007; Devictor *et al.* 2010). Indeed Dickinson *et al.* (2010) stated that many ecological processes occur at geographic scales that are beyond the reach of traditional research methods. They suggested that citizen science may be the only practical way to obtain distribution information at the scale required to detect species range shifts, population trends and impacts of environmental processes.

There are five established field methods for the indirect monitoring of squirrel populations, as described by Gurnell et al. (2001a). Drey counts, feeding-sign surveys and whole maize bait (where it is possible to determine whether squirrels or other rodents have fed on the maize) assume that just one species of squirrel is present in the study area whereas with visual transects and hairtube surveys it is possible to differentiate between squirrel species detected. Visual transects are restrictive both in the type of habitat and time of year they can be carried out. Therefore in the current study hairtube surveys, combined with a sightings survey which targeted professionals and drew upon citizen science, were chosen as the optimum methods for indirectly determining which species of squirrel were present across a wide area and a range of survey sites.



Figure 2.1 Red squirrel distribution (left) and grey squirrel distribution (right) in Ireland in 1997 (taken from O'Teangana *et al.* 2000) \Box = site of grey squirrel introduction in 1911



Figure 2.2. Distribution of pine marten in 10km grid squares in the Republic of Ireland during 1978–1980 (O'Sullivan 1983) (a) and from a re-survey in 2005–2006 (O'Mahony *et al.* 2006) (b). Total sample size of 10km national grid squares available for comparison n = 183. Taken from O'Mahony et al. (2012).

In the nineteenth and twentieth centuries, the European pine marten (*Martes martes*) population in Ireland experienced widespread decline as a result of habitat loss (large-scale deforestation) and heavy persecution (O'Sullivan 1983). Population censuses in the 1980s (O'Sullivan 1983) and again in 2005 (O'Mahony *et al.* 2006) revealed that pine marten distribution in Ireland was still mainly concentrated around core populations in the west and midlands, along with several smaller populations in the south west and south east of the country. However, the pine marten population in the west and midlands of Ireland had undergone a range expansion in recent decades, as a result of increased habitat availability and connectivity through afforestation, and importantly protection by law (Figure 2.2) (O'Mahony *et al.* 2006). The most recent population estimate for the island of Ireland was 3060 individuals (O'Mahony *et al.* 2012).

Indirect monitoring methods for pine marten include scat transects (O'Sullivan 1983; Birks *et al.* 2005; O'Mahony *et al.* 2006) snow tracking (Zalewski 1999), camera trapping (Rosellini *et al.* 2008; Manzo *et al.* 2012) and hair trapping (Lynch *et al.* 2006; Mullins *et al.* 2010). Snow track counts are not feasible in Ireland due to unsuitable climactic conditions and camera trapping is prohibitive in terms of the expense of materials. Scat transects, although used in previous Irish distribution surveys, also have several inherent limitations, the most notable of which being poor reliability when population density is low or where distribution is uneven (Birks *et al.* 2005).

The use of hairtraps in evaluating both presence and abundance of pine marten (Lynch *et al.* 2006) and for collecting hair samples for genetic analysis (Mullins *et al.* 2010) has been used successfully in Ireland and thus was selected as the optimum method, alongside the sightings survey as above, for detecting pine marten presence across the variety of survey sites.

2.2 Aims

The first aim of this study was to determine the current distribution of the red squirrel, grey squirrel and pine marten in the midland counties of Laois and Offaly, and to investigate the extent of the range retraction of the normally invasive grey squirrel, which had been reported in Carey *et al.* (2007). A second aim was to establish whether correlations in distribution exist between the three species.

2.3 Methods

2.3.1 Sightings surveys

Animal sightings were collected between January 2010 and December 2012. Three study areas were targeted consecutively as Laois and Offaly (referred to hereafter as the midlands study area), and Wicklow (referred to hereafter as the eastern study area) in 2010 and the buffer zone study area (which consisted of a 30km radius zone surrounding the midlands study area, incorporating Westmeath, Kildare, Carlow, North Tipperary and partially incorporating Longford, Meath and Kilkenny) in 2011 (Figure 2.3). The survey was advertised primarily by means of colour posters placed in population centres throughout the study areas, and also local media such as newspapers and radio stations. Foresters, ecologists and wildlife rangers were targeted for their knowledge on local species' distributions. The National and Regional Association of Game Councils (NARGC) supplied their member clubs with sightings survey forms and there was also national newspaper and television coverage of the survey.

A dedicated website was created which provided detailed descriptions and photographs of the target species, and an online survey form in which respondents were asked to provide details of the species seen, date and location of sighting, habitat and surrounding land use information, as well as contact information, which

was necessary for follow-up queries on selected sightings. Strict validation criteria were adhered to, where only those sightings that were either accompanied by photographic evidence, described as stationary (roadkill) animals or came from a professional (e.g. forester, ecologist) were classified as valid. Those sightings that could not be validated, but had sufficient information to be deemed as reliable were also included in the survey. Those that did not have sufficient contact information, location information or were unclear about the species seen were not included. All sightings were assigned a grid reference, and any sighting reported within a 1 km radius of another was considered a duplicate and therefore not included in the analysis. As such each sighting used was based on a unique point location.

2.3.2 Hairtube surveys

18 sites were selected for hairtube survey (consisting of eight in the midlands, five in the eastern and five in the buffer zone study areas) (Figure 2.3). Between 14 and 30 squirrel hairtubes and 7 and 15 pine marten hairtubes were installed at each site for a period of 14 days.

Squirrel hairtubes consisted of rectangular PVC piping (300 mm x 65 mm x 65 mm), secured horizontally just above chest height to the tree, baited with whole maize on the top surface as a visual lure and a mix of peanuts and hazelnuts inside the hairtube. At each end of the hairtube a removable wooden block with three 10 x 15 mm glue patches was attached by a metal clip, so that a dorsal hair sample from any mammal entering was collected. Hairtubes were installed at a density of one per hectare in a continuous grid where possible, but often in two to three smaller grids within the wood (Figure 2.4).

Pine marten hairtubes consisted of a 250 mm length, 118 mm diameter standard sewer piping with a 30 mm width section cut longitudinally to allow firm attachment to the tree trunk. Hairtubes were attached vertically at around chest height, baited with chicken pieces and open at the bottom end only, so that the



Figure 2.3 Locations of the 18 squirrel and pine marten hairtube surveys that took place between February 2010 and May 2012 in the midlands, east and buffer zone study areas.

animal had to climb up into the hairtube in order to retrieve the bait. Two 10 x 14 mm glue patches were positioned inside the hairtube on 20 x 25 mm pieces of corrugated plastic, which was attached to the tube with pressure sensitive scotch tape. Pine marten hairtubes were installed at a density of one per two hectares within the same grid system as the squirrel hairtubes (Figure 2.4).



Figure 2.4 Position of squirrel (n = 28) and pine marten (n = 14) hairtubes at site 3. Squirrel hairtubes were placed at a density of one per hectare and pine marten hairtubes at one per two hectares in three adjacent grids.

2.3.3 Laboratory Analysis

Hair samples were removed from the glue patches by being soaked in vegetable oil, and then rinsed in distilled water and histological cleaning agent (Histo-Clear, National Diagnostics, Hessle Hull, UK) and dried. A sub sample of hairs from each sample were analysed using cuticular analysis, by examining gelatine moulds of the hair cuticle at 100x magnification according to Teerink (1991). Hair samples were then classified as either squirrel, pine marten or other mammal. To distinguish between the two squirrel species, cross sectional analysis as per Teerink (1991) was carried out. The widest part of the red squirrel guard hair has a concavo-convex shape which is clearly distinguishable from the much more rounded grey squirrel hair cross section, which allowed for confident differentiation between hairs from the two species.

2.3.4 Data Analysis

Arcview 9.3 was used to map the point for each unique sighting location (min 1 km radius) and a 10 km radius kernel density was then used to display patterns of distribution and abundance of sighting locations for each species.

The number of unique sighting locations per species were counted for each hectad within the midlands (n = 44 hectads), east (n = 27 hectads) and buffer zone (n = 85 hectads) study areas. As data was normal, paired sample t-tests were then used to test for significant differences in the number of locations from which each species was reported per cell, for each study area.

The overall frequencies of sightings for each species were calculated for each study area as the (number of sightings per species)/(number of sighting locations) and thus differences in sightings frequencies between study areas were determined using a Chi square test for homogeneity. Non-parametric tests were carried out on hairtube data. The number of hair samples collected for each species at each site was expressed as a portion of the available hairtubes, and then compared between species over the 18 sites using the Wilcoxon test for matched pairs. Spearman Rank correlation was used to investigate correlations in species detection or non-detection throughout the study areas. It was assumed that the target species would not enter a hairtube once the bait had been removed by another animal, therefore rendering the previously visited hairtube unavailable for the purpose of the survey. This was estimated to have occurred on average half way through the study period, and so the number of available hairtubes was calculated as (no. installed) – (no. used by bird or other mammal species/2).

To estimate the extent of changes in red and grey squirrel distribution (in km²) published information on historical distribution (O'Teangana *et al.* 2000) was used.

2.4 Results

2.4.1 Sightings surveys

A total of 1136 animal sightings were collected from 835 unique sighting locations between January 2010 and December 2012. Red squirrel sightings (n = 424) comprised 37% of overall sightings, grey squirrel (n = 264) 23 % and pine marten (n = 448) 40% of overall sightings at various frequencies throughout the three study areas (Figure 2.5).

When the number of sightings per hectad were compared, both pine marten (\bar{x} = 4.34, t = 9.477, df = 43, p < 0.001) and red squirrel (\bar{x} = 4.14, t = 8.522, df = 43, p < 0.001) were reported from a significantly higher number of locations than the grey squirrel (\bar{x} = 0.66) in the midlands study area. In the eastern study area, there was no significant difference between the mean number of red and grey squirrel sightings however the pine marten (\bar{x} = 2.27) was reported from significantly fewer

locations than either the red squirrel ($\bar{x} = 3.88$, t = 0.2926, df = 25, p < 0.01) or the grey squirrel ($\bar{x} = 4.08$, t = 2.31, df = 25, p < 0.05) in this region. In the buffer zone, there were significantly more pine marten ($\bar{x} = 2.13$) than both red squirrel ($\bar{x} = 1.37$, t = -4.198, df = 84, p <.001) and grey squirrel ($\bar{x} = 1.4$, t = -2.722, df = 84, p < 0.01).



Figure 2.5. The percentage of grey squirrel, red squirrel and pine marten sightings in the midlands (n = 370 animal sightings in 279 locations, area = 44 hectads), east (n = 279 animal sightings in 199 locations, area = 26 hectads) and the buffer zone (n = 487 animal sightings in 357 locations, area = 85 hectads).

Paired sample correlation tests for each study area found a positive correlation to exist between the number of pine marten and red squirrel sightings per hectad (midlands P = 0.678, east P = 0.718, buffer zone P = 0.572, p < 0.001) throughout the three study areas (Figure 2.6). No further correlations between species (i.e. red

squirrel and grey squirrel or pine marten and grey squirrel) in terms of sightings per hectad were found.





Figure 2.6 Scatter graphs showing correlations between the number of red squirrel and pine marten sighting locations per hectad in **(a)** the midlands **(b)** the east and **(c)** the buffer zone study areas

Species specific differences in sightings frequencies between the three study areas were identified, with significantly more locations having red squirrels reported in the midlands and in the east than in the buffer zone ($\chi^2 = 17.39$, df = 2, P < 0.01). Grey squirrel sightings were significantly fewer in the midlands than in the east or the buffer zone ($\chi^2 = 69.61$, df = 2, P < 0.01) and pine marten sightings were significantly fewer in the buffer zone ($\chi^2 = 13.58$, df = 2, P < 0.01).

Distribution and sightings density patterns for each species based on 10 km radius kernel density are displayed in Figure 2.7.
2.4.2 Hairtube surveys

17 of the 18 hairtube surveys were successful in detecting either one or a combination of the target species. In the midlands, only red squirrel and pine marten were detected, whereas in the eastern and buffer zone study areas all three species were detected in various proportions (Figure 2.8).

The pine marten was found to have a significantly higher detection rate than either the red or grey squirrel in the study as a whole (Wilcoxon's test for matched pairs, p < 0.05). A strong negative correlation was found to exist between detection of grey squirrel and pine marten within sites ($r_s = -0.750$, n = 18, P < 0.001), where only one site (Ballygannon, Wicklow) tested positive for both species (n = 3/7 pine marten, n = 1/14 grey squirrel). A negative correlation was also found to exist between the detection of red and grey squirrels within sites ($r_s = -0.472$, n = 18, P < 0.05).



(a) Red squirrel



(b) Grey squirrel



(c) Pine marten

Figure 2.7 (a) Red squirrel **(b)** grey squirrel and **(c)** pine marten distribution and densities throughout the study areas as determined by sightings reported between 2010 and 2012 using sighting locations and 10km radius kernel density.



Figure 2.8 Proportion of available hairtubes containing red squirrel, grey squirrel or pine marten hair samples from the 18 sites throughout the midlands (Laois and Offaly), east (Wicklow) and buffer zone study areas.

2.4.3 Changes in distribution

The grey squirrel is now rare in the midland counties of Laois and Offaly and some parts of the buffer zone including North Tipperary and Westmeath (Figure 2.7), which accounts for approximately 9,000 km² of its former range (see Figure 2.1). The western-most point at which the grey squirrel population is abundant in the study area is east Kildare, and the species is very common in east Wicklow. The red squirrel has naturally recolonised approximately 72% of the midlands and buffer zone study areas or around 6,500 km² of its historic range, and is common and widespread throughout Laois, Offaly and north-west Tipperary. The red squirrel persists and appears to be common in the Wicklow mountains. The pine marten is common and widespread throughout Laois and Offaly, north-west Tipperary, Westmeath and north-west Kildare. There is also a population in the Wicklow mountains. Red squirrel and pine marten populations overlap extensively however grey squirrel and pine marten overlap appears to be limited.

2.5. Discussion

2.5.1. Distribution in the midlands

The grey squirrel is now rare in much of the midlands region of Ireland. Grey squirrel sightings accounted for less than 8% of animal sightings in counties Laois and Offaly, which is remarkably low considering that they are a much less elusive species than either the red squirrel or the pine marten, and are also more commonly associated with human settlements. A total of 28 grey squirrel sightings were received over the three year study in Laois and Offaly which were all followed up and found to be infrequent or one-off sightings and thus not representative of normal populations.

Whilst hairtube surveys can be used to confirm presence of species at woodland level, and if surveyed more intensively perhaps absence (e.g. Mortelliti and Boitani 2008), the technique used in the current study was not suitable to infer absolute absence, rather relative detectability.

The findings of the hairtube surveys further support those of the sightings survey, in that the grey squirrel was not found to be present in detectable levels at any of the midlands sites. Information gathered (when available) from woodland owners, foresters and gamekeepers indicated that seven out of the eight midlands sites had previously been colonised by grey squirrels (between the 1960's and the 1990's) and that occasional, localised control programs had taken place in some sites. However, culling programs at the scale that would be necessary to suppress the midlands population have never been carried out. In fact, the most recent grey squirrel control program in any of the midlands hairtube survey sites was carried out more than 10 years ago. Thus it is unlikely that culling has been an influencing factor in shaping contemporary grey squirrel distribution in the midlands study area.

In contrast to the grey squirrel, the red squirrel and pine marten were common and widespread throughout counties Laois and Offaly and each accounted for 46% of sightings respectively in the region. When survey respondents who had lived in the midlands study area for more than two decades were questioned, it appeared that the red squirrel had begun to recolonise Laois and Offaly from the late 1990's onwards. Whilst information on the timing of this phenomenon may be anecdotal, the sightings and hairtube survey results provide unequivocal evidence that the native red squirrel is now common and widespread in the region, which was once dominated by the introduced grey squirrel. This process has occurred naturally without any significant human intervention or management of either squirrel species.

2.5.2 Distribution in the east

Species distributions in the east were significantly different than to those in the midlands. In the east both the red and grey squirrel were reported in similar frequencies and the pine marten less frequently than either squirrel species. Hairtube survey results also revealed differences in distribution and various combinations of the three species were detected across the eastern sites as opposed to only red squirrel and pine marten in the midlands sites. One site in the east detected both grey squirrel and pine marten and this represented the only hairtube survey site in the entire study which detected both species together. Thus the pine marten and grey squirrel were found to have a strong negative correlation in detection at woodland level across the study areas. A negative, albeit weaker, correlation was also found to exist between the red and grey squirrel in terms of detection at woodland level, as would be expected due to the effects of competition. Extensive research has been carried out on the relationship between these two species, and it is now well established that the red squirrel population usually declines in both numbers and range as a result of competitive replacement by the grey squirrel (Kenward and Holm 1993; Wauters et al. 2002a; Gurnell et al. 2004), however in this case only two sites (12 and 17) had grey but no red squirrels.

2.5.3. Distribution in the buffer zone

The buffer zone study area appears to be divided between regions where pine marten are frequently reported (north and south west of the buffer zone) and regions where grey squirrels are frequently reported (east and south east) (Figure 2.7). The decline in grey squirrel range and recolonisation by the red squirrel population falls entirely within the core range of the pine marten population in the midlands and buffer zone study areas. Since the grey squirrel's introduction to Ireland, the river Shannon has been considered a barrier to its dispersal into the west of Ireland. Indeed, the eastern side of the Shannon has historically

represented the western most extent of the grey squirrel's range in Ireland (O'Teangana *et al.* 2000) (Figure 2.1). The current study has found the western boundary of an abundant grey squirrel population is now in the region of Kildare and Carlow, which represents a shift of more than 100 km east as the crow flies, and around 9,000 km² in terms of land coverage within the overall study area. This shift eastwards also suggests the Shannon, although undoubtedly important as a physical barrier, may not have been the sole barrier to the spread of the grey squirrel into the west of Ireland.

2.6 Conclusion

At landscape level it appears that where the pine marten is abundant, the grey squirrel is no longer present in an invasive manner. During the course of the study it was rarely seen by members of the public, woodland owners, foresters and gamekeepers alike, and was not detectable by hairtube surveys. At woodland level, the strong negative correlation between detection of the pine marten and the grey squirrel means their interactions (if any) will be difficult to investigate. In contrast, the red squirrel and the pine marten have positively correlated distributions and the red squirrel has now recolonised a large part of its former range in the midlands. The species is commonly seen throughout Laois and Offaly as is the pine marten and both species were detected in all suitable habitat by hairtube surveys. Distribution patterns are less clear in the east, where pine martens are less frequently seen and where they were also less frequently detected by hairtube surveys. The pine marten population in the east does not appear to have reestablished in the same manner as the population in the midlands and this is likely to have resulted from a smaller population persisting in the east historically, in comparison to the core population in the west. It is possible that the current population in the east is a mixture of a small remnant eastern population and animals which have more recently dispersed eastwards from the expanding midlands population.

3. Squirrel Population Studies

3.1 Introduction

The North American grey squirrel (*Sciurus carolinensis*) is classified as an invasive alien species in Ireland, the UK and Italy for two reasons: They cause serious damage to native hardwood trees by bark-stripping, and negatively impact native red squirrel (*Sciurus vulgaris*) populations as a result of competition and disease (Lowe *et al.* 2004). The rate at which the red squirrel is replaced by the grey depends largely upon the type of habitat available, with large seeded trees, in particular oak woodlands, being most favourable for grey squirrels (Kenward and Holm 1993) and small seeded trees such as sitka spruce being the least favourable (Wauters *et al.* 2000). An average time for local replacement, once the grey squirrel arrives in a previously "red-only" area, has been calculated to be 15 years (Pepper and Patterson 1998), however the local extinction of a red squirrel population can occur up to twenty times faster when disease is a contributing factor (Rushton *et al.* 2006).

