The use of baited hair traps and genetic analysis to determine the presence of Pine marten.

by

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Declaration

This thesis is my own work, except where explicit reference is made to the work of others. It has not been submitted for another qualification to this or any other institution.

Signed : _____

Date : _____

To Betty and Nick

(Kathleen and John)

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Abstract

The pine marten (*Martes martes)* is one of Ireland's most elusive mammals. Between July 2003 and July 2005, hair tubes attached to trees with adhesive patches inside, were used to detect presence of marten in Portlaw Woods, County Waterford. Screening tests of potential lures to assess attractiveness and suitability were carried out on a variety of substances on free-living and captive pine marten. The use of chicken as a short-term lure was successful in attracting pine marten to the hair tubes. Peanut butter and marmalade scored highly as potential long-term lures. There was an increase in the number of visits to the hair tubes, which may mean that pine marten 'learn' where the hair tubes were. Samples retrieved were DNA species-typed to verify presence /absence of target animals and DNA sex-typed for gender distribution. A strong gender bias in favour of males was discovered. Findings have implications for field studies and how data is gathered for such studies while also important for conservation population management schemes.

Chapter 1

General Introduction

1. General introduction

1.1 Mustelidae

The mustelids are members of the class Mammalia and of the order Carnivora. Hosoda *et al* (2000) suggest that the family Mustelidae comprise of 23 genera and 65 species. They occupy a wide range of habitats from aquatic systems (otters and mink) to prairies (black-footed ferret, *Mustela nigripes*, and North American badger, *Taxidea taxus*), steppes (steppe polecat, *Mustela eversmanni*), treeless tundra (wolverine, *Gulo gulo*), and forests (most mustelids). Some species, such as striped skunk (*Mephitis mephitis*) in the United States and beech marten (*Martes foina*) in Europe abound in urban areas. Mustelids occupy all continents except Antarctica. From Hosoda *et al* (2000) it is suggested that the mustelids evolved into three major divisions: running and climbing types (e.g. weasels and martens), digging types with large claws (e.g. badgers) and swimming types (e.g. otters).

The majority of the species are of small-to-medium stature characterized by long tubular shape or stocky build with short limbs, small heads, and habits that are either terrestrial or semi-aquatic. Although of the order Carnivora, many of the family Mustelidae are omnivorous (Warner and O'Sullivan 1982, Pulliainen *et al* 1996). Body size varies from 11– 26 cm in the Least weasel (*Mustela nivalis*), which is the smallest mustelid, to 100–150 cm in the giant otter (*Pteronura brasiliensis*). Generally there are five fingers and toes with non-retractile claws and all species have anal scent glands, some having chest or neck glands also (Hutchings *et al* 2000). All species have strong canine teeth for capturing and killing prey.

Only one species of mustelid, the giant otter, is monogamous; all other Mustelidae will mate with numerous members of the other sex. Pair bonds are typically short. Known as embryonic diapause, the implantation of the blastocyst into the uterine wall is delayed in many species. The embryo is maintained in a state of dormancy for a number of months, however, little is still known about entry into diapause. Females have one litter per year, which usually comprise of between one and three young, called kits. Males do not provide care for the young.

Some mustelids have exquisite furs which have been sought after for many centuries — the american mink (*Mu. vison*), the sable (*Ma. americana*) and the

ermine (*Mu. erminea*) are all members of the family. This has led to the hunting of these animals, however many species are threatened because of habitat loss. Black-footed ferrets are globally the most endangered of all mustelids. In Ireland, the Eurasian badger (*Meles meles*), the Eurasian otter (*Lutra lutra roensis*), the Irish stoat (*Mustela erminea hibernica*) and the European pine marten (*Martes martes*) are all protected whereas the American mink (*Mustela vison*), an introduced pest species, is not.

Among the members of this family, the genera *Martes* (martens) and *Mustela* (weasels and minks) are the most dominant (having the greatest number of subspecies), each having differentiated into various species and expanded their distribution across the entire Palaeartic region and the American continent.

The *Martes* lineage is comprised of seven extant species and have adapted to an arboreal habitat (climbing types). Three subgenera are recognised: the fishers (*Martes pennanti*), the yellow-throated marten (*Martes flavigula*) and the true marten (pine marten *Martes martes*, sable *Martes zibellina*, Japanese marten *Martes melampus*, American marten *Martes Americana* and beech marten *Martes foina*).

1.2 Mustelids in Ireland

Ireland is home to five members of the family Mustelidae: the introduced American mink (*Mustela vison*), the European pine marten (*Martes martes*), the Eurasian badger (*Meles meles*), the Eurasian otter (*Lutra lutra roensis*) and the Irish stoat (*Mustela erminea hibernica*).

Both the otter and stoat have been given subspecies status. According to Lynch *et al* (1996), the otter's status is due to morphological variations, in particular the cranial features and differences in sexual dimorphism. *Mustela erminea hibernica* is the Irish subspecies of stoat and is differentiated by having an irregular dividing line on the flank between dorsal and ventral colouration (back-belly line), and characteristically it does not go white in winter (Fairley 1981). It appears to have colonized naturally accumulating genetic mutations (greater haplotype diversity) during their nationwide population expansion (Martinkova *et al* 2007).

1.3 Pine marten (*Martes martes*)

1.3.1. Description

The pine marten, *Martes martes*, is one of Ireland's most exquisite and elusive mammals. The Irish name for pine marten is *cat crainn*, meaning *tree cat*. Although described as 'pine' marten, the use of pine forests is far from exclusive – in actual fact, marten seem to prefer mixed woodland; also the word 'marten' is an old Germanic name for a weasel, presumed reference to body form and/or undulating movement. This movement can be seen when they zig-zag in search of prey, possibly to avoid over head predatory birds (Sleeman 1983) or when striding in undulating bounds. When bounding at speed, pine marten can have stride lengths of up to 70cm in snow or up to 90 cm on firm ground (Birks 2002).

Pine marten are small cat-sized mammals measuring roughly 70cm from nose to tip of tail, with males being slightly longer than females. The ears are large and rounded and a fawn colour inside. The snout is quite pointed. Their coats are dense with guard hairs present (Toth 2002) and coloured a rich brown. A distinctive cream patch is present on the throat (Fairley 2001), extending down between the front legs (Figure 1.1). This patch varies in shape and size and can be used to distinguish between individuals (Balharry 1993). European relatives, the beech marten, have a similar patch but theirs is a pure white, un-tinted throat-'bib' (Sleeman 1989).

Marten tend to have relatively large paws, with hair between the pads and possess semi-retractile claws, allowing for grasping the smoothest of branches. The tail is long and bushy and is used to aid in arboreal navigation. They are capable of aerial bounds of almost four metres; hence pine marten are the only mustelid capable of aerial pursuits of red squirrel (Strachan *et al* 1996).

Scent marking is the primary form of communication for mustelids, with each species employing different scent marking strategies. For example, faeces may be deposited in a latrine as in the badger or singly on elevated sites such as on stones or grass tussocks as in by pine marten. The anal gland is common to all European mustelids and the secretions are characterized by the presence of sulphur-containing compounds (Hutchings and White 2000). Male pine martens possess an abdominal scent gland, situated on the underside (ventrum region) in front of the penis (Balharry

1993). The ventral gland secretions are thought to contain different chemical compounds (Brinck *et al* 1983).

Figure 1.1 Pine marten – Body shape, claws and throat patch clearly visible (Reproduced with permission from ionalister.com)

1.3.2. Pine marten ecology

The pine marten is a habitat specialist according to Brainerd (1990). However, it is more likely that the pine marten is a highly adaptable species rather than a specialist. It is to be found in a variety of habitats and when resources such as denning sites and food availability are low, this species can be very adaptable as discussed below.

An arboreal mammal, marten are frequently found in mixed conifer-deciduous forests, being strongly associated with old stands (Clevenger 1994). This can depend on a variety of factors such as topography and availability of denning sites coupled with food availability and population dynamics (De Marinis *et al* 1995 and Pulliainen *et al* 1996).

In Irish mixed conifer-deciduous forests, over-story canopy species include Sitka spruce, *Picea sitchensis,* Scots pine, *Pinus sylvestris*, Norway spruce, *Pinus abies*, Holly, *Ilex acquifolium* interspersed with deciduous species such as Oak, *Quercus rober*, Beech *Fagus sylvatica*, and Mountain ash, *Sorbus aucuparia* (Warner *et al* 1982). Common under-story species often include Hazel, *Corylus avellana*,

Rhododendron species, Bilberry, *Vaccinium myrtillus* and many of the Rosaceae family (Warner and O'Sullivan 1982).

Pine marten tend to prefer areas with over-head cover and would appear to avoid clear-fell areas or open tracts of land (Fairley 2001). This may be due to predator avoidance. Clevenger (1994) states that pine marten may not exhibit clear preferences for forested (with over-head cover) over non-forested areas (coastal shrublands). This may, however, be due to the topography of the location.

In places such as the windswept Highlands of Scotland and the barren karst landscape of the Burren in Co. Clare, Ireland, they have been quite often sighted and recorded (Fairley 2001). Many of the sightings have been in sites under bare slabs of rock in the Burren (Sleeman 1989, Birks 2002), presumably due to a lack of mature trees containing potential denning areas. The lack of readily available sites has led pine marten to encroach on human habitation. Rather than occupying squirrel dreys, they may occupy derelict or even farm outbuildings (MacDonald and Baker 2005). Clevenger (1995) states that pine marten occasionally forage through abandoned or seldom used farmlands and orchards, and use relatively open habitats, but this may be due to the fact that the location is devoid of marten predators. Birks *et al* (2005) suggests that 44.3% of dens are associated with trees, 27.6% with rocks and 13.8% with buildings. A total of 69.6% of dens were elevated and typically in structures offering limited shelter, with only 9.8% of all dens surveyed, in elevated tree cavities.

Feeding ecology studies show it to be a generalist predator, taking a variety of birds and mammals, while varying its diet according to local and seasonal food availability (De Marinis *et al* 1995). Pine marten have regularly been recorded taking mammal species such as the Woodmouse, *Apodemus sylvatica*, a range of passerine birds and numerous invertebrates from the coleoptera (year round) and hymenoptera (summer) groupings (Warner and O'Sullivan 1982). Studies show they will take berries of mountain ash, *Sorbus aucuparia*, bramble, *Rubus fruticosus agg*., hazel, *Corylus avellana* and ivy, *Hedera helix*, when they are in season (Fairley and O'Gorman 1974, Warner and O'Sullivan 1982).

Generally speaking, the mustelids are solitary foragers, living within an intrasexual territorial system (Powell 1979). Males tend to be dispersed with respect to females (with female territories inside male boundaries, Figure 2.2) and females dispersed with respect to food supply (Balharry 1993). When foraging, pine marten can cover up to 12.7 km per day, however a more normal daily average being

approximately 5 km, depending on seasons, with winter distances being shorter (Zalewski *et al* 2004). From the Pine Marten survey of England and Wales, Strachan *et al* (1996) state that the male pine marten is usually most active at night during the winter months whilst becoming both crepuscular and nocturnal during the summer. The females tend to be more active during the day especially if rearing young. Both sexes show continuous activity patterns though on occasion rests may be taken back at the main den or at a temporary 'hover'.

Pine marten are solitary and territorial animals occupying home ranges from 1.5 to 32.9 km^2 (O'Mahony *et al* 2006). Within marten systems, there is a clear division between intra-sexual territories, where solitary females defend against other females and solitary males against other males (MacDonald 1983). According to Powell (1979), males can have female territories within their own and will mate with the females on their 'patch'. This is called a 'dispersed harem system' (Figure 1.2).

The spatial distribution of adult martens conforms to an intra-sexual territorial pattern. These territories are not static and boundaries appear to change with season, resource availability or the death of a neighbour. Pine marten young (kits) who fail to secure a territory in adequate terrain are very much at risk and only likely to survive if a neighbour is killed, enabling them to take over. Such animals are usually males. They may end up with small bachelor territories and are unlikely to mate.

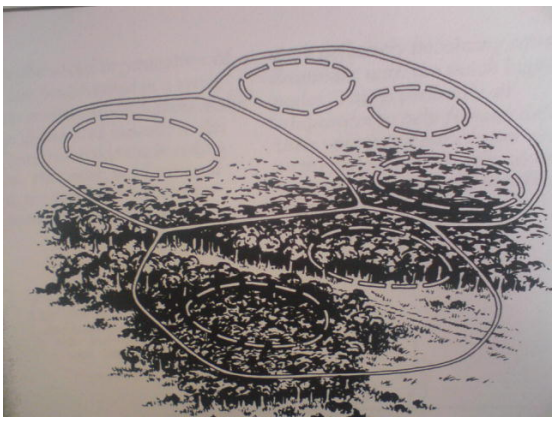

Figure 1.2 Males territories (solid lines) encompassing female territories (Powell 1979)

It has been found that when a male was killed, leaving a territory vacant, another male did move in. Balharry (1983) states that young non-breeding males may occupy

over-lapping ranges whereas breeding males occupy exclusive ranges and defend their territories ferociously.

1.3.3. Distribution

The pine marten can be found from the Boreal Regions to the Mediterranean Sea and on many of the islands that lie therein. Yet despite such diversity of range, the pine marten is listed as endangered in certain countries (if not already locally extinct) and as a result it has gained protected status in many countries.

The current decline in population numbers appears predominantly due to habitat loss due to increased urbanisation, increasing density of roads and forest fragmentation. In the United Kingdom, pine marten are almost extinct in England, with the exception of isolated pockets and they remain relatively stable in Scotland, even appearing to be on the increase as a result of The Wildlife and Countryside Act 1981 (Messenger and Birks 2000). This has banned all trapping or persecution, except under license. In Ireland it is protected under the Wildlife Act 1976. The pine marten is also listed in Appendix III of the Convention on the Conservation of European Wildlife and Natural Habitats (the Bern Convention) which requires that signatory states should (i) regulate the exploitation of, and (ii) ban certain means of capture or killing of, any species listed in Appendix III.

Historical persecution of the pine marten in Ireland (being poisoned with strychnine as vermin and hunted for its pelt) has also led to a decline in numbers. However, from the National Pine Marten Survey of Ireland 2005, it would appear that many of the localised populations that had declined are on the increase; particularly in counties Clare, Kerry, Mayo and Galway and in parts of Co. Waterford (Figure 1.3).

The report appears to indicate that this increase may be due to natural expansion from core populations, allowed by the increased rates of afforestation, which is creating suitable habitats and connecting isolated populations. These natural increases, in combination with reintroduction projects, such as the Killarney National Park initiative, where the pine marten have been reinstated, ensure pine marten not only retain their current population status but also manage to increase in numbers. Encouragingly, it has become government policy in the Republic of Ireland to increase the area under forest cover. The overall aim is to have 17% of the land under

forest cover by 2030. Figure 1.3 shows encouraging increases - from 26 occurrences (blue dots) to 35 (from O'Mahony *et al* 2005).

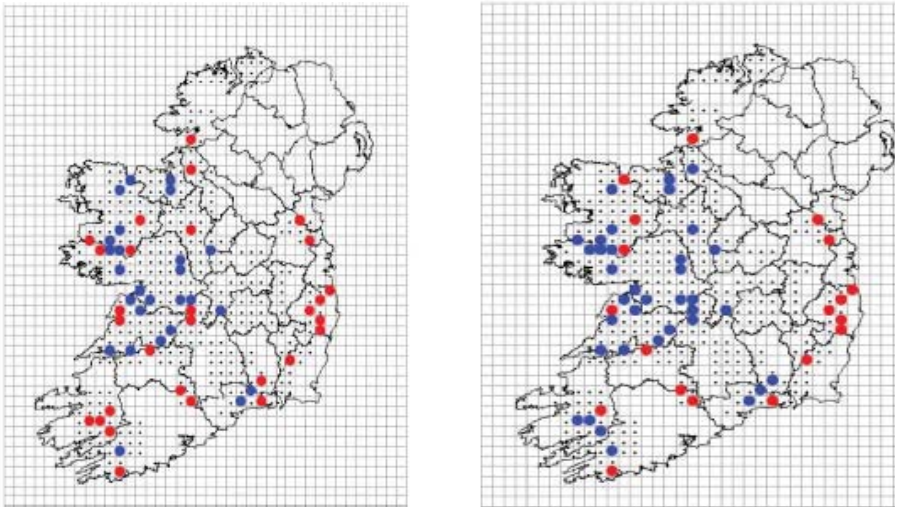

Figure 1.3 Map of Ireland with 10 km^2 National Grid overlay, indicating the location of survey squares completed in the National Pine Marten Survey 2005 and their status (occurrence indicated by blue dots, non-occurrence indicated by red dots) according to O'Sullivan (1983) (a) and their status as determined by the National Pine Marten Survey of Ireland 2005 (b). Also indicated are the locations of the remaining 10 km^2 National Grid survey squares (total n = 428; indicated by black dots) completed by O'Sullivan (1983) (O'Mahony *et al* 2005).

O' Sullivan (1983) and Fairley (2002) refer to old pine marten sightings and record them as present in forest around Portlaw (Figure 1.4). More recently marten road kills have been found at Ballmacarbry (S1515), Lismore (X0499), Kilclooney Bridge (S3410) and Carrick on Suir (S4120) (Smiddy and Berridge 2002). Studies show the pine marten being localised in its distribution status and this is true for the Waterford area ranging from the city boundaries to the Comeragh Mountains (O' Sullivan 1983). Localised records of pine marten in Waterford have come from sightings and road kill around the Curraghmore estate and Portlaw areas.

