

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



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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, the remainder occurring on the Isle of Man. 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 1km<sup>2</sup> of 10km grid squares across County Galway. Each tube was 20cm long, with a diameter of 52mm. A total of 600 tubes were laid out by 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 38 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.

# Acknowledgements

I wish to thank sincerely all the volunteers who met me, some on repeated occasions in the many car parks throughout the county, accepted crates of plastic tubes stuffed with rabbit meat, and carefully placed them in the backs of their cars for later distribution. On a hot summer's day, this was quite an undertaking. So too was the retrieval of these tubes from the countryside many days later, when they had acquired additional aromas.

The volunteers were:

Steve Bass, Dermot Breen, Sinead Biggane, Elizabeth Byrnes, Martin Byrnes, Cat Carlin, Helen Carty, Joe Costelloe, Tom Cuffe, Mags Flaherty, John Francis Flaherty, Martin Gammell, Marie Louise Heffernan, John Higgins, Michelle Hughes, Pdraig Keirns, Colin Lawton, Hilda MacLochlainn, Colm MacLochlainn, Pete Maguire, Eoin Mc Greal, Eugene Mc Keown, Ronan Mc Keown, Caelainn Mc Mullen, James Moran, Frank Murphy, Jacinta Murphy, Muireann Ní Cheallachain, Aonghus O'Donaill, Margaret O'Keefe, Ger O'Regan, Elaine O'Riordan, Norma Owens, Chris Peppiatt, Carole Reynolds, Pat Roberts, Lucy Roberts, Fergus Roberts, Cathy Seale, Neil Sharkey, Peggy Sharkey, Emma Sheehy, Raymond Stephens, Peter Stuart, Rebecca Teesdale, Pete Tyndall, Chris Tyndall, Marianne Ten Cate, Catherine Waters, Ronan Whilde.

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This project would not have been possible without the help of my colleague, John Messenger, who not only researched the design and deployment of the hair tubes, but who also made 180 tubes and prepared an excellent training DVD.

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## 1. Introduction

### 1.1. Background to The Vincent Wildlife Trust

The Vincent Wildlife Trust (VWT) undertook a pilot project on the Irish stoat (*Mustela erminea hibernica*) 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.

### 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 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, due 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 hair 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 of 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

(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) 1km<sup>2</sup> 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).

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## 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 advertised on the VWT'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 1km<sup>2</sup>, 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](http://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 holding the tube securely in place. 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. On four occasions volunteers stored the hair tubes in a freezer for a number of days, until they were able to lay them out.



Figure 3 Hair tube in a hedgerow

## 2.4 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 pair of 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 surveyor 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, such as landowner's details (where applicable), loss of tubes, notes on surrounding habitat, etc., was entered on a field record sheet. All samples and field notes were returned to the project coordinator.



Figure 4 Examining a tube for hairs

## 2.5 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).

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## 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; these included four sites that had not been surveyed during the previous county hedgerow surveys (Spiddle, Ballinaboy, Gorumna Island and Brackloon). 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 sent to WIT, 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 Hedgerows & stone walls sampled

A total of 48 hedgerows of the five vegetation types identified for the county were surveyed: species-poor hawthorn hedges (n = 33); holly-dominated hedgerow (n = 2); willow-dominated hedgerows on wet ground (n = 2); hazel-dominated hedgerows (n = 8); gorse-dominated hedgerows (n = 3). 12 stone walls were surveyed. The breakdown of roadside versus off road hedgerows was 26 and 34 respectively.

### 3.4 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. Figure 5 shows the results on a county-wide basis, with the exception of the four additional areas that were surveyed, Table 1 outlines the areas where stoat was detected, and Appendix A gives full details of all positive records.

68 samples were classified as 'not determined' due to poor quality DNA, but it is possible that some of these were from stoats. Wood mouse was found in fourteen samples, corresponding to twelve different areas, while one sample proved positive for rat.

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 = 2); hazel-dominated hedgerows (n = 3); gorse-dominated hedgerows (n = 1); and from along stone walls with and without vegetation (n = 5). No positive results were obtained from tubes laid

in the fifth hedgerow type, characterised by the presence of holly. Stoa hairs were found in tubes with both rabbit and chicken bait, and in one set of tubes that had been frozen prior to deployment.

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 ( $p = 0.39$ ,  $p > 0.05$ ). There was also no significant difference in the detection rate of stoat from the different hedgerow types, between hedgerows and stone walls, or between the west and east of the county.