The process of replacement and its causal factors have been the subject of much research to date (e.g. Kenward and Holm 1993; Kenward *et al.* 1998; Wauters *et al.* 2000; Wauters *et al.* 2002a; Gurnell *et al.* 2004). A factor considered to be of major importance in deciduous woodland in the presence of oak (*Quercus* sp) is the ability of the grey squirrel to exploit acorns as a food resource more efficiently than the red squirrel (Kenward & Holm, 1993). Grey squirrel abundance is correlated positively with acorn abundance, and their ability to increase productivity with acorn crops results in higher densities, and thus more rapid replacement of sympatric red squirrel populations (Kenward *et al.* 1998). Grey squirrels also exploit the seed caches of red squirrels where their populations overlap, and this is considered a causal factor in reduced red squirrel body mass in spring, leading to reduced fecundity at the individual level and reduced reproductive success in the

population (Wauters *et al.* 2002b). Recruitment and juvenile survival has been found to be dependent on grey squirrel density, and the proportion of females that breed twice per year is also lower when grey squirrels are present (Gurnell *et al.* 2004). Whilst broadleaved and in particular oak dominated woodlands in the species invasive range facilitate a more rapid replacement of the native red squirrel, the grey squirrel has been found to outcompete the red squirrel in coniferous woods also (Wauters *et al.* 2000), where a reduction in juvenile fitness inevitably leads to a reduction in recruitment, which over time causes the population to decline.

The impact of disease on the Irish red squirrel population is unknown and as such replacement of red squirrels in Ireland is thought to have occurred largely as a result of competition for resources, as squirrel pox virus was not detected on the island until 2011 (McInnes *et al.* 2012). The grey squirrel has been present in Ireland for approximately 100 years and has consistently spread, from their point of introduction in the midlands, throughout much of the north, east and south of the country, replacing the red squirrel in the process (O'Teangana *et al.* 2000), although in that study anecdotal reports of a decline in grey squirrel numbers in parts of Cavan and Monaghan were noted. In 2007, the Irish Squirrel Survey (Carey *et al.*), comprised of squirrel sighting reports from all 32 counties, found that in certain midland regions (Laois, Offaly and parts of Cavan and Monaghan), the grey squirrel was being seen much less frequently than in previous decades and the red squirrel was reported as having reappeared, often after an absence of several decades.

3.2 Aims

The overall aim of this study was to determine red and grey squirrel population dynamics in the midlands of Ireland with the following objectives:

Firstly, to determine whether the grey squirrel population is behaving in an invasive manner as expected of the species (i.e. a highly fecund, high density population which outcompetes red squirrels for resources) or whether the decline in range

observed in Chapter 2 is reflected in the demographics of the grey squirrel population at woodland level, compared to those in a control site. A second objective is to investigate claims that the red squirrel has reappeared in a woodland where it was once considered locally extinct (between 1960's and late 1990's), and to investigate whether the red squirrel population is suffering from the effects of competition with the grey squirrel as would be expected when the two species co-occur, or whether they are showing signs of competitive release.

3.3 Methods

3.3.1 Site selection and study areas

Charleville Forest was selected as a representative site for the Laois/Offaly area where red squirrels had been observed in recent years after an absence of several decades. Tomnafinnoge was selected as a control site, and is situated in the east of Ireland, where the grey squirrel is now more common than the red (Carey *et al.*, 2007). Both woodlands are grey squirrel favourable habitats in that large seeded trees, in particular oak (*Quercus* spp) are the dominant species present.

Both sites are classified as semi-natural woodlands according to the Heritage Council classification (Fossitt 2000). Charleville Forest is approximately 110 ha in size, dominated by mature pedunculate oak (*Quercus robur*), beech (*Fagus sylvatica*) and ash (*Fraxinus excelsior*) with a hazel (*Corylus avellana*) understorey. Tomnafinnoge is approximately 70 ha in size and dominated by mature sessile oak (*Quercus petraea*) with some beech, Douglas fir (*Pseudotsuga* spp) and ash, with a hazel understorey and well developed bilberry (*Vaccinum myrtillus*) shrub layer in much of the wood.

In Charleville Forest, 30 traps were installed at a density of 1 ha⁻¹ in three adjacent trapping grids (Figure 3.1). In Tomnafinnoge, 40 traps were installed at a density of 2 ha⁻¹ in two adjacent trapping grids (Figure 3.2).

3.3.2 Trapping and handling squirrels

The traps consisted of modified mink traps, with a wooden base and nest box attached. Traps were positioned above ground level on trees or tree stumps and baited with a 4:1 mixture of whole maize and peanuts which was placed on top of the nest box and on the base and entrance to the trap. Hazelnuts were then placed beyond the treadle. Each trapping session was 8 days long (with the exception of two 7 day sessions). Traps were pre baited for 4 days and set for 4 days at sunrise (but no earlier than 6am) and then checked after a minimum period of 4 hours.

Once captured, squirrels were transferred to a hessian sack and then into a handling cone, where their weight, sex, reproductive status and shin bone length were recorded. At first capture each animal was tagged by means of a Passive Integrated Transponder or microchip with a unique identification code.

In total 16 trapping sessions were carried out in Charleville on a monthly basis from September 2010 to October 2011 (with the exception of December 2010) and then on a bi-monthly basis from January 2012 to May 2012. A total of 7 trapping sessions were carried out in Tomnafinnoge, on a monthly basis from October 2011 to May 2012, with the exception of December 2011.



Figure 3.1 Squirrel traps in Charleville (1 trap ha⁻¹)



Figure 3.2 Squirrel traps in Tomnafinnoge (2 traps ha⁻¹)

3.3.3 Demography

To determine squirrel density Minimum Number Present was first calculated for each month, by counting the number of squirrels trapped that month, along with any squirrels that had been previously captured and were then recaptured at a later date (i.e. all squirrels that were known to be alive for each month), and then applying to the trapping area for each species.

In order to determine the effective trapping area, the edge effect was accounted for with information on home range for each species in deciduous woodlands. Although home ranges of individuals are known to vary depending on season, sex and habitat type (Wauters and Dhondt 1992), an average home range radius for grey squirrels in deciduous woods of 100m was used (Kenward *et al.* 1998) and for red squirrels an average home range radius of 100.9m was calculated from a mean home range value of 3.2 ha for red squirrels in deciduous habitat in Ireland (Waters 2012).

When edge effect was taken into consideration, in Charleville, the effective trapping area was 54 ha until March 2011 when the grids were modified slightly resulting in a 48 ha effective trapping area for the remainder of the study. In Tomnafinnoge the effective trapping area was 31 ha which remained the same for the duration of the study.

The density range for the red squirrel population in Charleville was compared to density ranges from previous studies on Irish red squirrel populations.

3.3.4 Body size and fitness

Body size is expected to have an influence on body mass. In order to remove any such effect, thus to solely examine individual fitness, each individual adult was assigned a fitness index value (Wauters and Dhondt 1995; Wauters *et al.* 2000). These fitness values were derived by calculating a linear regression of body mass on

shin bone length for the red squirrel population in Charleville (n = 41) and the grey squirrel population in Tomnafinnoge (n = 57), which was then used to derive residuals of body mass for each squirrel within each population. Individual residual values represent how much higher or lower an individual's body mass is, relative to their body size, when compared to the values obtained for their population through the regression equation. These residual values were then used as fitness values. The Charleville grey squirrel fitness values (n = 4) were derived from the Tomnafinnoge regression equation.

Residuals were tested for normality and two way ANOVAs were used to investigate variation in fitness against sex and trapping month within the Charleville red squirrel and the Tomnafinnoge grey squirrel population. A Mann Whitney U test was used to investigate whether a significant difference in fitness existed between the grey squirrel population in Charleville (n = 4 adults) and Tomnafinnoge (n = 58 adults). Data from females were then removed and male grey squirrel fitness was compared separately.

For comparative purposes, and to investigate for effects (or lack of) of competition on the fitness of the red squirrel population in Charleville, the linear regression equation was applied to mean shin bone and body mass measurements obtained from previous studies on Irish red squirrel populations to obtain mean fitness values for these populations. Data was only available for Irish red squirrel populations where grey squirrels have never been present.

Statistical analysis was carried out using IBM SPSS Statistics 20.

3.3.5 Reproduction

In order to determine breeding activity, only information from adult squirrels was included. In Charleville Forest, red squirrels weighing under 240g were classified as subadults (the weight under which no animals showed signs of reproductive activity), and were classified as adults thereafter. Weight was less consistent in the grey squirrel populations, and so those in Charleville with a body size (shin bone

measurement) of less than 75.4mm that showed no signs of reproductive activity (males only, testes not developed) were classified as subadults, as any squirrels measuring greater than 75.4 mm displayed signs of sexual development. In Tomnafinnoge, grey squirrels with a shin bone length of less than 74.4mm at first capture (the length under which no animals showed signs of sexual development), were classified as sub adult until such a time that they showed signs of reproductive activity.

Adult females were classified as being of positive or negative breeding status by the presence of one or a combination of the following: swollen vulva, swollen nipples, balding around nipples. Negative reproductive status was determined by the absence of swollen nipples or swelling of the vulva. Breeding activity was measured by the percentage of adult females in positive reproductive status for each month and breeding rate was measured for each season by the percentage of adult females lactating. A fecundity value for each adult female was determined by calculating the portion of breeding seasons which she was present for, that she was found to show signs of lactation (after Wauters et al. 2001; Gurnell et al. 2004). The population fecundity value was then determined for the grey squirrel population in Tomnafinnoge and the red squirrel population in Charleville as the mean fecundity value for all adult females. The number of weaned juveniles per year was estimated as 1.5 times the number of lactating females (Wauters et al. 1993). Recruitment for the population in Charleville, was measured as the number of subadults and new adults recorded between May 2011 and May 2012. The sex ratio in both woods was determined from the sex of each squirrel included in MNP calculations.

3.3.6 Habitat quality

In January 2011 and January 2012 in Charleville Forest, and in January 2012 in Tomnafinnoge, $1m^2$ quadrats were used to count the number of seeds that had fallen from the oak, beech and hazel trees. One quadrat was carried out per 4 hectares of the trapping area in each site in the month of January as 95% of the

previous autumn's acorns should have fallen from the trees by this time (Kenward *et al.* 1998; Shaw 1968). Seed crop densities were estimated by (mean no. of seeds counted per quadrat) x (percentage of total canopy cover occupied by that tree species). Habitat was then classified as good or poor according to the availability of acorns and beech nuts per metre squared (Wauters and Lens 1995), with > 30 beechnuts per m² and/or >10 acorns per m² classified as good squirrel habitat.

3.4 Results

3.4.1 Squirrel density

Red squirrel density in Charleville Forest was consistently higher than grey squirrel density for the duration of the study (Figure 3.3). Minimum density of 0.06 ha⁻¹ was recorded on the first trapping month i.e. September 2010 and a maximum density of 0.33 ha⁻¹ was recorded in May 2011, with the mean density for the entire study calculated to be 0.23 ha⁻¹. The minimum number of red squirrels known to be present ranged from 3 individuals in the first trapping month to 16 individuals in May 2011, with a mean value of 11.3 red squirrels over the course of the study. Red squirrels were trapped in all 16 trapping months. Grey squirrels were trapped in 12 of the 16 trapping months.

Grey squirrel density in Charleville Forest ranged between 0 ha⁻¹ and 0.1 ha⁻¹ with a mean value of 0.035 ha⁻¹ calculated for the duration of the study. The highest number of grey squirrels found was in the summer of 2011 when 5 squirrels (2 adults and 3 subadults) were



Figure 3.3 Red and grey squirrels densities in Charleville between September 2010 and May 2012 as determined by Minimum Number Present



Figure 3.4 Grey squirrel density in Tomnafinnoge between October 2011 and May 2012 as determined by Minimum Number Present

known to be present. A total of 4 adult grey squirrels, 3 male and 1 female, were trapped in the course of the study, with the remaining 4 squirrels, all of which were male, being trapped at subadult stage only, in the summer and autumn of 2011 and not again.

Grey squirrel density in Tomnafinnoge ranged from 0.35 ha⁻¹ to 1.45 ha⁻¹ with a mean value of 1 ha⁻¹, and numbers recorded ranged from 11 to 45 per month, with a mean value of 33 squirrels (Figure 3.4). No red squirrels were trapped in Tomnafinnoge.

3.4.2 Sex ratio

There was a total of 19 female and 26 male red squirrels trapped in Charleville, and the proportion of males present (using MNP) ranged from 0.5 to 1.0 with a mean of 0.68 (\pm 0.06, 95% CI). Only 1 female, and 7 male grey squirrels were trapped in Charleville and the proportion of males present ranged from 0.5 male to 1.0 with a mean of 0.82 (\pm 0.13, 95% CI) over the study period.

In Tomnafinnoge, a total of 29 female and 42 male grey squirrels were trapped, and the proportion of males present ranged from 0.45 to 0.64, with a mean of 0.55 (±0.06, 95% CI).

3.4.3 Breeding and recruitment

In Charleville Forest, male red squirrels showed signs of breeding activity in 13 of the 16 trapping months with peaks in February and April/May of 2011 and in January and May of 2012. Between March and October 2011, 9 female red squirrels were trapped whilst lactating, with 3 of these showing evidence of lactation in both spring and summer months suggesting that they had bred twice in that year. The estimated number of weaned juveniles was therefore 18. From that, recruitment was estimated at 0.67, as there were 5 subadults and 7 new adults captured between May 2011 and May 2012. There was just one female grey squirrel caught over the course of the study in Charleville Forest. She was trapped

monthly between May and October 2011, and showed signs of lactation in 4 of the 6 months suggesting that she potentially weaned two litters in 2011. There were 4 subadults caught in the summer of 2011, but not again, and 1 new adult caught in May 2012.

In Tomnafinnoge, male grey squirrels showed signs of breeding activity in each of the 7 trapping months, peaking in January and in particular May, when all 26 adult males trapped showed signs of breeding activity. Female grey squirrels in Tomnafinnoge also showed signs of breeding activity in each trapping month, with peaks in February/March and again peaking in May when 14 of the 15 adult females trapped were lactating. The trapping period of 7 months was not long enough to identify new adults in the population. Data gathered on the reproductive success of the populations from both woods are described in Table 3.1. As the study period in Tomnafinnoge was less than one year, not all parameters could be measured.

3.4.4 Body size

Mean adult red squirrel shin bone length ranged from 60.98mm to 71.7mm (n = 41, $x = 68.19 \pm 0.7$, 95% Cl) in Charleville forest, and there were no significant differences between male and female squirrels (F = 0.486, p = 0.49). Adult grey squirrel shin bone length in Charleville Forest ranged from 75.4mm to 80.3mm (n = 4, x = 77.85 ± 3.46, 95% Cl). Mean adult shin bone length in Tomnafinnoge ranged from 74.6mm to 79.7mm (n = 58, x = 77.04, ± 0.3, 95% Cl) and likewise no significant differences between the sexes were found (F = 0.56, p = 0.46).

3.4.5 Fitness

The linear regression equations for Charleville (Figure 3.5) and Tomnafinnoge (Figure 3.6) and mean fitness values for adult male and female squirrels are described in Table 3.2. Overall adult red squirrel fitness values in Charleville ranged from – 40 to + 31.9 (\bar{x} = - 0.03 ± 5.41, 95% Cl, n = 41). A two way ANOVA found no

significant variation in fitness between the sexes or trapping months in the Charleville red squirrel population (F = 0.905, p = 0.556). In Tomnafinnoge, fitness ranged from – 72.11 to 71.18 (\bar{x} = 0.019 ± 9.54, 95% CI, n = 57). Overall adult female grey squirrels were in significantly better condition than males (F = 31.837, df = 1, p < 0.001) and fitness in January was significantly higher regardless of sex (F = 2.203, p < 0.05, df = 6).

The Charleville adult grey squirrels were found to be in significantly poorer condition than those in Tomnafinnoge (p < 0.006), and when tested separately, the males even more so (p < 0.002).

Table 3.1 Sex ratios, reproductive activity and recruitment for the red and grey squirrel population at Charleville and the grey squirrel population at Tomnafinnoge. Breeding rate was calculated as the percentage of adult females that were lactating for each season. Population fecundity was calculated as the mean fecundity value for all females after individual fecundity was calculated as the portion of breeding seasons that each female was present for in which signs of lactation were observed.

		Charleville	Charleville	Tomnafinnoge
			Sept 10 – May	Oct 2011 - May
		Sept 10 – May 12	12	12
Species		Red	Grey	Grey
N		45	8	71
Sex ratio (% M)		0.68	0.82	0.55
Subadults				
trapped	2011	5	4	na
	2012	0	0	10
New adults				
trapped	2012	7	1	na
Litters (all year)	2011	12	2	na
(first half of				
year)	2012	4	0	19
		0.59		0.87
Fecundity		(± 0.21, 95% CI)	na	(± 0.13, 95% CI)
Breeding rate (F)	Spring 2011	62.5%	na	na
	Summer 2011	87.5%	na	na
	Spring 2012	50%	na	91 %
No. of litters per	0	25%	na	na
female in 2011	1	50%	na	na
	- 2	25%	na	na
	2	23/0	ila	IId



Figure 3.5 Linear regression on body mass (g) and shin length (mm) of adult red squirrels in Charleville y = 4.601x - 22.261, F = 13.633, $R^2 = 0.259$, p < 0.001



Figure 3.6 Linear regression on body mass (g) and shin length (mm) of adult grey squirrels in Tomnafinnoge, y = 21.065x - 1042.4, $R^2 = 0.394$, F = 35.833, p < 0.001)

Table 3.2 Fitness values (± 95 % confidence intervals) calculated as residuals from regression equations on body mass Vs shin bone length for adult male and female red squirrels in Charleville and Tomnafinnoge

Site & species	Linear regression	Mean fitness F	Mean fitness M
Charleville - red	y = 4.601x - 22.261	1.44 (±3.018, n=19)	-1.31 (±7.42, n=22)
Tomnafinnoge - grey	y = 21.065x - 1042.4	15.68 (±14.06, n=24)	-11.37 (±11.82, n=33)
Charleville - grey	as per Tomnafinnoge	-22.42 (n=1)	-87 (±90.38,n=3)

3.4.6 Habitat quality

In 2010 there was an abundance of acorns available as a food resource in Charleville Forest. 2011 had a more moderate tree seed crop but the availability of acorns and beech nuts per m² meant it was still classified as a good year in terms of red squirrel requirements. In Charleville in 2010, the average number of acorns per m² under oak canopy (n=6 quadrats) was 140 per m² compared to 36 per m² for 2011 (84 per m² and 22 per m² for the wood respectively). Beech mast was estimated to be 435 per m² in 2010 (overall value of 130.5 beech nuts per m² for the wood) compared to 309 per m² in 2011 under beech canopy (n=3 quadrats, overall value of 92.7 per m² for the wood) and hazelnuts were estimated to be available at a density of 23 per m² in 2010 and 22.5 per m² in 2011 in the parts of the wood containing hazel (n = 2 quadrats).

The seed counts in Tomnafinnoge, for oak and beech in 2011 produced an estimation of 9.6 acorns per m^2 under oak canopy (overall value of 7.2 acorns per m^2 in the wood) and 90.5 beech nuts per m^2 under beech canopy (overall value of 10 beech nuts per m^2 in the wood).

Therefore both 2010 and 2011 were classified as good quality habitat for red squirrels in Charleville and 2011 was poor in comparison in Tomnafinnoge (Figure 3.7).



Figure 3.7 Annual seed count data for large seeded trees in Charleville and Tomnafinnoge

3.5 Discussion

In both their natural range in the eastern United States, and their introduced range in Ireland, Britain, Italy and Canada, normal grey squirrel density is several squirrels per hectare in large seeded broadleaved woods (Thompson 1978; Gurnell 1996; Kenward *et al.* 1998; Don 1983; Gurnell *et al.* 2001b; Lawton and Rochford 2007). The grey squirrel density of 0.035 ha⁻¹ recorded in Charleville is therefore atypical for the species, particularly considering they have been established in the woodland since the 1960's. In contrast, the red squirrel population is living at normal density, despite an absence from Charleville of more than 30 years. The red squirrel population in Charleville is therefore in competitive release from the grey squirrel, in a woodland that it had once been considered locally extinct.

