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A Comparison of Nest Searches, Bait tubes and Live Trapping for Monitoring Harvest Mice (*Micromys minutus*) and Other Small Mammals

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## Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>1</td>
</tr>
<tr>
<td>Methods</td>
<td>2</td>
</tr>
<tr>
<td>Sampling Strategy</td>
<td></td>
</tr>
<tr>
<td>Field Methods</td>
<td>2</td>
</tr>
<tr>
<td>Laboratory Methods</td>
<td>3</td>
</tr>
<tr>
<td>Results &amp; Analysis</td>
<td>4</td>
</tr>
<tr>
<td>General Results</td>
<td>4</td>
</tr>
<tr>
<td>Derivation of Response Variables</td>
<td>4</td>
</tr>
<tr>
<td>Harvest Mouse Results</td>
<td>6</td>
</tr>
<tr>
<td>Results for All Species</td>
<td>7</td>
</tr>
<tr>
<td>Discussion</td>
<td>9</td>
</tr>
<tr>
<td>Harvest Mice</td>
<td>9</td>
</tr>
<tr>
<td>All Species</td>
<td></td>
</tr>
<tr>
<td>Comparison between Transect Lengths</td>
<td>10</td>
</tr>
<tr>
<td>Cost-effectiveness of Bait Tubes Versus Trapping</td>
<td>10</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td></td>
</tr>
<tr>
<td>References</td>
<td>11</td>
</tr>
</tbody>
</table>
**Introduction**

This pilot study formed part of a contract awarded to a collaboration between The Mammal Society (TMS) and the Wildlife Conservation Research Unit (WildCRU) of Oxford University. In addition, TMS obtained funding from Natural England (NE) to support the citizen science aspects of their pilot study by the use of TMS volunteers. This contract funded Phase I of a proposed three-phase study into the ecology, distribution and abundance of the harvest mouse in the UK.

There were two main requirements of Phase I:

- To undertake a stakeholder workshop to explore and summarise the current state of knowledge of harvest mouse ecology. This was run jointly by both organisations in May 2008.
- To undertake field trials of methods to monitor harvest mouse presence and abundance.

The two organisations had different requirements for monitoring methodologies. In Phases II and III, it was anticipated that WildCRU would use an experimental approach on a relatively small number of intensively studied sites. This might involve habitat manipulation or different management regimes. Consequently, the field methods would be designed for a small number of expert ecologists, working intensively over a period of two to three years. Furthermore, factors such as field placement of traps (on-ground / above-ground) and location of sites needed to be investigated. WildCRU undertook this field trial, comparing two field methods (live trapping and nest searches) which has been reported separately by Riordan et al (2009).

In contrast, TMS would contribute to Phases II and III of the project with two extensive, national surveys for harvest mice carried out at five or six year intervals. The main purpose of the TMS pilot was to investigate methods for detecting harvest mouse presence and abundance that would be suitable for a long-term, volunteer-based monitoring programme. Consequently, the pilot differed from the WildCRU approach by using a number of volunteers, mostly with a high level of experience of small mammal fieldwork, but generally not professional ecologists.

The other main difference between the two pilot studies was that the TMS pilot compared three different field methods. A previous study by Poulton & Stone (2008) had included the use of bait tubes to collect faeces. This was based on work by Carter & Churchfield (2006) who used bait tubes in a national survey of water shrews (*Neomys fodiens*) [see also Churchfield et al (2000)]. Unfortunately, identification of faeces to species proved impossibly time-consuming, resulting in considerable loss of information from the bait tube method. However, practical developments by the Waterford Institute of Technology (WIT) in the use of polymerase chain reaction and DNA amplification, meant that a relatively cheap, quick and “non-expert” method of identifying the source of faeces samples was now available. So the TMS pilot was designed to compare harvest mouse nest searches, live trapping and DNA analysis from bait tubes.

The basic results from Poulton & Stone (2008) indicated that harvest mice would form only a small component of the small mammal fauna recorded by these methods. It was always intended, therefore, that the two multi-species methods (trapping and bait tubes) would be analysed separately, for all species, to obtain information on both the efficacy of bait tubes compared to live trapping, but also the overall costs of the two methods.
Methods

Sampling Strategy

For the purposes of this trial, the Primary Sampling Units (PSUs) were defined as OS tetrads. Volunteers were asked to select a suitable tetrad in the vicinity of their homes. In a national monitoring scheme, the PSUs would probably be sampled in a more objective way and then assigned to volunteers as they became available.