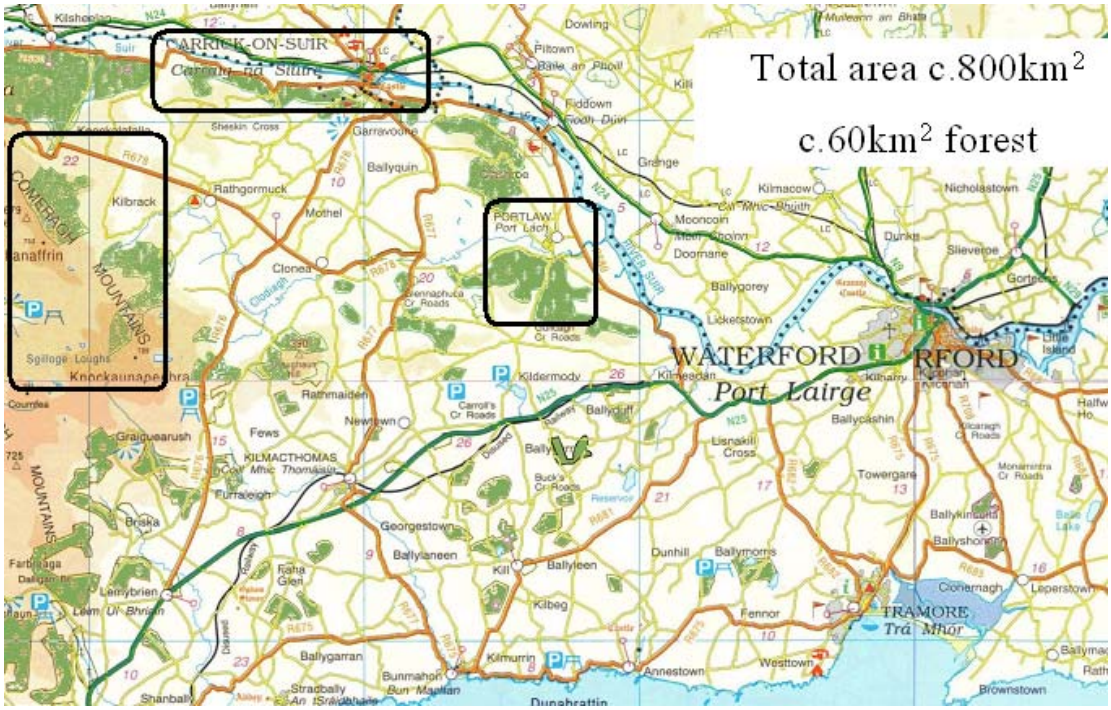

Figure 1.4 County Waterford showing location of some pine marten sightings

1.4 Non-invasive sampling techniques used in pine marten surveys

Pine marten field study methods are many and varied and determining the abundance of this species with any degree of accuracy can be difficult (Pigott and Taylor 2003). Traditional methods of estimating abundance have been based on direct observational counts or on indirect signs such as faecal deposits or footprint identification but for highly elusive populations, this is difficult (Slauson *et al* 2002). The collection of hair and faecal samples in combination with molecular analysis can give a better account of presence or absence of a target species and may give an estimation of abundance. For sensitive species, invasive sampling is to be avoided due to potential damaging of populations and/or their dynamics (Pigott *et al* 2003). Hoyle *et al* (1995) recorded that when trapping the highly endangered hairy-nosed wombat, *Lasiorhinus krefftii*, the animals lost weight between trappings and had a reduced population-size estimate. This could suggest the animals have temporarily or even permanently left the area, hence it can have an adverse effect on populations.

There has been little critical evaluation of whether hair-tube sampling is actually efficient at detecting medium-sized terrestrial mammals. This is important to know, because the results of hair-tube surveys are increasingly being used to

influence conservation management of these groups of animals across forested landscapes (Mills *et al* 2002).

Pine marten are nocturnal and/ or crepuscular species and are rarely sighted. Monitoring schemes to retrieve samples should be easily operated, favourable in terms of cost, apply to a variety of habitats and workable over a long period of time (Harris and Yalden 2004). Non-invasive techniques are of great value as capture is not required, the cost is low and no harm comes to individual animal (Foran *et al* 1997). These monitoring schemes could be categorized as direct or indirect, with direct monitoring requiring contact with the target species and indirect (non-invasive) allowing for the accumulation of information without any contact (Lynch *et al* 2006).

The use of sightings and reports of road kills are methods of gaining insight into the presence or absence of a population in an area. Sighting surveys do require their confirmation and some level of verification (hair, scat or body) or very careful questioning of the observer. They may be reliable enough to give a 'defendable record' of a target species presence but are not reliable enough to give accurate numbers of animals.

Remote cameras at bait stations can provide standardized detection methods but can prove too costly to be used to monitor large areas (McDaniel *et al* 2000). Infra-red, break-beam devices linked to a multi-shot, 35mm camera would be one of the more sophisticated in use but 'home-made' single-shot systems can be assembled using an 'instamatic'-type camera triggered by a line attached to the bait (Messenger and Birks 2000).

Track plates have been used in many studies to record footprints, whether they are soot covered, left in mud or in sand (Gomez-Moliner *et al* 2004). Snow tracking is occasionally used (Zalewski 1999) but within the United Kingdom and Ireland snow rarely lies for long, except over very high ground (Messenger and Birks 2000) and may not provide irrefutable evidence of marten occurrence.

The use of non-invasive techniques to collect samples, especially as a source of DNA, is highly desirable. Hair samples have been used in the study of various mammal species and several sampling methods have been tested. Examples include Suckling *et al* (1978), Lindenmayer *et al* (1999) and Mills *et al* (2002) surveying small mammals in Australia, Mowat *et al* (2000) surveying grizzly bear populations in British Columbia and Alberta and Boersen *et al* (2003) monitoring black bear populations in Louisiana, Unites States of America and Lynch *et al* (2006) surveying

pine marten in Ireland. Appropriate analyses of those samples can extend the utility of the samples, including identification of the species and gender of the animal (Kohn and Wayne 1997). Hair samples have proven to be very reliable in genetic analysis, and have been used in the study of several species of mammals.

1.4.1. Scat collection

The collection of faeces or 'scats' (Figure 1.5) is a more productive method of sampling, as a much greater quantity of data can be gleaned from individual scats (Clevenger 1994). Pine marten use scats to communicate a variety of signals amongst populations. Scent marking is believed to be important for individual recognition and publicizing status in the hierarchy (Sleeman 1989), whereas scat deposition is believed to demarcate territories. Scats may be a means of avoiding encounters between individuals or as a method of sending 'details' of reproductive status (Hutchings and White 2000).

Scat surveys have been used across Europe (Clevenger 1994 and 1995) and in the UK and Ireland (Messenger and Birks 2002, Turner *et al* 2002) for many years to gain insight into *Martes* populations. However it doesn't always give a conclusive estimate of overall density or population size (Birks *et al* 2004).

Searches for scats are conducted along linear features, such as forest trails and paths. This technique arose from the work of Lockie (1964), who first suggested a relationship between the numbers of martens in an area and their scats. These surveys involve scanning the ground, as the scats are often deposited in visible locations such as on logs, rocks or grass tussocks (Pulliainen 1982) which allows for easy collection of samples.

It must be noted that pine marten activity may be predominantly arboreal rather than terrestrial. In the Netherlands, for example, marten scats are concentrated on branches and beneath woodpecker holes (Birks et al 2004). Also, where abundances of martens are low, the need for territorial marking is greatly reduced and consequently they may not defecate on or near these 'open' areas. For the surveyor, this could mean recording a negative scat survey result leading to the incorrect assumption that pine marten are not present in a given area. On the other hand, high-

density trail-marking behaviour may be a feature of strongly territorial populations of martens (Balharry *et al* 1986), particularly those in breeding condition, giving a much greater number of scats, but this may potentially lead to an over estimation of population density.

Seasonality may be an issue in scat collection rates, with fewer scats being observed in winter. Velander (1986) reported that scat densities may be as much as 100 times greater in July than in January. It is difficult to get a realistic view of pine marten status based on scat surveying alone. Scats can be quite moist and will easily wash away if a deluge hits the scat before it has been recorded. Heavy rain may wash away scats, especially fruit-laden scats in autumn. Also, slugs have been known to consume an entire scat in 24 hours in times of high precipitation (Davison *et al* 2002). This could lead to the inference that pine marten are absent from an area giving a negative scat survey result. The risks of inferring absence falsely from negative scat surveys are emphasised by Velander (1983), where negative scat surveys resulted even though positives were recorded for marten sightings and carcasses.

It is likely that seasonal changes in marten numbers, general activity levels and the intensity of social marking behaviour contribute to the observed pattern of month to month variations in pine marten scatting rates (Helldin and Lindstrom 1995) and these have to be taken into account when surveying pine marten population densities on the basis of scat depositions alone.

It is also possible that the scat may not be of the species targeted. Oftentimes, pine marten scats have been mistaken for fox faeces. Some studies refer to the sweet, musky odour as being critical to the correct identification of pine marten scats. Bright and Harris (1994) stated that "marten scats were recognised by their distinctive sweet odour and frequent characteristic long, twisted shape; they were not recognised on the basis of shape alone". Odour does appear to be a factor in pine marten scat recognition but the scat has to be relatively fresh for this to be apparent.

Morphologically, scats can be confused with other mammals. On the smaller end of the scale, scats can be confused with stoat, *Mustela erminea*, and on the other extreme can be confused with fox, *Vulpes vulpes*. Recent work carried out by Davison *et al* (2002) reported that even in the case of experienced fieldworkers, misidentification of scats occurred 20% of the time. Reports of misidentifications of up to 97% in areas of low abundance in England and Wales have been recorded. Although morphology and odour of scats can give quite a good indication of species,

it is suggested that DNA analysis should be used to prove species type (Davidson *et al* 2002).

Figure 1.5 Pine marten (*Martes martes*) scats

DNA testing is an invaluable means of obtaining reliable data for estimating distribution and abundance of target species (Mowat *et al* 2000). Mitochondrial D-loop amplification and sequencing from scat and hair samples make it possible to genetically analyse and identify many species (Davison *et al* 2002, Statham *et al* 2006). Unfortunately DNA identification of species from faecal (scat) samples is problematic due to the small quantities and poor quality of the DNA isolated from such samples. However, recent advances in DNA analysis such as the use of Real-time Polymerase Chain Reaction (PCR) in species typing has increased results from a 53% scat identification rate (Davidson *et al* 2001) to 94.6% (O' Reilly *et al* 2007). This gives future DNA species testing a far more promising outlook.

1.4.2. Hair retrieval

Hair collection is a popular method of non-invasive sampling. This technique has been used widely in conducting mammal surveys (Suckling 1978, Finnegan *et al* 2007), including pine marten surveying (Lynch *et al* 2006). Many different methods of hair retrieval have been designed and used.

Dilks *et al* (1996) found partially camouflaged tunnels with bait no more effective than visible ones, and single tunnelled traps caught as many stoats, *Mustela erminea,* as double ended ones. Hence, trap design and position do not appear to have a bearing on the attraction of stoats to traps. The hair was removed with double sided sticky tape, as used in many other studies, although Mowat and Strobeck (2000) have removed grizzly bear, (*Ursus arctos*) hair by snagging it on barbed wire and Lynch *et al* (2006) used recoiling springs within hair tubes to pluck hair from pine marten.

In Australia, Mills *et al* (2002) used large-diameter pipes (hair tubes) and tapered hair funnels to sample their target species. Both 'traps' were lined with double-sided adhesive tape to catch the animal's hair. Lindenmayer *et al* (1999) defines hair tubes as a 'remote' sampling technique that can detect mammals by attracting them to an open cylinder containing food bait held within a closed chamber. When in the tube (Figure 1.6), some of the animal's hair is removed, adhering to adhesive patches (Figure 2.5).

Figure 1.6 Hair tubes used in Portlaw Woods devised by Dr. Peter Turner, Waterford Institute of Technology (Photo. Dr. Peter Turner)

Although there are variations within hair sampling techniques, a plucked hair tuft from the animal can be used to verify species type and gender and possibly even identify individuals. This technique has been used, as pine marten have been attracted to and successfully recorded as having visited hair tubes (Messenger and Birks 2000, Mowat and Paetkau 2002, Lynch *et al* 2006).

The advantages of hair tubes are that they are cheap to manufacture, light (transport and set up), compact and highly durable. They can be left for years in the field if necessary. Lynch *et al* (2006) found that when surveying presence /absence of pine marten, scat collection is not always possible due to lack of paths, hence hair tubes can be used, even in the remotest of areas. They are seen as a real alternative for monitoring pine marten presence. As a result hair tubes are becoming increasingly used in conservation management studies, particularly when a species range is prone to change (Finnegan 2007).

1.4.3. Lure types

Hair tubes have to be baited with a suitable 'lure' to attract the target animal. Lures generally used to attract mammals into traps or hair tubes vary greatly depending on target species required. Catling *et al* (1997) used fresh meat and apple in one study to survey arboreal mammals, whereas Mills *et al* (2002) used peanut butter, rolled oats and honey to attract their target marsupial species, both in new South Wales, Australia. Dilks *et al* (1996) used eggs, possum flesh and synthetic lures to attract stoat, *Mustela erminea* whilst Suckling (1978) used golden syrup and oats to attract sugar gliders, *Petaurus breviceps*. The methodology by which pine marten are lured to hair tubes varies from survey to survey. In order to gain some insight into the efficacy of potential lures, scats might be analysed to record food types taken.

The lure material should ideally be non-toxic, stable enough not to decay too quickly, detectable from a distance and preferably be able to be left for long periods of time. The creation of a non- perishable or long lasting lure based on its dietary preferences is part of the main focus of this study. Meat based lures have been tried and found to be perishable in the short term and once taken, no longer provide an incentive to enter that tube. De Marinis *et al* (1995) suggest that forest-dwelling voles, *Clethrionomys sp.*, and field voles, *Microtus sp*., may form a large part of the pine martens diet Europe-wide, whereas O' Sullivan *et al* (1982) found that in Ireland, the main food species, due to lack of voles in this country, range from small *Passeriformes* to a variety of Coleoptera and Hymenoptera *sp.* with fruits forming a very large part of the pine marten diet, hence the use of fruits or other food types

could be a focus for lure choice. These preferences can vary seasonally or annually, due to some years having higher fruit cropping seasons or there being population surges in wood mouse numbers for example. In Ireland, Warner and O'Sullivan (1982) found the pine marten diet to be highly seasonal, with rates of bird, mammal and insect and fruit ingestion highly variable. None of the above appear conclusive as a definite lure type so if a non-perishable lure were provided, it would allow for constant but regular checks on the hair tubes, possibly for months at a time, potentially allowing the survey to be conducted on an annual basis.

'Long life' natural or synthetic lures have been tested using natural odours of prey species as an attractant, often as a method of population control. In a study of captive stoats, the smell of dead mice, dead day-old chicks, raw meat and raw hen eggs were highly attractive but the artificial odours and flavours tested were not (Spurr 1999). Clapperton *et al* (1994) found that when both natural and synthetic lures were used, 2-n-propylthietane (a component of stoat anal sac secretions) proved an effective ferret attractant however the synthetic scent lures did not match the stoat catch rates for egg bait. They also found that the synthetic lures did not appear to act as repellents to other species.

Commercial lures have been tested in low-density population management trials. McDaniel *et al* (2000) found that beaver, *Castor canadensis*, castoreum and imitation catnip oil had a much more positive result attracting lynx, *Lynx canadensis*, to hair snares than expected but found synthetic lures such as Cat Passion™ and Hawbacker's Cat Lure #1™ of no benefit. They found the attraction of bears to the lures to be problematic; hence it has been found that with commercial scent gland lures, it is important to refine the lure to attract as few non-target species as possible. It would be cost-inefficient to analyse non-target species hair, plus those species may deter the target species (McDaniel *et al* 2000).

The samples retrieved can give valuable data on the rates of entry to tubes and potential 'learning behaviour'. Hair collected can be forensically analysed to prove species type and sex of the individual; and using microsatellite analysis to differentiate between individuals, can gain insight into population structure and dynamics.

1.5 Genetic Analysis

Historically pine marten populations have been identified using scats – classified by their morphology, scent and contents (Gomez-Moliner *et al* 2004). In areas of low abundance, misidentifications (as high as 97%) are possible, even by proficient ecologists (Davidson *et al* 2002). They are primarily mistaken for fox and vice versa. It is possible to microscopically analyse samples of guard hairs and under-fur collected from the marten's pelage but it is both time consuming and confusing with pine marten having many closely related species and subspecies (Teerink 1991, Mowat and Paetkau 2002). Non-invasive genetic methods are becoming increasingly integrated into obtaining information on population size and genetic variability (Waits and Paetkau 2005).

1.5.1 Species-typing using mitochondrial DNA

Amplified DNA is traditionally species typed by DNA sequencing or restriction fragment length polymorphism (RFLP) analysis. PCR-RFLP markers prove useful for the identification of mustelid species, for example, as they are cost effective and suitable to work with low quality- low quantity DNA. This may also be important because routine DNA typing from scats can be difficult due to rapid degradation of scat DNA; however high copy number means that mitochondrial DNA may be most useful for surveying species, as it may even be used in relatively old and rain-washed samples (Davison *et al* 2001) and the target region analysed is a short fragment that can be amplified even in the case of partial sample degradation (Colli *et al* 2005). With the advent of new molecular data such as mitochondrial DNA sequences or microsatellites, scientists have been able to examine the levels of genetic diversity in their target species, compare its extent within and among populations as well as between species and test if the observed pattern was congruent with geography, known as phylogeography (Excoffier 2004).

Mitochondrial DNA is a small extrachromosomal genome, typically ~16 kb in size and the study of these genomes as they function in mitochondrial systems – 'mitochondrial genomics' – serves as a model for genomic studies. Replication of the

mitochondrial genome is initiated in the non-coding control region, termed the D-loop (Displacement loop) and this has a high mutation rate due to a lack of corrective enzymes. As a result the control region has high intra- and inter-specific variability, making it a useful genetic marker for inferring evolutionary relationships, since rearrangements appear to be unique, unlikely to arise independently in separate evolutionary lineages (Boore 1999). This may be particularly useful for differentiation between closely related subspecies. Also, because of this variability, the D-loop has been suggested as a good candidate for population analyses and for phylogenetic studies (Bellinvia 2004). It provides a method of differentiating between haplotypes even among closely related species and populations (Gomez-Moliner 2004).

Hansen and Jacobsen (1999) used PCR amplification and restriction enzyme digestion of part of the mitochondrial cytochrome *b* gene to differentiate between the mustelids -otter, mink and polecat while Riddle *et al* (2003) developed mustelid identification protocols using Fisher's marten, *Martes pennanti*, wolverine, *Gulo gulo*, American marten, *Martes americana*, mink, *Mustela vison* and skunk, *Mephitis mephitis* using known mitochondrial cytochrome *b* sequences. Statham *et al* (2005) developed a method for analysing mitochondrial d-loop sequences using restriction enzymes (RFLP analysis of the D-loop region) to differentiate between mustelids in Ireland.