3.5.1 Competitive release

Although the effects of competition are notoriously difficult to measure, several long term studies on sympatric red and grey squirrel populations, compared to "red squirrel only" sites, have identified the population parameters by which the effects of interspecific competition can be measured. As such, decreased juvenile recruitment (Wauters et al. 2000), and reduced fitness leading to reduced fecundity (Gurnell et al. 2004) in the red squirrel population have been found to be key factors in the usual replacement of red squirrels by greys, in particular in oak dominated habitat (Kenward et al. 1998) such as Charleville. During this study however, red squirrel population demographics did not indicate that there was any significant competitive pressure from the sympatric, low density, grey squirrel population. For example, the population fecundity value of 0.59 is more comparable to the value of 0.62 (found in a "red-only" population in north Italy) than 0.48 (found in a "red-grey" population in the same study) (Gurnell et al. 2004). Similarly, the breeding rates of 62.5% and 87.5% for spring and summer 2011 respectively in Charleville were more typical of the "red-only" site in the north Italy study as opposed to the "red-grey" sites, where summer breeding in particular was significantly lower in the "red-grey" sites.

3.5.2 Density and fitness

Red squirrel density and red squirrel fitness in Charleville were also comparable to those of several other Irish squirrel populations where grey squirrels had never been present (Table 3.3). Both density and fitness compared favourably with these "red-only" populations. Red squirrels would typically be expected to have lower densities than grey squirrels when their populations do overlap, but this is clearly not the case for the population in Charleville, where mean red squirrel density of 0.23 ha⁻¹ was more than six times greater than mean grey squirrel density during the course of the study.

In contrast, the grey squirrel density of 1 ha⁻¹ recorded in Tomnafinnoge is typical of expected densities for this type of habitat in Ireland (Lawton and Rochford 2007), and is < 28 times greater than that of grey squirrel density in Charleville. While there was only one female grey captured throughout the entire study period in Charleville, there were 29 females captured in Tomnafinnoge, despite the shorter study period, with an estimated 19 litters born, and a population fecundity value of 0.87.

Table 3.3 Fitness values and density ranges for Charleville^{*} and various other red squirrel populations in Ireland where grey squirrels were not present (fitness values derived from reported mean body weight and shin bone length using the regression equation from adult red squirrels in Charleville: y = 4.601x - 22.261)

Habitat type	Mean fitness value	Density range	Study
coniferous	+5.53	0.21 - 0.39	(Reilly 1997)
coniferous	+4.07	0.02 - 0.11	(Reilly 1997)
broadleaf	-0.03	0.20 - 0.33	Charleville*
mixed broadleaf	-5.02	0.24 - 0.44	(Waters 2012)
coniferous	-19.68	0.21 - 0.34	(Waters 2012)
mixed	-24.91	0.35 - 1.02	(Poole 2007)

Despite an abundance of available tree seeds in Charleville, when the fitness values for the grey squirrel population were compared to those in Tomnafinnoge, significant differences were found. Whilst body size values were very similar between the two populations, there was a considerable difference in body mass, thus the adult grey squirrels in Charleville, in particular males, were found to be in significantly poorer condition than those in Tomnafinnoge as a result (p < 0.002), despite the difference in sample size. Adult females in Tomnafinnoge were also found to be in significantly better condition than their male counterparts, however this is likely to have been influenced by a greater body mass in gestating females, as 91% of adult females showed signs of breeding activity at the time they were captured.

Notably, the four subadult grey squirrels that were captured in the summer of 2011 in Charleville were never detected again, suggesting they had either dispersed from the woodland, or died. This is unusual considering that the population is well below the normal carrying capacity, and as such recruitment of subadults into the population should be high.

The overall health and status of the grey squirrel population in Charleville was extremely poor, in stark contrast to the population in Tomnafinnoge who are thriving. Woodland size and tree species composition are very similar between the two woods, and tree seed counts determined that the habitat quality during the course of the study in Charleville was actually superior to that of Tomnafinnoge, in terms of squirrel requirements, suggesting habitat quality is not a contributing factor. Although it is unclear what is the cause of the poor health of the grey squirrel population in Charleville, what is evident from this study is that the red squirrel population is co-existing with this albeit low number of greys, without any evidence of competitive pressure from their invasive counterparts.

3.5.3 Squirrel and pine marten distribution

The concept of a localised grey squirrel population decline in Ireland was first suggested by O'Teangana et al. in 2000, as a result of anecdotal reports from the 1990's of grey squirrels declining, and red squirrels reappearing, in parts of Cavan and Monaghan. More recently, Carey at al. (2007) reported a similar phenomenon, not only in parts of Cavan and Monaghan, but also in Laois and Offaly. This study is the first to investigate the population dynamics of sympatric red and grey squirrels in a region where grey squirrels are believed to have gone into decline, and the findings confirm anecdotal reports that in certain parts of Ireland, the grey squirrel

is living at much lower densities than expected for the species, and the red squirrel population has re-established itself as a result.

A national pine marten distribution survey in the 1980's (O'Sullivan 1983), reported a major reduction in the species range had taken place in the 20th century, and that the pine marten in Ireland was largely confined to the west of the river Shannon, between Limerick and Sligo. A subsequent survey in 2005 found that the population had significantly increased as a result of reduced persecution and increased habitat availability (O'Mahony *et al.* 2006). Along with an increase in pine marten abundance in the stronghold of the west, and several smaller populations in the south west and the south east, the Slieve Bloom mountains (situated in counties Laois and Offaly) were identified as host to a recently expanded pine marten population.

In the 2007 survey on squirrel distribution, Carey et al. reported that the apparent reduction in grey squirrel numbers in Laois and Offaly coincided with an increase in pine marten sightings, suggesting there may be a link between the two species' distribution. Red squirrels and pine marten have co-existed in Ireland and many other parts of Europe for many millennia, and the red squirrel has appeared only as a very low frequency prey item in Irish pine marten dietary studies (Warner and O'Sullivan 1982; Lynch and McCann 2007), and indeed in other European studies, except in Russia and Sweden, where alternative small mammal prey are less abundant (De Marinis and Masetti 1995). What is yet to be established is the relationship between the European pine marten and the alien (North American) grey squirrel, as their natural ranges do not overlap. On a broad scale at least, there is still very little overlap between the pine marten and the grey squirrel populations in Ireland (Carey et al. 2007; O'Mahony et al. 2012; see also Chapter 2). The grey squirrel has never established itself in what is considered the pine marten's stronghold, the west of Ireland, despite having breached the Shannon on several occasions (O'Teangana et al. 2000; Carey et al. 2007), and they are now present only at very low densities in the midlands of Ireland where the pine marten population has recently re-established. It is possible that predation and/or the presence of a high density predator population could deter the alien squirrel

species from establishing in, or remaining in an area. However, it is not possible to determine in retrospect by what means the grey squirrel population in Charleville declined so significantly.

3.6 Conclusion

The grey squirrel population in Charleville is now present only in extremely low numbers and the red squirrel population is in competitive release. Grey squirrel recruitment is low and fitness is poor in comparison to the population in the east of the country. Sightings and hairtube surveys (Chapter 2) confirm this grey squirrel decline has occurred throughout Laois and Offaly and parts of the surrounding region over recent decades. Habitat quality does not appear to be a contributing factor. The possibility that the recently recovered European pine marten population may be influencing Irish squirrel population dynamics needs to be investigated further, as the relationship between the alien squirrel species and the native squirrel predator has yet to be established.

4. A non-invasive approach to determining pine marten abundance and predation

4.1 Introduction

In the nineteenth and twentieth centuries, the European pine marten (Martes martes) population in Ireland experienced widespread decline as a result of habitat loss (large-scale deforestation) and heavy persecution (O'Sullivan 1983). Population censuses in the 1980s (O'Sullivan 1983) and again in 2005 (O'Mahony et al. 2006) revealed that pine marten distribution in Ireland is still mainly concentrated around core populations in the west and midlands, along with several smaller populations in the south west and south east of the country. However, the pine marten population in the west and midlands of Ireland has undergone a range expansion in recent decades, as a result of increased habitat availability and connectivity through afforestation, and importantly protection by law (O'Mahony et al. 2006). The most recent population estimate for the island of Ireland is 3060 individuals (O'Mahony et al. 2012), although there is still relatively little known about Irish pine marten population densities in the western-most part of their European range. The European pine marten has traditionally been considered a forest specialist. Zalewski and Jedrzejewski (2006) estimated that 2 km² is the minimum area of forested habitat necessary to support an adult pine marten in the temperate forest zone. Despite an extremely fragmented forest landscape (with < 11% forested area, Ireland represents the lowest forested land cover in their range), previous population studies in Ireland (Lynch et al. 2006; Mullins et al. 2010) have found pine marten density can be higher in Ireland than is typical throughout their British and European ranges (Birks 2002; Zalewski and Jedrzejewski 2006; Mergey et al. 2011; Caryl et al. 2012a). The Irish studies were conducted on the smaller population pockets in the south west (Killarney, Co. Kerry) and the south east (Portlaw, Co. Waterford). A population density estimate for the core

population, and also the part of their range where they are believed to be much less common (i.e. the east of the country), has yet to be determined.

In Ireland and Scotland, it has been anecdotally suggested that the recovering pine marten population may be inhibiting the spread of the invasive North American grey squirrel (*Sciurus carolinensis*), and indeed that the grey squirrel population has retracted in the presence of pine martens in both countries (Carey *et al.* 2007; Paterson and Skipper 2008). Published information on red (*Sciurus vulgaris*) and grey squirrel distribution in Ireland in 2007 (Carey *et al.*) and more recent studies on squirrel distribution (Chapter 2) have found the grey squirrel to be rare in the midlands of Ireland, but their range potentially overlaps with that of the pine marten in the east. It has been suggested that the European pine marten preys preferentially on the larger, less arboreal of the two squirrel species, however, there has been no evidence to date of the European pine marten predating upon the North American grey squirrel anywhere in its invasive range, which only overlaps to a small extent with that of the pine marten (see maps in Carey *et al.* 2007 and O'Mahony *et al.* 2012).

Non-invasive genetic studies to identify species distribution and population size have become important tools to aid the study of wild, and particularly elusive, carnivore populations such as martens (Mowat and Paetkau 2002; Williams *et al.* 2009). Hair sampling is commonly used to non-invasively survey for the presence of mammal species. Lynch et al (2006) found hair traps (fur-snagging devices) both quick and reliable in detecting pine marten presence in broadleaved woodlands. Subsequently, Mullins et al. (2010) optimised a panel of microsatellite loci to identify unique genotypes within the Irish pine marten population, thus enabling distribution and abundance to be established reliably through non-invasive field studies such as hair trapping. The use of molecular techniques in the analysis of carnivore diet has also become popular in recent years (Deagle *et al.* 2005; Dunshea 2009; Shehzad *et al.* 2012), as prey DNA found in scats can be identified to taxon and species level and is not dependent on hard parts surviving digestion. Molecular techniques have recently been optimised to specifically detect the presence of mammalian prey in the diet of the Irish pine marten (O'Meara *et al.* 2013).

4.2 Aims

Using these recently developed non-invasive techniques, this study firstly aims to quantify pine marten abundance in the fragmented forest habitat in their core range in the midlands of Ireland and in the east of the country where they are considered to be less common (O'Mahony *et al.* 2006; O'Mahony *et al.* 2012). Secondly, the study aims to quantify the frequency of occurrence of small mammals in the diet with emphasis on red and grey squirrels. In the process, we aim to quantify scat density in the midlands and eastern regions and to compare the findings of both molecular and macro dietary analysis techniques.

4.3 Methods

4.3.1 Field methods

The primary study area consisted of counties Laois and Offaly, in the midlands of Ireland, and the secondary study area was county Wicklow, in the east of the country, where the pine marten population is considered to be less abundant (O'Mahony *et al.* 2012; Chapter 2).

4.3.1.1 Abundance study

Five sites were selected, a broadleaved and a predominantly coniferous woodland from each study area (with two broadleaved woods examined sequentially in Co. Wicklow). Site 1, Charleville Forest, Co. Offaly, is a mature broadleaved wood (c.113 ha) in which oak (Quercus robur) is the dominant tree species. Site 2, Clonad (c.143 ha), Co. Offaly, is a mixed, mainly coniferous woodland situated 1.5 km from Charleville, where Norway (Picea abies) and sitka spruce (Picea sitchensis) are the dominant tree species. Site 3, Cloragh (c.160 ha) is located in Ashford, Co. Wicklow. The dominant tree species present are sitka spruce and Douglas fir (*Pseudotsuga* spp). Site 4, Knocksink nature reserve (c. 60 ha), is located in Enniskerry, Co. Wicklow. It consists of mature oak (Quercus petraea) and mixed woodland. Site 5, Tomnafinnoge (c.80 ha) in south Wicklow, is a broadleaved woodland consisting mainly of mature oak (Quercus petraea) (Figure 4.1). Sites 2 and 4 are considered discrete woodlands as there is no forested habitat within 1 km of these sites. Site 1 is separated from adjacent forest habitat by a new primary road. Site 3 is separated from adjacent forestry by the Vartry river. Site 5 is not separated from adjacent forestry by either natural or man-made boundaries, and as such represents the only site which is indiscrete in this study (Figure 4.1). Spring based hair traps as described by Messenger and Birks (2000) were installed in sites 1 (n = 5), 2 (n = 7), 3 (n = 8), and 4 (n = 4) in March 2011 for a period of 14 months, with the exception of site 4, where the hair traps were moved to site 5 in October 2011. Each trap was

baited once per month with chicken and the tree trunk was smeared with marmalade. Traps were positioned 450m apart at a density of one trap per 20 ha throughout the sites as previous home range sizes for the pine marten in Ireland have been reported as $\geq 0.2 \text{ km}^2$ (Birks 2002). Animals that were genetically identified in three or more months (including at least one month between November 2011 and April 2012) were assumed to be resident adults. Abundance values were then obtained by applying the number of residents adults identified at each site to the corresponding forested sampling area.



Figure 4.1 Location of the study area in Ireland (inset) and the locations of the hair trap sites within the fragmented forested landscape. Non forested land cover is white, forested habitat is grey and hair trap sites are represented in black. Scat survey sites are indicated by open diamonds. The position of each hair trap within the study sites are also shown
4.3.1.2 Dietary study

Scats were collected between March 2010 and August 2012 from 23 sites throughout the midlands and the east of Ireland (Figure 4.1) and stored at -20°C. In order to ensure reliability and validity of both scat density and dietary analysis, all scats collected as part of this study were subjected to DNA analysis in order to confirm they were of pine marten origin.

In order to determine whether a potential prey species was being preyed upon, it was necessary to establish first of all that it was available as a prey item. Of the 23 scat collection sites, 17 were classified as being either red squirrel or grey squirrel positive sites in accordance with the findings of concurrent squirrel distribution studies (Table 4.1) (see also Chapter 2). A sample of scats from the west of Ireland that were collected during the course of a red squirrel population study (Waters and Lawton 2011) were also included. Woodlands where both grey squirrels and pine marten were confirmed as being present together were identified as key sites and revisited where possible to increase the sample size of scats collected from these zones. In August 2012, a scent detection dog, specially trained to detect pine marten scat, carried out searches in three woodlands (including abundance study site 5, Tomnafinnoge and two further sites in the east). With the exception of the searches made by the scent detection dog, and a few scats that were collected opportunistically, the distance covered during the course of scat collection was used to estimate scat density, where scat density = (no. of scats collected) /(distance walked). All scats were collected by the author and transects were walked once per month in the abundance study sites, and in the rest of the sites either once or twice in total. A Fisher Exact test was used to test for an overall difference in accuracy in scat identification in the field between the midlands and eastern sites, and to test for a significant difference in the frequency of occurrence of red and grey squirrels in the diet.

Table 4.1 Scat surveys in sites where squirrel distribution studies had taken place (seeChapter 2; Waters and Lawton 2011) resulted in a total of 361 scats for analysis of squirrelin the diet *site re-visited with scent detection dog. RS = red squirrel, GS = grey squirrel,M = midlands, W = west, E = east, Y = squirrel species detected during field survey, N =squirrel species not detected during field survey

Site	Location	RS	GS	RS site scats	GS site scats	RS prey	GS prey
Charleville	М	Y	Ν	162		4	
Clonad	М	Y	Ν	109		3	
Abbeleix Demesne	М	Y	Ν	7		1	
Birr Castle	М	Y	Ν	1			
Ballykilcavan	М	Y	Ν	16			
Cappard	М	Y	Ν	6			
Emo Park	М	Y	Ν	9			
Ballyteige	М	Y	Ν	0			
Garryhinch	М	Y	Ν	11			
Derryclare	W	Y	Ν	6			
Croneybyrne	E	Y	Ν	1			
Clara	E	Y	Ν	1			
Tomnafinnoge*	E	Ν	Y		20		5
Dollardstown*	E	Ν	Y		8		
Ballygannon	E	Ν	Y		4		
Mullaghreelan*	E	Ν	Y		0		
Oakpark	E	Ν	Y		0		
Jenkinstown	E	Ν	Y		0		
Total				329	32	8	5
					2	2.4% FO	15.6% FO

4.3.2 Laboratory methods

4.3.2.1 Abundance study

Molecular analysis

Genomic DNA was isolated from (n = 158) hair samples using The ZR Genomic DNA II Kit^M (ZYMO Research, CA, USA) using the protocol for hair extraction (ZYMO RESEARCH Cat no. D3040). The DNA was eluted with 100 µl of deionised water. Real-time PCR was used for species (targeting mitochondrial DNA) and sex determination (targeting ZFX and ZFY sequences on the X and Y chromosome) of the hair samples as described by Mullins et al (2010). The Ct value for the ZFX gene was used to screen the samples for genotyping suitability. Samples with a Ct value less than 36 were deemed to contain adequate quantities of nuclear DNA for genotyping.

Genotyping

Samples that were deemed suitable for genotyping were screened in duplicate at seven loci (Ma2-mini, Mel1, Gg7-mini, Mvi1341, Mvi1354, Mvis075, Ggu234). As the samples used for genotyping came from a non-invasive source (remotely plucked hairs), each sample was independently genotyped twice. Scores were only recorded if they were observed twice and exactly matched. Samples that were not replicated after the first two PCR's were repeated. Details of primers and multiplex setup are provided in Table 4.2. Fragment Analysis was conducted on an ABI PRISM 310[®] Genetic Analyser (Applied Biosystems) according to the manufacturer's instructions with the standard run module. Alleles were scored with GS500 LIZ[™] size standard using GeneMapper software v3.7 (Applied Biosystems). Two authors independently called alleles.