Field Methods

Within their PSU volunteers were asked to select two transect locations, at least 500m apart, in habitat suitable for harvest mice. This could include reedbeds or ditches containing Phalaris, Phragmites and possibly Typha spp. or hedgerows with rank grasses (e.g. Dactylis, Ahrenatherum, etc.) and very often bramble (Rubus agg.).

One transect (I) measured 100m in length and the other (II) was 200m long. Each transect was divided into 10 sections, each 10m long in Transect I and 20m in Transect II (Figure 1). The three different field methods were applied, simultaneously (Table 1) in each transect in the following way;

a) Live Trapping. 40 Longworth traps were set in ten groups of four, one group in each section. The traps were all located on the ground, within dense vegetation, each group within an area of approximately 2m². They were provided with adequate bedding and blowfly pupae (fisherman’s casters), rolled oats and/or pieces of fruit for food. The traps were set on the morning of Day 1 and checked on Day 1 pm, Day 2 am, Day 2 pm and Day 3 am. All captured animals were recorded and identified to species. Animals caught in the first two sessions were marked with a fur clip on their left-hand sides and those caught during the final two sessions on their right-hand sides, even if already marked from the first two sessions. (In reality, animals caught in the last session did not need to be marked as they would not be recaptured.) The traps were collected on the morning of Day 3 (Session 5).

b) Bait tubes. 30 bait tubes were set in ten groups of three, one group in each section. The tubes were all located on the ground, in a similar way to the traps, but interspersed between them, so that the trap groups tended to one half of the section, with the tube groups in the other. The tubes were baited with casters only and set on the morning of Day 1. One tube from each group (designated Tube A) was collected on the morning of Day 3 (Session 5). On the morning of Day 5 (Session 6), another tube was collected from each group and designated Tube B. Finally, on the morning of Day 7 (Session 7) the last tube (C) was collected. In this way, three batches of tubes were collected after three, five and seven days in the field. Any signs of activity in the tubes, such as the casters having been eaten or the muslin chewed, were recorded. All faeces in the tube were collected, as a single sample, into clean snap-lock sample bags. These were stored in refrigerated conditions and sent to the laboratory as a single batch at the end of the field season.

c) Nest Searches. On the morning of Day 5 (Session 6), after the bait tubes were collected, the first nest search was undertaken. Each of the ten sections was searched for harvest mouse nests, spending between five and ten minutes on each section. The number of nests in each section was recorded. On the morning of Day 7 (Session 7), a second search was carried out on an adjacent zone of similar habitat, either parallel (such as the hedge or ditch on the opposite side of the road) or continuing from one end of the first transect. This zone was the same length and also divided into ten sections. The number of harvest mouse nests in each section was recorded.
Laboratory Methods

As the amount of material differed between samples, with some samples containing just fragments of faecal pellets while others contained upwards of 10, all faecal material present was used for DNA extraction. This was done to avoid possible sub-sampling error, as two or more pellets present in a sample could have been produced by two different species.

DNA was extracted from faeces using the ZYMO ZR Genomic DNA II Kit (Product No. D3007) according to the manufacturer’s instructions, but using half the recommended volumes because of the small size of the samples. DNA extracts were stored at –20°C until further use.

Samples were amplified with each species-specific primer set in 10μl reactions containing 5 μl Power SYBR Green I PCR Master Mix (ABI), 200 nM of each primer and 4.2 μl DNA solution (1:100 dilution). Positive controls included 4.2 μl DNA extracted from tissue (1:100 dilution) and negative controls included 4.2 μl sterile H2O. All real-time PCR amplifications were performed using two Applied Biosystems 7300 Real-Time PCR Systems. Amplifications were conducted using the following default profile: 2 minutes at 50°C, 10 minutes at 95°C and then 40 or 50 cycles of 15 seconds at 95°C, 60 seconds at 60°C. C_T values were determined using the default threshold level of 0.2. Final dissociation steps for melt-curve analysis were also included after each PCR, which determined whether amplification was specific or non-specific.