1.5.2 Determination of sex using zinc finger genes

Sex identification using DNA, from non-invasively collected samples such as hair, provides new opportunities to improve census methods and determine the sex composition of social groups – demographic composition (sex ratio) and potential gender bias in population management conservation schemes. Nuclear DNA is required for sex determination. Mammals can be sexed using nuclear DNA as the female possesses two X chromosomes and the male possess one X and a smaller Y chromosome.

Molecular sexing is the process of sexing individuals based on variation in DNA between sexes. Methods include amplification of Y-specific fragments, usually based on the sex-determining region of the Y-chromosome (*SRY* gene), and

amplification of homologous fragments (the *ZFX–ZFY* genes in mammals) on both X and Y chromosomes. Molecular sexing has been widely used to sex foetuses in humans, other primates and livestock and to a lesser extent in sexing birds, whales, seals and fish, which are difficult or impossible to sex visually (Vidya *et al* 2003). Morin *et al* (2005) states that SRY gene detection via polymerase chain reaction has become widely used in gender determination across a variety of mammalian species.

Eutherian mammals have one copy of the gene on each sex chromosome (*ZFX* and *ZFY*). Analysis of the zinc finger genes, have been widely used in sexing studies. Amplification of Y-specific fragments has been based on the Y chromosomal sex-determining region (SRY) gene, with positive amplification indicating male identity. One demonstration of DNA-based sex assignment in mustelids has been gender determination (Lynch and Brown 2006) by PCR amplification of the chromosome Y-linked SRY gene however this system produces male-only specific amplifications. Non-amplification of the target Y fragment should indicate a female but doesn't always, as PCR amplification may fail for many reasons, such as poor quality or low quantity of DNA (Ortega *et al* 2004).

ZFX-ZFY primers are more reliable than SRY markers particularly in non-invasive genetics because they minimise the risk of false negatives (Mucci *et al* 2007). For molecular sexing, PCR primers can be designed to amplify *ZFX* and *ZFY* genes, which are distinguished by PCR product size polymorphism or restriction fragment length polymorphism (RFLP). Conserved regions allow amplification from a wide range of species (different regions of the zinc finger gene have been used to sex animals to date) but as the product size is often the same for *ZFX* and *ZFY* genes, sequencing followed by restriction enzyme digestion is often required.

The advantage of using the homologous *ZFX-ZFY* gene is that the *ZFX* can be used as an internal positive control for PCR amplification. If both the X and Y fragments amplify, the sample is male whereas if only the X amplifies the sample is female.

1.5.3 Real-time analysis

Polymerase Chain Reaction (PCR) is a process where by tiny amounts of DNA can be enzymatically amplified to produce many thousands of copies. This has

revolutionised the detection of DNA because even a single copy of a particular sequence can be specifically amplified and detected. DNA polymerase enzyme copies the region of DNA determined by two primers, with the addition of deoxynucleotides, magnesium, template DNA and buffers. After 25 to 40 repeats the target sequence is amplified giving a large product. In theory there is a quantitative relationship between the starting target sequence and the amount of PCR product given.

Real-time PCR gives the ability to monitor the progress of the PCR as it occurs. Data is collected throughout the PCR process rather than at the end of the PCR. The product accumulation can be viewed in real time using fluorescent signals. The higher the starting copy number of the nucleic acid target, the sooner a significant increase in fluorescence is observed. Analysis is carried out by computer software.

There are three phases to the PCR; the product doubling phase (after every cycle if at maximum efficiency), the linear phase and the plateau phase where amplification ends as reaction components are used up. Real-time PCR detects product accumulation during the exponential phase after each cycle. In real-time PCR, reactions are characterised by the point in time during cycling when amplification of a target sequence is first detected (the threshold cycle or Ct) rather than the amount of target accumulated after a fixed number of cycles. This threshold is usually set by the software to be above background fluorescence but in the exponential amplification phase, hence fluorescence increases with product formation. The fractional cycle number at which the sample fluoresces reaches a threshold value called the cycle threshold (Ct) value. It provides a measure of the initial DNA concentration, as a sample with many copies of the DNA sequence required, will reach the threshold faster than a sample with low DNA yields. Also, the Ct levels are inversely proportional to the amount of target nucleic acid in the sample (i.e. the lower the Ct value the greater the amount of target nucleic acid in the sample).

There are two main chemistries used in real-time PCR – intercalating dyes (usually SYBR Green 1) and fluorogenic probes. The SYBR Green binds to all DNA present so as the amount of DNA accumulates the level of fluorescence increases proportionally. The fewer cycles it takes to reach a detectable fluorescence, the greater the amount of target DNA present (Higuchi *et al* 1993). A disadvantage to this technique is that the lack of dye specificity can mean false signals (for specific and non-specific products) can be produced.

1.5.3.1 Taqman chemistry

Real-time systems for PCR were improved by the introduction of fluorogenic-labelled probes that use the 5' nuclease activity of DNA polymerase. Holland *et al* (1991) were the first to show cleavage of a target probe by the 5' nuclease activity of Taq DNA polymerase and that it could be used to detect amplification of the target-specific product. The TaqMan probe enabled the detection of a specific PCR product as it accumulated during PCR (advantageous in real-time PCR). This avoided the need for electrophoresis and allowed the selection of short sequences for amplification, which may be critical when obtaining DNA from deteriorated samples (Lopez-Andreo *et al* 2005).

Fluorogenic probes complementary to the target DNA region have been developed with both reporter and quencher dyes attached (Lee *et al* 1993, Livak *et al* 1995). If a target sequence is present, the probe anneals between the primer binding sites and is cleaved by the 5' nuclease activity of the Taq DNA polymerase as this primer is extended. Cleavage of the probe separates the reporter dye from the quencher dye, increasing the reporter dye signal and removes the probe from the target strand, allowing primer extension to continue to the end of the template strand. The reporter dye emits a fluorescence detected by the computer software to signal the presence of target DNA.

TaqMan minor groove-binding (MGB) probes are commonly used as they contain a non-fluorescent quencher at the 3' end to remove background fluorescence, which might interfere with detection of the reporter dye signal and they stabilise the probe to the template strand allowing the design of shorter probes that can specifically discriminate between two sequences that differ by a single nucleotide polymorphism (SNP).

1.6 Aims of this Study

The aims of this study were -

1. to determine the presence of pine marten in Portlaw Woods, Co. Waterford. Although it is generally known from sightings and road kill (neither being common place) that a population of pine marten do reside in the woods in Portlaw, a baseline study survey needed conducting to verify this. From other studies it is known that pine marten can have large territories (especially males) it is presumed that the population here must be small and potentially limited.

2. to determine where in the woodland would be most suitable to locate hair tubes to collect data? Hair tubes would need to be placed on sturdy trees, away from public view but accessible for baiting and pine marten entry.

3. to conduct field trials to assess which type of lure might be used to attract pine marten to the hair tubes on a regular basis? Meat-based lures and chemical (both natural and synthetic) lures have been used in surveys for other species such as the stoat; hence a selection of these was tested for use in attracting pine marten.

4. to devise a non-invasive method of removing hair samples from the coats of the animals for collection and analysis. From the various hair retrieval techniques that have been used in previous studies, one was selected and field tests carried out to monitor its use and perfect where necessary.

5. to monitor pine marten movements or locate potential territories by using coloured pellets in the lures.

6. to genetically test the collected hair samples to confirm pine marten presence and then sex the samples to give a greater indication of the gender ratio of the population. This could potentially be used to map territories and/or prove very useful in monitoring population dynamics for conservation management schemes, especially if the ratio was high in favour of males.

Chapter 2

Materials and Methods

2. Materials and Methods

2.1 Hair tube location

In June 2003, Portlaw Woods, Co. Waterford, were assessed for habitat suitability for the location of the hair tubes. Pine marten scat heavily where their own trails bisect man-made ones, along other linear features such as streams and on open sites such as logs and rocks (Birks *et al* 2002), hence in this study, man-made tracks and streams are featured along the survey area. Many studies construct transects in straight lines but in this case the study takes a looped format and encompasses large areas of Portlaw Woods (Figure 2.1). This is part of a large (c.1000 ha) mixed deciduous-coniferous woodland and is for the most part, Coillte Forestry Service managed. Part of the forest belongs to the Curraghmore estate, and access to this was not possible.

The hair tubes were numbered, labelled and placed approximately 350 footpaces apart to give adequate coverage of the terrain. The study was carried out to prepare a base line, to investigate the presence of pine marten in the area.

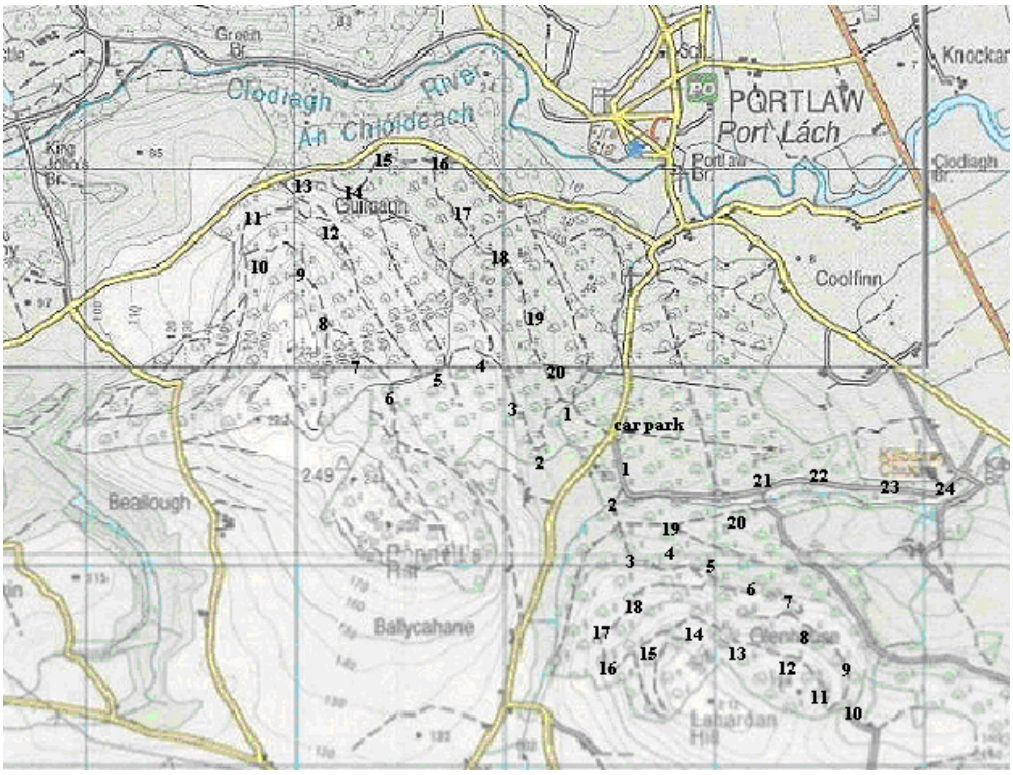

Figure 2.1 Portlaw Woods, Co. Waterford showing location of hair tubes

The tubes were placed ~1 metre from the edge of the service tracks. These are used by the Coillte forestry service for forest maintenance and by the public as functional leisure routes (Figure 2.2). The tubes were wired 1 to 1.5 metres up the side of the tree and were faced into the undergrowth, in order to be inconspicuous to minimise human interference (Figure 2.3a).

Figure 2.2 Track used by Coillte and the public, adjacent to which tubes were located

Figure 2.3(a) Hair tube wired inconspicuously to tree and **(b)** the method of attachment to tree to allow for loosening as tree's girth expands

The tube body is a 250mm length of standard 118mm diameter sewer pipe. A section approximately 3cm wide is cut from it longitudinally. This allows better hold on the tree when in place and allows the pine marten access to grip the tree trunk. Holes are drilled near the top of the tube for the fixing wire and to allow the tube to be wired for bait (Figure 2.4). The cap is a standard sewer end plug and fits over the top.

The hair tubes were wired to trees though holes in the tubes and were tied such that the galvanised wire could be loosened as the tree's girth grew. This is important so as not to damage the trees.

Figure 2.4 Hair tube design showing fixing holes and with bait inside

Once the tubes were *in situ*, sticky patches were then placed in the tubes along with the chicken bait as shown above. Two types of sticky patch were used during this study – a double-sided sticky tape patch and later a stickier glue mousetrap patch, the latter designed by Dr. Peter Turner, Waterford Institute of Technology. The double-sided sticky tape patch was used to collect hair initially. The patches were cut from a sheet of corrugated plastic, approximately 4mm thick, into ~ 2 cm^2 pieces. The block was wrapped with Scotch 'pressure sensitive' tape (stock reference no: 465; North British Tapes Ltd; Killingworth, Tyne & Wear, UK). Double-sided sticky tape was wrapped around this and then covered to maintain adhesiveness until ready for use. These were similar to the patches used by Mowat and Paetkau (2002) when collecting hair samples of *Martes americana* in Canada.

The patches were placed 7 to 10 cm up into the tube and on opposite inner walls. It was ensured that there was no contamination around the tube entrance. Any moisture or grease is dried thoroughly with a clean paper towel. The patch is pressed into place with the corrugations running along the length of the tube. This is imperative as it allows the block to take the curvature of the tube. The patch is firmly pressed into the tube, ideally using enough force to make the corrugations collapse

and being careful to press on the paper covering the patch and not to sticking your finger or thumb to the patch.

The chicken was pierced with a piece of wire and dangled from the top of the hair tubes just under the lid – lid removable for access. Once baited and patches in place, the tubes were left *in situ*. The tubes were checked approximately every 3 days and the chicken and sticky patches renewed as required. As the patches were collected, they were placed in plastic universal tubes with the tube number and date retrieved recorded. The samples were returned to the laboratory and stored at room temperature for later genetic analysis.

During the June 2003 survey, it was noted that the double-sided sticky patches lost their adhesiveness in a number of incidences, so for the following study in 2004, the patches were upgraded to the more effective glue mousetrap patch (Figure 2.5).

The mousetrap glue patches were assembled by wrapping the block in pressure sensitive tape allowing a moderate overlap and left covered with the backing layer. A mousetrap patch was stuck on the block ensuring that the cardboard backing is stuck to the block and the waxed paper side is uppermost. The cardboard is identifiable by the printing and by being thicker. The adhesive glue section was cut from a Mouse glue trap (Product code: STV182 STV International Ltd., Forge House, Little Cressington, Thetford, Norfolk, IP25 6ND, England – obtained from Solway Feeders Ltd., Dundrennan, Kirkcudbrightshire, Scotland).

Figure 2.5 Mousetrap glue patch used in hair tubes

On completion of the June 2003 baseline study, the wire on the 20 tubes was checked for tightness and loosened if it appeared to be constricting the trees. The

tubes were cleaned thoroughly to remove residues. The hair samples were returned to the laboratory and stored at room temperature.

2.2 Evaluation of the effect of lures on captive pine marten

To test the efficacy of a number of different lures, a study was undertaken with two captive pine marten in Wildwoods Animal Park, Kent, England. The pine marten, being solitary animals, are housed separately in large enclosures (Figure 2.6). They have a nest box for a den and have created a number of routes of preferred travel in their enclosures.

Figure 2.6 Pine marten in enclosure in Wildwoods Animal Park, Kent, England

Lures were offered using wooden stakes, approximately 18 cm long and 2 cm square. Each had a hole drilled 4 – 5 cm's down into the centre. The lure was liquefied if necessary and poured into the hole in the top of the stake. The stake was left to allow the lure to permeate through the wood, except for the blank, which remained empty. Ten different lures were used – mink oil, chicken fat, fish oil, duck fat, blackberry juice, ivy berry juice, beef oxo, chicken oxo, peanut butter, sardine oil and one blank.

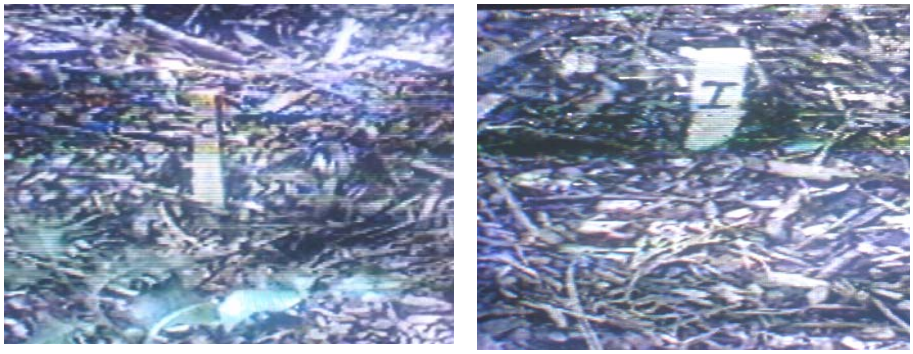

Figure 2.7 Wooden stakes in floor of pine marten's enclosure

The stakes (Figure 2.7) were hammered into the floor of the animal's enclosure (spatially approximately 1 metre apart) close to the animal's tracks. Two different pine martens were studied and their behaviour towards the lures recorded over a two day period. It was ensured that disruption to the animal's routine was avoided where possible, while recording any reactions to the new scents (lures) in their enclosures.

2.3 Testing the efficacy of four different lures in the field

In July 2004, a twenty four hair tube study was drawn up in the area of forest opposite the previous study in Portlaw Woods (Figure 4.4). The area was assessed for suitable hair tube sites and the tubes were set up as for previous studies. This survey took place over four weeks and three different lures were used plus a blank. The lures used were fish oil (a Blue Dragon product purchased in the local supermarket), chicken (a wing piece) and Mink Gland lure (purchased from Kishel's Quality Animal Scents and Lures, P.O. 162, East Aurora, NY 14052, U.S.A.). The blank remained empty. The lures were put into the first four tubes then repeated for the next four and so on along the transect line. These were rotated over the four weeks so that each tube will have contained each lure at one point.