Locus	Primer sequence 5' – 3'	Size range	Reference
Ma2-mini	F: YAK-CCATGTACTTTTCCTATCTTTTAGGA	131-141	O'Reilly (This study)
	R: ATCTTGCATCAACTAAAAAT		Davis and Strobeck (1998)
Mel1	F: FAM-CTGGGGAAAATGGCTAAACC	106-116	Bijlsma et al. (2000)
	R: GCTCTTATAAATCTGAAAATTAGGAATTC		Mullins et al. (2010)
Gg7-mini	F: FAM -GTTTTCAATTTTAGCCGTTCTG R: GCTCTTCACTCTGTGTGGCATCTAC	132-140	Davis and Strobeck (1998) O'Reilly (This study)
Mvi1341*	F: PET -GTGGGAGACTGAGATAGGTCA R: GTTTCTTGGCAACTTGAATGGACTAAGA	164-178	Vincent et al. (2003)
Mvi1354*	F: FAM -CCAACTGGAGCAAGTAAAT R: GTTTCTTCATCTTTGGGAAAGTATGTTT	200-212	Vincent et al. (2003)
Mvis075*	F: FAM -GAAATTTGGGGAATGCACTC R: GTTTCTTGGCAGGATAGGATGTGAGCT	145-155	Fleming et al. (1999)
Ggu234	F: PET -TTACTTAGAGGATGATAACTTG R: GAACTCATAGGACTGATAGC	84-90	Duffy et al. (1998)

Table 4.2 Microsatellite primers used in pine marten genotyping. Reverse primers marked with an * were modified with a 5' sequence of GTTTCTT to promote non-templated nucleotide addition (Brownstein et al. 1996). The Ma2-F and Gg7-R primers were redesigned to produce a smaller product. The primers were used in two multiplex mixes. Mix A contained Gg7-mini and Mvi1354 and Mix B contained all the other primers. Each primer pair was at a final concentration of 0.5 μ M. Microsatellite amplifications were performed in a total volume of 10 μ l with 4 μ l DNA extract, 1 μ l primer mix and 5 μ l GoTaq[®] Hot Start Green Master Mix (Promega). The PCR conditions were 95°C for 5 min followed by 30 cycles of 95°C for 30 s, 60°C for 90 s and 72°C for 30 s, followed by 72°C for 30 min

Data Analysis

The two genotyping replicates were compared to assess the data for genotyping errors including the presence of allelic drop out and false alleles using GIMLET version 1.3.4 (Valière 2002). PCR success rates were also calculated using GIMLET version 1.3.4 (Valière 2002). The occurrence of repeated genotypes was identified using GENALEX version 6.4.1 (Peakall and Smouse 2006) and the number of replicates or individual recaptures was recorded. A final dataset was created with duplicated data removed and MICROCHECKER version 2.2.3 (Van Oosterhout *et al.* 2004) was used to further identify possible genotyping errors, including the presence of null alleles, large allele dropout, and scoring errors as a result of stutter peak (using default settings).

Gametic phase linkage disequilibria by Fisher's method (1000 dememorizations and 5000 iterations) and deviations from Hardy-Weinberg equilibrium were assessed (default settings, exact tests) using GENEPOP version 4.0.10 (Rousset 2009). Observed (H_0) and expected (H_E) heterozygosities and the number of alleles (a), were calculated using GENALEX version 6.4.1 (Peakall and Smouse 2006), and allelic richness (R_s) was estimated using FSTAT 2.94 (Goudet 1995).

4.3.2.2 Dietary study

Molecular analysis

Approximately 0.2g of scat was used for DNA extraction as described in O'Reilly et al (2008), and using the ZR Genomic DNA II Kit[™](ZYMO Research, CA, USA). Pine marten DNA was verified as described above. All samples with a Ct value lower than 32 were classified as pine marten and those with a greater Ct value were classified as non pine marten and excluded from further analysis. To test for prey DNA in the confirmed pine marten scats, species-specific Taqman assays designed

to detect red and grey squirrel DNA were used. All PCR reactions and probes were as described by O'Meara et al. (2012). A sub-sample of 160 scats (80 from sites 1 and 2 respectively) were also tested for small mammal prey DNA; woodmouse (*Apodemus sylvaticus*), bank vole (*Myodes glareolus*), pygmy shrew (*Sorex minutus*), and greater white toothed shrew (*Crocidura russula*) (O'Meara *et al.* 2013). Samples with Ct values of 36 or higher were discounted and positive results were replicated for verification. Percentage frequency of occurrence (%FO) in the diet for each prey species was calculated as the number of scats in which the species' DNA was amplified/total no. of scats tested*100.

Macro analysis

A sub-sample of 110 scats were subjected to traditional hard part analysis to identify mammalian prey using keys to identify mammal bones (Yalden and Morris 1990) and hairs (Teerink 1991). The subsample of 110 scats comprised 40 scats from both the Charleville and Clonad subsamples respectively (which had been tested for squirrel and other small mammalian prey DNA) and a further 30 scats from the grey squirrel positive sites (which had been tested for squirrel DNA only). The results from molecular and macro analysis were compared and then combined to determine an overall frequency of occurrence in the diet for each prey species. A chi square test was used to investigate significant differences in the frequency of occurrence of each species according to molecular, macro and combined results. Regression analysis was used to investigate whether a relationship exists between the frequency of occurrence of prey items using molecular and macro techniques. %FO for mammalian prey species was calculated as in molecular analysis and percentage relative biomass of prey ingested (%BPI) was calculated as: weight of dried remains for each species/total weight of dried remains. Previous studies investigating the contribution the main food groups make in terms of biomass to the diet have used pre-established correction factors in such estimations (Lynch and McCann 2007; Caryl et al. 2012b); these correction factors were derived from feeding trials in which the weight of the food item eaten was divided by the dry

weight of undigested matter later identified in scats (Lockie 1961; Balharry 1993; Jedrzejewska and Jedrzejewski 1998; Lanszki *et al.* 2007). Individual correction factors for the mammalian prey species investigated in the current study were not available as they are usually simply grouped together in feeding trials as 'small mammals'.

4.4 Results

4.4.1 Abundance study

A total of 157 hair samples were collected out of 273 baited hair traps. Sites 1 and 2 in the midlands yielded the highest success rates with 91% and 78% respectively. In site 3, 37% of potential trapping events yielded hair samples. In site 4, one hair sample was obtained out of a possible 24, and this was the only hair sample in the study to test as negative for pine marten DNA. Site 5 yielded nine hair samples, a success rate of 37.5%. A further hair sample was collected from a roadkill animal in July 2011, c. 3 km from site 2, bringing the total number of hair samples to 158.

Of the 158 hair samples, 157 were successfully genetically identified as pine marten and 139 were successfully sex-typed. 109 samples had a ZFX Ct value \leq 36 and of these, 104 were successfully genotyped (at six or more loci) (i.e. 95% of samples that passed the screening process, or 66% of all hair samples collected). This success rate varied between individual sites (Figure 4.2).



Figure 4.2 The number of successful hair trapping events and the portion of hair samples successfully genotyped from each site

A total of 25 individual genotypes were obtained from the 104 samples; one from the roadkill animal near site 2, and 24 from the abundance study sites (site 1, n = 6 pine marten detected with five hair traps; site 2, n = 10 pine marten detected with four to seven hair traps; site 3, n = 6 pine marten detected with eight hair traps; site 5, n = 2 pine marten detected with four hair traps). Pine marten abundance ranged from 0 to 3.54 per km² including adult residents only (Table 4.3). The number of hair samples genotyped from each individual ranged from 1 to 14 with a mean value of 4.29 (± 1.6, 95% CI) and the number of months each animal was captured ranged from 1 to 7, with a mean value of 3.29 (± 0.96, 95 % CI) (Table 4.4). One individual (a male) was detected in both site 1 and site 2 (Jan 2012 and May 2011 respectively). These sites are located 1.5 km apart (Figure 4.1) and together they comprise less than 3 km² of forested habitat. In total, eight adult residents were

	Site name	Site area	Total PM identified	Mean Captures	No. adult residents	No. adult residents per km ²
Midlands	1. Charleville	1.13 km ²	6	4.67 (0.98)	5	4.42
	2. Clonad	1.43 km ²	10	2.8 (0.79)	3	2.10
East	3. Cloragh	1.58 km²	6	2.83 (0.75)	2	1.27
	4. Knocksink	0.6 km ²	0	0.00	0	0.00
	5. Tomnafinnoge	0.8 km ²	2	2.5 (1.5)	1	1.25
Total	All	5.54 km ²	24	3.29 (0.46)	11	1.99
	Midlands	2.56 km ²	16	3.56 (0.63)	8	3.13
	East	2.98 km ²	8	2.75 (0.62)	3	1.01

Table 4.3 The total number of pine marten identified by unique genotypes at each site, in total (All), in sites 1 and 2 combined (Midlands) and in sites 3, 4 and 5 combined (East). Mean Captures = mean number of times each animal was captured per site (Std Er). No. adult residents = number of animals that were detected in \geq 3 months including at least one month during Nov 2011 and April 2012

Animal	Site	Sex	GT	Months
CVF01	1. Charleville	Female	1	1
CVF02			3	3
CVF03			10	7
CVF04			6	4
CVM01		Male	9	7
CVM02			14	6
CDM05*			1	1
CDF01	2. Clonad	Female	4	4
CDF02			1	1
CDF03			3	3
CDF04			1	1
CDM01		Male	1	1
CDM02			3	2
CDM03			1	1
CDM04			12	8
CDM05*			1	1
CDM06			7	6
WWF01	3. Cloragh	Female	2	2
WWF02			3	3
WWM01		Male	7	5
WWM02			1	1
WWM03			6	5
WWM04			1	1
TFF01	5. Tomnafinnoge	Female	4	4
TFF02		Male	1	1
Roadkill			1	
Total			104	

Table 4.4 The site each pine marten was recorded at and the sex assigned through DNAanalysis. GT = the number of hair samples successfully genotyped for each animal. Months= the number of months each animal was identified. *Animal was captured at two sites

detected in the two sites and a further eight that are assumed to be either subadult or non-resident individuals were also detected. With the exception of the one animal there was no further crossover of individuals detected between these two woodlands, despite their close proximity to one another and the lack of surrounding forested habitat.

4.4.1.1 Assessing genotyping errors

No ambiguous genotypes were detected in the replicated genotypes. The proportion of positive PCRs ranged from 90 – 100% across loci and from 86 to 100% across samples. Analysis of genotyping error revealed the presence of allelic dropout rates of 0.08 at locus Ma2-mini, 0.10 at locus Ggu234 and 0.46 at locus Mvis354, with no false alleles detected. The overall allelic dropout error rate across all loci was 0.09, 0.32 across all samples, and 0.08 across all PCRs. There was no systemic evidence of scoring errors and the data was not shown to be affected by the systemic presence of null alleles or large allelic dropout.

4.4.1.2 Genetic variability

The number of alleles was low ranging from 2 at Ggu234 and Mvis075 to 4 at Gg7mini (Table 4.5). Low levels of allelic richness per locus and per sample were also observed from 2 at Ggu234 and Mvis075 to 3.69 at Gg7-mini. Expected levels of heterozygosity ranged from 0.106 at Mvis1354 to 0.570 at Ma2-mini, and observed levels of heterozygosity ranged from 0.111 at Mvis1354 to 0.708 at Gg7-mini and Ma2-mini (Table 4.5). There were no significant deviations from Hardy–Weinberg expectations at any loci

Table 4.5 Descriptive statistics for microsatellite analysis of pine martens in four study sites in Ireland: N = the number of individuals that successfully amplified at each locus, a = number of alleles per locus, $R_s =$ allele size range, $A_s =$ allele size, $H_E =$ expected heterozygosity, $H_O =$ observed heterozygosity. There were no significant deviations from Hardy-Weinberg equilibrium

	Ggu234	Mel1	Gg7-mini	Ma2-mini	Mvis075	Mvis1341	Mvis1354	Mean
Ν	24	24	24	24	24	24	18	23.14
а	2	3	4	3	2	3	3	2.86
Rs	2.0	2.99	3.69	3.0	2.0	2.75	3.0	
as	87-93	108-116	132-142	131-137	151-153	170-180	200-208	
Η _E	0.353	0.379	0.548	0.570	0.305	0.442	0.106	0.386
Ho	0.458	0.417	0.708	0.708	0.292	0.542	0.111	0.462
Hw	0.145	0.861	0.775	0.889	0.834	0.632	0.996	

4.4.2 Dietary study

A total of 517 scats were collected between March 2010 and August 2011. Four hundred of these were collected in the midlands (n = 9 sites) and 117 were collected in the eastern sites (n = 14 sites) (Figure 4.1). Overall, 86% of scats collected in the midlands tested positive for pine marten DNA compared to 39% in the east. As such, accuracy in the field was found to be significantly lower in the eastern region (p < 0.001, Fisher exact). The majority of scats were included in scat density calculations as distance walked was known (333 out of 344 and 38 out of 46 in midlands and eastern sites respectively). Thus scat density was estimated to be 1.745 scats per km in the midlands and 0.221 scats per km in the east. Although scat density was found to be higher in areas of higher pine marten occupancy, no statistically significant relationship was found between scat density and pine marten abundance. The scent detection dog succeeded in collecting seven pine marten scats from two of three woods visited over a two day period. When scats were categorised into red squirrel and grey squirrel sites (as per Chapter 2) a total of 329 were classified as coming from red squirrel positive sites, and 32 from grey squirrel positive sites (Table 4.1). Squirrels appeared in the diet as prey items at low frequencies during nine months of the year, spring and early summer being most common (Table 4.6).

Table 4.6 Squirrels as detected as prey items in molecular and macro analysis of pinemarten scats, including the date and site at which the scat was collected.GS = grey squirrel, Y = positive, N = negative (blank = not tested)

Species	Date	Site	Molecular	Macro
RS	2010-03-22	Abbeyleix	Y	
RS	2010-11-16	Clonad	Y	Ν
RS	2011-03-31	Charleville	Y	Y
RS	2011-04-01	Charleville	Y	
RS	2011-05-11	Charleville	Y	
RS	2011-06-07	Clonad	Y	Y
RS	2011-06-07	Clonad	Ν	Y
RS	2011-09-30	Charleville	Y	
GS	2011-10-01	Tomnafinnoge	Y	
GS	2012-02-03	Tomnafinnoge	Ν	Y
GS	2012-03-13	Tomnafinnoge	Y	Y
GS	2012-04-18	Tomnafinnoge	Ν	Y
GS	2012-05-18	Tomnafinnoge	Y	Ν

4.4.2.1 Molecular and macro analysis

Regression analysis found a linear relationship to exist between the %FO of mammalian prey items as detected by molecular and macro analyses ($\gamma = -1.694 + 1.969x$, $R^2 = 0.895$, p < 0.05) (Figure 4.3). The woodmouse featured more frequently in the diet than any other mammal species in both the molecular ($\chi^2 = 41.17$) and the macro ($\chi^2 = 67.58$) analyses (df = 4, p < 0.01) (Figure 4.4). Grey squirrels featured more frequently than red squirrels as prey items in both analyses, significantly so in the molecular analysis, despite the considerable difference in sample size (p < 0.05, Fisher exact).

When results from the reduced sample (n = 110) that was the subject of both molecular and macro analyses were combined, the %FO increased for all species, however there was very little effect on the low frequency prey items (red squirrel, bank vole and pygmy shrew). The %FO for woodmouse and grey squirrel increased more considerably when results from both methods were combined, but not significantly so (Figure 4.5). %BPI also found the woodmouse to be the most important prey species in the diet of the pine marten, followed by the grey squirrel where it was available, although c.30% of mammalian remains could not be identified to species level (Figure 4.6).



Figure 4.3 A linear relationship was found to exist between the frequency of occurrence of mammalian prey items as determined using molecular and macro analyses (y = -1.694 + 1.969x, $R^2 = 0.895$, p < 0.05)



Figure 4.4 Overall results for molecular and macro analysis of mammalian prey species in pine marten diet (n = number of pine marten scats tested where that item was available to the pine marten). Those subject to macro analysis are a random sub-sample of the molecular samples except the sample from the grey squirrel positive sites. The woodmouse features significantly more frequently than any other prey species (molecular and macro analysis) and macro analysis detected significantly more woodmouse in the diet than molecular analysis (p < 0.01, Fisher exact). Grey squirrels were more frequently detected than red squirrels as prey items (molecular analysis: p < 0.05, Fisher exact)



Figure 4.5 Comparison of results from both molecular and macro analysis of pine marten scats, where all samples were subjected to both techniques (n = sample size). Frequency of occurrence was higher for all species detected when results were combined, and significantly so for the woodmouse ($\chi 2 = 6.04$, df = 1, p < 0.05)



Figure 4.6 The percentage relative biomass (%BPI) of mammalian prey items ingested as determined by macro analysis of pine marten scats. WM = woodmouse, R = rat, BV = bank vole, PS = pygmy shrew, RS = red squirrel, GS = grey squirrel

4.5 Discussion

4.5.1 Pine marten abundance

This study has provided an index of abundance for the pine marten population in both their core range and a considerably less populated part of their range in the east of Ireland. Abundance values are not directly comparable to European studies where density values were obtained from radio-tracking or snow tracking e.g. Zalewski and Jedrzejewski (2006) and Mergey et al.(2011), but are comparable to Irish studies that have used a combination of hair trapping and live-trapping to determine population density estimates. The abundance values obtained in this study suggest that the population in the midlands of Ireland (mean value of 3.13 adult residents per km²) is currently living at a higher density than previously reported for the species in Europe (0.01 to 1.75 per km²) or Ireland (0.5 to 2 per km²) and thus quite possibly represents the highest density in their natural range. It is unclear why the European pine marten reaches these relatively high numbers in Ireland, particularly when their favoured habitat is so sparse and fragmented. In a review of available literature on European pine marten densities, Zalewski and Jedrzejewski (2006) found that between 41° and 68°N densities declined exponentially with decreasing winter temperature and increasing seasonality, and suggested that both winter severity and availability of rodents are limiting factors on populations. Thus it is possible that Ireland's relative lack of seasonality and mild winters (the moderating influence of the Atlantic gulf stream results in mean minimum winter temperatures of 2 to 6°C) (MetEireann website) contribute to the observed high pine marten abundance. Other contributory factors may include lack of competition and lack of predators, with the red fox (*Vulpes vulpes*) representing the pine marten's only real competitor or predator in Ireland.

Zalewski and Jedrzejewski (2006) estimated that 2 km² is the minimum area of forested habitat necessary to support an adult pine marten in the temperate forest zone. However, this does not appear to apply to the core Irish pine marten population and both midlands sites, Charleville (1.13 km²) and Clonad (1.43 km²), sustain relatively high pine marten numbers in comparison to other extensively forested parts of Europe (Zalewski and Jedrzejewski 2006). The European pine marten has traditionally been considered a forest specialist, however recent studies have found the species to be less restricted to large forests than previously believed and highlighted the importance of the surrounding landscape not only in providing habitat corridors but also in providing essential food resources and den sites throughout fragmented landscapes (Clevenger 1994; Pereboom et al. 2008; Mergey et al. 2011; Caryl et al. 2012a). In the current study, hair traps were only placed within the forested habitat and thus no data was obtained on the use of the surrounding landscape, or in the case of site 5, the adjacent forestry. Abundance values per km² are thus only applicable to forested area as use of surrounding landscape is not accounted for with this sampling technique. The fact that only one animal was detected both in sites 1 and 2 despite their close proximity to one

another, supports the theory that these small woodlands can be considered as relatively discrete in terms of pine marten occupancy, however it is likely that surrounding non-forested landscape is also used to some extent.

4.5.2 Non-invasive techniques

When compared to other methods of determining pine marten density, such as scat transects (O'Mahony et al. 2006), snow-tracking (e.g. Zalewski 1999) (not feasible in Ireland), camera-trapping (e.g. Manzo et al. 2012) and live trapping, there are certain benefits and limitations to genetic tagging through hair samples. Genetic tagging does not provide specific biological information such as weight, reproductive activity and condition, nor does it provide detailed spatial information on home range size or territoriality which is achieved through radio-tracking. It is however the only non-invasive method that confidently differentiates between individual animals. The data collection process was non intrusive to the animal and time-efficient in terms of data collection. The hair traps themselves are inexpensive to construct and maintain. However it is clear from our data that hair trap density should be increased to optimise information gathered on the population. In the midlands sites there were more individuals detected per site over the study period than there were hair traps available per month; in site 1, six pine marten were detected using five hair traps and in site 2, ten pine marten were detected with as few as four hair traps (over the course of the sampling period, hair traps were persistently stolen from this site). It is possible that this resulted in an underestimation of the abundance value at this site as the number of hair traps available was not sufficient to give each individual present the opportunity to use a hair trap each month, in particular during the winter months when residency was being determined.