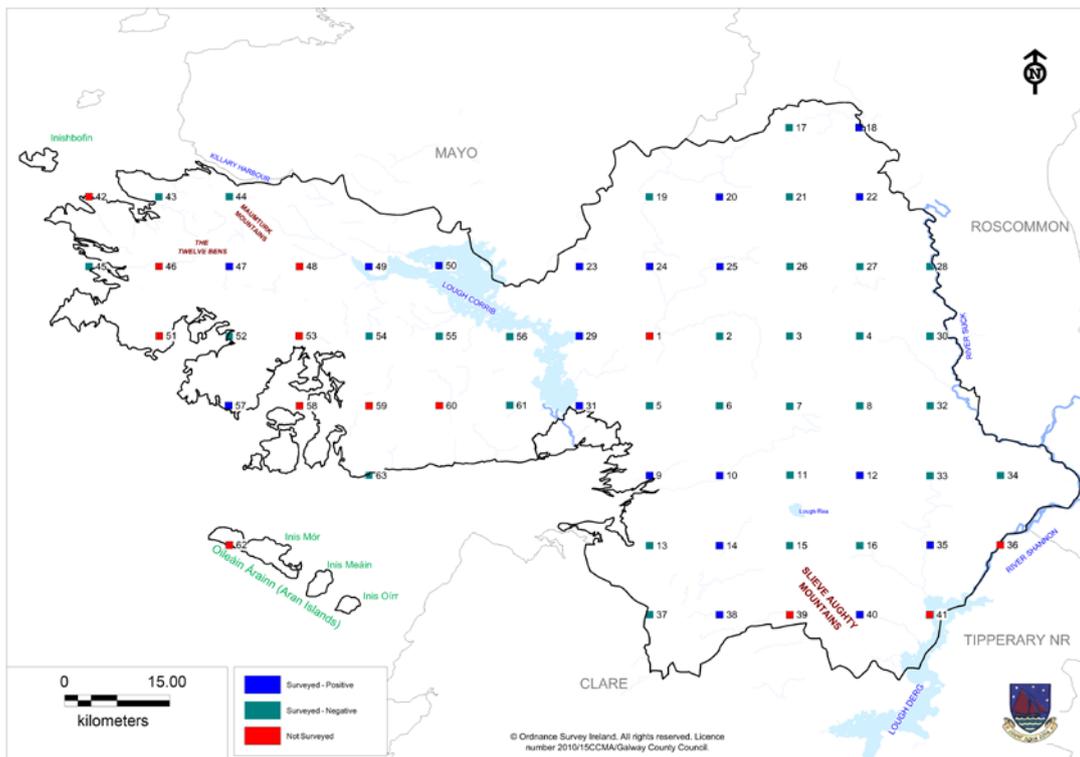


Figure 5 Distribution of positive and negative results for stoat

Area Number	Nearest town/village	Grid Reference
09	Clarinbridge	M 40 20
10	Craughwell	M 50 20
12	Kilreekill	M 70 20
14	Laban	M 50 10
18	Ballymoe	M 70 70
20	Dunmore	M 50 60
22	Gortnadeeve	M 70 60
23	Caherlistrane	M 30 50
24	Belclare	M 40 50
25	Barnaderg	M 50 50
29	Grange	M 30 40
31	Menlough	M 30 30
35	Killimor	M 80 10
38	Kilbecanty	M 50 00
40	Woodford	M 70 00
47	Recess	L 80 50
49	Cornamona	M 00 50
50	Clonbur	M 10 50
57	Carna	L 80 30
----	Gorumna Island	L 80 20
----	Brackloon	M 30 50

Table 1 Areas where stoat was detected

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## 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. Although this methodology has been used extensively in other countries for stoats, notably New Zealand (Byrom *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.

Stoats were detected throughout the county, at 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 and will form the basis for a flyer on the Irish stoat, funded by Galway County Council. All positive records have been submitted to the National Biodiversity Data Centre and can be viewed by visiting the data centre's website: [www.nbdc.ie](http://www.nbdc.ie), selecting *Access Data*, and entering *Stoat* into the *Species Search* box.

The high number of samples in which stoat was not detected, despite the presence of hairs, may have arisen due to interference by the presence of the rubber band and glue during the laboratory analysis. It was not considered feasible to ask volunteers to remove hair from the bands when they retrieved the tubes, due to the extra time this would take. The use of a purification kit may detect more positive results from poorer DNA samples and also overcome the inhibition caused by the bands and the glue, but this would add to the cost.

The access to detailed maps and aerial photographs from previous hedgerow surveys was a key factor in the success of this project because it made it extremely simple for volunteers to locate a study site in their area. As this project 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, which can be time consuming. In those cases where hedgerows on private land were sampled, landowners were happy to grant access and appeared genuinely interested in the study.

It is important to note that a negative result for a square does not mean that the Irish stoat was not present in that square, but that its presence was not detected during the sampling period.

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## 6. Appendix

Volunteers	Area No.	Hedge No.	Date checked	Tube Nos.	Species
M & E Byrnes	09	07	Aug 31	10, 60, 61, 63, 64	Stoat & woodmouse
P Keirns	10	08	Sept 2	78,79	Stoat
M & E Byrnes	12	06	Oct 20	173	Stoat
R Stephens	14	04	July 16	100	Stoat
C Carlin & M Gammell	17	08	Oct 9	156	Woodmouse
C Lawton & C Waters	18	07	Oct 22	23,24,28	Stoat & woodmouse
J Higgins & E McGreal	20	10	June 29	21	Stoat
C Lawton & C Waters	22	01	Oct 22	63,63	Stoat & woodmouse
J Costelloe	23	06	June 9 & 24	79, 77, 80	Stoat & woodmouse
N & P Sharkey	24	Gorse hedge	July 13	60	Stoat
J Higgins & E McGreal	25	02	July 29	161,162,163, 165,166	Stoat
P Tyndall	29	08	Oct 3	86	Stoat
M O'Keefe	31	05	July 17	23	Stoat
H Carty	35	08	Aug 3	37,38	Stoat & rat
J Moran & S Biggane	38	08	Aug 4	95	Stoat & woodmouse
J Murphy	40	01	Sept 3	149	Stoat
D Breen	45	Stone wall		103	Woodmouse
M Heffernan	47	Stone wall	Aug 29	47	Stoat
C Reynolds	49	Stone wall	Aug 12	171,178	Stoat & woodmouse
J Higgins & E McGreal	50	Gorse hedge	Oct 26	30, 38	Stoat & woodmouse
D Breen	57	Stone wall	Aug 30	90	Stoat
A O'Donail	Gorumna	Stone wall	Aug 3	90	Stoat & woodmouse
S Bass	Brackloon	Hawthorn hedge	Oct 3	29	Stoat

Details of areas with positive results for stoat, wood mouse and rat

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An Chomhairle Oidhreachta  
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