In order to get the liquid lure into the tubes, a cotton wool pad was soaked in the lure and the cotton wool was forced into the corrugations in the sticky patches. Hence the patches smelled of a particular scent. This did not appear to affect the

adhesiveness of the patches in the short term. These were set up and checked every day for 3 days. The tubes were cleared out for two days to allow the scent to dissipate and the lures rotated. For example tube number 1 had mink gland oil in week one, chicken in week two, fish oil in week three and was empty for week four.

The hair patch samples collected were put into plastic tubes with the date of collection and tube number put on it. The samples were returned to the laboratory and stored at room temperature with previous samples. After completion of the study the tubes were emptied and cleaned but left *in situ* in the forest.

2.4 Efficacy field study of six different lures

Meat-based lures are known to be excellent lures (Dilks *et al* 1996) and a predominant pine marten food item (M De Marinis 1995, Pulliainen *et al* 1996). By experiment, if chicken is used, and taken, it no longer becomes useful as a lure in a long-term study. It was hoped that the use of beef oxo cube paste, the juice of blackberries and ivy berries combined, Blue Dragon fish oil, Bovril (a yeast based meat substitute), smooth (nut-free) peanut butter and marmalade might give a result that could be used as a long-term long-life lure. Peanut butter was included because it was the lure, which gave the greatest result in the captive pine marten study in Wildwoods in April 2004.

This survey hoped to give a definitive result for some other lure type that could be used instead of chicken in long-term field studies. Six different lure types were used in 18 of the tubes from the 24 tube transect in the previous study. The first 18 tubes were used as very little activity had been recorded in the last 6 tubes. Also the study area was too long for one person to survey alone. This survey was carried out over a 40 day period from February to April 2005.

Each of the 6 lures was pasted into the lids of the hair tubes in around the rims. The next 6 tubes got the same 6 lures etc. The lures were left in place for 3 nights and then cleaned out. They were left for 1 full day, re-baited the next, the lure being moved on to the next tube so that each tube contained each lure at one time. This was done on a rotating basis similar to the previous study. On completion of the study, the

tubes were emptied and cleaned out and left *in situ* for further use. As before, the samples collected were recorded and stored in the laboratory at room temperature.

2.5 Bait marking survey to monitor pine marten activity

The development of new techniques that aid the study of free-living animals is a constant challenge for mammal ecologists however if the study animals can be persuaded to eat prepared baits, then various marker substances can be incorporated. Marked bait is widely used in studies of the ecology of the European Badger (*Meles meles*), but despite this very little information is available on the origins, applications, methodology and limitations of the 'bait-marking' technique (Delahay *et al* 2000).

The July 2005 study was carried out over a 23 day period. Every second tube from the previous 18 tube transect was used, hence 9 tubes were baited. Peanut butter with a smear of marmalade was used as the lure. Peanut butter was rolled on a bed of flour and had ~ 250 propylene-based 1 to 2mm pellets incorporated into the 'bait ball'. Each bait ball contained its own single colour, non-toxic pellets, tiny enough to pass through the animal's digestive system. The pellets were from Masterplast Ltd., Monread Industrial Estate, Monread Road, Co. Kildare. The peanut butter was wedged around the inside of the lid of the hair tube and smeared with a little marmalade and the lid replaced. The tubes were checked approximately every second day and the bait replaced as was required. Each hair tube was given bait with a particular coloured pellet inside (Figure 2.8)

The route was scanned for scats containing the coloured pellets (Figure 2.9). The location of any scat found with pellets and the colour(s) of the pellets it contained was recorded. The scats were collected, washed and the pellets removed and counted. The numbers of pellets per scat (and collected overall) was recorded (Table 3.14). On completion of this study all hair tubes were removed from the woods, the hair samples were returned to the laboratory and stored dry as before.

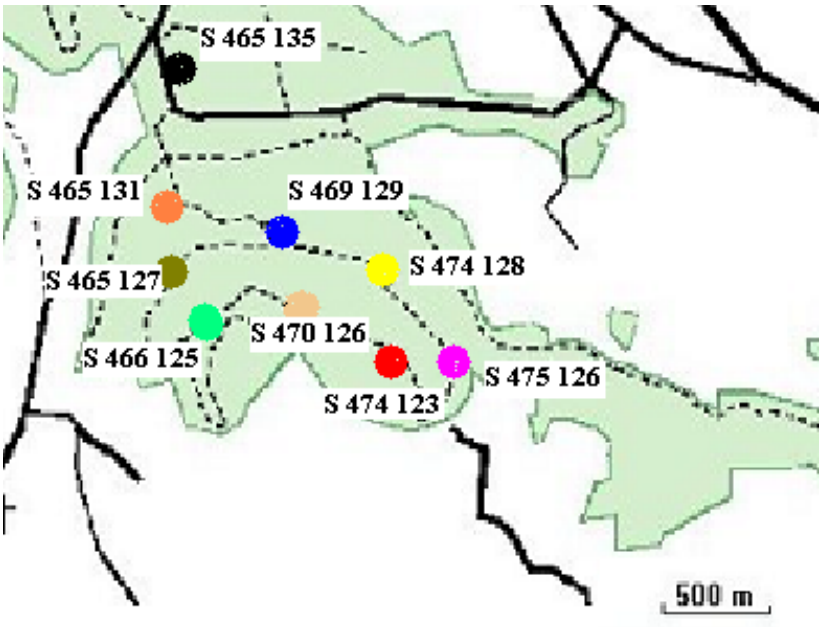

Figure 2.8 Location of the 9 hair tubes used (including grid references), each containing a single coloured pellet as per diagram.

Figure 2.9 Rain drenched scats showing incorporated plastic pellets.

2.6 Genetic analysis of hair samples

2.6.1 DNA extraction

DNA was isolated from hair using an adaptation of the Chelex method (Walsh *et al* 1991). The protocol for the extraction of DNA from hair was followed and the

supernatants, with the isolated DNA, carefully removed and frozen at -20^{o}C before analysis.

Materials used per extraction

200 µl 10% w/v Chelex solution – made up in sterile H_2O

1 µl Proteinase K 20ug/ul

7 µl 1M (DTT) Dithiotreitol

1.5ml micro centrifuge tubes

When extracting DNA from pine marten hair, the scissors, forceps etc. are sterilised before use and between samples by flaming in alcohol. Approximately 15 hairs with follicles were removed from the glue patch. This was done while the forceps was still hot as this melts the glue and aids in the removal of the hair from the patch. The 10% Chelex (BIORAD 143-2832) was re-suspended by shaking and 200 µl was added per tube. Proteinase K (Sigma cat. No. P2308) and DTT (Molekula, Dorset Prod. no. M62236573) were then added and the tubes incubated at 56^{o}C for 3 hours. The tubes were inverted every 30 minutes. The tubes were incubated in the heating block at 100^{o}C for 8 minutes after which they were centrifuged in the microcentrifuge at 13,000 rpm for 3 minutes. The supernatant was pipetted off very carefully so as not to disturb the pellet at the bottom of the tube. Supernatants were stored at -20^{o}C.

2.6.2 Real-time PCR

To species- and sex-type DNA samples using real-time PCR, primers and probes were designed by Dr. Catherine O' Reilly, Waterford Institute of Technology using Primer Express 2 software (Applied Biosystems). All reactions were carried out on an Applied Biosystems 7300 sequence detection system using Applied Biosystems Sequence Detection Software (SDS 1.2).

A commercial PCR mastermix (Taqman Assay Mastermix – Applied Biosystems) was used which contained the necessary components for the PCR excluding primers and probes. In preparing the real-time PCR, a complete working

mastermix (12.5 µl) was prepared and dispensed into each well of the 96-well reaction plate (Tables 2.1 and 2.2). The probe(s) and primers were added and DNA added last before sealing the plate. DNA dilutions varied depending on the process – 1 in 20 for species typing and 1 in 10 for sex typing but these concentrations depended greatly on the results of the first PCR. Two No-Template Control blanks were used to verify the reaction was free of contamination. Two known controls of species- and sex were also used (pine marten DNA) to ensure the PCR worked. If these gave a correct value, any negative results obtained were sample-specific not due to the components added.

Component	Sequence	Quantity
Taqman mastermix		12.5 µl (4304437)
Stoat PM Forward	5'-CTG CCC CAT GCA TAT AAG CA 3'	1.0 µl (~200 nM)
Stoat PM Reverse	5'-GGC CCG GAG CGA GAA G 3'	1.0 µl (~200nM)
PM3 probe	5'- 6FAM-CGTGCACCTCACTTAG-3'-MGB	1.0 µl (~200 nM)
DNA		9.5 µl

Table 2.1 Reaction mix for species-typing assay

Component	Sequence	Quantity
Taqman mastermix		12.5 µl
PMZF –SNP1F	5'-AGC CAA CAA AAT GCA CAA GTG TAA A-3'	1.0 µl
PMZF – SNPIR	5'-CCA AAA GGT GGC GAT TCA ACA A-3'	1.0 µl
X probe (ZFX)	5'-Vic-CCT TGT TCG GCT GTC T-3'-MGB	1.0 µl
Y probe (ZFY)	5'-6Fam-CCC TTG TTC AGC TGT CT-3'-MGB	1.0 µl
DNA		8.5 µl

Table 2.2 Reaction mix for sex-typing assay; concentrations as before (PMZF - Pine marten zinc finger, SNP1F - snip 1 forward primer, SNP1R – snip 1 reverse primer, SNP - single nucleotide polymorphism)

The forward primers were labelled with a fluorescent dye (Vic or 6Fam) and was synthesised by Applied Biosystems. The reverse primers were unlabelled and synthesised and supplied by MWG Biotech, Germany. Run conditions using the

standard run for Absolute Quantitation were set by the instrument (Table 2.3). Forty cycles were used for species typing and fifty cycles used for sex typing.

Step	Repeat	Temperature	Time
Incubation	1	50 ^{o}C	2 minute
Polymerase activation	1	95 ^{o}C	10 minute
Denaturation	40	95 ^{o}C	15 seconds
Annealing		60 ^{o}C	1 minute

Table 2.3 Run conditions for above real-time PCR assays

Chapter 3

Results

3. Results

The survey in Portlaw Woods, Co. Waterford was undertaken from July 2003 to July 2005. Initial baseline work was carried out to determine the presence of pine marten in the area. It was found that pine marten did visit the hair tubes, bait was taken, hair samples were collected and genetically analysed.

3.1 Baseline study to determine hair tube use by pine marten

In June 2003 the forest in Portlaw was trekked along Coillte paths and suitable habitats for hair tubes were chosen. The topography varied from low-lying, wet and dry areas of mixed dense cover (contour line 70), to the bleaker top of the hill (Figure 3.1)

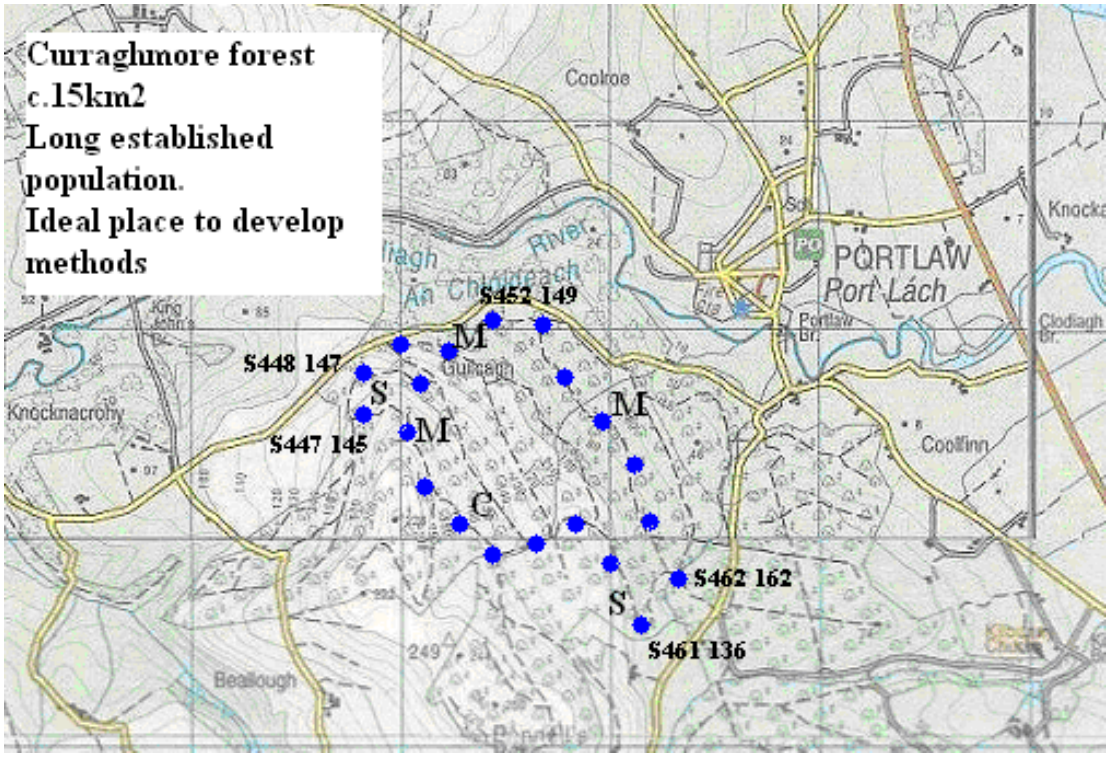

Figure 3.1 Map of Portlaw wood showing tube locations and types of habitat S – spruce plantation, M – mixed woodland, C – areas of clear fell

The terrain varied from dense spruce plantation, clear-fell areas of logged spruce with new saplings up to 1 metre high, to mixed evergreen (spruce, holly and rhododendron) and deciduous areas (Table 3.1). Twenty tube sites were chosen and were paced apart by footfall. Point zero (approx S463 150) was taken as the centre of the car park (Figure 3.1) and the first tube was set 347 paces from here. The other tubes were placed as grid referenced (Table 3.1).

Tube number	Habitat type	Tube grid reference	
1	Dense Spruce canopy, bramble	S462	162
2	Spruce, birch mixed canopy, grasses	S461	136
3	Isolated Rhododendron thicket	S460	113
4	Dense Sitka spruce, bramble and grasses	S457	141
5	Mixed deciduous woodland	S456	139
6	Exposed Sitka spruce, rocky, grasses	S453	140
7	Birch, bramble, ditch between pasture and clear-fell	S451	142
8	Lone Holly, felled area, sapling spruce	S450	143
9	Rhododendron thicket	S450	145
10	Willow stand, bramble and grasses	S447	145
11	Dense Sitka spruce, bramble, moss	S448	147
12	Mixed oak, rowan, fern	S451	147
13	Dense Sitka spruce, bare needle floor	S449	148
14	Mixed rowan, birch fern	S451	149
15	Mixed willow, rowan, fern and grasses	S452	149
16	Holly, oak, fern, bramble and grasses	S455	150
17	On tree-stump above bramble thicket	S457	148
18	Airy open alder, birch grove with fern	S459	146
19	Waterlogged, Alder Birch ferns and moss	S461	143
20	Flooded, Alder Birch Rowan fern, moss	S462	140

Table 3.1 Hair tube habitats and grid references in Portlaw Woods.

The majority of the terrain is covered by mixed woodland - dense, mixed over story of deciduous (e.g. mountain ash, hazel, birch, alder) and evergreen (e.g. spruce, rhododendron, holly) woodland. The under-story comprised of grass and fern with bramble throughout under the deciduous areas whereas mainly needles and moss under the spruce and bare floor under the rhododendron canopy.

The tubes were baited with a piece of chicken and double-sided sticky patches inserted. A "visit" to the hair tube was determined by the presence of hair on the sticky patches. In the July 2003 study, the hair tubes were left in place for 28 nights, then checked and re-baited every 3 to 4 days giving a total of 7 baiting sessions. 27 visits to the tubes were recorded. The most frequently visited tubes were in the area of tubes 15 to 18 (Figure 3.2 and Table 3.2).

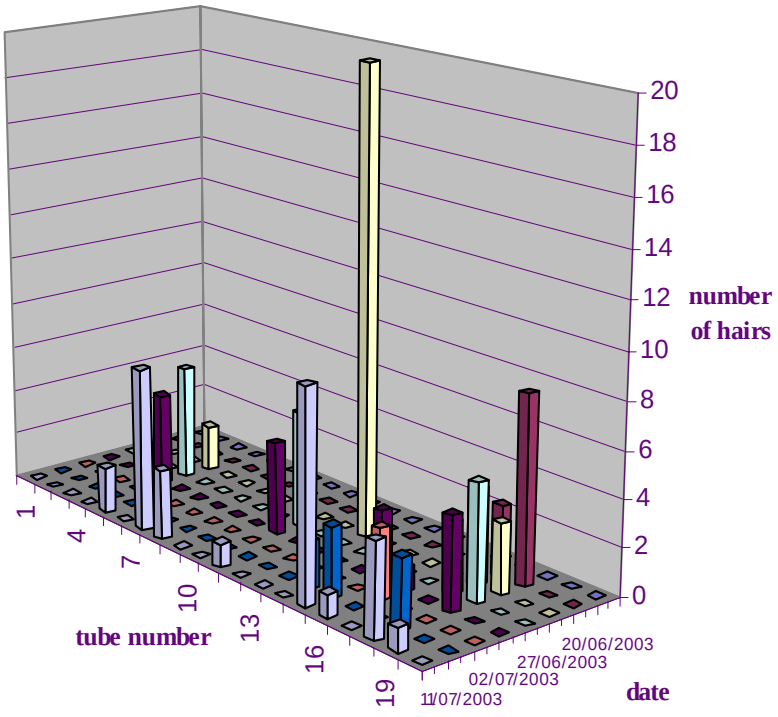

Figure 3.2 Numbers of hair collected per sticky patch

Figure 3.2 shows tubes 15 to 18 have the highest numbers of hair retrieved per patch. There were 113 hairs gathered and ranged from 1 to 20 hairs per patch (Table

3.2), averaging 4.19 hairs per visit. There was a definite lag time in the uptake of bait from the tubes.