The proportion of hair samples collected that were successfully genotyped (66%) could also be improved in future studies. A relationship has been found to exist between the number of hairs in a sample, and the probability it will be successfully genotyped (Mowat and Paetkau 2002). In the majority of hair trapping events in

the current study more than 10 hairs were captured, thus providing a relatively high amount of DNA. However, the samples were left in situ for a period of up to one month, which may have caused the DNA to degrade due to relatively high ambient temperatures and humidity. Lynch et al. (2006) suggested a survey period of six days is sufficient to detect pine marten presence (in lowland broadleaf woods), so the hair samples could alternatively be collected approximately one week after hair traps are set. Screening the quantity of nuclear DNA in the samples using the sex typing assay prior to genotyping helped to increase the genotyping success (95%) as the samples that were deemed to have insufficient high quality DNA did not proceed to the genotyping stage. This also helped reduce the overall cost and this technique combined with shorter sampling periods could substantially help improve the overall success rate in future studies.

The overall number of alleles and levels of heterozygosity in this study were very low. Mullins et al. (2010) also recorded low levels of genetic diversity in the Irish pine marten population, and this could be due to the low number of individuals that the current pine marten population have re-established themselves from. Furthermore, there has only been one mitochondrial DNA haplotype found in the contemporary Irish population (Jordan *et al.* 2012). The long-term effects of such low levels of genetic diversity in an expanding population are not known. However, the low diversity found in this study may also be partially due to the microsatellites used, as they were originally developed for use with other mustelids. This was also discussed as a reason for lower levels of genetic variability in the Iberian pine marten population by Ruiz-González et al. (2013b).

An alternative form of quality screening to the method used in the current study involves preliminary analysis with a sub-group of microsatellites, as was undertaken by Ruiz-González et al. (2013). Samples that amplified well (>50 % positive PCRs) with the sub-group were then taken to the next stage of analysis. The approach taken in the current study may be more useful as data not used for genotyping at least provides further information on species and sex. The pre selection of DNA samples for genotyping removes samples that are unlikely to replicate or may cause

Chapter Four

a higher occurrence of genotyping error (Zhan *et al.* 2010) and thus may be more efficient.

Low genotyping error rates were reported in this study, and were at the lower end of the level of error when compared to other non invasive genetic studies using DNA extracted from hair (Broquet *et al.* 2007). Genotyping errors are easier to control and account for in small studies with fewer samples (Zhan *et al.* 2010). Mullins et al. (2010), also working with a relatively small dataset, similarly reported low genotyping errors. The high number of recaptures reported in the current study further supports the low occurrence of genotyping errors, and helps to validate the genotyping results. If an erroneous individual had been detected within the dataset, this individual would not affect the overall abundance estimates, as only animals identified within a site on at least three separate months were included in the abundance estimates.

Future studies could investigate the potential of using faecal DNA for genotyping in similar studies (see Ruiz-Gonzalez et al. 2013). Genotyping errors are more likely to arise with faecal derived DNA however, and approximately only 50% of samples can be successfully genotyped. There is also a higher risk of contamination and more replicates are needed in order to be sure that the genotyping results are correct (Ruiz-González *et al.* 2013b and references within). The genotyping screening approach taken in this study could be applied to a faecal DNA genotyping study in the future, as it could be used to select the faecal DNA samples with the highest quantity of nuclear DNA, and that are more likely to amplify. A possible benefit of using a combination of faecal and hair DNA to sample the population, may be the opportunity to identify a higher portion of the true population. It is possible that some animals may avoid using hair traps and that other animals may not mark their territory as widely or actively as others. Thus it is possible that more accurate density estimates might be gained through combination studies, and the feasibility of using both techniques together should be investigated in the future.

4.5.3 Scat density

There are inherent problems with surveying for pine marten scats in areas of low population density (Birks *et al.* 2005); most notably misidentification of scats in the field, even by experienced surveyors. This problem is addressed in modern surveys by the use of genetic tests to confirm pine marten origin (O'Reilly *et al.* 2008; Balestrieri *et al.* 2011; Caryl *et al.* 2012b). The current study found that in the east of Ireland, where pine marten abundance is lower, of those scats that were collected, significantly fewer were confirmed as pine marten than those collected in the midlands, where abundance is higher. A factor that is likely to have contributed to this result is the likelihood that the surveyor was less discriminate about which scats were collected in the lower scat density sites. When scats are scarce in the field, any opportunity to collect a potential scat is likely to be taken, even if the scat has been weathered and has lost the often distinctive pine marten odour, or the ability to extract amplifiable DNA. In areas where scats are more abundant, key features such as smell and shape are taken into account, and the surveyor is likely to be more critical regarding the quality of the scat collected for the survey.

Furthermore, in areas of low pine marten population density, territorial scent marking behaviour may be greatly reduced (Macdonald *et al.* 1998). Lockie (1964) was the first to suggest that a relationship exists between the number of scats and pine marten abundance, however in a review of nine previous scat surveys in the UK and Spain, Birks et al. (2005) found that the field relationship between scat abundance on transects and marten numbers was yet to be established. Indeed, whilst the current study found scat density to be higher in areas of higher pine marten abundance, regression analysis failed to define this possible relationship.

Scent detection dogs are increasingly being used in the study of elusive carnivores (Smith *et al.* 2003; Long *et al.* 2007; Reed *et al.* 2011), and have been found to have a superior detection rate to that of humans. In the current study, the scent detection dog was used over a two day period, and succeeded in collecting a total of eleven scats, seven of which were confirmed as pine marten through molecular

analysis. Those that tested negative for pine marten DNA also tested negative for fox DNA (the species that pine marten scat is most likely to be misidentified as in Ireland), which suggests that the quality of the DNA in those samples was too degraded for genetic species identification. As such, it is not possible to determine whether the scats detected by the dog that tested negative for pine marten DNA were true or false negatives. Only one pine marten scat was detected during the two day survey without the aid of the dog, suggesting that the use of scent detection dogs in areas of low pine marten and low scat density can greatly improve sampling efficiency.

4.5.4 Dietary analysis

The absence of grey squirrel in the diet of the pine marten in the midlands most likely reflects their lack of availability as a prey item (Carey et al. 2007; Chapter 2). Molecular and macro analysis produced an overall frequency of occurrence for grey squirrel of 9.4% and 10% respectively in sites where grey squirrels are known to be present, that increased to 15.6% when results from molecular and macro analysis were combined. The relative biomass of grey squirrels gave a similar estimate (13% BPI). These figures are based upon a relatively small sample size however, and must be interpreted with caution as small sample sizes can cause a prey item to be either under or over represented in dietary analysis (Trites and Joy 2005). However, they do confirm that the North American grey squirrel forms part of the European pine marten diet when the two species' ranges overlap. Throughout the course of the current study, the grey squirrel was only confirmed as an available prey item in areas of low scat density, which made scat collection for dietary analysis in these areas very challenging. A larger sample size of scats, perhaps collected with the aid of a scent detection dog, will allow for a more robust dietary analysis in areas of low density, and will thus be useful in determining differences in pine marten feeding ecology between areas of high and low density, as well as predation on the alien grey squirrel. At low numbers it is unlikely that a pine marten population could significantly impact grey squirrel numbers by predation,

and it remains to be established what impact, if any, an increase in pine marten numbers will have on the grey squirrel population in the east. In contrast, the sample size of scats collected where red squirrels were confirmed as present was adequate to detect with confidence the frequency at which the red squirrel occurs in the diet. Red squirrels and pine marten have co-existed in Ireland and many other parts of Europe over many millennia, and the red squirrel has also appeared only as a very low frequency prey item in previous Irish pine marten dietary studies (Warner and O'Sullivan 1982; Lynch and McCann 2007; O'Meara *et al.* 2013). Red squirrels have been recorded at higher frequencies in Russia and Sweden, however, where other small mammal prey are less abundant (De Marinis and Masetti 1995). Red squirrels live at lower densities than grey squirrels (Gurnell 1987) and would therefore be numerically less available as prey items. They are also lighter than the grey squirrel, capable of reaching the outermost branches of trees, and spend the vast majority of their foraging time in the canopy, whereas grey squirrels spend around 85% of their foraging time on the ground (Kenward and Tonkin 1986).

In this study, both molecular and macro analyses detected prey species in similar proportions, therefore molecular techniques can be accepted as a reliable method to detect mammals as prey items in pine marten diet. This is a useful tool in determining the small mammal composition of carnivore diet and also the spread (and possible decline) of both invasive and native mammal species in Ireland. However the macro analysis was significantly more sensitive in the detection of the woodmouse, which was the most frequently consumed mammal in the diet. As such it is recommended that any study aiming to determine more exact frequencies of a species in the diet (as distinct from determining prey species presence or absence) be validated with traditional hard part (macro) analysis. In this study, a standard DNA extraction for both the species and dietary analysis was used (a cost effective strategy). However, to improve the molecular dietary detection of prey DNA, future molecular studies might increase the detection rate by sampling a larger amount of scat, extracting multiple samples from the same scat, or homogenising the scat prior to DNA extraction (see King et al. 2008). As expected, the greater white toothed shrew was not detected as a prey item in the subsample

examined, as scats were collected outside the described range of the species in Ireland (Montgomery *et al.* 2012). Similarly, the bank vole was found at a very low frequency and only in site 2, which is located at the edge of their current range (Montgomery *et al.* 2012). The woodmouse was found to occur in 31.8% of scats tested , which is similar to frequencies found in Northern Spain and Tuscany (De Marinis and Masetti 1995). Previous studies in Ireland have found the woodmouse to occur at around 13% frequency (Lynch and McCann 2007) and 14.7% (O'Meara *et al.* 2013) in pine marten scats. In Scotland pine marten have been found to show a seasonal preference, regardless of abundance, for field voles (Caryl *et al.* 2012b), a species which is not available to the Irish pine marten.

Biomass or %BPI values could be better estimated for both macro and molecular analyses if feeding trials were conducted with captive pine marten to determine the appropriate correction factors for (a) the detection rates of the various mammalian prey species DNA after known amounts have been consumed and (b) the relationship between weight of dried remains and fresh weight ingested for red and grey squirrels as distinct from each other and from the 'small mammal' grouping.

4.6 Conclusion

As expected, this study confirms that the grey squirrel does not feature in the pine marten diet in the midlands of Ireland, which is most likely explained by their rarity as a prey species in the region. In Ireland and Scotland it has been speculated that the pine marten population has inhibited the grey squirrel population from spreading, and has even caused the grey squirrel population to crash in areas where they were once established (Carey *et al.* 2007; Caryl 2008; Paterson and Skipper 2008). No grey squirrel control measures had been carried out in any of the Irish midlands sites surveyed since the 1990's, therefore human management of the alien squirrel population is not an explanatory factor in their rarity Habitat is not a factor either, as red squirrel populations are found in the woodland, as until relatively recently were high numbers of grey squirrels. Whether predation was a factor in the retraction of the grey squirrel range historically is not possible to determine in retrospect, but evidence of predation on the alien squirrel species in the east confirms that the pine marten will indeed prey upon the grey squirrel, where it is available. This study introduces the possibility that there could be some form of density dependent avoidance behaviour taking place on the part of the normally invasive grey squirrel, where areas of high predator abundance might be discouraging the grey squirrel from remaining in, or establishing in a woodland in the first place. The possibility that pine marten density may be influencing squirrel distribution warrants further investigation.

Future studies on the pine marten population in Ireland should also address the lack of information on home range size and territoriality. In particular, differences in home range sizes and territoriality between the core population in the midlands, and the less abundant population in the east, will reveal density related differences in spatial ecology. What effect an increase in pine marten numbers in the east of Ireland will have on grey squirrel distribution and abundance merits further investigation as a matter of priority, as such information will be vital in order to establish the relationship between the native squirrel predator and the invasive squirrel species.

5. Grey squirrel sensitivity to predator odour

5.1 Introduction

The European pine marten is a known predator of the Eurasian red squirrel. Indeed the range of the pine marten overlaps almost entirely with that of the western portion of the red squirrel's range (Figure 5.1). In Ireland, several studies on pine marten diet have been carried out in areas where the red squirrel is present as a prey item. The percentage frequency of occurrence (%FO) in the diet has ranged from 0.1 to 2.4% (Warner and O'Sullivan 1982; Lynch and McCann 2007; O'Meara *et al.* 2013, see also Chapter 4). In Scotland %FO of red squirrel in pine marten diet has been found to range from 0 to 0.8% (Balharry 1993; Gurnell and Lurz 1997; Caryl *et al.* 2012b). In Spain and Switzerland the red squirrel has been found to occur at a higher frequency in Sweden (0 – 51%) and Russia (1.7 – 48.8%) (Yurgenson 1947) where other small mammal prey species are less abundant (De Marinis and Masetti 1995).

In contrast, little is known about marten predation on the North American grey squirrel. The natural range of the grey squirrel in North America (where it is known as the Eastern gray squirrel) only overlaps with that of the American marten (*Martes americana*) and fisher (*Martes penanti*) in a very small part of its north eastern and north western natural range limits (Figure 5.2). As a result of this limited range overlap, few dietary studies have been carried out in areas of grey squirrel sympatry with either marten species. A study in Michigan on marten trapping seasons) found the grey squirrel to occur in 17% of 151 GI tracts examined (Hales *et al.* 2008). A study on fisher diet in Maine (n = 68 scats collected between December and March) found the grey squirrel occurred at a 16% FO in the diet (Arthur *et al.* 1989). The lack of further evidence of the grey squirrel in either

marten or fisher diet likely results from their lack of availability as a prey item in much of the American marten species ranges.





Figure 5.1 The natural ranges of (a) the European pine marten and (b) the Eurasian red squirrel. The range of the European pine marten overlaps extensively with that of the Eurasian red squirrel.



Figure 5.2 The natural ranges of (a) the fisher (b) the American marten and (c) the grey squirrel in North America and Canada. The natural range of the grey squirrel overlaps only in a small part of its north eastern and north western limit with those of the marten species

Whilst both the American and the European pine marten prey upon sympatric squirrel species e.g. in America the pine squirrel, *Tamiasciurus hudsonicus*, Douglas squirrel, Tamiasciurus douglasii, and flying squirrel, Glaucomys sabrinus, and in Europe the red squirrel (Sciurus vulgaris), there is no evidence in the literature of martens being a limiting force on tree or flying squirrel populations (Gurnell 1987; Smith 2007). Indeed the agility, the feeding habits and the densities at which these squirrels typically exist (pine squirrel: 0.3 – 2.ha⁻¹; Douglas squirrel: 0.2 – 0.5.ha⁻¹; flying squirrel: 0.1 - 3.3.ha⁻¹; red squirrel: 0.3 - 1.5.ha⁻¹) (Kenward and Tonkin 1986; Gurnell 1987; Steele 1998, 1999; Smith 2007) are likely to lead to low vulnerability to predation by martens. These aspects of their ecology are likely to be a result of their habitat adaptations to mainly coniferous forests in North America, Canada and Eurasia, and may not necessarily represent predator avoidance techniques. Nonetheless, as a result of their typical population densities and their habitat preferences tree squirrels such as the Eurasian red squirrel and the American *Tamiascirus* spp.are suitably adapted as elusive, low frequency marten prey species and have a history of successsful sympatry with the European and American martens.

In contrast, the grey squirrel typically lives at relatively high densities $(2 - 16.ha^{-1})$ (Gurnell 1987) and spends the majority of its foraging time on the ground (Kenward and Tonkin 1986). Therefore they should be theoretically more vulnerable to predation by martens. However, they exist in and are adapted to the hardwood forests of eastern North America, where martens are all but absent. This lack of exposure to marten species may have resulted in grey squirrels being 'predator naive' to martens in terms of evolution. Prey species have been found to show specific adaptations to predators that allow recognition, avoidance and defence against predator derived odours (Apfelbach *et al.* 2005). Prey defence mechanisms can be described as primary (that is they operate on a continuing or chronic basis e.g. animals that live in herds) or secondary (they come into effect when a predator is actually present e.g. flight response in ungulates). Along with visual and sound cues, predator odour can trigger primary and secondary defensive

response behaviours including inhibition of activity or alterations in activity patterns (e.g. Fenn and MacDonald 1995), a reduction in non-defensive behaviours such as foraging, grooming and reproduction (e.g. Koivisto and Pusenius 2003) and shifts in habitat to locations or habitat types where the risk of predation is reduced (e.g. Dickman 1992). It is possible that predator naivety could manifest in a lack of sensitivity in the grey squirrel to marten odour as that of a potential predator's.

Extensive experiments on mammalian prey species response to predator odour, in particular rodents, have been carried out both in field and laboratory environments. The effects tested have included behaviour (e.g. Jędrzejewski *et al.* 1993), trapping rate (e.g. Dickman 1992), feeding rate (e.g. Koivisto and Pusenius 2003), space use (e.g. Dickman 1992), endocrinology and reproduction (e.g. Zhang *et al.* 2003; Ylönen and Ronkainen 1994).

Although grey squirrel response to the threat of predation by martens has not been studied, their assumingly innate behaviour in response to predation risk in general has been investigated in several studies to date. In studies investigating grey squirrel foraging behaviour in relation to predation risk Lima *et al.* (1985; Lima and Valone 1986) established that a trade-off exists between foraging efficiency and predation risk, such that at a given distance from cover a grey squirrel is more likely to carry larger food items back to cover before consumption. Paradoxically, for any given food item size, a squirrel was less likely to carry it with increasing distance to cover. Thus a trade-off was found to exist between foraging efficiency and predation risk. It was logically assumed that risk of predation increased with increasing distance from cover (i.e. a tree) and predators present in the study site were domestic dog, cat and one red-tailed hawk (*Buteo jamaicensis*) although no incidences of predation were observed.

The influence of distance to refuge on flight initiation distance in the grey squirrel has also been investigated. Dill and Houtman (1989) found that flight initiation distance in response to a predator (in this case a motorized model cat) increased as distance to refuge increased. Similarly to the former study, distance to refuge was defined as distance to the nearest tree. Vigilance during food handling has also

been investigated in the grey squirrel (Makowska and Kramer 2007), where it was found that grey squirrels will shift their position in order to enhance vigilance whilst handling large food items but not when handling smaller, low-energy food items. This suggests that vigilance may be sacrificed if it leads to relatively high foraging costs.

However, just one study has investigated the potential effects of predator odour on grey squirrel behaviour. Rosell (2001) tested the foraging behaviour (consumption of butternuts) in a population of grey squirrels in an urban green space (cemetery) in New York. The experiment involved placing cotton squares with predator and control scent (urine) in close proximity to butternuts in a number of experimental feeding patches. The predator odours utilised for the experiment were those of the species known to predate on grey squirrel that were present in the study site: red fox (*Vulpes vulpes*), raccoon (*Procyon lotor*) and human. Both red fox and raccoon odours resulted in a significant decrease in foraging (consumption of butternuts) when compared to non-predator control treatments. The red fox, *Vulpes vulpes*, is known to opportunistically predate upon the grey squirrel in its native and introduced range (Cook and Hamilton 1944; Hockman and Chapman 1983; Doncaster *et al.* 1990), however the frequency of occurrence in the diet is low and red fox abundance is not considered an influencing factor in grey squirrel distribution.

The current study also focuses on predator odour in particular. Pine marten scent marking behaviour (in terms of prominent deposition of scats) has been found to be higher in areas of higher population abundance (Lockie 1964; Birks *et al.* 2005; Chapter 4) although the relationship between scat density and pine marten density has yet to be established (Birks *et al.* 2005, see also Chapter 4). Pine marten and grey squirrel detectability in Irish woodlands have been found to be negatively correlated (see Chapter 2) and it is thought that pine marten abundance may be influencing the grey squirrel's success or failure to establish or remain established in Irish woodlands. However, no causal mechanisms have been investigated to date.

