

A pilot study to test the use of hair tubes to detect the Irish stoat along hedgerows in County Galway.



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for The Heritage Council.

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Summary

In Ireland the Irish stoat is considered to be a near-endemic subspecies, with >90% of the global population estimated to occur in the country. Although believed to be common and widespread, no systematic survey has been conducted to date. This study, funded by The Heritage Council, involved laying plastic baited hair tubes, 10 per 200 metres at 20m intervals, along hedgerows in the bottom left 1km² of 10 km grid squares across County Galway. Each tube was 20cm long, with a diameter of 52mm. A total of 600 tubes were laid out by 50 volunteers from June to October during 2010. 338 samples were collected, of which 174 were suitable for testing using DNA sequencing. Stoats were detected in 36 samples from a variety of hedgerow types, as well as stone walls (with and without vegetation). These results show that hair tubes are an effective method for detecting stoats in the Irish countryside.

Acknowledgments

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1. Introduction

1.1. Background to the VWT

The Vincent Wildlife Trust (VWT) undertook a pilot project on the Irish stoat (*Mustela erminea Hibernia*) in County Galway during 2010 under the Heritage Research Grants Scheme 2010. This project drew on the experience gained by the Trust over many years in the UK where it has conducted surveys on a range of small mammals. The VWT is a charity specialising in mammal research and conservation in Britain and Ireland. For more than 30 years, it has made major contributions to the conservation of many of the rarer mammals, including the otter, dormouse, water vole, pine marten, polecat and the bats. The Trust also has strong links with the mustelids and currently has active research projects investigating the distribution of the pine marten in England and Wales, and the abundance of the polecat.

1.2. The Irish stoat

The Irish stoat is recognised as a distinct subspecies, confined to Ireland and the Isle of Man. The main difference lies in the shape of the dividing line between the chestnut dorsal fur and the cream ventral fur; this line is almost always straight on British stoats, but usually irregular on the Irish subspecies. Also, the hair on the upper lip is white in Britain, but brown in Ireland, and there is a white edge to the ear of the stoat in Britain, which is absent in the Irish stoat (Fairley, 2001).

The Irish stoat is a protected species in the Republic of Ireland, under the Wildlife Act, 1976 and the Wildlife (Amendment) Act, 2000. The habitat of the Irish stoat was studied by Sleeman (1993) using radio telemetry and showed that, when available, woods are a preferred habitat for the this species.

1.3. Aim of the project

The Irish stoat is described as widespread in Ireland, and while there is no knowledge of its population, there is no evidence of a decline (Marnell et al, 2009). However, no systematic survey has been conducted to date, primarily to the elusive nature of the animal. The Galway project was a pilot study to investigate if the simple field method of using sticky patches in baited tubes placed in hedgerows could be used to obtain hairs that could then be identified as stoat using DNA sequencing. If successful, this method has the potential to provide a reliable and non-invasive way to detect the presence of stoats on a larger scale. A key element to the project was the involvement of volunteers throughout the county who could deploy the hair tubes in their local areas.

1.4. Study Areas

Hedgerows and to a lesser extent stone walls along roadsides or close to roads were chosen as study sites because (1) stoats are known to use both hedgerows and stone walls as cover to move about the countryside, (2) extensive data existed on both the location and nature of hedgerows in County Galway, and (3) such areas could be accessed safely, easily and quickly by volunteers. In the east of the county the hedgerows selected were those that had been previously surveyed during the East Galway hedgerow survey conducted by Fuller (2006), thus hedgerows in the south-western (bottom left) 1km² of each of the 10km National Grid squares were selected. A similar approach was attempted in west Galway, based on the West County Galway Hedgerow Survey and County Galway Townland Hedgerow Survey undertaken by Giorrai Environmental Services (2007).

2. Methods & Materials

2.1 Volunteer recruitment and training

Volunteers were recruited by advertising the project through BirdWatch Ireland (Galway Branch), the Irish Environmental Network website, the Hedgelaying Association of Ireland, the People and Nature Project, the Galway Naturalists' Field Club, the National Parks and Wildlife Service, National University of Ireland Galway, Galway Mayo Institute of Technology, and at public biodiversity events. The project was also featured on the Trust's website, using photographs of an Irish stoat taken by Carrie Crowley and kindly provided by *Crossing The Line Films*. Two training workshops were held in July, one in Coole Nature Reserve, Gort (Figure 1) and one in Connemara National Park, Letterfrack, at which volunteers chose the area they wished to survey.



Figure 1 Volunteers at the Gort workshop

For each 1km², the following were supplied by Galway County Council: a copy of the relevant six inch OS map (second series), the corresponding vector map, a vector map showing the locations of the hedges previously studied (Figure 2), and an ortho-rectified colour aerial photograph.

Contact was maintained with the volunteers by regular group emails and by phone calls to arrange the dropping off and collection of tubes throughout the summer. Volunteers received their prepared set of baited tubes in a plastic crate, which was inside a large plastic bag for ease of handling. All the sampling materials needed for the fieldwork were provided by the coordinator, as was personal protective equipment such as disposable gloves, antiseptic hand wipes, bandages and high visibility jackets.