Species-identification was conducted based on two criteria: T_M value and C_T value. Firstly, samples were identified based on specific amplification, which was determined using melt-curve analysis, i.e. where the T_M values of the amplicons were within ± 1.0°C of the T_M value of the positive control. Then, probable positive identification was concluded for those samples amplifying with a C_T < 35.00, whereas samples showing apparent specific amplification but with C_T values > 35.00 were identified as probable species.
Results & Analysis

General Results

A total of 40 volunteers worked individually or in groups in 13 tetrads (Figure 2). In one tetrad, only one transect was completed, giving a total of 25 completed transects. Although the three methods were all applied in the same transect during the same period, they did have different numbers of Field Survey Units (FSUs) which, in turn, yielded different types of primary data (Table 2). Firstly, within the 25 transects, a total of 1,000 traps were laid for two 24 hour periods each, giving 2,000 trap-nights. The primary records were captures (including recaptures) of which 326 were made. A total of 750 bait tubes were laid from which 280 faeces samples were collected. Finally, 500 sections were searched, within which 22 nests were found.

These highly different success rates emphasised two characteristics of the methods. Firstly, the trapping and bait tube methods were multi-species, whereas nest searching was constrained to harvest mouse nests. Secondly, the traps were laid for four sessions, which gave them the “opportunity” to make multiple captures. In contrast, bait tubes and sections were only used or searched once, and nests are relatively permanent signs compared to the ephemeral nature of faeces.

Derivation of Response Variables

The trapping and bait tube methods had supplementary factors built into the within-site experimental design. These were; a) the number of days that the traps had been set and b) the number of days the tubes had been laid before collection. In addition, trapping data could be treated in a number of ways; just counts of unmarked animals to give estimates of Minimum Number Alive (MNA) or using a Capture/Mark/Recapture technique to provide a Petersen Index estimate of population size. Similarly, the DNA analysis yielded a small proportion of uncertain identifications, so the effect of using “definite” v “possible” identifications needed to be investigated.

Furthermore, to allow a comparison between the three methods, the raw data obtained from each method needed to be transformed into a common response variable. A previous pilot study (Poulton & Stone; 2007) showed the benefit of converting different field methods to a count of transect sections in which a species was present. The summary data in Table 2 have been converted in this way (aggregating all species for trapping and bait tube data) and clearly show the relative paucity of nest searching compared to the two multi-species methods.

Trapping

The 326 captures comprised eight

<table>
<thead>
<tr>
<th>Type of FSU</th>
<th>Trapping</th>
<th>Bait-tubes</th>
<th>Nest Searches</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of FSUs</td>
<td>Trap-night</td>
<td>Tube</td>
<td>Section</td>
</tr>
<tr>
<td>Primary Records</td>
<td>Captures</td>
<td>Faeces Samples</td>
<td>Nests</td>
</tr>
<tr>
<td>N (%)</td>
<td>326 (16%)</td>
<td>280 (37%)</td>
<td>22 (4.4%)</td>
</tr>
<tr>
<td>Number of sections</td>
<td>250</td>
<td>166 (66%)</td>
<td>17 (3.4%)</td>
</tr>
</tbody>
</table>
species (Table 3). From these raw capture data, four response variables were derived, based on each species in each transect, giving 200 cases (Figure 3). All four distributions were dominated by the large number of zero records (62%). The main differences were that the two count variates had more severely extended right-tails, so very little information would appear to be lost in converting the capture data to section frequencies. Furthermore, the inclusion of all captures rather than just captures of new animals simply had the effect of increasing nine of the 200 frequencies by one.

The section frequencies could be further sub-divided by just taking the captures recorded from Day 1 and comparing them with the frequencies derived from captures from both days (Figure 4). The number of transect/species counts equal to zero decreased from 147 (73.5%) to 124 (62%) when both day’s captures were included. There was a highly significant increase in the median number of sections with captures when both days were used (Wilcoxon matched-pairs, $Z = 6.62$, $p \approx 0$).

**Bait Tubes**

Of the 750 bait tubes laid, 42 were not recovered or had been completely washed out by rain. Of these, 383 (51% of tubes laid) showed signs of activity, of which 280 provided faeces samples. Of these, 256 (34%) provided identifications of one or more species, leaving 24 samples (8.6%) which were unable to yield an identification.