Methods

A concurrent pine marten population study (see Chapter 4) confirmed through DNA analysis that 1 female pine marten was present in the study site for the duration of the study, with a second marten being detected in the final month, and five out of twenty pine marten scats collected contained either grey squirrel hair or DNA. One non-pine marten scat was confirmed through DNA analysis to be of fox origin, and this also contained grey squirrel DNA. Therefore both predator species were known to be present in the study site at low densities and were known to be preying upon the squirrel population.

5.2 Aims

The aim of this study is to determine the sensitivity of the North American grey squirrel to European pine marten odour, in a population of grey squirrels in their introduced range in the east of Ireland by testing the trapability of grey squirrels in relation to pine marten odour. Three possible outcomes are predicted: 1. The grey squirrel exhibits the same reaction to pine marten scent as it does to that of the red fox odour (null hypothesis). 2. The grey squirrel exhibits less sensitivity to pine marten odour than it does to fox odour as it does not recognise it as a potential predator. 3. The grey squirrel exhibits more sensitivity to pine marten odour than it does to that of the fox.

5.3 Methods

5.3.1 Field Methods

The study took place in the east of Ireland in Tomnafinnoge wood, Tinahely, Co. Wicklow, which is described in detail in Chapter 3. Grey squirrels were first reported here in the late 1990's and have now entirely replaced the red squirrel population (see Chapter 3). Both pine marten and fox were confirmed as present at the site in low densities.

40 traps were installed at a density of 2 ha⁻¹ in two adjacent trapping grids, which were each divided into two contiguous grids containing ten traps each (Figure 5.3). Trapping was conducted as described in Chapter 3. Each trapping session was 8 days long (with the exception of two 7 day sessions). Traps were pre baited for 4 days and set for 4 days at sunrise (but no earlier than 6am) and then checked after a minimum period of 4 hours.



Figure 5.3 Locations of squirrel traps (n = 40) in Tomnafinnoge. Traps were positioned at a density of 2.ha-1 in two adjacent grids which were further subdivided into contiguous grids for the purpose of the experimental odour treatments.

A total of 7 trapping sessions were carried out in Tomnafinnoge, on a monthly basis from October 2011 to May 2012, with the exception of December 2011. In February 2012 (the fourth trapping month), various odour treatments were introduced to the traps. Cotton filters containing the respective odours were placed in small capsules (with multiple piercings that allowed the odour to escape) and attached securely approximately midway along the inner base of the cage trap, in front of the treadle. The four odour treatments were pine marten, red fox, vegetable oil and no odour (no capsule). In order to reduce potential temporal or spatial bias in capture rate, each grid was assigned a particular odour per trapping session, such that over the four sessions each grid contained a different odour treatment. Fresh odours were placed in traps on each trapping day so that the potency was consistent throughout the trapping session. No odours were used in the pre-baiting period.

Red fox odour used was 100% natural fox urine, purchased from Kishels Quality Animal Scents and Lures Inc. (East Aurora, NY 14052). Pine marten odour was obtained by expressing the content of anal scent glands that were removed from roadkill animals. Standard vegetable oil was used as a neutral control.

5.3.2 Statistical Analysis

A capture event was defined as the capture of the target species i.e. a grey squirrel. On the occasion that traps were missing (stolen) or in disrepair and therefore not set, the number of available traps for statistical analysis was reduced accordingly. On the occasion that a trap was mis-triggered or captured a non-target species, this was estimated to have occurred on average half way through the study period, and so the number of available traps was calculated daily for each grid as (total number of traps set) – (no. mis-triggered or used by non-target species/2).

The capture rates from the first trapping month were excluded from statistical analysis as the first trapping month is expected to have a lower capture rate than
any proceeding trapping months. A capture rate was calculated per day for each trapping grid from November to May. Further analysis was then carried out for the experimental months of February to May. Capture rates per odour treatment (between February and May) were compared individually and then grouped as

either treated with predator odour (i.e. fox or pine marten odour) or not treated with predator odour (i.e. vegetable oil or no odour).

Comparisons were carried out using one way ANOVAs to test for significant variation in capture rate between trapping days, between trapping sessions and between treatments. Statistical analysis was carried out using IBM SPSS Statistics 20 software.

5.4 Results

A total of 71 grey squirrels were captured over the course of the study, during 372 trapping events. The number of squirrels captured during each trapping session for each odour treatment is listed in Table 5.1.

One way ANOVAs found no significant variation in capture rate between trapping days (F = 0.6, p = 0.93), trapping sessions (F = 1.883, p = 0.21), trapping grids (F = 1.258, p = 0.226) or individual odour treatments (F = 1.463, p = 0.110) for the November to May period. Nor was there any significance when odour treatments were grouped into predator and non-predator treatments during the February to May period (F = 1.04, p = 0.6) (Figure 5.4). Capture rate (per grid day) ranged from 0 to 1 with a mean value of 0.39 (\pm 0.04, 95% CI, n = 104). Capture rate for the predator odour treatment group (Feb – May) ranged from 0.11 to 0.9 (\bar{x} = 0.4649 \pm 0.111, 95% CI, n = 14) and capture rate for the non-predator odour treatment group (Feb – May) ranged from 0.22 to 0.7 (\bar{x} = 0.4645 \pm 0.0848, 95% CI, n = 14).

Table 5.1 The number of traps available during each monthly trapping session for each odour treatment and the corresponding number of squirrelscaptured

Treatment	None		Vegetable		Pine Marten		Fox	
	Traps	Captures	Traps	Captures	Traps	Captures	Traps	Captures
Oct	158	22						
Nov	160	58						
Jan	139	59						
Feb	25	7	27	7	27	8	26.5	10
Mar	20	10	20	6	23	10	22	8
Apr	39	20	38.5	22	38	20	39.5	18
May	32.5	21	38.5	20	39.5	22	39	24
Total	116.5	58	124	55	127.5	60	127	60

Results

As expected, the lowest capture rate was observed in October. However, when October was excluded, the lowest capture rate per treatment group was observed on the first trapping day of February, when predator odours were first introduced (both pine marten and fox odour treated grids had 1 out of 9 traps used each i.e. a 0.11 capture rate). The second lowest was observed on the first trapping day of March (the pine marten odour treated grid had 2 out of 7.5 traps used and the fox odour treated grid had 1 out of 7 traps used, i.e. a capture rate of 0.2). The highest capture rate (0.9) was recorded on day 3 in May, when traps were left open for approximately twice the usual duration. The outliers are displayed in Figure 5.4. Figure 5.5 shows the monthly variation in capture rates across treatment groups.



Figure 5.4 The minimum, maximum, median, mean (\bar{x}) and inter-quartile range values for (a) all capture rates between November and May (b) predator odour treated grids (Feb – May) and (c) non-predator odour treated grids (Feb – May). One way ANOVAs found no significant variation between capture rates.



Figure 5.5 Variation in grey squirrel capture rate over the course of the study. D = trap day, Y = predator treated trapping grids (fox, pine marten), N = non-predator treated trapping grids (vegetable oil, no odour). Excluding the first trapping session, the lowest capture rate was recorded at the introduction of predator odour on trap day 1 in February, followed by the second lowest capture rate on trap day 1 in March.

5.4 Discussion

No statistically significant variation in trapability was observed throughout the study i.e. the overall trapability of the grey squirrel population was not affected by the presence of pine marten or fox odour in the squirrel traps. However, there was a qualitative sensitivity observed on the first day that predator odour was introduced. This was represented by the fact that the lowest extreme in capture rates measured (out of 22 days that trapping took place) occurred on the very first day that predator odours were introduced to the traps. This sensitivity then waned over the proceeding days as capture rates returned to normal values. The second lowest capture rate was then observed on the first day of trapping in the second experimental month, but again, capture rates returned to normal by the next day.

There are a number of possible explanations for this outcome. Firstly, it is possible that an initial sensitivity to a familiar predator odour that was detected waned due to a perceived low threat of actual predation. Although both the red fox and the pine marten were both present in the study site, their densities were considered to be very low. Indeed there was just one pine marten detected in the site on a monthly basis between October and April, with a second animal detected in May. The pine marten was only detected during monthly sampling sessions in one section of the wood and it is possible that whilst Tomnafinnoge was within the marten's territory, she may not have been residing there or indeed using all of the woodland, and hunting may not have taken place on a regular basis. Furthermore, very few pine marten scats were collected from Tomnafinnoge (n = 20) over the course of the study as obvious scent marking with faecal deposits was not taking place. Territorial scent marking in marten populations may be greatly reduced when densities are very low (Macdonald et al. 1998). Fox density was assumed to be very low as likewise very few scats were collected. This may explain the fact that whilst the grey squirrel was sensitive to the odour of both predators that

frequented the wood, they did not show a strong aversion to their odour as the perceived threat of predation was low.

In many prey species, the perceived risk of predation is evaluated by the actual presence of the predator, or indeed by the actual act of predation (e.g. Fraser and Huntingford 1986). Previous studies on the house mouse, Mus domesticus, in Western Australia (Dickman 1992) found that there was little avoidance of either fox, Vulpes vulpes, or cat, Felix catus, in areas that were devoid of the predators. However, in the areas which the predator species were present, the mice avoided the predator odour treated traps and showed increased avoidance behaviour (habitat shift) when predator faeces density was increased experimentally. This indicates that the perceived risk of predation increased with signs of increased predator density (Dickman 1992). Prey species have also been found to discriminate between different predator species, such that the odour of one predator can invoke a stronger reaction than that of another. In the same study, Dickman found that the house mouse avoided cat and fox odour but not that of the western quoll, Dasyurus geoffroii. This was most likely explained by the fact that the western quoll tends to defecate in latrines, as opposed to the fox and cat which defecate frequently on paths along which they travel. Indeed foxes scent mark paths with urine and faeces in proportion to the frequency with which they travel them (MacDonald 1980). Therefore it should be selectively advantageous to avoid areas of heavy scent marking in these species, but less so in those of latrine users like the quoll. Korpimaki et al. (1996) found that the field vole, Microtus agrestis, which is an important prey item for both the least weasel, *Mustela nivalis*, and the kestrel, Falco tinnunculus, perceived the kestrel as a greater risk than the weasel, as demonstrated by preferential avoidance of kestrel foraging habitat.

Secondly, despite the grey squirrel's lack of exposure to the European pine marten (and to a large degree the American marten), the initial sensitivity observed in the current study may also have been a reaction to a general predator odour, rather than a specific reaction to the pine marten and fox treated traps. Many prey

species have shown responsiveness to scents from both sympatric and allopatric predator species, which suggests that there may be an innate reaction to a signal which is common in carnivores (Apfelbach *et al.* 2005). Nolte *et al.* (1994) hypothesised that the amount of meat in coyote diet would influence the effectiveness of coyote urine in repelling mountain beavers. The results suggested that diet composition and sulphurous metabolites of meat are important in repellency to prey species. It is not possible to deduce from the current study whether the grey squirrel's initial sensitivity to pine marten odour is an innate response to a general predator odour or a learned response to an active (albeit in low density) predator, due to the pine marten's presence in the study site, however should the experiment be replicated at a future time when pine marten numbers have increased, it may be possible to determine whether sensitivity or avoidance behaviour increases in relation to marten density i.e. with increased risk of predation.

Thirdly, in order to achieve a stronger response to the odour of fox and pine marten in the experiment, stronger odour concentrations may have been required. Takehashi *et al.* (2005) found that the quantity of cat odour influenced the degree to which rodents displayed avoidance behaviours. Indeed Burwash *et al.* (1998a, b) found that the roof rat, *Rattus rattus*, was sensitive to both mongoose, *Herpestes auropunctatus*, faeces and synthetic predator odours in laboratory trials, but failed to illicit a significant response in field trials in Hawaiian orchards. One of the proposed explanations by the authors for the lack of response in the field experiment was improper methodology to detect the response or effectiveness of the odour release devices used. Similarly, in the current study, there was no attempt to determine prior to the field experiment that the quantity of, or method of deploying, the predator odours was sufficient to induce a response from prey animals.

5.5 Conclusion

Although there was no significant variation in capture rate between traps that contained odour of pine marten, fox or non-predator odours, an initial sensitivity to the odour of both the pine marten and the fox was detected in the grey squirrel population at Tomnafinnoge. This may be due to the similarly low densities of both pine marten and fox in the study site, where as such the risk of predation by both species is perceived to be low. Alternatively, the squirrels may have been responding to a general predator odour, in which case recognition of or discrimination between predator species was not occurring in the first place. It is also possible that the weak response was an artefact of insufficient quantities of predator odours being used.

In theory, if pine marten and fox numbers were to increase at Tomnafinnoge, grey squirrel sensitivity to, or avoidance of, predator odour may increase more so for one predator than the other in accordance with perceived risk of predation. In order to test this experimentally in a field study, it would be necessary to locate a site where pine marten, fox and grey squirrel are all relatively abundant. However, to date, a strong negative correlation has been found to exist between the presence of pine marten and grey squirrel at woodland level (see Chapter 2). Thus finding a suitable study site for such an experiment may not be possible.

6 General Discussion

6.1 Population crashes in formerly invasive species

The results from the extensive sightings and field surveys (Chapter 2) provide unequivocal evidence that the typically invasive grey squirrel population has indeed retracted from the midlands region of Ireland. This is the first documented evidence of a grey squirrel population retracting, without any human intervention, subsequent to having established itself as an invasive species. In a review of population crashes of established introduced species, Simberloff and Gibbons (2004) define a "population crash" as one in which numbers or densities were believed to have fallen by at least 90% in less than 30 years. Under this criterion, the grey squirrel population crash" based on a density of 0.035 squirrels.ha⁻¹ (compared to a control population density in the east of at least 1 squirrel.ha⁻¹). However, as the current study found the population in the east of the country to be doing well, and a recent sightings and hairtube survey has confirmed that the grey squirrel continues to spread into the south and south west of the country (Goldstein *et al.* in review), the population crash must also be described as regional.

In their review, Simberloff and Gibbons found that such population crashes are not a common phenomenon, they have rarely been studied experimentally or quantitatively, and the causal factors often remain unidentified. Furthermore whilst quantitative data documenting perceived declines were exceedingly scarce, even more so was evidence as to how or why such population crashes came about, as the majority of proposed explanations were simply *ad hoc* suggestions with no actual supporting evidence. Simberloff and Gibbons discussed seventeen examples including invasive vertebrate, invertebrate and plant species' decline. Causal factors were suggested including competition with (subsequently) introduced species, parasitism by subsequently introduced species, exhaustion of resource, and

one case of adaptation by a native herbivore, however in the majority of cases the cause remained entirely unknown.

In the case of the grey squirrel's regional decline, certain theories can be ruled out with certainty, for example, competition with a subsequently introduced species. In Ireland the only species with which there is any significant competition for resources is the native red squirrel. All research to date on the population dynamics of these two squirrel species confirms that under normal circumstances, the grey squirrel is a superior competitor and inevitably outcompetes the red squirrel for resources (MacKinnon 1978; Wauters *et al.* 2000; Gurnell *et al.* 2004).

Squirrel numbers in any given year have been found to be closely linked with the previous year's tree seed crops (Gurnell 1983), and population densities fluctuate on a yearly basis in accordance with both tree seed crops and winter temperatures (Gurnell 1996). Exhaustion of resource or food scarcity can be ruled out for two reasons. Firstly, there has been no evidence of a tree seed crop failure in Ireland, nor specifically in the midlands region, that could conceivably lead to such a prolonged drop in grey squirrel numbers. Indeed, habitat analysis in Charleville Forest confirmed there was an abundance of tree seeds available in 2010 and 2011. Secondly, the red squirrel population were confirmed to be in good condition, with body condition index, breeding and recruitment all within the healthy range. Any impact of food scarcity, current or historic should in theory have also impacted the red squirrel, who is typically an inferior competitor, at least as significantly, if not more so, than its alien congener.

In the example of 'adaptation by a native herbivore' in Simberloff and Gibbons (2004), the regional population crashes of the invasive aquatic weed *Myriophyllum spicatum* in North America, were associated with a native weevil, *Euhrychiopsis lecontei*. The observed crashes typically occurred 10 - 15 years after the weed invaded the range of the weevil, as reportedly it takes this amount of time for the weevil to switch from its native host to the invasive host, and to reach numbers adequate to initiate population crash in the weed. It may be possible to draw some analogies between this finding and the current study's results in that the evidence

gathered in the course of the current project on both grey squirrel and pine marten distribution and abundance suggest that the success or failure of the grey squirrel population in Ireland may be linked to pine marten abundance more so than distribution alone.

6.2 Failure of the grey squirrel to establish in the west of Ireland

Historically, the grey squirrel has never established itself in the core range of the Irish pine marten population in the west, despite its close proximity to their point of introduction. The river Shannon has traditionally been considered a barrier to the grey squirrel's dispersal into the West of Ireland as the eastern banks of the Shannon were historically the western most extent of the grey squirrels range. Indeed this significant physical barrier could conceivably partially but not entirely hinder the progression of the invasive species range expansion. The grey squirrel is capable of swimming (Koprowski 1994), and has crossed other large rivers in Ireland (Carey et al. 2007). Furthermore, several areas along the banks of the river Shannon exist at which a squirrel could cross via the tree canopy from one side to the other. Carey et al. raised these points in 2007, and proposed that the lack of suitable habitat west of the Shannon was more likely to explain the failure of the grey squirrel to establish there, despite having crossed the river on several occasions. Whilst the lack of favourable habitat for dispersal along much of the western banks of the Shannon is likely to have contributed towards the grey squirrel's failure to establish in the west, the habitat theory does not account for the grey squirrel's failure to disperse westwards north of the source of the Shannon, where the habitat map in Carey 2007 shows both moderately and highly favourable habitat for dispersal exists.

It is plausible now to suggest, as a result of the findings of the current study (namely a negative correlation between grey squirrel and pine marten presence at woodland level, and the landscape level patterns in distribution and abundance) that the failure of the grey squirrel to establish in the west may also be related to pine marten distribution. O'Sullivan (1983) described the pine marten population

in Ireland in the mid 20th Century (the time at which the grey squirrel population was expanding) as being restricted to the west of the river Shannon, from Limerick to Sligo. Indeed distribution maps in both O'Sullivan (1983) and O'Mahony *et al.* (2012) indicate that the limit of the pine marten's range in the west both currently and historically reaches north of the source of the Shannon, and thus could well have been an inhibitor to the spread of the invasive species through this corridor of suitable habitat.

6.3 Range retraction of the grey squirrel

As the pine marten population has expanded from the west and the Slieve Bloom mountains, grey squirrel range has retracted. The information on the timing of the process is anecdotal to some degree, however there is also some useful information available from the literature on the subject. It was first noted by O'Teangana et al. (2000) where reports from the 1980's with regards to the Cavan/Monaghan region (north of the study area) suggested the grey squirrel population appeared not to be doing as well as expected, albeit at a very regional level. However at this point the grey squirrel was described as ubiquitous in the midlands (see Figure 2.1). The distribution map provided by Carey et al. shows the initial range retraction of the grey squirrel and as such it is possible to infer that the range of the grey squirrel population in the midlands began to noticeably decline between 2000 and 2007. The results from the current study clearly show the grey squirrel population in the midlands by 2012 has in fact collapsed. It was not until Carey et al. reported the decline in the Laois Offaly region in 2007 that the pine marten was implicated as a possible factor in the grey squirrel's decline. Interestingly, O'Mahony et al.'s National Pine Marten Survey in 2006 (including distribution map), published around the same time as Carey et al.'s Irish Squirrel Survey, illustrate the expansion of the pine marten population from the core range west of the Shannon (and a smaller population in the Slieve Bloom mountains in Laois and Offaly) into the greater midlands area. This expansion occurred, probably very gradually, between the 1980's and 2007 however the exact timing has not been documented.

Nonetheless, the current study has confirmed that by 2012 there was a high density native pine marten population in the midlands of Ireland, and only the highly reduced remnants of a once abundant alien grey squirrel population persist.