Tube number	Number of visits	Number of hairs collected per visit
1	0	0
2	0	0
3	0	0
4	3	2, 5, 4
5	1	2
6	0	0
7	1	7
8	1	3
9	0	0
10	2	5, 4
11	1	1
12	1	20
13	0	0
14	1	2
15	3	3, 3, 9
16	3	1, 3, 1
17	3	3, 4, 3
18	6	8, 3, 5, 4, 3, 4
19	1	1
20	0	0

Table 3.2 Number of visits and hairs retrieved in July ’03 field study

3.2 Efficacy of chicken as a lure using glue sticky patch

From January to March 2004, a repeat of the July 2003 study was carried out. This featured the new mousetrap glue patch (Figure 2.5) and extended over a longer period of time. The same 20 tubes were used; located as before. The tubes were left for 36 nights and re-baited every 5 to 7 days. There were 6 baiting sessions. 283 hairs were obtained in this study as opposed to 113 previously.

Table 3.3 shows the quantities of hair captured over the 36 nights. In total 35 visits were recorded. A lag time was evident (Figure 3.3) although both the numbers of hairs and frequency of tube visits were erratic.

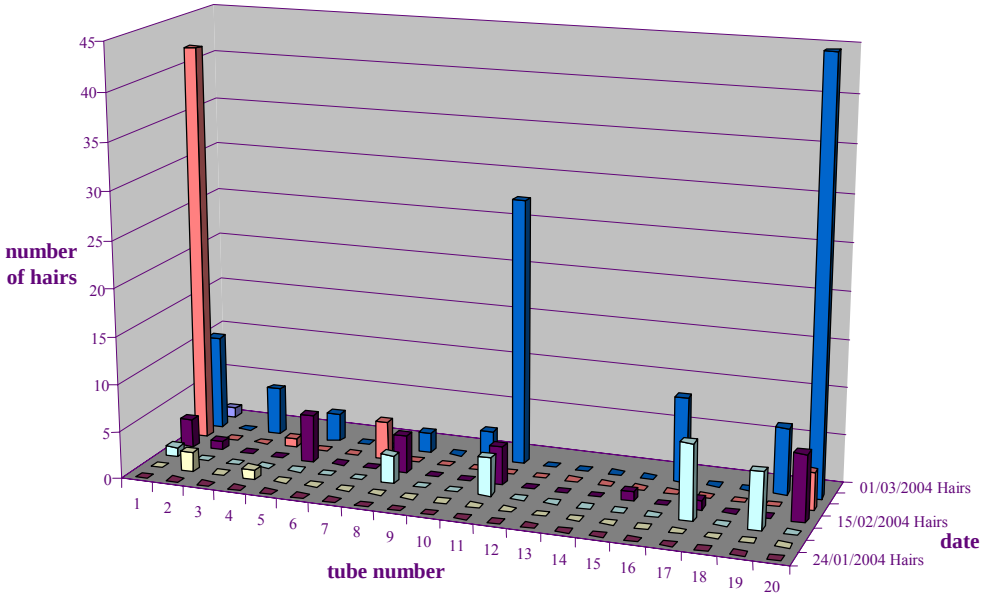

Figure 3.3 Number of hairs retrieved using the modified glue patch in the January/ March'04 study

Tube number	Number of visits	Number of hairs per visit
1	5	1, 3, 42, 10, 25
2	3	2, 1, 4
3	1	5
4	3	1, 1, 4
5	3	5, 3, 16
6	0	0
7	1	4
8	3	3, 4, 2
9	1	6
10	1	3
11	3	4, 4, 28
12	0	0
13	1	1
14	0	0
15	2	1, 6
16	1	16,
17	2	8, 1
18	0	0
19	2	6, 7
20	3	4, 7, 45

Table 3.3 Number of visits and hairs retrieved per visit January/ March 2004

3.3 Captive pine marten responses to lures

A study was carried out in Wildwoods Animal Park in Kent, England in April 2004 to assess pine marten responses to a variety of lures. The favoured lure would be used in field studies in Portlaw Woods to entice pine marten into hair tubes. Ten wooden stakes were hammered into the floor of two pine marten enclosures, each having been soaked in a different lure type (Figure 2.7). The animal's reactions were recorded over a period of two days. Normal routes around the enclosures were noted and if the animals deviated from this it was recorded. Observations were made at each enclosure (Table 3.4) and the responses converted to numerical data (Tables 3.5 and 3.6).

DAY 1	
11.15am	(male) "Fudge" in nest box
11.40am	sniffed at duck fat
11.41am	scent marked ivy berry stake; sniffed mink gland, sardine oil
11.44am	scent marked beef oxo stake
11.44am	scent marked corner near female enclosure
11.50am	too many people – "Fudge" hid in nest box
11.56am	out and scent marked peanut butter stake
12.06pm	stereotypical running round and round enclosure
12.20pm	keeper arrived with food
DAY 2 – stakes rearranged	
11.48am	most stakes sniffed at
11.50am	scent rubbed ivy berry
12.06pm	chewed peanut butter stake; mink gland stake scent marked

Table 3.4 Sample observations of captive pine marten reactions to lures.

Sniffing and nudging indicated little response. Chewing, scent marking and urinating required more deviation from the animal's normal path. Scent marking involved rubbing the underbelly glands against the stakes in the enclosures. Responses were given a value of 1 regardless of the response type; hence a value of 4 implies a lure was sniffed at 4 times over the course of the observation time.

Lure/ Reaction	Sniffed	Nudged	Chewed	Scent	Urinated
Mink oil	4	1	0	**13**	1
Chicken fat	3	0	0	0	0
Fish oil	1	0	0	3	3
Duck fat	1	0	0	0	0
Blackberry juice	4	2	0	2	0
Ivy berry juice	0	0	0	1	0
Beef oxo cube	4	3	0	1	0
Chicken oxo	1	2	0	0	0
Peanut butter	2	1	**1**	0	0
Blank	0	0	0	0	0
Sardine oil	2	0	0	0	0

Table 3.5 Reaction of "Fudge" to each lure; highlighted numbers elicited strongest response

Lure/ Reaction	Sniffed	Nudged	Chewed	Scent	Urinated
Mink oil	0	0	0	**12**	0
Chicken fat	0	0	0	0	0
Fish oil	0	0	0	0	0
Duck fat	0	0	0	0	0
Blackberry juice	3	2	1	2	0
Ivy berry juice	0	0	0	0	0
Beef oxo cube	1	0	0	0	0
Chicken oxo	0	0	0	0	0
Peanut butter	1	0	**1**	0	2
Blank	0	0	0	0	0
Sardine oil	0	0	0	0	0

Table 3.6 Reaction of "Poop" to each lure; highlighted numbers elicited the greatest response

The highest ranking lure was mink oil, with 13 and 12 responses respectively. Peanut butter elicited a variety of responses, the most dramatic being that the stake was pulled from the ground and chewed. Observations of both marten showed very similar results, with the mink oil being scent marked most often and peanut butter stake also being pulled from the ground and chewed.

3.4 Survey of the efficacy of lures in the field

In the July 2004 study, 24 hair tubes were set up in different locations (Figure 3.4) with habitat detail and grid references given in Table 3.7. The centre of the car park was taken as point zero (grid reference S465 137) and paces counted as before. The hair tubes were in place for 24 nights and the tubes were checked twice per baiting session (once during the baiting session and again at re-baiting).

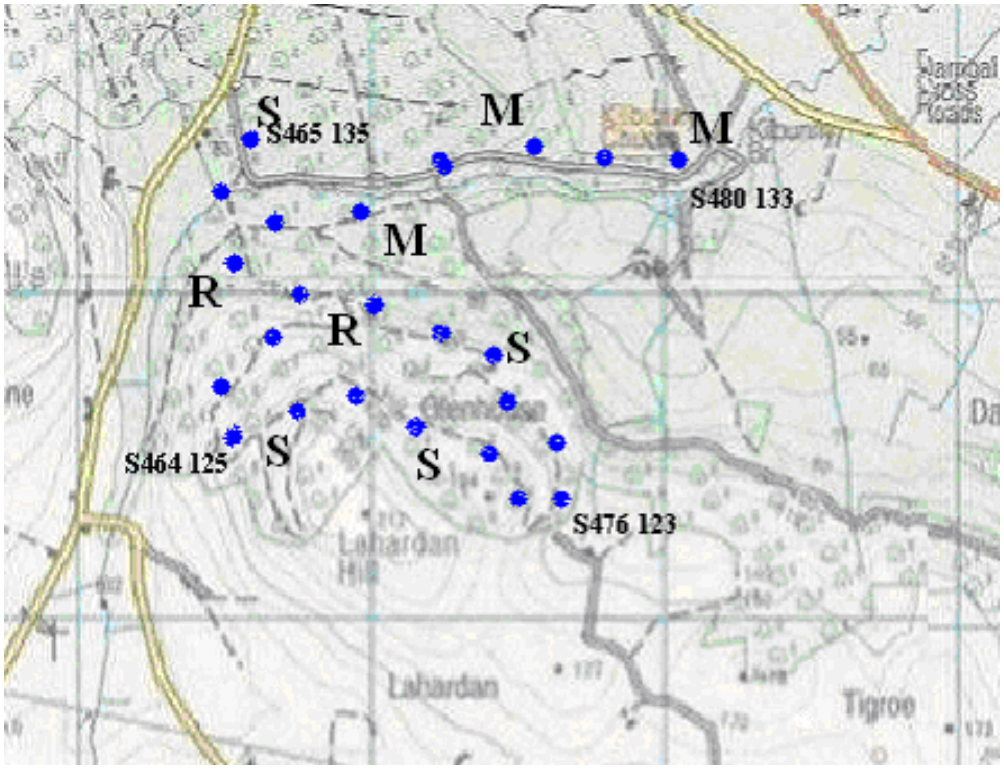

Figure 3.4 Area studied from July 2004 to July 2005 with grid reference points, showing hair tube location and habitat types; M - mixed woodland, S - Spruce, R-Rhododendron

There was an initial baseline study in which chicken alone was used in all 24 tubes. Four baiting sessions ensued. On the first rotation, the chicken was changed to mink oil, fish oil and chicken and a blank (taken as 4 lures) to see what affect this might have on the numbers of visits to the hair tubes. Each lure was rotated through the 24 tubes such that each tube received each 1 of the 4 lures and no tube had the

same lure for longer than 3 nights in the rotation. After 3 nights, each lure was removed, the tube cleaned and the lure moved ahead.

As before, visits were made to the hair tubes, which resulted in no hair being retrieved i.e. patches missing or bait gone but no hair on patches but only those visits that resulted in hair retrieval are recorded. During this study there were 67 visits to the hair tubes.

Tube	Habitat type	Grid Ref	
1	Dense Sitka spruce cover, needle floor	S465	135
2	Birch and Holly stand bramble and fern	S465	133
3	Rhododendron thicket with mossy floor	S465	131
4	Birch Rowan stand, bramble and fern	S467	130
5	Sitka spruce, Holly, bramble and fern	S469	129
6	Dense Rhododendron with mossy floor	S471	128
7	Sitka spruce, needle floor	S474	128
8	Holly stand, dense bramble and fern	S473	127
9	Beech stand, fern and moss under story	S475	126
10	Birch and Holly with bramble and fern	S476	123
11	Sitka spruce, needle floor	S474	123
12	Dense Holly stand, bramble and grass	S472	125
13	Sitka spruce, bramble and needle floor	S470	126
14	Oak tree, bramble, grass and moss	S468	126
15	Fallen oak surrounded by grasses	S466	125
16	Dense Sitka spruce with moss floor	S464	125
17	Open Birch stand, bramble, fern, grasses	S465	127
18	Dense Rhododendron stand, moss floor	S466	128
19	Oak tree, bramble and grass	S468	131
20	Dense holly cover with ferns and moss	S470	132
21	Beech tree stand bramble and grass cover	S472	134
22	Dense Laurel cover with grassy floor	S474	134
23	Mixed stand -Beech, Oak, Birch, grasses	S477	134
24	Mixed stand -Beech, Holly, bramble	S480	133

Table 3.7 Hair tube locations including habitat type and grid references

The July 2004 study showed 22 visits to the hair tubes when chicken was used. Ensuing rotations showed mink oil, fish oil and the tube with no lure (blank) were visited also. The chicken did bring animals to the hair tubes and the results show that with the addition of a number of different lures hair visits to the tubes continued and hair was still retrieved. Even with the change of lures, it is evident that chicken was the preferred lure with 60 out of 67 visits (Table 3.8). It is still evident that chicken out performs the other lures (Figure 3.5).

Lure/ Bait session	**Mink**	**Fish**	**Blank**	**Chicken**
Initial baiting	0	0	0	22
1	2	0	3	5
2	0	0	0	7
3	1	1	0	10
4	0	0	0	16
Total	3	1	3	60

Table 3.8 Number of visits each lure received in the four lure rotation study.

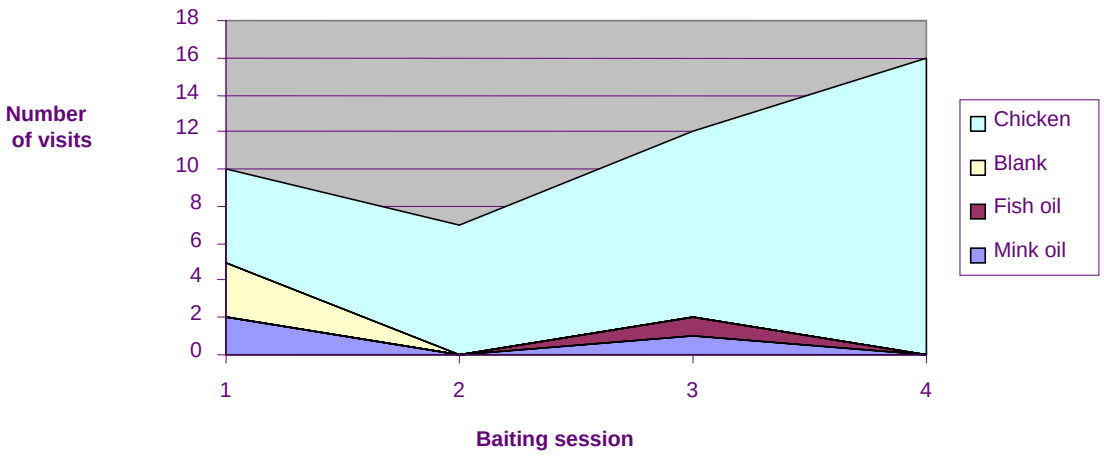

Figure 3.5 Comparison of number of visits to the 4 lures over the 4 baiting sessions is shown but the initial chicken-only baseline results are omitted

3.4.1 Statistical analysis of multiple lure rotation survey

The data was analysed as a two-factor ANOVA (Analysis of Variance). The statistics are based on the 4 lure rotation study with the initial baseline chicken results omitted. The factors were - Rotation (4 levels, because there were 4 changes to where the mink gland oil, fish oil, the blank and chicken were placed) and Bait (4 levels: mink gland oil, fish oil, blank, chicken). The dependent variable is number of visits. Descriptive results are displayed in Table 3.9.

Descriptive Statistics

Dependent Variable: number

rotation	bait	Mean	Std. Deviation	N
1	mink	0.67	0.577	3
	fish	0.00	0.000	3
	blank	1.00	1.000	3
	chicken	1.67	0.577	3
	Total	0.83	0.835	12
2	mink	0.00	0.000	3
	fish	0.00	0.000	3
	blank	0.00	0.000	3
	chicken	2.33	1.155	3
	Total	0.58	1.165	12
3	mink	0.33	0.577	3
	fish	0.33	0.577	3
	blank	0.00	0.000	3
	chicken	3.33	1.155	3
	Total	1.00	1.537	12
4	mink	0.00	0.000	3
	fish	0.00	0.000	3
	blank	0.00	0.000	3
	chicken	5.33	0.577	3
	Total	1.33	2.425	12
Total	mink	0.25	0.452	12
	fish	0.08	0.289	12
	blank	0.25	0.622	12
	chicken	3.17	1.642	12
	Total	0.94	1.577	48

Table 3.9 Analysis of the lure rotation study giving mean number of visits per lure type used (n = number of night in hair tube)

The ANOVA results reveal a statistically significant rotation /bait interaction ($p < 0.001$). This means that the effect of bait on the number of visits is significantly different, for different rotations. This can be seen from the table, where the average

number of visits for chicken is 5.33 on the final rotation but only 1.67 for the first. It is also notable that the average number of visits for chicken grows steadily over the four rotations, suggesting that there is a "learning" effect in operation, which will be discussed later.

3.5 Modified field study to show lure efficacy

Results to date show chicken is the preferred lure. In the February/ April 2005 study, chicken was removed in order to find another lure that might be favoured as highly. The first 18 of the previous 24 hair tubes (left *in situ* from July 2004) were used and the rotation carried out as before using 6 lures in this study. They were left for 42 nights, and checked every 3 to 4 days, cleaned out, left empty for a day and then re-baited.

Baiting session/ Lure	**Feb 26**	**Mar 5**	**Mar 12**	**Mar 19**	**Mar 27**	**Apr 3**	**Total visits**
Marmalade	1	0	3	1	4	2	11
Peanut	0	2	2	1	3	1	9
Beef oxo	1	0	1	1	0	1	4
Berry juice	0	1	2	1	2	1	7
Fish oil	0	1	0	0	1	0	2
Bovril	0	1	0	1	2	2	6

Table 3.10 Numbers of visits to each lure type over the 6 baiting sessions

In this study a number of modifications were made. These were (a) a reduction in tube numbers used, (b) an increase in the length of time and (c) no chicken was used as a lure. The number of visits to the hair tubes was 54 but only 39 contained hair on the sticky patches. Of the 39 visits (samples retrieved), results ranged from 11 visits for marmalade to 2 for fish oil (Table 3.10 and Figure 3.6). Pine marten appear

to have a preference for marmalade, with peanut butter second. The reaction of the pine marten to peanut butter in Wildwoods was dramatic and peanut butter also ranks highly here.