Each volunteer received an information pack that contained emergency telephone numbers, the VWT's Health and Safety Guide for Volunteers, a guide on using OS National Grid References, an introductory letter to present to the Gardai, landowners or interested members of the public; a

postcard of a stoat and a field record sheet. Each volunteer was also given a copy of a training DVD that detailed the making, setting up and deployment of the tubes, this was also available from the VWT's website www.vwt.org.uk



Figure 2 Vector map showing hedges surveyed by Fuller (2006)

2.2 Hair tube construction

Each hair tube was 52mm in diameter and 20cm long, with a 5mm diameter hole midway along the length and a slot cut half way through the tube 25mm from each end. The tubes were made from black plastic underground ducting pipe, cut to the required length using a hand operated mitre saw. A drill was used to make the hole in the tube to allow the bait wire to pass through. The bait wire was a 30cm length of galvanised garden wire, 1.5-2.0mm diameter. Care was taken to remove any rough edges created during the cutting and drilling steps.

Skinned rabbit meat was used as bait on all but eleven occasions, when uncooked chicken was used. The wire was poked through the bait and bent around to hold it in place, then the wire was inserted into the tube through one of the ends, passed out through the central hole, and finally wound around the outside of the tube, with the loose end secured safely inside the wound section.

Once the bait was in place, Size 31 rubber bands were placed into the end slots, any twists that formed were carefully removed and the tension of the band adjusted to ensure that a stoat could enter the tube without having to force its way against the band. Finally, a small amount of strong adhesive glue was applied to both sides of the rubber bands.

2.3 Hair tube deployment

Within each study area, ten tubes were placed at 20m intervals on the ground along a hedgerow or occasionally along a stone wall, in the absence of a suitable hedgerow, at approximately fortnightly intervals from the end of June until mid October. When a hedgerow was less than 200m long, two separate sections of 100m were used, but this was the exception. The tubes were anchored in the ground using a 22cm long tent peg that was hammered into the ground and to which a cable tie was attached. The tube was placed through the cable tie and the latter then pulled tight, so that the tube would not wobble. Loose vegetation was cleared away from the ends of the tube to prevent it being dragged in and adhering to the glue (Figure 3). In some cases it was possible to secure the tube by attaching the cable tie to wire fencing, horizontal branches or tree roots. The tubes were left in place for a minimum of seven days.



Figure 3 Hair tube in a hedgerow

2.3 Sample collection

At the end of the sampling period, tubes were checked for the presence of hairs (Figure 4). When hairs were present, a forceps was used to hold the rubber band securely while a scissors was used to cut out the hair sample. The sample (section of rubber band plus hair) was then placed into a small plastic centrifuge tube and sealed. This was then placed into a sealable plastic bag (with writeable window) on which the date of collection, tube number, site identification code and volunteer name were recorded. Where hairs were found at both ends of a tube, a separate centrifuge tube was used for each sample. Forceps and scissors were wiped clean after each sample was collected to prevent cross contamination. Additional information was entered on a field record sheet, such as landowner's details (where applicable), loss of tubes, notes on surrounding habitat, etc. All samples and field notes were returned to the project coordinator.



Figure 4 Examining a tube for hairs

2.4 Laboratory analysis

Samples were posted on a regular basis to the Department of Chemical and Life Sciences in Waterford Institute of Technology (WIT) where DNA was isolated from hair using an adaptation of the Chelex 100 method (Walsh *et al.* 1991) as described in Statham, 2005. Stoat DNA was identified using a real-time PCR TaqMan™ MGB® probe assay (C. O'Reilly, unpublished). It was decided to also test for rat (*Rattus norvegicus*), wood mouse (*Apodemus sylvaticus*), and pygmy shrew (*Sorex minutus*) using a SYBR green species specific real-time PCR assay, as described by Moran et al (2008).

3. Results

3.1 County coverage

Of the 63 10km grid squares that comprise the county, 50 were surveyed, 37 in the east of the county and 13 in the west, with an additional four sites that had not been surveyed during the previous county hedgerow surveys (Gorumna Island, Brackloon, Ballinaboy and Spiddle). Four squares were surveyed more than once - due to the availability of both suitable hedgerows and volunteers. The 13 non-surveyed sites were mainly those without hedgerows or road access.

3.2 Volunteer effort

A total of 600 tubes were laid out by 50 volunteers from June to October, 430 tubes in East Galway and 170 in West Galway. 338 samples were collected of which 174 were suitable to test for the presence of stoat.

Approximately 240 volunteer hours (30 days) were spent on this project.

3.3 Stoat detection

The Irish stoat was detected throughout County Galway during the sampling period. 36 samples proved positive for stoat DNA, corresponding to 21 different squares throughout the county, 16 in East Galway and 5 in West Galway, the latter primarily along stone walls. Wood mouse was found in fourteen samples, corresponding to twelve different areas, rat in one, with 133 unidentified samples.