At woodland level, the hairtube surveys consistently found that where pine marten were present in detectable levels, grey squirrels were not, not only in the midlands sites but also in the buffer zone and in the east. The landscape level distribution patterns further highlight the negative association between pine marten and grey squirrel abundance.

6.4 Why has the grey squirrel population declined?

It is not possible to determine in retrospect by what means the grey squirrel population in the midlands has declined, however the population in Charleville forest might offer some clues as to what processes may have lead to the current situation. The eight individual grey squirrels that were detected there between 2010 and 2012 were living at extremely low density despite ample food supply. Body condition was also poor, such that adult body size (albeit from the small sample of adults captured, n = 4) appeared to be normal, but body mass was significantly lower than the eastern population.

At the same time, five adult pine marten were identified as being resident in the woodland (a density of 4.42 marten per km²). It is possible that the high density predator population in Charleville may be influencing foraging behaviour, body condition, reproductive activity or density in the grey squirrel population. Since there have been no previous studies on sympatric European pine marten and North American grey squirrel populations, it is only possible to theorise. However, there is a considerable body of literature documenting the effects of predator presence (or predator related cues) on prey populations (Apfelbach *et al.* 2005). The effects on females can be behavioural (e.g. avoidance of copulation, Ronkainen and Ylönen 1994) or physiological (e.g. suppressed oestrus, Apfelbach *et al.* 2001). A reduction in time spent foraging can lead to decreased body mass and potentially failure to

achieve critical breeding mass (e.g. Ylönen 1994). Effects on the endocrine system in males have been found to include raised cortisol levels, larger adrenal glands and smaller thymus glands (e.g. Zhang *et al.* 2003) which could lead to suppressed immunity. The effects on subadults have been found to include a reduced body weight during development. Carlsen *et al.* (1999) found that subadult field voles, *Microtus agrestis*, exposed to higher predation risk exhibited significantly less weight gain than those with low predation risk as a result of decreased feeding effort. Several other studies with field voles have demonstrated reduced feeding effort in higher risk predation situations (e.g. Koivisto and Pusenius 2003).

Whatever the cause of low adult body weight, it is possible that the critical breeding weight is not being reached, which results in reduced breeding activity, leading to reduced recruitment of juveniles into the population. There was only one adult female captured during the Charleville population study however, and it is therefore not possible to deduce that adult females are not reaching a critical breeding weight from that one individual. Indeed, that individual was within the normal weight range and showed signs of successfully breeding twice in 2011, although none of her offspring were recruited into the population.

It is possible that the observed population crash may have been disease or parasiteinduced. Disease ecology is cryptic and difficult to study in natural communities (De Castro and Bolker 2005). Simberloff and Gibbons (2004) suggest that unexplained population declines are often attributed to pathogens in the absence of alternative explanations. Perhaps the pine marten is a vector for pathogens or parasites that do not affect the native red squirrel but have deleterious effects on the alien grey, or perhaps the grey squirrel has been affected by a disease that is independent of the pine marten population. However, any such (theoretical) effect of pathogens or parasites is only occurring regionally, does not affect sympatric red squirrels and is only found in areas that the pine marten population is abundant.

Culling of the grey squirrel population can be ruled out as a causal factor in their regional decline. To date some, but relatively sporadic and *ad hoc*, grey squirrel control has been carried out in Ireland. For example, woodland level control

General Discussion

programs have been carried out by foresters in order to protect trees from damage by bark-stripping however, no national grey squirrel control policy exists in the Republic of Ireland (Lawton 2003). In recent years a government incentive to control grey squirrel numbers involved the payment of a small bounty for each grey squirrel tail returned. As a result, in 2011 and 2012 the total number of grey squirrels returns received for the Republic of Ireland by the NARGC (including those that were culled as part of the bounty scheme) was 1568 and 1675 respectively. Whilst a temporary impact at woodland level may be possible through such control measures, this level of control would certainly not be sufficient to have any impact on the overall grey squirrel population in Ireland. Furthermore, whilst numbers culled in the east of Ireland were several hundred over the course of 2011 and 2012, a total of just 5 grey squirrels were recorded as having been culled in the midlands study area of Laois and Offaly.

There was no evidence of pine marten predation on grey squirrels in any of the scats collected from Charleville forest or indeed any of the midlands sites. This was not surprising due to the rarity of the grey squirrel as a prey item at woodland and landscape level in the midlands region. In a study of the effects of White-tailed sea eagles, *Haliaeetus albicilla*, on an alien American mink, *Neovison vison*, population Salo *et al.* (2008) found that the risk of predation from the eagle led to reduced movement by the mink which could ultimately lead to reduced population growth. As a result the authors propose that nonlethal predation effects may have stronger impacts on prey populations than direct predation impacts, and that the consequences of such interactions become particularly important if invasive species act as prey.

However, little is known about how alien prey species affect native predators. In a review on the subject, Carlsson *et al.* (2009) examine evidence that native predators may adapt to use alien prey more efficiently and propose that native predators could be important in regulating the long term population dynamics of invasive species. There is no evidence to suggest from the current study however,

that the grey squirrel is affecting the pine marten population in Ireland. The pine marten population has been expanding steadily from their core range in the west as a result of increased habitat availability, and most importantly, protection by law. The expansion of the pine marten population from their core range in the west cannot be attributed to any adaptation to utilising an alien prey species, as the grey squirrel has never established itself in the west of Ireland and therefore could not have played a role. Whilst some predation on grey squirrels may have taken place in the midlands as the population of pine marten was increasing, the extensive dietary study between 2010 and 2012 has proven that the grey squirrel does not currently feature in the diet, and therefore is not a significant prey species for the high density pine marten population.

In contrast to the high density pine marten population in the midlands, where detectable levels of predation on grey squirrels were not found, the low density pine marten population in the east appears to predate upon the alien grey squirrel more so than on the native red squirrel where it is available. The sample size of scats from Tomnafinnoge was small, and therefore must be interpreted with caution. However the %FO in the diet of 15.6% was similar to the %FO of grey squirrel in larger samples of American marten (17%) and fisher diet (16%), in the very limited areas that their natural ranges overlap. Despite this apparent higher rate of predation on the alien squirrel species, it would be unlikely that a low density pine marten population could impact a high density grey squirrel population by direct predation alone. Thus in order to establish the nature of the relationship between the native predator and invasive squirrel species, observing the effects of an increase in pine marten density on an established grey squirrel population will be fundamental. There are few examples in the literature of native predators suppressing exotic species, and it is a difficult process to monitor, in particular if direct predation is not a significant factor. Carlsson et al. (2009) suggest that the historic human overexploitation of predator populations, indeed the decimation of predator populations in many cases, could actually facilitate biological invasions by leaving ecosystems open to invasion by non-native species. The presence of native

predator populations can supply biotic resistance by preventing alien species from becoming established or if already established, from becoming hyper successful.

6.5 Implications for the rest of Ireland, Britain, and Europe.

Although evidence thus far has been anecdotal, it has been suggested that a similar phenomenon is occurring in Scotland to that which has occurred in Ireland; that is the failure of the grey squirrel to establish in the region of the core pine marten population in the north west of Scotland and a subsequent retraction of the grey squirrel's range when the pine marten population has expanded (Paterson and Skipper 2008). Furthermore, the grey squirrel has flourished (at the expense of the native red squirrel) in the absence of a pine marten population in England and Wales. Habitat loss and particularly human persecution are believed to be responsible for what is now described as a "functionally extinct" pine marten population in England and Wales, and it is possible that the absence of the native squirrel predator has allowed the alien squirrel species to become hyper-successful since its introduction in the 19th century.

It is possible that the current range expansion of the pine marten in Scotland (Croose *et al.* 2013) could perhaps result in the eventual re-establishment of a pine marten population in England and Wales. However whether the pine marten population will reach similar densities to those of the Irish population remains unknown, as does whether they might cause a subsequent decline in the numbers or range of the invasive squirrel species.

In Italy, the grey squirrel has also successfully established as an invasive species, and has caused the local extinction of native red squirrel populations in Piedmont, north-west Italy. Since their introduction near Turin in 1948, they have spread to an area greater than 2000 km², and subsequently the red squirrel population has undergone local extinction in c. 62% of the area which is now occupied by the invasive squirrel species (Bertolino *et al.* 2013). The grey squirrel is now also spreading into the hilly region of Piedmont, approaching Alpine valleys where

continuous forest cover may facilitate both an increase in the population range and the rate at which they spread. This represents a serious threat to the long term survival of the red squirrel in Europe, as the dispersal of the grey squirrel beyond the Italian border could lead to the species establishment and continued spread throughout mainland Europe to the detriment of the red squirrel (Lurz *et al.* 2001; Bertolino and Genovesi 2003; Bertolino *et al.* 2008; Bertolino *et al.* 2013).

Recent studies have found the pine marten population in the region of Piedmont may also be increasing. The pine marten in this area is typically associated with forests situated between 1000 and 2000 metres above sea level (in Balestrieri et al. 2010), however roadkill pine marten have recently been reported in the nearby lowland agricultural landscape in the western part of the River Po plain. Data on pine marten distribution and abundance in Italy is limited however they are believed to have increased in recent decades as a consequence of a combination of factors including protection by law and habitat restoration (Balestrieri et al. 2010). Population densities in the region are not known but appear to be low from the records to date. To date just one population density estimate has been obtained for pine marten in Italy, where 0.34 pine marten.km² were estimated to be present in 18km² of forest in central Italy by means of camera trapping (Manzo *et al.* 2012). This is considerably lower than the pine marten density in the midlands of Ireland where density is high and indeed the east of Ireland where density is considered relatively low. The fact that the grey squirrel population in the east of Ireland appear to be thriving, despite the presence of a comparatively low density pine marten population, further suggests that pine marten abundance more so than presence could be a critical factor.

Similarly to Britain, it is not known what population density the pine marten could potentially reach in Italy, as the historic decimation of predator populations means information on contemporary density capacities are unavailable. The potential role of the Italian and indeed the Swiss and French pine marten population in preventing the spread of the grey squirrel further into mainland Europe therefore should be investigated.

LIST OF REFERENCES

- Apfelbach R, Blanchard CD, Blanchard RJ, Hayes RA, McGregor IS (2005) The effects of predator odors in mammalian prey species: A review of field and laboratory studies. Neuroscience & amp; Biobehavioral Reviews 29 (8):1123-1144
- Apfelbach R, Wiest H, Vasilieva NY (2001) Ferret (*Mustela putorius f. furo*) odor affects the estrous cycle in Campbell's hamster females (*Phodopus campbelli*). Wiss Mitt Niederoesterr Landesmuseum 14:147-152
- Arthur SM, Krohn WB, Gilbert JR (1989) Habitat use and diet of fishers. The Journal of Wildlife Management:680-688
- Balestrieri A, Remonti L, Ruiz-González A, Gómez-Moliner BJ, Vergara M, Prigioni C (2010) Range expansion of the pine marten (*Martes martes*) in an agricultural landscape matrix (NW Italy). Mammalian Biology - Zeitschrift für Säugetierkunde 75 (5):412-419
- Balestrieri A, Remonti L, Ruiz-González A, Vergara M, Capelli E, Gómez-Moliner B, Prigioni C (2011) Food habits of genetically identified pine marten (*Martes martes*) expanding in agricultural lowlands (NW Italy). Acta Theriologica 56 (3):199-207
- Balharry D (1993) Factors affecting the distribution and population density of pine martens (*Martes martes* L.) in Scotland. PhD, University of Aberdeen, Aberdeen, Scotland
- Barrington On the introduction of the squirrel to Ireland. In, 1880. Scientific Proceedings, Royal Dublin Society. pp 615-631
- Bertolino S, Genovesi P (2003) Spread and attempted eradication of the grey squirrel (*Sciurus carolinensis*) in Italy, and consequences for the red squirrel (*Sciurus vulgaris*) in Eurasia. Biological Conservation 109 (3):351-358
- Bertolino S, Lurz PW, Sanderson R, Rushton SP (2008) Predicting the spread of the American grey squirrel (*Sciurus carolinensis*) in Europe: A call for a coordinated European approach. Biological Conservation 141 (10):2564-2575

- Bertolino S, Montezemolo N, Preatoni D, Wauters L, Martinoli A (2013) A grey future for Europe: *Sciurus carolinensis* is replacing native red squirrels in Italy. Biological Invasions:1-10
- Bijlsma R, Van De Vliet M, Pertoldi C, Van Apeldoorn R, Van de Zande L (2000) Microsatellite primers from the Eurasian badger, *Meles meles*. Molecular Ecology 9 (12):2215-2216
- Birks J (2002) The Pine Marten. The Mammal Society. SP Press, London
- Birks J, Messenger J, Braithwaite T, Davison A, Brookes R, Strachan C (2005) Are Scat Surveys a Reliable Method for Assessing Distribution and Population Status of Pine Martens? Martens and Fishers (*Martes*) in Human-Altered Environments:235-252
- Broquet T, Ménard N, Petit E (2007) Noninvasive population genetics: a review of sample source, diet, fragment length and microsatellite motif effects on amplification success and genotyping error rates. Conservation Genetics 8 (1):249-260
- Burwash MD, Tobin ME, Woolhouse AD, Sullivan TP (1998a) Field testing synthetic predator odors for roof rats (*Rattus rattus*) in Hawaiian macadamia nut orchards. Journal of Chemical Ecology 24 (4):603-630
- Burwash MD, Tobin ME, Woolhouse AD, Sullivan TP (1998b) Laboratory evaluation of predator odors for eliciting an avoidance response in roof rats (*Rattus rattus*). Journal of Chemical Ecology 24 (1):49-66
- Carey M, Hamilton G, Poole A, Lawton C (2007) Irish Squirrel Survey. COFORD, Dublin
- Carlsen M, Lodal J, Leirs H, Jensen TS (1999) The effect of predation risk on body weight in the field vole, *Microtus agrestis*. Oikos:277-285
- Carlsson NO, Sarnelle O, Strayer DL (2009) Native predators and exotic prey-an acquired taste? Frontiers in Ecology and the Environment 7 (10):525-532
- Caryl F (2008) Pine marten diet and habitat use within a managed coniferous forest. PhD thesis, University of Stirling,
- Caryl FM, Quine CP, Park KJ (2012a) Martens in the matrix: the importance of nonforested habitats for forest carnivores in fragmented landscapes. Journal of Mammalogy 93 (2):464-474. doi:10.1644/11-mamm-a-149.1

- Caryl FM, Raynor R, Quine CP, Park KJ (2012b) The seasonal diet of British pine marten determined from genetically identified scats. Journal of Zoology 288 (4):252-259
- Clevenger A (1993) Spring and summer food habits and habitat use of the Europena pine marten (*Martes martes*) on the island of Micorca, Spain. Journal of Zoology 229 (1):153-161
- Clevenger AP (1994) Habitat characteristics of Eurasian pine martens *Martes martes* in an insular Mediterranean environment. Ecography 17 (3):257-263
- Cook DB, Hamilton WJ, Jr. (1944) The Ecological Relationships of Red Fox Food in Eastern New York. Ecology 25 (1):91-104
- Cooper CB, Dickinson J, Phillips T, Bonney R (2007) Citizen science as a tool for conservation in residential ecosystems. Ecology and Society 12 (2):11
- Croose E, Birks J, Schofield H (2013) Expansion zone survey of pine marten (*Martes martes*) distribution in Scotland. Scottish Natural Heritage Commissioned Report No. 520.
- Davis C, Strobeck C (1998) Isolation, variability, and cross-species amplification of polymorphic microsatellite loci in the family Mustelidae. Molecular Ecology 7 (12):1776-1778
- Davison A, Birks JD, Brookes RC, Messenger JE, Griffiths HI (2001) Mitochondrial phylogeography and population history of pine martens *Martes martes* compared with polecats *Mustela putorius*. Molecular Ecology 10 (10):2479-2488
- De Castro F, Bolker B (2005) Mechanisms of disease-induced extinction. Ecology Letters 8 (1):117-126
- De Marinis AM, Masetti M (1995) Feeding habits of the pine marten (*Martes martes* Lineaus 1758) in Europe: a review. Hystrix 7:143-150
- Deagle BE, Tollit DJ, Jarman SN, Hindell MA, Trites AW, Gales NJ (2005) Molecular scatology as a tool to study diet: analysis of prey DNA in scats from captive Steller sea lions. Molecular Ecology 14 (6):1831-1842
- Devictor V, Whittaker RJ, Beltrame C (2010) Beyond scarcity: citizen science programmes as useful tools for conservation biogeography. Diversity and Distributions 16 (3):354-362

- Dickinson JL, Zuckerberg B, Bonter DN (2010) Citizen science as an ecological research tool: challenges and benefits. Annual review of ecology, evolution, and systematics 41:149-172
- Dickman CR (1992) Predation and Habitat Shift in the House Mouse, Mus domesticus. Ecology 73 (1):313-322
- Dill LM, Houtman R (1989) The influence of distance to refuge on flight initiation distance in the gray squirrel (*Sciurus carolinensis*). Canadian Journal of Zoology 67 (1):233-235
- Don BAC (1983) Home range characteristics and correlates in tree squirrels. Mammal Review 13 (2-4):123-132
- Doncaster CP, Dickman CR, MacDonald DW (1990) Feeding Ecology of Red Foxes (*Vulpes vulpes*) in the City of Oxford, England. Journal of Mammalogy 71 (2):188-194
- Duffy A, Landa A, O'Connell M, Stratton C, Wright J (1998) Four polymorphic microsatellites in wolverine, *Gulo gulo*. Animal Genetics 29 (1):63
- Dunshea G (2009) DNA-Based Diet Analysis for Any Predator. PLoS ONE 4 (4):e5252
- Fenn MG, MacDonald DW (1995) Use of middens by red foxes: risk reverses rhythms of rats. Journal of Mammalogy:130-136
- Finnegan LA, Edwards CJ, Rochford JM (2008) Origin of, and conservation units in, the Irish red squirrel (*Sciurus vulgaris*) population. Conservation Genetics 9 (5):1099-1109
- Fleming MA, Ostrander EA, Cook JA (1999) Microsatellite markers for American mink (*Mustela vison*) and ermine (*Mustela erminea*). Molecular Ecology 8 (8):1352-1355
- Fossitt JA (2000) A Guide to Habitats in Ireland. The Heritage Council
- Fraser DF, Huntingford FA (1986) Feeding and avoiding predation hazard: the behavioral response of the prey. Ethology 73 (1):56-68
- Goldstein E, Lawton C, Sheehy E, Butler F (in review) A comparison of two methods to locate a species invasion front: citizen science projects and indirect field surveys. Submitted to 'Wildlife Research'

- Goudet J (1995) FSTAT (version 1.2): a computer program to calculate F-statistics. Journal of Heredity 86 (6):485-486
- Gurnell J (1983) Squirrel numbers and the abundance of tree seeds. Mammal Review 13 (2-4):133-148
- Gurnell J (1987) The natural history of squirrels. Christopher Helm London
- Gurnell J (1996) The Effects of Food Availability and Winter Weather on the Dynamics of a Grey Squirrel Population in Southern England. Journal of Applied Ecology 33 (2):325-338
- Gurnell J, Lurz P (eds) (1997) The Conservation of Red Squirrels, *Sciurus vulgaris* L. People's Trust For Endangered Species, London
- Gurnell J, Lurz P, Pepper H (2001a) Practical techniques for surveying and monitoring squirrels. Forestry Commission Practice Note 11:1-12
- Gurnell J, Pepper H (1993) A critical look at conserving the British Red Squirrel Sciurus vulgaris. Mammal Review 23 (3-4):127-137
- Gurnell J, Wauters L, Lurz P, Tosi G (2004) Alien species and interspecific competition: effects of introduced eastern grey squirrels on red squirrel population dynamics. Journal of Animal Ecology 73 (1):26-35
- Gurnell J, Wauters LA, Preatoni D, Tosi G (2001b) Spacing behaviour, kinship, and population dynamics of grey squirrels in a newly colonized broadleaf woodland in Italy. Canadian Journal of Zoology 79 (9):1533-1543
- Hales AL, Belant JL, Bird J (2008) Effects of Sex and Age on Winter Diet of American Martens in Michigan. The Ohio Journal of Science 108 (4):60-64
- Hockman JG, Chapman JA (1983) Comparative Feeding Habits of Red Foxes (*Vulpes vulpes*) and Gray Foxes (*Urocyon cinereoargenteus*) in Maryland. American Midland Naturalist 110 (2):276-285
- Jedrzejewska B, Jedrzejewski W (1998) Predation in vertebrate communities: the Bialowieza Primeval Forest as a case study, vol 135. Ecological Studies. Springer, Berlin
- Jędrzejewski W, Rychlik L, Jędrzejewska B (1993) Responses of bank voles to odours of seven species of predators: experimental data and their relevance to natural predator-vole relationships. Oikos 68:251-257