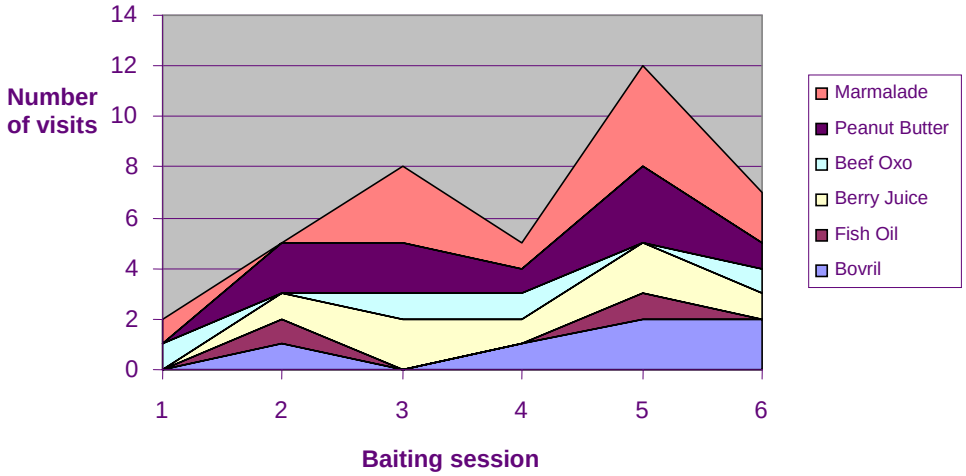

Figure 3.6 Number of visits to each lure type over 6 baiting sessions

3.5.1 Statistical analysis of modified field lures efficacy study

As before, the data was analysed as a two-factor ANOVA. In this case the interaction term (bait by rotation) is not significant, although nearly so ($p = 0.056$). The two main effects (bait and rotation) are both significant ($p < 0.001$ in both cases), so that there is strong statistical evidence of differences in the mean number of hits for different baits, and also for different rotations.

Tables 3.11, 3.12 and 3.13 summarise the analysis and effects results. Unlike above where there was an interaction effect so that the individual factors were not considered separately, it now makes sense to provide results for each factor separately. The preferred lures were marmalade (mean 0.583) and peanut butter (mean 0.444). These rank the highest in the tables but this is not significant as beef oxo has a mean of 0.417. However, as the two highest ranking lure types, marmalade and peanut butter were used in the hair tubes in ensuing studies.

Descriptive Statistics

Dependent Variable: number of visits

rotation	bait	Mean	Std. Deviation	N
1	marmalade	0.2	0.40	3
	peanut butter	0.0	0.00	3
	beef oxo	0.3	0.52	3
	berry juice	0.0	0.00	3
	fish sauce	0.0	0.00	3
	bovril	0.2	0.41	3
	Total	0.1	0.32	18
2	marmalade	0.2	0.41	3
	peanut butter	0.3	0.52	3
	beef oxo	0.2	0.41	3
	berry juice	0.2	0.41	3
	fish sauce	0.2	0.41	3
	bovril	0.2	0.41	3
	Total	0.2	0.41	18
3	marmalade	0.5	0.55	3
	peanut butter	0.3	0.52	3
	beef oxo	0.2	0.41	3
	berry juice	0.5	0.55	3
	fish sauce	0.0	0.00	3
	bovril	0.0	0.00	3
	Total	0.2	0.44	18
4	marmalade	0.7	0.58	3
	peanut butter	0.7	0.58	3
	beef oxo	1.0	0.00	3
	berry juice	0.7	0.58	3
	fish sauce	0.0	0.00	3
	bovril	1.0	0.00	3
	Total	0.7	0.49	18
5	marmalade	1.0	0.00	3
	peanut butter	1.0	0.00	3
	beef oxo	0.5	0.55	3
	berry juice	0.3	0.52	3
	fish sauce	0.2	0.41	3
	bovril	0.3	0.52	3
	Total	0.6	0.50	18
6	marmalade	1.0	0.00	3
	peanut butter	0.3	0.58	3
	beef oxo	0.3	0.58	3
	berry juice	0.7	0.58	3
	fish sauce	0.0	0.00	3
	bovril	0.7	0.58	3
	Total	0.5	0.51	18
Total	marmalade	0.5	0.51	18
	peanut butter	0.4	0.50	18
	beef oxo	0.4	0.49	18
	berry juice	0.3	0.48	18
	fish sauce	0.1	0.25	18
	bovril	0.3	0.49	18
	Total	0.3	0.48	108

Table 3.11 Analysis of lure rotation showing mean number of visits, with marmalade proving the most successful lure**.**

Dependent Variable: number of visits

rotation	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
1	0.11	0.07	0.02	0.24
2	0.19	0.07	0.06	0.33
3	0.25	0.07	0.12	0.38
4	0.67	0.09	0.48	0.85
5	0.56	0.07	0.42	0.69
6	0.50	0.09	0.31	0.69

Table 3.12 Effects of rotation. Note that rotations 4, 5, 6 have higher mean number of visits. This may imply a learning effect.

Dependent Variable: visits

bait	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
marmalade	0.58	0.08	0.43	0.74
peanut butter	0.44	0.08	0.29	0.60
beef oxo	0.42	0.08	0.26	0.57
berry juice	0.39	0.08	0.24	0.54
fish sauce	0.06	0.08	1.00	0.21
bovril	0.39	0.08	0.24	0.54

Table 3.13 Effects of bait. Marmalade has the highest mean number of visits, others much the same except for fish sauce.

3.6 Multiple lure survey using bait marking

In June and July 2005 a survey was undertaken using a similar procedure to the previous studies and using 9 of the 18 tubes from the previous study. In this study 1 – 2mm coloured non-toxic plastic pellets (which would move freely through the animal's digestive system) were added to the bait to hopefully gain some insight to the movements of the pine marten (see pellets in scat in Figure 2.9). Each tube had its own specific coloured plastic pellet embedded in the peanut butter lure (Figure 3.7).

Peanut butter was rolled into a ball in flour and had ~ 250 pellets incorporated into the ball. The bait was left for 23 nights. The tubes were re-baited 11 times over the 23 day period (every second day) this gave a total of 24,750 plastic pellets used in

the survey. In this study hair patches were collected as before. Sixty eight had hair attached to the patches even though almost every single tube had been visited on each check.

The forest tracks were examined for scats containing pellets. Over the 23 days only 9 scats containing pellets were found. The scats were collected, washed and the pellets counted (Table 3.14). In the 9 scats collected only 451 pellets were recovered. As pine marten do scat on routes where their paths intersect human routes and scat at hair tubes, it was assumed that there would be a higher pellet recovery rate. However, as only 451 pellets were recovered, this only gave a 1.8% recovery rate.

Date	**Numbers of pellets per scat**		
22/06	11 (4.4%)	90 (36%)	17 (6.8%)
24/06	0	0	0
26/06	0	0	0
27/06	0	0	0
29/06	11 (4.4%)	0	0
02/07	76 (30%)	0	0
04/07	0	0	0
07/07	52 (21%)	0	0
11/07	0	0	0
13/07	55 (22%)	62 (25%)	77 (31%)
15/07	0	0	0

Table 3.14 The 2005 dates the 9 scats were retrieved, pellet numbers within the scats and in parenthesis the percentages of overall pellets recovered

The colours of the pellets in the scats and where they were dropped gave an indication of where the animals may have travelled over the 23 nights. The results are shown in Figures 3.7 (scats with only one pellet colour present) and Figure 3.8 (pellets of different colours). There was a 2 night interval between hair tube baitings; therefore it is not certain how many animals left the scats or whether just one animal left them all.

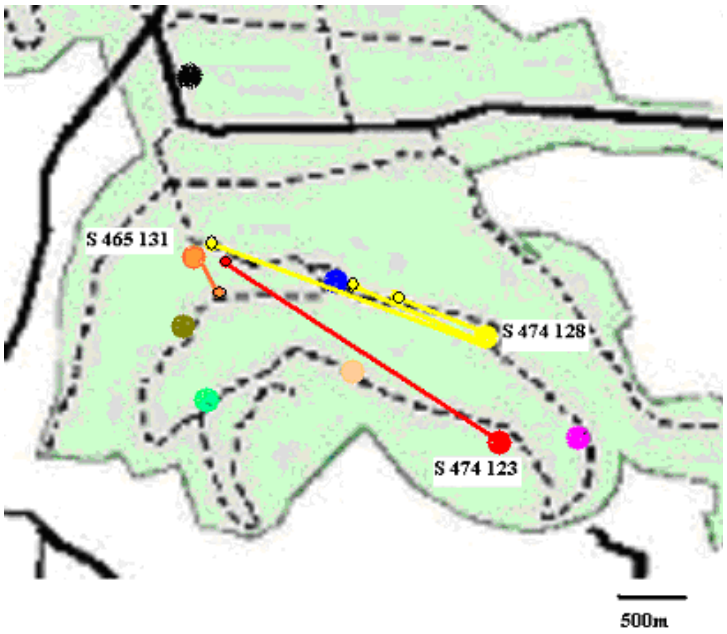

Figure 3.7 Scats with single coloured pellets and where they were found. Large circles represent the colour of the pellets in the hair tubes, small black encircled colours give the location of the deposited scat (tube grid references Fig. 2.8)

Figure 3.8 Scats retrieved with more than one coloured pellet. Colours denote hair tubes and black circled colours show the location of the scats. 3 scats contained pellets from 2 hair tubes and 1 scat contained 3 different coloured pellets.

3.7 Comparison of hair tube visits and habitat preferences

The number of visits over the duration of the entire two year study is shown in Table 3.15. The 2003/ 2004 results show an increase in the number of visits to the hair tubes over the study period. When the hair tubes were moved to a different location, a drop occurred in the number of visits initially but the number of visits increased with time. The July 2003 baseline study did attract pine marten to the tubes and an increase was seen in the numbers of visits to the hair tubes over the duration of the study. Statistics appear to show a 'learning' behaviour in pine marten (Table 3.9, 3.12 and 3.13); Table 3.15 would concur with this.

Date	Bait sessions	Number of visits
July 2003	7	27
Feb /Mar 2004	6	35
July 2004	4	67
Feb /Apr 2005	6	54
July 2005	11	68

Table 3.15 Total number of visits to hair tubes over the 2 year study period.

Pine marten show a preference for areas of cover over exposed areas. The preferred canopy was mixed deciduous /evergreen woodland or closed rhododendron canopy during the summer months and dense spruce or rhododendron cover during the winter (Table 3.16).

Date	Tube visited most often	Habitat preference
July 2003	15, 16, 17, 18	Mixed deciduous woodland
Feb/ Mar 2004	1, 2, 4, 5, 20	Dense spruce or rhododendron cover
July 2004	3, 4, 13, 17	Varied –mixed deciduous and evergreen cover
Feb/ Apr 2005	3, 10, 12, 17	Tube 3, 10, 12 dense spruce or rhododendron cover Tube 17 deciduous flanked by evergreen cover
July 2005	3, 5, 7, 9, 11, 13, 17	Varied –mixed deciduous and evergreen cover

Table 3.16 Comparison of tubes most frequently visited and habitat in which located

3.8 Genetic analyses of samples collected

The hair samples collected were analysed using Real-time PCR. To check for the target species DNA when species typing, stoat PM forward and stoat PM reverse primers and pm3 probe were used. The primers amplify both pine marten and stoat DNA; however the pm3 probe only binds to a specific part of the DNA and fluoresces if pine marten DNA is present, acting as a 'pine marten-or-not' reaction.

Species- typing gave pine marten Ct values ranging between 23.01 and 36.96, averaging 28.61. An example of the resultant Ct values is presented in Table 3.18 (taken from Appendix A). Ct values below 29 are strong positive reactions indicative of abundant target nucleic acid in the sample. Ct values of between 30 and 37 are positive reactions indicative of moderate amounts of target nucleic acid and Ct values of between 38 and 40 are weak reactions indicative of minimal amounts of target nucleic acid, which could represent sample contamination.

The wide range of Ct values seen in the sample set reflects variations in the DNA concentrations due to the variations in the number, quality and quantity of hairs used originally to produce the DNA.

Tube number	Date	Number of hairs	Ct
2	23.2.04	1	24.15
5	23.2.04	3	23.19
11	23.2.04	63	26.73
1	3.7.04	65	29.18
17	3.7.04	47	28.81
2	5.3.05	1	28.08
12	3.4.05	100+	24.78
3	27.6.05	2	27.57
1	7.7.05	2	29.38

Table 3.17 Sample of species-typed Ct values from Appendix A data set

There is no correlation between the number of hairs and the Ct value given as 1 hair gave a value of 24.15 and a tufted sample of over 100 hairs gave a similar value (24.78). Only one sample from the total 187 samples did not give a pine marten positive result when species typed. When the 187 samples were sex-typed, 28 (15%) were female, 142 (76%) were male and 17 (9%) did not give a result. The female samples gave only a value for the X chromosome and an undetermined (no value) for the Y. The female values ranged from 29.57 to 40.79. Male data recorded both the X and Y chromosome and values for the X ranged from 27.46 to 44.82 and the Y ranged from 28.50 to 43.54. In some cases only the Y chromosome gave a value, this is deemed to be male but was re-run for verification.

Tube number	Date	Y Fam	X Vic	Sex of animal
5	6.7.04	39.21	37.22	Male
15	6.7.04	Undet	31.05	Female
21	26.7.04	Undet	31.04	Female
5	27.7.04	34.86	34.41	Male
7	26.2.05	Undet	32.25	Female
3	26.6.05	31.08	30.61	Male
15	26.6.05	29.29	29.65	Male

Table 3.18 Sample of sex-typed values from Appendix B showing values for Y and X chromosomes, undetermined value in Y if female.

Study period	Number of visits	Number species-typed	Number sex-typed
Jan/ Mar ‘04	35	20	18
July ‘04	67	60	58
Feb/ Apr ‘05	54	39	36
July ‘05	68	68	58

Table 3.19 Total number of visits with numbers of species-typed and sex-typed samples recorded. The July ’03 samples were mislaid hence no results.

Table 3.19 shows the rise in visits over the entire study with activity peaking in the summer months. Mapping the data where the samples were collected may give an idea of territory layout. Sleeman (1989) has shown that territories can be found one within another, female(s) within male boundaries (Figure 1.2). There appears to be a strong gender bias towards males. The location of the male and female samples collected and speculative maps of territories have been drawn below.

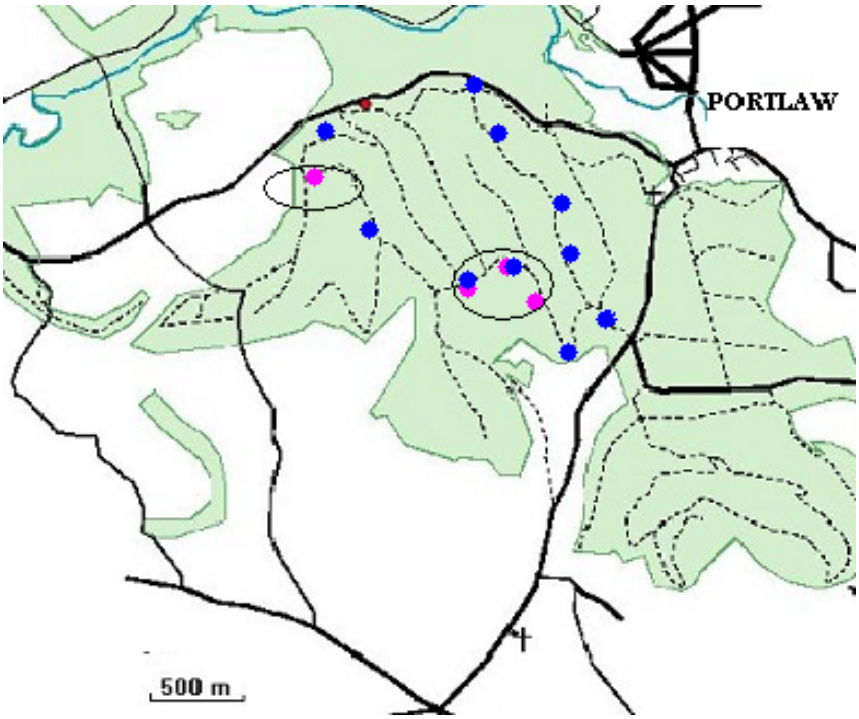

Figure 3.9 Location of sex-typed samples (blue –male, pink – female) showing gender distribution Jan/ Mar ’04 with circles showing potential female territories

The January /March 2004 distribution showed a strong bias toward males (Figure 3.9). It is not known how many males or females were in the area.

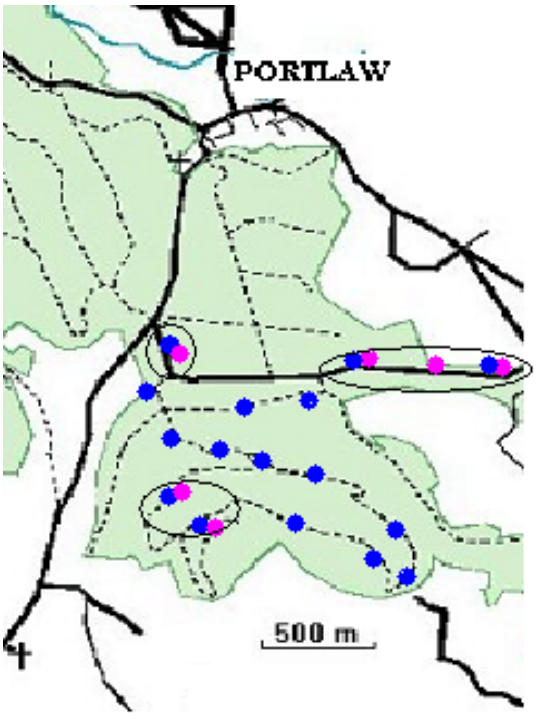

Figure 3.10 Location of sex-typed samples July ’04 showing gender distribution with strong bias toward males (blue – male, pink – female).