From these successful samples, 336 species identifications were made. All eight species that were recorded in the traps were also recorded from the bait tubes (Table 4). Of these identifications, only 47 (14%) were

![Graphs](image-url)

**Figure 3.** Frequency histograms of four derived response variables for trapping data.

<table>
<thead>
<tr>
<th>Species</th>
<th>All</th>
<th>New (MNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Apodemus flavicollis</em></td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td><em>Apodemus sylvaticus</em></td>
<td>136</td>
<td>95</td>
</tr>
<tr>
<td><em>Micromus minutus</em></td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td><em>Microtus agrestis</em></td>
<td>29</td>
<td>23</td>
</tr>
<tr>
<td><em>Myodes glareolus</em></td>
<td>77</td>
<td>56</td>
</tr>
<tr>
<td><em>Neomys fodiens</em></td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td><em>Sorex araneus</em></td>
<td>56</td>
<td>41</td>
</tr>
<tr>
<td><em>Sorex minutus</em></td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>326</td>
<td>242</td>
</tr>
</tbody>
</table>

**Table 3.** Summary of captures by species.
classified as “possibles”, although as these constituted all the records for two species they have been included in subsequent analyses.

As with the trapping data, frequency distributions of counts of identifications showed a very long right-tail and very little information appeared to be lost when they were expressed as section frequencies (Figure 5). A remarkably similar number of zero counts (127 or 63.5%) was recorded.

To investigate the effect of the time for which the tubes were laid on the identification rate, the section frequencies were recalculated separately, from each batch of tubes. This was complicated slightly by the fact that in one site, the volunteer had misunderstood the instructions and collected and relaid all 30 tubes at each session. These have been excluded from this comparison. A non-parametric repeated-measures ANOVA (Friedman’s test) showed a significantly lower mean number of sections with DNA identifications from the three different batches ($\chi^2(2) = 39.7, p \approx 0$). This was primarily due to the lower mean number of sections collected from Batch A (after two nights) compared to the other two batches, although there was also a significant difference between Batches B and C ($\chi^2(1) = 8.02, p < 0.005$).

However, these results could have been due to the fact that Batch A took place simultaneously with the trapping, so that activity in bait tubes was curtailed by the traps. To test this, the data from the erroneous site where all 30 bait tubes were collected at each session provided three batches of 30 tubes, each laid for two nights; during the trapping, one day after and three days after the trapping sessions. A similar Friedman ANOVA showed no significant differences between these three batches, suggesting that the actual time elapsed, rather than the coincidence with trapping was the significant effect.

**Harvest Mouse Results**

The harvest mouse data were extracted for separate analysis because they were the only species for which all three methods were used. Exploratory analyses showed that relatively low recording rates were achieved with all three methods.

**Nest Searches**

The 13 sites contained 25 transects, each of which comprised two nest Searches. Of the 50 searches, 12 (24%) had one or more sections with nests (Figure 6). Nine out of the 25 transects (36%) recorded at least one nest in one or other search.

There was no relationship between the number of sections with nests in each of the two searches (Spearman $R_{s(24)} = 0.349, p \approx 0.095$). In fact only three of the 25 transects had nests in both searches.

At the transect level, in the twelve tetrads that included both transects there was a marginally significant correla-
tion between the two (Spearman $R_{(12)} = 0.44$, $p \approx 0.031$). Again, only three of the tetrads had positive counts in both transects. Finally, there was no evidence that nests were more frequently found in the 200m long transect than the 100m; Wilcoxon signed-rank ($Z = 0.93$, $p \approx 0.35$).

**Bait Tubes**

The presence of harvest mouse DNA was only recorded in three of the 25 transects (Section counts; 1, 2 & 2). No tetrads had positive records in both transects.

**Trapping**

Harvest mice were only captured in three of the 25 transects (Section counts; 1, 2 & 3). No tetrads had positive records in both transects.

**Comparison of Methods**

Using the section counts from Search 1, two analyses were undertaken. Firstly, a Spearman rank-order correlation matrix showed only a marginal correlation between bait tubes and traps ($R_{(24)} = 0.405$, $p \approx 0.045$), with neither of the other two being significant. In other words, the presence of harvest mice revealed by one method in a transect did not ensure that they would be recorded by one of the other methods. Secondly, the mean number of sections in which harvest mice were recorded did not differ between the three methods (Friedman non-parametric ANOVA; $\chi^2_{(1)} = 0.889$, $p \approx 0.64$)

**Results for All Species**

A second subdivision of the data extracted all species using just the bait tube and trapping field methods. Following the analyses presented in the Derivation of Response Variables, trapping results from both days were used, expressed as numbers of sections with captures, and bait tube results utilising both definite and probable identifications from Batch C, expressed as numbers of sections per transect were used. The erroneous site was included as the section counts for Batch C were not significantly different from all other sites, and the site comprised 8% of the available data.