- Jordan N, Messenger J, Turner P, Croose E, Birks J, O'Reilly C (2012) Molecular comparison of historical and contemporary pine marten (*Martes martes*) populations in the British Isles: evidence of differing origins and fates, and implications for conservation management. Conservation Genetics 13 (5):1195-1212
- Kelly J, Tosh D, Dale K, Jackson A (2013) The economic cost of invasive and nonnative species in Ireland and Northern Ireland. Invasive Species Ireland. The Northern Ireland Environment Agency and National Parks and Wildlife Service, Ireland
- Kenward R, Holm J (1993) On the replacement of the red squirrel in Britain. A phytotoxic explanation. Proceedings of the Royal Society of London Series B: Biological Sciences 251 (1332):187-194
- Kenward R, Tonkin J (1986) Red and grey squirrels: Some behavioural and biometric differences. Journal of Zoology 209 (2):279-304
- Kenward RE (1983) The causes of damage by Red and Grey squirrels. Mammal Review 13 (2-4):159-166
- Kenward RE, Hodder KH, Rose RJ, Walls CA, Parish T, Holm JL, Morris PA, Walls SS, Doyle FI (1998) Comparative demography of red squirrels (*Sciurus vulgaris*) and grey squirrels (*Sciurus carolinensis*) in deciduous and conifer woodland. Journal of Zoology 244 (1):7-21
- King R, Read D, Traugott M, Symondson W (2008) Molecular analysis of predation: a review of best practice for DNA-based approaches. Molecular Ecology 17 (4):947-963
- Koivisto E, Pusenius J (2003) Effects of temporal variation in the risk of predation by least weasel (*Mustela nivalis*) on feeding behavior of field vole (*Microtus agrestis*). Evolutionary Ecology 17 (5-6):477-489

Koprowski JL (1994) Sciurus carolinensis. Mammalian Species (480):1-9

- Korpimaki E, Koivunen V, Hakkarainen H (1996) Microhabitat use and behavior of voles under weasel and raptor predation risk: predator facilitation? Behavioral Ecology 7 (1):30-34
- Lanszki J, Zalewski A, Horváth G (2007) Comparison of red fox *Vulpes vulpes* and pine marten *Martes martes* food habits in a deciduous forest in Hungary. Wildlife Biology 13 (3):258-271

- Lawton C (2003) Controlling grey squirrel damage in Irish broadleaved woodlands. COFORD Connects, Silviculture/Management No. 7, Dublin
- Lawton C, Rochford J (2007) The recovery of grey squirrel (*Sciurus carolinensis*) populations after intensive control programmes. Biology and Environment: Proceedings of the Royal Irish Academy 107B (1):19-29
- Lima SL, Valone TJ (1986) Influence of predation risk on diet selection: a simple example in the grey squirrel. Animal Behaviour 34 (2):536-544
- Lima SL, Valone TJ, Caraco T (1985) Foraging-efficiency-predation-risk trade-off in the grey squirrel. Animal Behaviour 33 (1):155-165
- Lockie J (1961) The food of the pine marten *Martes martes* in west Ross-shire, Scotland. Journal of Zoology 136 (2):187-195
- Lockie JD (1964) Distribution and Fluctuations of the Pine Marten, *Martes martes* (L.), in Scotland. Journal of Animal Ecology 33 (2):349-356
- Long RA, Donovan TM, Mackay P, Zielinski WJ, Buzas JS (2007) Comparing Scat Detection Dogs, Cameras, and Hair Snares for Surveying Carnivores. The Journal of Wildlife Management 71 (6):2018-2025
- Lowe S, Browne M, Boudjelas S, De Poorter M (2004) 100 of the World's Worst Invasive Alien Species: A selection from the Global Invasive Species Database. Invasive Species Specialist Group (ISSG). Hollands Printing, Auckland
- Lurz P, Garson P, Ogilvie J (1998) Conifer species mixtures, cone crops and red squirrel conservation. Forestry 71 (1):67-71
- Lurz P, Rushton S, Wauters L, Bertolino S, Currado I, Mazzoglio P, Shirley M (2001) Predicting grey squirrel expansion in North Italy: a spatially explicit modelling approach. Landscape Ecology 16 (5):407-420
- Lurz PWW, Garson PJ, Rushton SP (1995) The ecology of squirrels in spruce dominated plantations: implications for forest management. Forest Ecology and Management 79 (1–2):79-90
- Lurz PWW, Gurnell J, Magris L (2005) Sciurus vulgaris. Mammalian Species (769):1-10

- Lynch Á, Brown M, Rochford J (2006) Fur snagging as a method of evaluating the presence and abundance of a small carnivore, the pine marten (*Martes martes*). Journal of Zoology 270 (2):330-339
- Lynch Á, McCann Y (2007) The diet of the pine marten (*Martes martes*) in Killarney National Park. Biology & Environment: Proceedings of the Royal Irish Academy 107 (2):67-76
- MacDonald DW (1980) Patterns of scent marking with urine and faeces amongst carnivore communities. Symposia of the Zoological Society of London 45:107-139
- Macdonald DW, Mace G, Rushton S (1998) Proposals for future monitoring of British mammals. Department of the Environment, Transport and the Regions, London, UK
- MacKinnon K (1978) Competition between red and grey squirrels. Mammal Review 8 (4):185-190
- Makowska IJ, Kramer DL (2007) Vigilance during food handling in grey squirrels, Sciurus carolinensis. Animal Behaviour 74 (1):153-158
- Manzo E, Bartolommei P, Rowcliffe J, Cozzolino R (2012) Estimation of population density of European pine marten in central Italy using camera trapping. Acta Theriologica 57 (2):165-172
- Marchesi P, Lachat N, Lienhard R, Debieve P, Mermod C (1989) Comparaison des régimes alimentaires de la fouine (*Martes foina* Erxl.) et de la martre (*Martes martes* L.) dans une région du Jura suisse. Revue Suisse de Zoologie 96 (2):281-296
- McCormick F (1999) Early evidence for wild animals in Ireland. The Holocene history of the European vertebrate fauna: modern aspects of research. Verlag Marie Leidorf GmbH, Rahden, Germany
- McInnes CJ, Coulter L, Dagleish MP, Deane D, Gilray J, Percival A, Willoughby K, Scantlebury M, Marks N, Graham D, Everest DJ, McGoldrick M, Rochford J, McKay F, Sainsbury AW (2012) The emergence of squirrelpox in Ireland. Animal Conservation 16 (1):51-59
- Mergey M, Helder R, Roeder J-J (2011) Effect of forest fragmentation on space-use patterns in the European pine marten (*Martes martes*). Journal of Mammalogy 92 (2):328-335

Messenger JE, Birks JDS (2000) Monitoring the very rare pine marten populations in England and Wales. Management and conservation aspects of small carnivores: human interactions. Backhuys, Leiden, the Netherlands

MetEireann (2012) Temperature in Ireland. http://www.met.ie/climate/temperature.asp?prn=1. Accessed 23 May 2013

- Montgomery W, Lundy M, Reid N (2012) 'Invasional meltdown': evidence for unexpected consequences and cumulative impacts of multispecies invasions. Biological Invasions 14 (6):1111-1125
- Mortelliti A, Boitani L (2008) Inferring red squirrel (*Sciurus vulgaris*) absence with hair tubes surveys: a sampling protocol. European Journal of Wildlife Research 54 (2):353-356
- Mowat G, Paetkau D (2002) Estimating marten *Martes americana* population size using hair capture and genetic tagging. Wildlife Biology 8 (3):201-109
- Mullins J, Statham M, Roche T, Turner P, O'Reilly C (2010) Remotely plucked hair genotyping: a reliable and non-invasive method for censusing pine marten (*Martes martes*, L. 1758) populations. European Journal of Wildlife Research 56 (3):443-453
- Nolte D, Mason JR, Epple G, Aronov E, Campbell D (1994) Why are predator urines aversive to prey? Journal of Chemical Ecology 20 (7):1505-1516
- O'Mahony D, O'Reilly C, Turner P (2006) National Pine Marten Survey of Ireland 2005. Coford Connects. Environment 7:1-8.
- 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. Mammalian Biology 77 (5):351-357
- O'Meara D, Sheehy E, Turner P, O'Mahony D, Harrington A, Denman H, Lawton C, MacPherson J, O'Reilly C (2013) Non-invasive multi-species monitoring: realtime PCR detection of small mammal and squirrel prey DNA in pine marten (*Martes martes*) scats. Acta Theriologica:1-7. doi:10.1007/s13364-013-0155-8
- O'Meara D, Turner P, Coffey L, O'Reilly C (2012) TaqMan assays for species identification of the red squirrel (*Sciurus vulgaris*) and the grey squirrel (*Sciurus carolinensis*). Conservation Genetics Resources 4 (3):603-604

- O'Reilly C, Statham M, Mullins J, Turner P, O'Mahony D (2008) Efficient species identification of pine marten (*Martes martes*) and red fox (*Vulpes vulpes*) scats using a 5' nuclease real-time PCR assay. Conservation Genetics 9 (3):735-738
- O'Sullivan PJ (1983) The distribution of the Pine marten (*Martes martes*) in the Republic of Ireland. Mammal Review 13 (1):39-44
- O'Teangana D, Reilly S, Montgomery W, Rochford J (2000) Distribution and status of the red squirrel (*Sciurus vulgaris*) and grey squirrel (*Sciurus carolinensis*) in Ireland. Mammal Review 30 (1):45-56
- Paterson WD, Skipper G (2008) The diet of pine martens (*Martes martes*) with reference to squirrel predation in Loch lomond and The Trossachs National Park, Scotland. BSc Thesis, University of Glasgow, Glasgow
- Peakall ROD, Smouse PE (2006) genalex 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes 6 (1):288-295
- Pepper HW, Patterson GS (1998) Red squirrel conservation. Forestry Authority, Edinburgh
- Pereboom V, Mergey M, Villerette N, Helder R, mi, Gerard J-F, ois, Lod, Thierry (2008) Movement patterns, habitat selection, and corridor use of a typical woodland-dweller species, the European pine marten (*Martes martes*), in fragmented landscape. Canadian Journal of Zoology 86 (9):983-991
- Poole A (2007) An investigation of translocation as a technique to conserve the red squirrel (*Sciurus vulgaris*) in Ireland. National University of Ireland, Galway, Galway
- Reed SE, Bidlack AL, Hurt A, Getz WM (2011) Detection Distance and Environmental Factors in Conservation Detection Dog Surveys. Journal of Wildlife Management 75 (1):243-251
- Reilly S (1997) Aspects of the ecology of the red squirrel, *Sciurus vulgaris*, in commercial conifer forests. Ph.D, Trinity College, Dublin
- Ronkainen H, Ylönen H (1994) Behaviour of cyclic bank voles under risk of mustelid predation: do females avoid copulations? Oecologia 97 (3):377-381
- Rosell F (2001) Effectiveness of predator odors as gray squirrel repellents. Canadian Journal of Zoology 79 (9):1719-1723

- Rosellini S, Osorio E, Ruiz-González A, Piñeiro A, Barja I (2008) Monitoring the smallscale distribution of sympatric European pine martens (*Martes martes*) and stone martens (*Martes foina*): a multievidence approach using faecal DNA analysis and camera-traps. Wildlife Research 35 (5):434-440
- Rousset F (2009) genepop'007: a complete re-implementation of the genepop software for Windows and Linux. Molecular Ecology Resources 8 (1):103-106
- Ruiz-González A, Madeira MJ, Randi E, Abramov AV, Davoli F, Gómez-Moliner BJ (2013a) Phylogeography of the forest-dwelling European pine marten (*Martes martes*): new insights into cryptic northern glacial refugia. Biological Journal of the Linnean Society 109 (1):1-18
- Ruiz-González A, Madeira MJ, Randi E, Urra F, Gómez-Moliner BJ (2013b) Noninvasive genetic sampling of sympatric marten species (*Martes martes* and *Martes foina*): assessing species and individual identification success rates on faecal DNA genotyping. European Journal of Wildlife Research 59 (3):371-386
- Rushton S, Gurnell J, Lurz P, Fuller R (2002) Modeling impacts and costs of gray squirrel control regimes on the viability of red squirrel populations. The Journal of Wildlife Management 66 (3):683-697
- Rushton S, Lurz P, Gurnell J, Nettleton P, Bruemmer C, Shirley M, Sainsbury A (2006) Disease threats posed by alien species: the role of a poxvirus in the decline of the native red squirrel in Britain. Epidemiology and Infection 134 (03):521-533
- Salo P, Nordström M, Thomson RL, Korpimäki E (2008) Risk induced by a native top predator reduces alien mink movements. Journal of Animal Ecology 77 (6):1092-1098
- Seebeck J (1984) The eastern grey squirrel, *Sciurus carolinensis*. Victorian Naturalist 101:61-66
- Shaw MW (1968) Factors Affecting the Natural Regeneration of Sessile Oak (*Quercus Petraea*) in North Wales: II. Acorn Losses and Germination Under Field Conditions. Journal of Ecology 56 (3):647-660
- Shehzad W, Riaz T, Nawaz MA, Miquel C, Poillot C, Shah SA, Pompanon F, Coissac E, Taberlet P (2012) Carnivore diet analysis based on next-generation sequencing: application to the leopard cat (*Prionailurus bengalensis*) in Pakistan. Molecular Ecology 21 (8):1951-1965

- Simberloff D, Gibbons L (2004) Now you see them, now you don't!-population crashes of established introduced species. Biological Invasions 6 (2):161-172
- Smith DA, Ralls K, Hurt A, Adams B, Parker M, Davenport B, Smith MC, Maldonado JE (2003) Detection and accuracy rates of dogs trained to find scats of San Joaquin kit foxes (*Vulpes macrotis mutica*). Animal Conservation 6 (4):339-346
- Smith WP (2007) Ecology of *Glaucomys sabrinus*: habitat, demography, and community relations. Journal of Mammalogy 88 (4):862-881
- Steele MA (1998) Tamiasciurus hudsonicus. Mammalian Species (586):1-9
- Steele MA (1999) Tamiasciurus douglasii. Mammalian Species (630):1-8
- Takahashi LK, Nakashima BR, Hong H, Watanabe K (2005) The smell of danger: A behavioral and neural analysis of predator odor-induced fear. Neuroscience & Biobehavioral Reviews 29 (8):1157-1167
- Teerink BJ (1991) Hair of west-European mammals. 1st edn. Cambridge University Press,
- Thompson DC (1978) Regulation of a Northern Grey Squirrel (*Sciurus carolinensis*) Population. Ecology 59 (4):708-715
- Thornington RW, Hoffmann RS (2005) Family Sciuridae, vol 2. Mammal Species of the World: a taxonomic and geographic reference (Wilson DE and Reeder DM eds.), 3 edn. John Hopkins University Press, Baltimore, Maryland
- Trites AW, Joy R (2005) Dietary analysis from fecal samples: how many scats are enough? Journal of Mammalogy 86 (4):704-712
- Valière N (2002) GIMLET: a computer program for analysing genetic individual identification data. Molecular Ecology Notes 2 (3):377-379
- Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) micro-checker: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes 4 (3):535-538
- Vincent I, Farid A, Otieno C (2003) Variability of thirteen microsatellite markers in American mink (*Mustela vison*). Canadian Journal of Animal Science 83 (3):597-599

- Warner P, O'Sullivan P (1982) The food of the pine marten *Martes martes* in Co. Clare. Transactions of the International Union of Game Biology 14:323-330
- Waters C (2012) Post-release monitoring of two translocated red squirrel (*Sciurus vulgaris* L.) populations. PhD Thesis, National University of Ireland, Galway
- Waters C, Lawton C (2011) Red Squirrel Translocation in Ireland. Irish Wildlife Manuals, vol 51. National Parks and Wildlife Service, Department of the Environment, Heritage and Local Government, Dublin, Ireland
- Wauters L, Bijnens L, Dhondt A (1993) Body mass at weaning and juvenile recruitment in the red squirrel. Journal of Animal Ecology 62 (2):280-286
- Wauters L, Dhondt A (1992) Spacing behaviour of red squirrels, *Sciurus vulgaris*: variation between habitats and the sexes. Animal Behaviour 43 (2):297-311
- Wauters L, Dhondt A (1995) Lifetime reproductive success and its correlates in female Eurasian red squirrels. Oikos 72 (3):402-410
- Wauters L, Gurnell J, Martinoli A, Tosi G (2002a) Interspecific competition between native Eurasian red squirrels and alien grey squirrels: does resource partitioning occur? Behavioral Ecology and Sociobiology 52 (4):332-341
- Wauters L, Lens L (1995) Effects of Food Availability and Density on Red Squirrel (*Sciurus Vulgaris*) Reproduction. Ecology 76 (8):2460-2469
- Wauters L, Lurz P, Gurnell J (2000) Interspecific effects of grey squirrels (*Sciurus carolinensis*) on the space use and population demography of red squirrels (*Sciurus vulgaris*) in conifer plantations. Ecological Research 15 (3):271-284
- Wauters L, Tosi G, Gurnell J (2002b) Interspecific competition in tree squirrels: do introduced grey squirrels (*Sciurus carolinensis*) deplete tree seeds hoarded by red squirrels (*S. vulgaris*)? Behavioral Ecology and Sociobiology 51 (4):360-367
- Wauters LA, Gurnell J, Preatoni D, Tosi G (2001) Effects of spatial variation in food availability on spacing behaviour and demography of Eurasian red squirrels. Ecography 24 (5):525-538
- Williams BW, Etter DR, Linden DW, Millenbah KF, Winterstein SR, Scribner KT (2009) Noninvasive Hair Sampling and Genetic Tagging of Co-Distributed Fishers and American Martens. The Journal of Wildlife Management 73 (1):26-34

Yalden D (1999) The history of British mammals. Poyser, London

Yalden DW, Morris PA (1990) Analysis of owl pellets. The Mammal Society, London

- Ylönen H (1994) Vole cycles and antipredatory behaviour. Trends in Ecology & Evolution 9 (11):426-430
- Ylönen H, Ronkainen H (1994) Breeding suppression in the bank vole as antipredatory adaptation in a predictable environment. Evolutionary Ecology 8 (6):658-666
- Yurgenson P (1947) Sexual dimorphism in feeding as an ecological adaptation of a species. Byulleten Moskovskogo Obshchestva Ispytatelei Prirody 52:33-35
- Zalewski A (1999) Identifying sex and individuals of pine marten using snow track measurements. Wildlife Society Bulletin 27 (1):28-31
- 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 (1):31-43
- Zhan X, Zheng X, Bruford MW, Wei F, Tao Y (2010) A new method for quantifying genotyping errors for noninvasive genetic studies. Conservation Genetics 11 (4):1567-1571
- Zhang J-X, Cao C, Gao H, Yang Z-S, Sun L, Zhang Z-B, Wang Z-W (2003) Effects of weasel odor on behavior and physiology of two hamster species. Physiology & Behavior 79 (4):549-552