Stoats were recorded from four of the five native hedgerow types found in the county: species-poor hawthorn hedges (n = 10); willow-dominated hedgerows on wet ground (n = 3); hazel-dominated hedgerows (n = 3); gorse-dominated hedgerows (n = 1); and from along stone walls with and without vegetation (n = 4). No tubes were laid in the fifth hedgerow type, that characterized by the presence of holly. Stoat hairs were found in tubes with both rabbit and chicken bait.

In areas where it was not possible to locate a 200m length of roadside hedgerow, volunteers placed tubes along hedgerows away from roads (off road). A Fisher Exact Test on the detection rate of stoats at roadside hedgerows versus off road hedgerows shows there is no significant difference in the detection rate ($p = 0.39$, $p > 0.05$). Table 1 gives details of the areas with positive stoat records.

Grid Reference	Nearest town/village
M 40 20	Clarinbridge
M 50 20	Craughwell
M 70 20	Kilreekill
M 50 10	Laban
M 70 70	Ballymoe
M 50 60	Dunmore
M 70 60	Gortnadeeve
M 30 50	Caherlistrane
M 40 50	Belclare
M 50 50	Barnaderg
M 30 40	Grange
M 30 30	Menlough
M 80 10	Killimor
M 50 00	Kilbecanty
M 70 00	Woodford
L 80 50	Recess
M 00 50	Cornamona
M 10 50	Clonbur
L 80 30	Carna
L 80 20	Gorumna Island
M 30 50	Brackloon

Table 1 Areas with positive stoat records

4. Discussion

The primary aim of this study was to determine if stoats could be detected along hedgerows using hair tubes and this was achieved. Stoats were detected from all parts of the county, from hedgerows along roads, off road and also from along stone walls. The results of this study provide new information on the fauna associated with hedgerows in Galway County. Although this methodology has been used extensively in other countries for stoats, notably New Zealand (Bryom et al 2010), this is the first time that a systematic survey using hair tubes has been undertaken for stoats in Ireland. This study also demonstrated the valuable and generous contribution made by volunteers.

The high number of samples in which stoat was not detected, despite the presence of hair, may have arisen due to some interference by sections of rubber band during the laboratory analysis, and this warrants further investigation. It was not considered feasible to ask volunteers to remove hair from bands when they were collecting the tubes, due to the extra time this would take.

The access to detailed maps and aerial photographs from previous hedgerows surveys was a key factor in the success of this project because it made it extremely simple for the volunteers to locate a study site in their area. As this study was dependent on volunteers, it was also considered essential that study sites were low risk environments in which to work and, as far as possible, situated in public areas, thus removing the need to gain permission to access private land. In those cases where access was needed, landowners granted this and appeared to be genuinely interested in the study.

The success of this pilot project has already generated interest amongst mammal workers in Britain and elsewhere in Europe who wish to develop cost-effective methods for detecting and monitoring small mustelids; mammal researchers from Southampton University have asked for information on how to deploy hair tubes for stoats and this study was discussed at the 28th Mustelid Colloquium held in the Netherlands during November 2010 (J. Birks, pers. comm.). A proposal to present the results of this study as an oral paper at the Easter Meeting of The Mammal Society in Nottingham, 2011 has been officially accepted.

5. References

- Byrom, A., Howitt, R., and Gleeson, D. DNA for population estimation: stoats in a New Zealand beech forest. <http://www.ecogene.nz> (accessed March 2010)
- Fairley, J. (2001) *A basket of weasels*. Private Publication. Belfast, Northern Ireland.
- Fuller, J. (2006) East Galway Hedgerow Survey. Report prepared by Galway County Council. www.hedgelaying.ie
- Giorrai Environmental Services. (2007) West County Galway Hedgerow Survey and County Galway Townland Hedgerow Survey. Report prepared for Galway County Council. www.hedgelaying.ie
- Lindenmayer, D.B., R.B. Cunningham, R.D. Incoll, M.L. Pope, C.F. Donnelly, and B.E. Triggs. (1999) Comparison of hairtube types for the detection of mammals. *Wildlife Research* 26: 745-753.
- Marnell, F., Kingston, N., and Looney, D. (2009) Ireland Red List No. 3: Terrestrial Mammals. National Parks and Wildlife Service. www.npws.ie
- Moran, S., Turner, P. and O'Reilly, C., (2008) Non-invasive genetic identification of small mammal species using real-time polymerase chain reaction. *Molecular Ecology Resources* 8, 1267-1269.
- Sleeman, D. P. (1993) Habitats of the Irish stoat. *Ir. Nat. J.* Vol. 24. No. 8.
- Statham MJ (2005) Development and use of DNA identification techniques for Irish Mustelids. Dissertation, Waterford Institute of Technology, Ireland.
- Walsh, P.S., Metzger, D.A. and Higuchi, R. (1991) Chelex-100 As a Medium for Simple Extraction of DNA for PCR-Based Typing from forensic material. *Biotechniques* 10, 506-513.