**Bait Tubes**

Of the 250 bait tubes laid in Batch C, 117 (47%) provided one or more identification of any species. However, 41 tubes provided more than one identification (Table 5) giving a total of 162 species identifications. As each section had only one tube, this equates to a return-rate for identifications of 65% — in other words, for every 100 sections there were 65 species identifications.

**Trapping**

Of the 250 transect sections, 133 (53%) achieved one or more captures. When expressed as species identifications, 47 sections provided multiple records (Table 6a), giving a total of 190 species identifications. This equates to a 76% return-rate for species identifications.

However, because animals were marked before release, the number of individuals could also be calculated, which allowed the frequency distribution of individual identification per section to be derived (Table 6b). When the frequency counts are multiplied through, the 326 individual capture records are attained.
Comparison between Methods, Transects and Species

A logistic regression model was constructed using three main factors; Transect, Method and Species and their two-way interactions. All factors were treated as fixed-effect and no account was taken of the repeated-measures nature of the Method or Species factors, resulting in an intrinsically less powerful model. Furthermore, due to the complete absence of field voles in Batch C of the bait tubes, this species had to be excluded from the model. This left an unbalanced, but at least full-rank model which could be satisfactorily resolved.

Of the three main effects, Transect and Method were non-significant. Not surprisingly, Species was highly significant (Wald $\chi^2_{(6)} = 166$, $p \approx 0$), with four distinct groupings (Figure 7). The Transect $\times$ Method interaction was not significant but both species interactions were significant. Firstly, the Species $\times$ Transect interaction showed marginal significance (Wald $\chi^2_{(6)} = 14.56$, $p \approx 0.024$), with a significantly greater proportion of sections per transect (0.27) recording common shrews on the 200m transects than the 100m (0.15), whereas no other species showed this distinction. A separate logistic regression of this species alone showed highly significant differences between both transects and methods, but no interaction. Consequently, the combination of “best” method and transect (bait tubes on 200m transects) gave a mean number of positive sections of 33%, whereas the opposite combination gave a mean of only 10%.

The Species $\times$ Method interaction was very highly significant (Wald $\chi^2_{(6)} = 33.7$, $p < 0.001$; Figure 8). Wood mice and bank voles were recorded in a significantly greater proportion of sections using trapping than with bait tubes. This pattern was reversed for common shrews and, although not separately significant, for pygmy shrews as well.

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<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
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<td>86</td>
<td>38</td>
<td>8</td>
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<td>2</td>
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<td>86</td>
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<td>56</td>
<td>326</td>
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</table>

**Table 6. Frequency breakdowns of species and individual identifications from trapping sections.**

![Figure 7](image-url) Means and 95% C. I. of proportion of sections per transect for each species (excluding field voles).

![Figure 8](image-url) The interaction between Species and Method.
Discussion

**Harvest Mice**

The results from this pilot support the findings of previous studies showing that harvest mice are not commonly encountered during widespread surveys (Poulton & Stone; 2008, Riordan et al; 2009). All three methods resulted in at least 75% absence from transects, with no significant differences between them. Furthermore, where one method did record the presence of harvest mice, there was no correlation with the other two methods. In addition, there was no relationship between harvest mouse presence and the length of the transect, suggesting that the small-scale distribution of harvest mice was very clumped; resulting in binary rather than quantitative data.

These factors mitigate against an easy, efficient, volunteer-based survey for harvest mice alone. As two of the three methods trialled here also gather considerably more data on other small mammal species, this leaves two approaches for the future;

- To obtain quantitative data on harvest mouse distribution and abundance, autecological studies will have to be intensive, focussed on a few sites and involve frequent visits to pick up short-term fluctuations in numbers.
- Longer-term and more extensive monitoring is unlikely to generate sufficient returns from nest searching alone to keep volunteers interested. Consequently, a national survey should include a number of methods, including multi-species techniques such as bait tubes and live trapping. This predicates a survey for all small mammal species rather than just harvest mice.