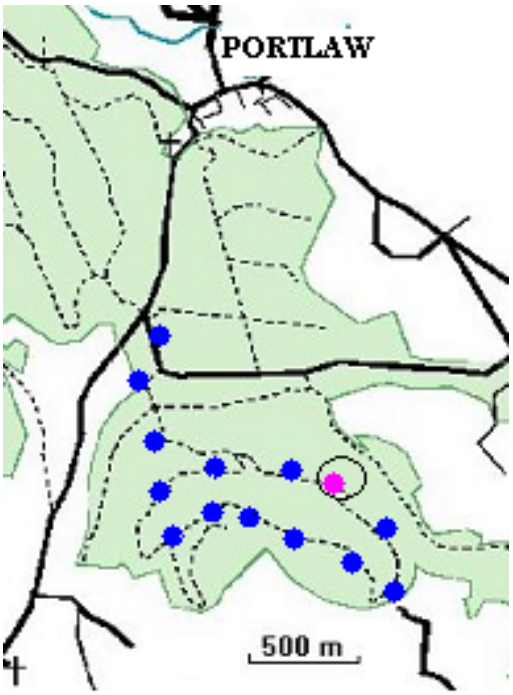

Figure 3.11 Gender distribution of sex-typed samples for Feb/ Apr ’05

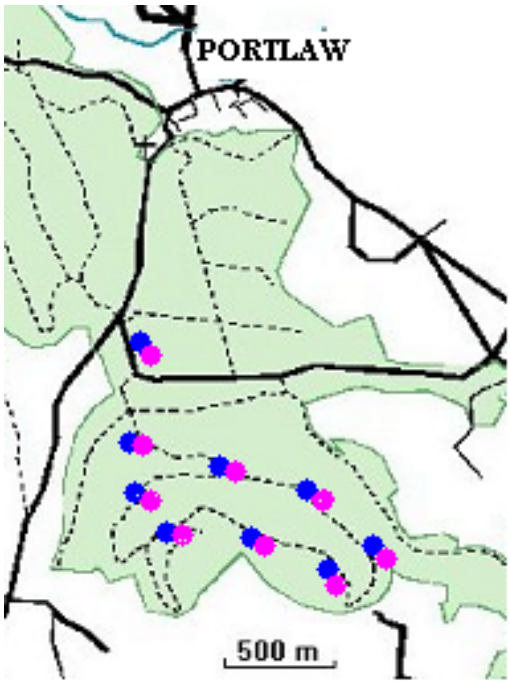

Figure 3.12 Distribution of sex-typed samples for July '05

Comparing gender distribution over the studies January /March 2004 to July 2005, it is clear that there is a strong bias toward males.

	Jan/ Mar 2004	July 2004	Feb/ Apr 2005	July 2005
Male	13 (72.2%)	49 (84.5%)	35 (97.2%)	45 (77.6%)
Female	5 (27.8%)	9 (15.5%)	1 (2.8%)	13 (22.4%)
Samples verified	18	58	36	58
Total number	20	60	39	68

Table 3.20 Results for sex-typing showing gender bias in favour of males.

Chapter 4

Discussion

4. Discussion

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The aims of this study were to locate suitable habitats to erect hair tubes in order to attract pine marten and hence prove their presence or absence in Portlaw Woods; to carry out field studies to determine which lures might attract the pine marten to the hair tubes; to successfully collect hair samples non-invasively and to DNA species and sex-type the collected samples. These aims were successfully completed as set out below.

4.1 Pine marten habitat preferences

As an endangered species, it is necessary to conserve mature forest stands because martens have been shown to be highly sensitive to the loss and fragmentation of mature forest. This loss has led to a dramatic decline throughout much of its historical distribution (Slauson *et al* 2002). In assessing suitable habitat locations for the monitoring of pine marten, where the hair tubes were located did appear to make a difference.

O' Sullivan (1983) suggested that pine marten had become extinct in various locations in Waterford, such as the Comeragh mountains. It is hoped that the population in Portlaw is not isolated to the extent that inbreeding will lead to the demise and collapse of this population. There is a wooded corridor along the Suir River valley, creating a link with suitable cover and food between Portlaw and Clonmel, County Tipperary so it is possible that pine marten may move between these areas. Current on-going studies in these areas will hopeully open up the true movements and dynamics of the pine marten in this part of the South East.

Strachan *et al* (1996) suggested that preferred habitat types in England and Wales would be areas of mixed coniferous woodland. Warner and O' Sullivan (1982) undertook a dietary analysis of the pine marten in areas of mixed deciduous /coniferous regions of Ireland. Consistent with these, the habitats assessed in Portlaw Woods (Figures 3.1 and 3.4) were similar (mixed deciduous/ evergreen woodland

inclusive of spruce plantation with rocky crags and outcrops, varying through wet and dry terrain and from low-lying topography to hilltop) and deemed very suitable areas within which to find pine marten.

4.1.1 Habitat preference comparison

In the July 2003 study, the tubes, which had the greatest number of visits, were in areas of mixed woodland – deciduous species (willow, *Salix* sp., beech, *Fagus sylvatica* and birch, *Betula* sp.) interspersed with evergreen species (holly, *Ilex sp.*, spruce, *Pinus sp.* and rhododendron species). These were tubes 15, 16, and 18 (hidden in the undergrowth) and 17 (unusually, on an exposed log) whereas those visited in the January / March study were tubes 1, 2, 4, 5 and 20 all hidden in undergrowth and all in evergreen stands.

Of these two studies only tube 6 (grid reference S453 140) was never visited. This tube was located on an exposed bend in the path on a spruce trunk open for 3 to 4 metres from ground level to the first branches and close to pasture, looking onto a clear fell area. Fairley (2001) states that pine marten tend to avoid open clear fell areas and this is consistent with the above results.

In the July 2004 study tubes 3, 4, 13 and 17 were visited most often. Tube 3 was tied to the trunk of a large rhododendron giving dense overhead cover with open under story. Tubes 4 and 17 were in mixed deciduous canopy close to the path and almost visible to anyone walking the path. Tube 13 was on the edge of a dense spruce plantation backing onto the path.

February /March 2005 saw tubes 3, 10, 12 and 17 visited most often but as for this study and July 2005 nearly every hair tube was being visited regardless of habitat type. The lure and/ or patches were gone many times but no hair was gleaned from the tube. More work will have to be done in the area of hair retrieval.

The second set of studies (2004 and 2005) had a sole hair tube (number 16, grid reference S464 125) that was never visited. This tube was on the periphery of the wood in a sparse spruce stand close to pasture and was within hearing distance of human habitation (dogs audible). It was assumed this tube was too exposed and noisy for marten habitation.

Visits to hair tubes would appear to be influenced by the presence of a dense over story canopy. These studies show that tubes, which had the greatest number of visits, were located in areas where the canopy cover was maximised. In most cases this was deciduous woodland with mixed evergreens such as holly and rhododendron, which gave abundant cover. A number of the hair tubes were attached to large rhododendron trunks and although these are an invasive pest species, they would appear to benefit the existence of pine marten as the rhododendron give a year round dense overhead canopy.

The tubes attached to deciduous species would have become exposed in the autumn at leaf fall; hence during the winter months pine marten move to seek cover (Table 3.16). Thus seasonality must influence the tubes which are visited with pine marten moving from predominantly deciduous areas to deeper evergreen cover over the winter months (where the canopy fluctuates little seasonally giving a relatively stable year round environment). However the tubes in all areas were not consistently visited (occasional erratic results) until closer to the end of the studies where nearly all tubes were visited on a regular basis. If this type of study were undertaken over a much longer period of time more definitive results may be obtained.

4.2 The use of lures to attract pine marten

The diet of the marten should give some insight as to the nature of the lure to be used but as yet there is no definitive lure to attract pine marten to hair tubes. The lure would preferably be something the target species will find irresistible. This may or may not form part of the animal's natural diet. The marten's diet is predominantly carnivorous, becoming seasonally omnivorous, and this was addressed over the course of these studies.

Meat-based lures are not good long term lures as once taken there are no longer available. This can be advantageous, in that the tubes will not attract other animals and hence have only one animal's hair will be collected on the glue patch, but for use as a long-term attractant, meat is not viable. Chicken was used in the July 2003 and February /March 2004 studies. Tables 3.2 and 3.3 show the tubes visited

using chicken as a lure. In both cases pine marten were lured to the tubes with number of visits increasing in the second study.

In July 2003, chicken was the lure used in the short-term baseline study to verify the presence or absence of pine marten in Portlaw Woods. As it proved successful as a lure (the number of visits increased from 27 to 35), it was used again in the January /March 2004 study. The major drawback was found to be that the chicken decomposed and /or became maggot infested (fly-blown) thereby being eaten away and rapidly needing replacement. Chicken, though could be used in short-term studies, was discounted as a long-term lure. Dilks *et al* (1996) mentions similar flyblown lures and their replacement requirement in warm summer months.

Wildwoods Animal Park, Kent, England was visited in April 2004 to test the efficacy of a variety of lures on two of their captive pine marten. Ten stakes, soaked in lures were hammered into the floors of two of the pine marten enclosures (Figure 2.7). Mink scent oil, chicken fat, fish oil, duck fat, blackberry juice, ivy berry juice, beef oxo paste, chicken oxo paste, peanut butter, sardine oil and a blank were used, giving a variety of natural and synthetic, meat and fruit based scents. Both marten were observed over the two day period. Excessively long tests are to be avoided because in long tests there will be a greater proportion of random encounters rather than active locations. The two pine marten, 'Fudge' and 'Poop', had regular paths worn in their enclosures but did deviate from them to investigate the stakes. Tables 3.5 and 3.6 give the responses to the lures. Both animals pulled up and chewed the peanut butter filled stake. This would appear to have been the preferred lure type for these pine marten.

In synthetic lure studies on stoats, Spurr (1999) found that artificial odours, such as acetamide (mousy odour), trimethylamine (synthetic meat oour) and isopentenyl methyl sulphide (synthetic mustelid anal gland secretion) gave 0% attraction rates. Mink anal gland secretion oil was obtained and used in Wildwoods to test its efficacy as a lure for pine marten. This proved successful in obtaining a response from the marten. The response however, was a rubbing, scent marking response (Table 3.5 and 3.6). This response-type was not appropriate enough to consider mink gland oil as a lure type.

To verify or discount it as a lure, mink gland secretion oil was used in a field trial in Portlaw woods in the hair tube studies in July 2004. It was compared with chicken and fish oil to test for lure preference using a blank as a control. Table 3.9

shows chicken ranked highest with 38 out of 45 visits (84%) to the hair tubes whilst the mink gland oil received 3 (0.07%) visits thus discounting it as a potential lure, along with fish oil (1 visit – 0.02%). Figure 3.5 depicts this. Statistical analysis verifies this with the mean visit per tube for mink oil being 0.25 whereas chicken was 3.17.

Some limitations are obvious in the Wildwoods studies. Studying only two captive pine marten gives minimal data and results of these observations cannot be used to accurately represent all pine martens lure/ food preferences. One of the lures used was a mink oil anal sac secretion, successfully used in the United States as a lure in hunting. In the captive study the pine marten merely scent rubbed over the mink oil. It would appear as though they were masking over the mink oil with their own scent. No indication was given by the animals that this could be successful as a lure to hair tubes other than to scent rub the spot. This could not guarantee their entry into the hair tubes so mink oil was discounted as a lure (Table 3.17). Also, pen test studies cannot take the place of field studies but they do offer a way to substantially reduce the time, effort and money needed to develop effective lures (Jolly and Jolly 1992).

Fruit and insects form a large part of the marten diet (Sleeman 1989, Warner *et al* 2002) and hence in the January / March 2005 study, fruit juices were incorporated to investigate the efficacy of these and other lures to attract pine marten to hair tubes. The berry juice was a combination of ivy berry, *Hedera helix* (which may not have been palatable as they were collected the previous autumn and due to seasonality of study, stored dry) and blackberries (from frozen produce). Insects not being viable as a lure type, being too small and rapidly perishable, were not used. Beef oxo cube paste, fish oil, Bovril, smooth (nut-free) peanut butter and marmalade were the other lures used. In this study, marmalade and peanut butter both ranked best as pine marten lures (Figure 3.6 and Table 3.11). The data shows that the marmalade and peanut butter results are not statistically significant as beef oxo paste, berry juice, fish oil and Bovril all rank too close (Table 3.11). As the lures were rotated through the hair tubes over 4 baiting sessions, the effect of the lure type (Table 3.13) on the number of visits was not statistically significant either but may infer a 'learning effect' is in operation.

It is noted that strawberry jam sandwiches have been used by the Forestry Commission in North York moors, England while in an article in the Scotsman newspaper (Wednesday 7th June 2006), Peter Ranscombe states that the Scottish pine

marten seem to have a penchant for peanuts and peanut butter. A survey in the Marble Arch caves in County Fermanagh, Ireland found that on offering peanut butter, jam and cheese to the marten, cheese was the preferred food. Other Irish personal observations (local residents in County Roscommon) found that bread and jam (unspecified) work well to encourage pine marten into their gardens. While cheese was not offered in these studies, it is clear that without the presence of meat in the hair tubes the next preferred food type is fruit; whether berries or nuts.

Peanut butter smeared with marmalade was used in the hair tubes in the July 2005 study and it was found that the visit frequency rose to 55 visits (Table 3.15). This study incorporated pellets into the lure to carry out a multi-faceted study. The peanut butter and marmalade was very successful at luring pine marten to the hair tubes as nearly every re-baiting revealed a visit had occurred (further work, as mentioned, needs to be done on hair retrieval). The deposition of the pelleted scats, it was hoped, would give an insight into pine marten dynamics.

It was noted that pine marten deposit scats in exposed areas such as paths and at the base of hair tubes. When plastic pellets were introduced to the lure and the scats searched for, only 9 scats with pellets were located. Of the total, this gave a highly significant recovery rate of only 1.8% (Table 3.14). From the 9 scats located, 5 had 1 colour pellet, 3 had 2 different coloured pellets and 1 had 3 different coloured pellets within. The single coloured pellets (Figure 3.7) can show movement and direction but inclusion of multiple colours (Figure 3.8) in the scats reveals more about the overall pattern of marten movement through their habitat. The poor recovery rate of pellets (1.8%) could render scat surveying unsupportable and this would concur with Birks *et al* (2004) where the reliability of scat surveys in distribution and population management of pine marten is questioned. Lockie (1964) initially suggested a relationship between numbers of scats and martens but this presumed approach to surveying may involve assumptions about habitats utilised, spatial patterns of scat deposition and field surveyors' identification skills. Nevertheless, even with genetic verification, scat abundance figures could be meaningless if seasonal variation in scat deposition patterns is not controlled for (Birks *et al* 2004). If the results of the scat locations are combined with the results of the genetic analysis of the hair collected during the July 2005 study, a better indication of the dynamics of pine marten movement may reveal itself. Further work on micro satellite analysis to determine

kinships and eventually individual genotypes need to be done to achieve reliable results.

4.2.1 Significance of rotating lures

The July 2004 and February /March 2005 studies were similar to the previous studies, however a few key factors changed. Firstly, the area where the hair tubes were positioned was across the road (Figure 3.4) from the previous site (Figures 3.1). Secondly, the number of hair tubes varied (the July 2004 study used all 24 hair tubes but the February/ March study used only the first 18 tubes) and thirdly, there was more than one lure used in each study (July 2004 used 3 lures and a blank whereas the February /March 2005 study used 6 lures). The July 2004 study proved emphatically that chicken was the preferred attractant to pine marten (even though in a different area of the woods). This was set up again in spring 2005 without chicken and it was seen that marmalade and peanut butter were the top attractants.

Statistically the ANOVA results reveal a significant rotation /bait interaction. This means that the effect of the lure on the number of visits is significantly different, for different rotations. The average number of visits for chicken is 5.33 on the final rotation but only 1.67 for the first (Table 3.11). Chicken has proven a significant lure to pine marten to hair tube regardless of site however it is also notable that the average number of visits for chicken grows steadily over the four rotations, suggesting that there is a "learning" effect in operation (Figures 3.12 and 3.13).

4.2.2 Learning behaviour in pine marten

The number of visits to the hair tubes rose over time (Table 4.16) and this may well mean that the pine marten are an intelligent species. It is possible that they have been learning where the tubes were or that when foraging, finding a hair tube means food is present. Results show that the July 2003 and February /March 2004 studies (Table 4.1) had only had a slight increase, from 27 visits to 35 which is not significant. Further studies, from July 2004 to July 2005 show figures climbing to 68 visits (Table 3.15). The drop in February /March 2005 could have been due to kits

maturing and moving /being forced away at the end of the rearing season or seasonal variations in populations. These figures show increases in visits to hair tubes overall and could imply that pine marten have an acute sense of smell to be able to pick out a new food scent in the air or imply a learning effect is in operation.

The two processes above may be connected. Chicken was used initially but a variety of lures were tested in latter experiments. ANOVA tests reveal a statistically significant rotation/bait interaction ($p < 0.001$). Initially chicken had a mean visit rate of only 1.67 but this rose to a mean of 5.33 visits by the end of the 4 lure rotation survey (Table 3.9). Mink gland oil, fish sauce and the blank had means of 0.25, 0.08 and 0.25 respectively, thereby of no significant value as attractants to hair tubes. The high chicken mean may imply that the pine marten are learning to search the tubes for chicken.

Statistical analysis of February /March 2005 data (Table 3.11) show that when multiple lures were set out in the hair tubes and rotated around the study area, the mean number of visits per lure fluctuated as the lures were moved. In this study chicken was not available as a lure. As mentioned above, pine marten appear to have a sweet tooth and this is borne out by marmalade having the highest mean thereby the most successful lure in this survey. It would appear that the marten were learning where the tubes were and/ or that they represented a food source (Table 3.13) because as the lures were rotated, the rotation mean rose significantly. This could have implications for 'random' field surveys, as they no longer become random when the pine marten 'realise' where tubes are or that hair tubes mean food. Further work will need to be done in this area to test if a learning behaviour exists.

4.3 Hair retrieval methodologies

How the hair is retrieved varies from study to study. Lynch *et al* (2006) collected hair from pine marten, which had been snagged in coiled springs. Hair patches similar to those used in this study were used by Gurnell *et al* (2004) and Finnegan *et al* (2007) monitoring red and grey squirrels in Britain and Ireland respectively.

Double-sided sticky-tape patches were used initially, which in July 2003 led to the collection of 113 hairs. The new mousetrap glue patch increased this to 283 hairs (January/ March 2004 study). The adhesiveness of the original patch was fair but over time the level of adhesion dwindled. This appeared due to dampness in the tubes. The mousetrap glue patches were much more successful at trapping hair despite adhering dust, leaves, ants and flies in the summer months.