**All Species**

Three of the seven species used in the comparison between Bait tube and Trapping Transects showed very low overall recording rates of between 0.1 and 0.2. This means that for these scarce species (yellow-necked mouse, harvest mouse and water shrew) between five and ten transects of either type would be required for a single record. This concurs with the findings of (Poulton & Stone, 2008) that sample sizes of many hundreds, if not thousands, of transects would be required to detect changes in these species over time. Pygmy shrews were recorded on average in around 6% of sections (significantly more than the scarce species), implying a positive section in every two transects. Bank voles were significantly more frequently recorded in around 12% of sections. Finally wood mice and common shrews were, on average across both methods, recorded on around 20% of sections, suggesting two positive sections per transect.

The interaction between method and species in this comparison showed highly significant differences between methods for the three most frequently recorded species (Figure 8). Wood mice and bank voles showed about twice the recording rate for trapping than for bait tube transects, whereas common shrews showed the reverse effect. This could have been for two reasons. Firstly, the only type of food used in bait tubes was casters, which would have been attractive to insectivorous shrews, but maybe less so for omnivorous mice and bank voles. Therefore, the overall type and quantity of food may have been insufficient to keep mice and voles in the tubes long enough to defecate. The fact that herbivorous field voles were not recorded in the Batch C tubes used for this comparison supports this suggestion. The second reason could be that the smaller and lighter shrews were more readily recorded in bait tubes than Longworth traps, especially if they had not been set carefully to trap lighter animals. The bait tubes could have been recording the true presence of shrews, whereas traps were consistently under-recording due to lack of sensitivity. This is speculative and is not supported by the very low bait tube recording rate for harvest mice which are a similar weight to shrews, but it is recommended that further research be carried out into the efficacy of live-trapping light-weight animals.
Comparison between Transect Lengths

Although there was no overall difference between recording rates for the two different transects, there was a marginally significant interaction with species. This effect was largely due to the response of common shrews and for this species, there could be an argument for using 200m transects rather than 100m.

Cost-effectiveness of Bait Tubes Versus Trapping.

The ten tubes in each bait tube transect took about one hour to prepare, set, retrieve and collect faeces. In addition two journeys to the site were required taking, on average, about 30 minutes each. So each bait tube transect would take about two hours to complete. In contrast, the Intensive trapping method required five visits (2.5 hours), about four hours of fieldwork and one hour preparation; 7.5 – 8 hours in total. So, although the trapping transects did provide a slightly higher proportion of sections with species identifications (76% compared to 65%), they took four times as long to complete as the bait tube transects. Given that several bait tube transects could be run simultaneously on one site, it would be reasonable to assume that around five bait tube transects could be completed in the same time that it takes to complete one trapping transect.

Without undertaking the sort of detailed analysis of information content that Poulton & Stone (2008) presented, it is difficult to ascertain the sensitivity to change that these two methods provide. However, in terms of section identifications, bait tubes would yield about four times as much information as the Intensive trapping transects. However, the latter also provide individual identifications, which the bait tube transects do not. This would allow population estimates, such as MNA, which could provide greater sensitivity to change. So, a purely subjective assessment might suggest that bait tube transects provide about twice as much monitoring information as intensive trapping transects for a given amount of volunteer time.

The capital costs involved in the two methods are also very different. The current cost of Longworth traps is over £60 each which, in turn, generates carriage and handling costs as traps usually have to be borrowed and returned by volunteers. But Longworth traps are robust, so assuming a lifetime of 100 sessions the full cost of 40 traps amortises to £24 per transect. Add to this approximately £20 for carriage, plus staff time for packing and despatch, equals very approximately £75 per transect. The equivalent capital costs for bait tubes is negligible; about £2 for 50 tubes (£10 amortised over five sessions) plus about £3 for muslin and rubber bands. So a batch of 50 bait tubes would cost about £5 to produce.

However, the consumable costs are very different in the opposite way. Both methods require casters and other food or bait, and although traps require bedding, this is a negligible cost. But the laboratory costs for the DNA analysis for the bait tubes are high; currently around £6 per sample for reagents and about £4 per sample for labour. Assuming a 65% return-rate, the 50 tubes would yield about 32 good faeces samples, therefore costing approx. £320 to analyse. Although there may be economies of scale if thousands, rather than hundreds, of samples are analysed, this method is still about four times the cost per equivalent unit (£325 / £75). As described above, the bait tube transects yield about twice the amount of information, so they are currently twice as expensive.
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