Comparing the data between July 2003 and January/ March 2004, the number of nights the tubes were *in situ* increased, as did the number of visits but when the new glue patch was introduced, the average numbers of hairs collected per night increased more than two fold so this would appear to be an effective method of hair retrieval (Table 4.1).

Date	Number of Hairs	Number of visits	Average hairs per visit	Number of bait sessions	Patch used
July '03	113	27	4.19	7	Double sided sticky tape
Jan/ Mar '04	283	35	8.08	6	Glue patch

Table 4.1 Comparison of hair capture results in July '03 against January /March '04

Seasonality affects the rate of hair retrieval and quantities of hair collected by the patches. Pine marten moult in April in Britain and Ireland, the thick winter coat being renewed for the sleeker summer coat; hence the rate of hair retrieved should increase with seasonal changes. The studies were carried out in approximately February and July over 2 years and the July studies yielded more hair, with large tufts being noted (visually, though not counted in latter parts of the study) whereas the February hairs retrieved tended to be short (guard) hairs. As the number of visits to the hair tubes rose, the quantity of hair recovered rose, particularly when the glue patch was introduced (Table 4.1). Thus seasonality does influence the rate and quantity of hair recovered due to their activity periods and the moulting period varying with season.

4.4 Genetic analysis

Using hair (inclusive of follicles) for species verification represents a better, cleaner source of DNA, when compared to DNA analysis of collected scats. Hair samples were stored individually to prevent cross contamination and kept in relatively dry climatic conditions (Pigott *et al* 2003).

In this study, the samples gathered were analysed using Real-time PCR. It is convenient that when running a 96-well plate that species and sex typing can be done at the same time. Multiplexing is a very convenient method of analysing results, carrying out different methodologies within the same reaction while reducing the cost of the analyses and the time taken to get workable results.

Here 187 samples were genetically analysed to verify species type and to determine the sex of the individuals to gain some insight into the gender distribution of pine marten in the woods in Portlaw. When species typed for pine marten all but 1 sample (3.13.7.5 – tube 3 gathered 13th of July 2005) proved to be pine marten. This 1 sample did not record a Ct value thus may not be pine marten. Other researchers have collected rat and stoat DNA in hair tubes and it is possible that the hair may be from either of these species. The methodology used to verify species type is highly specific. If a result is given, the species is deemed to be pine marten. If an 'undetermined' value was recorded, the sample may have needed to be analysed using other methodologies, not included here, to species identify it. The range of Ct values is quite large – from 23.01 to 36.96 with an average of 28.61. The lower the Ct value, the greater the amount of target nucleic acid in the sample hence it is preferred to have the Ct values in the 23.0 to 28.0 range. A number of reasons may be attributed to receiving higher values such as a high level of pigmentation within the hairs or poor quality or degraded DNA.

When the hair samples were sex-typed, it was discovered that there was a significant gender bias in favour of tube useage by males in Portlaw Woods. With sex-typing, one notable issue was that if the Y chromosome did not amplify, then the sample would appear female though could actually have been male. For example in

July 2004, 84.5% of the samples collected that summer were from males and only 15.5% from females.

A potential source of error scored is allelic dropout (the failure of one allele to amplify) that leads to heterozygotes being mistaken for homozygotes. Occasionally the X chromosome doesn't get amplified. This cannot conclusively state that the sample is male. If just the X chromosome appears it is still not clear as to the sex of the individual. Erroneously low heterozygosity levels can lead to false interpretations with regard to in-breeding and population structure (Taberlet *et al* 1999). In non-invasive genotyping, PCR replicates can alleviate this issue and give reliable sex identifications (Lynch and Brown 2006) and the specificity of the *ZFX*-*ZFY* primers can minimize the risk of false negatives (Mucci et al 2007).

When the results of this study are mapped by gender, they show a biased distribution in Portlaw (Figures 3.10, 3.11, 3.12 and 3.13). There would appear to be 'pockets' where females appear. From Sleeman (1989), female territories can exist within male territories and that a dominant male will maintain his harem within his boundaries (Figure 1.2). It is difficult to know how many males or females are present. In Figure 3.10, a circle has been incorporated to suggest such a potential female territory in a male dominated area. Suggested indications of territories are possible but without microsatellite analysis separating one individual from another this is highly speculative. The February /April study indicates 1 hair tube visit by a sole female during that study. Male and female data may not be solely from individual adult animals. Mothers and kits may be present during the summer months, which may affect sample analysis and territory plotting.

Genetic improvements are ongoing to distinguish between individuals. Micro-satellite analysis of hair samples could be used to give the numbers of individuals in an area. This would be of use in conservation /population management schemes, to establish individual identity, paternity and kinship (Pigott *et al* 2003) and to determine if viable populations exist in any given location. Current works monitoring the pine marten along the Suir valley and in Portlaw may provide samples that may be genetically analysed to give a greater understanding of the pine martens status in the region aiding in the conservation and population management of one of Ireland's finest yet elusive top carnivores.

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Appendix A

Ct values for Species-typed samples

Sample Jan/ Mar '04	Ct value	Sample July '04	Ct value	Sample Feb /Mar '05	Ct value	Sample July '05	Ct value
8.7.2.4	28.39	1.3.7.4	29.18	3.26.2.5	24.64	3.22.6.5	28.14
2.15.2.4	25.89	2.3.7.4	29.68	7.26.2.5	23.17	5.22.6.5	26.58
8.15.2.4	26.42	3.3.7.4	34.83	3.1.3.5	23.92	7.22.6.5	26.00
17.15.2.4	28.22	4.3.7.4	36.08	12.1.3.5	26.15	9.22.6.5	27.44
1.19.2.4	27.28	17.3.7.4	28.81	13.1.3.5	23.79	11.22.6.5	27.29
4.19.2.4	27.54	3.5.7.4	29.51	2.5.3.5	28.08	13.22.6.5	27.53
1.23.2.4	24.15	4.5.7.4	24.22	13.5.3.5	25.96	17.22.6.5	31.94
3.23.2.4	23.99	23.5.7.4	32.96	15.5.3.5	24.66	1.24.6.5	26.99
5.23.2.4	23.19	1.6.7.4	27.37	4.9.3.5	38.47	3.24.6.5	25.48
10.23.2.4	25.64	2.6.7.4	28.24	6.9.3.5	23.01	5.24.6.5	27.41
11.23.2.4	26.73	3.6.7.4	33.33	9.9.3.5	29.79	9.24.6.5	27.91
16.23.2.4	29.22	4.6.7.4	32.35	3.12.3.5	29.56	17.24.6.5	25.79
19.23.2.4	26.62	5.6.7.4	29.77	9.12.3.5	23.84	1.26.6.5	32.42
20.23.2.4	23.17	6.6.7.4	29.21	10.12.3.5	29.97	3.26.6.5	30.31
1.1.3.4	25.66	10.6.7.4	28.24	11.12.3.5	28.06	5.26.6.5	28.10
2.1.3.4	23.14	13.6.7.4	28.11	12.12.3.5	30.28	7.26.6.5	27.51
4.1.3.4	27.69	15.6.7.4	28.28	1.19.3.5	25.63	9.26.6.5	32.84
5.1.3.4	25.18	20.6.7.4	28.51	3.19.3.5	24.03	11.26.6.5	21.28
9.1.3.4	24.05	23.6.7.4	27.31	10.19.3.5	27.29	13.26.6.5	30.97
15.1.3.4	24.53	1.9.7.4	31.31	17.19.3.5	26.09	15.26.6.5	25.62
		3.9.7.4	34.63	18.19.3.5	27.37	17.26.6.5	26.39
		4.9.7.4	32.11	10.22.3.5	26.07	3.27.6.5	27.57
		20.9.7.4	32.26	11.22.3.5	29.03	9.27.6.5	28.92
		23.9.7.4	30.27	12.22.3.5	28.44	11.27.6.5	29.37
		4.10.7.4	30.75	14.22.3.5	25.17	13.27.6.5	30.92
		20.10.7.4	35.00	17.22.3.5	23.56	15.27.6.5	29.49
		4.11.7.4	32.01	18.22.3.5	26.52	3.29.6.5	28.06
		13.11.7.4	31.32	10.27.3.5	27.95	7.29.6.5	29.79
		15.11.7.4	28.60	11.27.3.5	27.62	9.29.6.5	25.29
		20.11.7.4	34.69	12.27.3.5	28.57	1.2.7.5	25.51
		21.11.7.4	34.64	14.27.3.5	24.37	3.2.7.5	29.88
		3.14.7.4	27.98	15.27.3.5	27.43	5.2.7.5	31.56
		17.14.7.4	30.66	17.27.3.5	25.27	7.2.7.5	29.68
		3.16.7.4	32.07	9.3.4.5	28.42	9.2.7.5	31.17
		15.16.7.4	29.70	11.3.4.5	28.21	11.2.7.5	29.81
		3.17.7.4	28.04	12.3.4.5	24.78	13.2.7.5	30.42
		19.17.7.4	31.74	13.3.4.5	30.54	15.2.7.5	24.72
		23.17.7.4	34.78	17.3.4.5	25.32	17.2.7.5	27.31

	6.19.7.4	29.33	18.3.4.5	30.17	11.3.7.5	30.35
	6.20.7.4	28.61			3.4.7.5	32.41
	10.20.7.4	29.76			5.4.7.5	31.42
	21.20.7.4	26.06			7.4.7.5	33.33
	6.22.7.4	32.59			9.4.7.5	28.37
	10.22.7.4	30.38			13.4.7.5	25.75
	17.22.7.4	31.50			15.4.7.5	34.22
	18.22.7.4	28.43			1.7.7.5	29.38
	22.22.7.4	31.63			3.7.7.5	31.30
	5.25.7.4	30.59			5.7.7.5	31.81
	9.25.7.4	25.02			7.7.7.5	29.86
	13.25.7.4	26.93			9.7.7.5	34.72
	17.25.7.4	26.76			11.7.7.5	30.12
	21.25.7.4	26.64			13.7.7.5	30.09
	13.26.7.4	25.42			15.7.7.5	34.05
	17.26.7.4	29.53			1.11.7.5	34.02
	21.26.7.4	26.63			3.11.7.5	36.35
	1.27.7.4	34.35			13.11.7.5	27.32
	5.27.7.4	29.68			17.11.7.5	27.73
	9.27.7.4	27.03			1.13.7.5	29.52
	11.27.7.4	36.96			3.13.7.5	Undetermined
	13.27.7.4	28.95			5.13.7.5	30.17
					7.13.7.5	28.01
					9.13.7.5	32.81
					11.13.7.5	31.06
					13.13.7.5	26.94
					17.13.7.5	29.73
					9.15.7.5	27.41
					11.15.7.5	32.92
					17.15.7.5	25.88

Figures show sample numbers (8.7.2.4 – Tube 8, sample collected on 7th of February 2004) and the samples Ct values. Sample 3.13.7.5 is undetermined indicating that it is species unknown.

Appendix B

Sex- typed results 2004

January /March 2004			July 2004		
Sample number	**Y - Fam**	**X - Vic**	**Sample number**	**Y- Fam**	**X - Vic**
8.7.2.4	41.61	undet	1.3.7.4	31.87	31.86
2.15.2.4	41.09	undet	2.3.7.4	35.29	33.58
8.15.2.4	38.49	37.64	3.3.7.4	35.25	35.17
17.15.2.4	32.01	31.85	4.3.7.4	35.03	35.36
1.19.2.4	37.75	27.46	17.3.7.4	undet	32.66
4.19.2.4	undet	32.24	3.5.7.4	38.27	39.86
1.23.2.4	42.83	32.24	4.5.7.4	30.08	29.99
3.23.2.4	undet	32.97	23.5.7.4	undet	29.57
5.23.2.4	undet	36.48	1.6.7.4	33.66	32.15
10.23.2.4	undet	38.51	2.6.7.4	39.15	36.9
11.23.2.4	34.59	36.12	3.6.7.4	42.26	undet
16.23.2.4	undet	31.69	4.6.7.4	30.26	30.39
19.23.2.4	36.23	37.2	5.6.7.4	39.21	37.22
20.23.2.4	32.05	32.13	6.6.7.4	32.73	31.28
1.1.3.4	37.19	35.6	10.6.7.4	43.03	43.53
2.1.3.4	35.27	34.76	13.6.7.4	34.12	32.84
4.1.3.4	33.23	32.79	15.6.7.4	undet	31.05
5.1.3.4	42.51	34.37	20.6.7.4	34.88	34.44
			23.6.7.4	41.17	30.08
			1.9.7.4	37.54	36.24
			3.9.7.4	34.41	34.05
			4.9.7.4	31.64	31.78
			20.9.7.4	36.93	37.88
			23.9.7.4	undet	34.06
			4.10.7.4	33.83	33.52
			20.10.7.4	34.25	34.26
			13.11.7.4	35.73	34.02
			15.11.7.4	35.21	33.98
			21.11.7.4	43.54	35.28
			3.14.7.4	33.22	31.77
			17.14.7.4	35.01	34.79
			3.16.7.4	34.21	34.05
			15.16.7.4	undet	32.19
			3.17.7.4	30.17	30.13
			19.17.7.4	35.06	36.21
			23.17.7.4	39.38	31.38
			6.19.7.4	35.96	35.34
			6.20.7.4	33.29	33.04
			10.20.7.4	35.23	34.95
			21.20.7.4	34.43	32.88
			6.22.7.4	29.99	29.62

	10.22.7.4	34.02	32.54
	17.22.7.4	44	33.45
	18.22.7.4	33.77	32.74
	22.22.7.4	undet	31.66
	5.25.7.4	35.07	33.07
	9.25.7.4	33.46	32.08
	13.25.7.4	31.94	30.23
	17.25.7.4	32	31.06
	21.25.7.4	undet	32.52
	13.26.7.4	31.46	30.15
	17.26.7.4	31.11	30.16
	21.26.7.4	undet	31.04
	1.27.7.4	undet	33.37
	5.27.7.4	34.86	34.41
	9.27.7.4	33.44	32.47
	11.27.7.4	39.35	38.28
	13.27.7.4	32.75	31.57

Appendix C

Sex –typed samples – 2005

February /March 2005			July 2005		
Sample number	**Y - Fam**	**X - Vic**	**Sample number**	**Y – Fam**	**X - Vic**
3.26.2.5	43.42	34.08	3.22.6.5	Undet	39.32
7.26.2.5	undet	32.29	5.22.6.5	29.37	30.07
3.1.3.5	37.06	37.08	7.22.6.5	28.5	29.16
12.1.3.5	37.03	39.19	9.22.6.5	undet	38.42
13.1.3.5	38.02	35.88	11.22.6.5	37.19	37.46
2.5.3.5	36.62	34.09	13.22.6.5	38.62	36.27
13.5.3.5	33	31.45	1.24.6.5	35.03	30.39
15.5.3.5	37.37	32.34	3.24.6.5	32.45	31.24
6.9.3.5	35.14	34.33	5.24.6.5	33.33	33.28
9.9.3.5	43.49	42.42	9.24.6.5	32.03	31.31
3.12.3.5	35.42	35.18	17.24.6.5	32.6	31.28
9.12.3.5	32.11	32.3	1.26.6.5	undet	38.71
11.12.3.5	36.06	37.02	3.26.6.5	32.47	33.13
12.12.3.5	34.31	34.36	5.26.6.5	undet	39.06
1.19.3.5	38.1	35.06	7.26.6.5	30.52	30.17
3.19.3.5	34.53	32.21	9.26.6.5	38.01	37.49
10.19.3.5	37.46	37.75	11.26.6.5	33.6	34.95
17.19.3.5	36.3	42.45	13.26.6.5	36.35	36.02
18.19.3.5	36.12	35.3	15.26.6.5	34.58	32.36
10.22.3.5	36.81	36.11	17.26.6.5	32.18	31.37
12.22.3.5	38.73	undet	3.27.6.5	30.81	30.11
14.22.3.5	37.72	undet	9.27.6.5	33.2	32.3
17.22.3.5	33.77	33.49	11.27.6.5	34.34	33.36
18.22.3.5	37.52	37.55	13.27.6.5	37.22	38.01
10.27.3.5	36.27	39.08	15.27.6.5	34.58	32.36
11.27.3.5	35.41	35.98	3.29.6.5	37.13	35.38
12.27.3.5	36.32	35.97	7.29.6.5	31.62	32.5
14.27.3.5	35.32	35.17	9.29.6.5	34.49	33.78
15.27.3.5	34.37	33.97	1.2.7.5	31.96	31.21
17.27.3.5	36.18	38.17	7.2.7.5	undet	39.28
9.3.4.5	36.16	38.09	9.2.7.5	34.49	33.78
11.3.4.5	37.72	44.82	11.2.7.5	34.36	34.04
12.3.4.5	32.62	32.36	13.2.7.5	37.39	37.54
13.3.4.5	37.89	40.76	15.2.7.5	36	38
17.3.4.5	36.21	39	17.2.7.5	33.44	32.2
18.3.4.5	34.38	35.06	11.3.7.5	34.54	34.26
			9.4.7.5	37.54	36.04
			13.4.7.5	undet	37.11
			15.4.7.5	undet	39.03
			1.7.7.5	32.65	31.5
			3.7.7.5	33.81	32.5
			5.7.7.5	40.91	

	7.7.7.5	39.35	31.91
	9.7.7.5	41	
	11.7.7.5	undet	40.79
	13.7.7.5	32.31	31.1
	15.7.7.5	undet	39.1225
	1.11.7.5	undet	39.6514
	3.11.7.5	undet	39.0723
	13.11.7.5	undet	38.0288
	17.11.7.5	32.51	31.35
	5.13.7.5	32.69	31.6
	7.13.7.5	34.52	33.12
	9.13.7.5	40	
	11.13.7.5	34.12	32.16
	13.13.7.5	38.64	38.05
	17.13.7.5	undet	33.49
	17.15.7.5	31.65	30.59

Male (Y – Fam) and female (X – Vic) sex typing results (2004 and 2005) including sample numbers (8.15.2.4 - sample from tube 8 was collected on the 15th of February 2004) and the sex-typed values across. Y- Fam undetermined and X- Vic value gives a female as they do not possess the Y chromosome (XX). Y value and X value implies male (XY).