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1 **A PIT-tag based method for measuring individual bait uptake in small**
2 **mammals**

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19 **Abstract**

- 20 1. Rodents and other small mammals cause an increasing number of negative economic and
21 environmental impacts worldwide. In the UK, the non-native grey squirrel has a significant impact
22 on the forestry industry and has caused the decline of the native red squirrel.
- 23 2. Baits are used to deliver biocides and contraceptives to reduce overabundant wildlife populations
24 and as vehicles for vaccines to control disease outbreaks. Bait-delivered contraceptives are also
25 being developed to manage grey squirrel populations in the UK. The effectiveness of bait-delivered
26 drugs on wildlife populations depends on the amount of bait consumed by individuals over time;
27 therefore, it is important to understand individual level bait uptake in order to optimise delivery
28 methods.
- 29 3. Passive Integrated Transponder (PIT) tags are increasingly used to mark and monitor animal
30 behaviour as they are cost-effective, have minimal negative welfare impacts and have a lower tag
31 loss rate than external tags, particularly in small animals.
- 32 4. The aim of this study was to design and test a novel bait hopper equipped with a PIT-tag reader and
33 bait weighing device, to record bait uptake by individual grey squirrels for optimising the delivery
34 of a contraceptive bait. The hopper was designed to overcome some of the limitations of traditional
35 PIT-tag systems, by improving battery life and the quality and quantity of data collected in the field.
- 36 5. In captive trials, the hopper proved to be highly effective at recording feeding visits by squirrels, as
37 95% of the visits could be attributed to a PIT-tag record. The hoppers measured the amount of bait
38 removed per feeding visit to an accuracy of 0.1 g, with 97% of the bait taken from six hoppers
39 attributed to a PIT-tag ID. In a field trial, the hoppers were effective at recording the feeding visits
40 by grey squirrels in two woods, with 47 of the 51 PIT-tagged grey squirrels entering the hoppers.
- 41 6. The adaptability of the hopper design means that it has wider applications for wildlife management;
42 in particular, efficacy studies for bait-delivered substances in the context of wildlife disease control
43 and/or population reduction.

44 Keywords: feeding behaviour, grey squirrel, contraceptives, biocides, microchip, *Sciurus carolinensis*,
45 wildlife management, remote sensing.

46

47 **Introduction**

48 Rodents and other small mammals cause an increasing number of negative economic and environmental
49 impacts worldwide, including losses to the food industry, damage to property and the transmission of
50 diseases (Messmer, 2019). For instance, damage by rats has been estimated to cost the United States
51 approximately \$19 billion per year (Pimentel et al., 2000). In the UK, the grey squirrel *Sciurus*
52 *carolinensis* causes an estimated £10 million in tree damage per annum (Derbridge et al., 2016) and is
53 also responsible for the decline of the native red squirrel *S. vulgaris* (Gurnell et al., 2016; Rushton et
54 al., 2006). Baits are used to deliver biocides and contraceptives to reduce wildlife populations (Townes
55 & Broome, 2003; Pyzyna et al., 2016) and as vehicles for vaccines to control disease outbreaks in
56 wildlife (Slate et al., 2005). Bait-delivered contraceptives could be used to manage grey squirrel
57 populations (Dunn et al., 2018) and this is a research objective in the current UK Government
58 management plan for the species (Forestry Commission, 2014).

59 Bait markers such as tetracycline (Algeo et al., 2013), iophenoxic acid (Marks & Bloomfield, 1999)
60 and rhodamine B (Baruzzi et al. 2017) can be used to assess bait uptake at a population level. However,
61 the effectiveness of rodenticides, oral vaccines and bait-delivered contraceptives depends on the number
62 of visits to bait stations by individual animals over time and the quantity of bait they consume at each
63 visit. Consequently, understanding how bait uptake differs between the individuals in a population is
64 important when optimising the delivery of contraceptives, vaccines and biocides.

65 Passive Integrated Transponder (PIT) tags are increasingly used to mark and monitor animals, as they
66 have minimal welfare impacts, are relatively cheap and easy to apply and generally have a lower tag
67 loss rate than external tags (Smyth & Nebel, 2013). Once a number of individuals have been PIT-tagged,
68 Radio Frequency Identification (RFID) technology can be used to create remote sensing stations that

69 record and monitor the presence of individuals via their PIT-tags. PIT-tags do not rely on an internal
70 power source, so can feasibly be used over the lifetime of an animal, providing an important
71 improvement in animal welfare by reducing the amount of trapping and/or handling required. Examples
72 of how this technology has been used to collect remote data on wildlife in the field include recording
73 the behaviour of wood ducks *Aix sponsaa* at nest boxes (Bridge et al., 2019), monitoring the home
74 ranges of wood mice *Apodemus sylvaticus* using bait stations (Godsall et al., 2014) and recording den
75 use by edible dormice *Glis glis* (Kukalová et al., 2013).

76 The aim of this study was to design and test a novel bait hopper, equipped with a PIT-tag reader and
77 bait weighing device that could record the frequency of feeding visits and amount of bait consumed per
78 visit by individual grey squirrels in a natural environment. The hopper was tested in a laboratory, in a
79 trial using captive grey squirrels and then used to collect data on the feeding behaviour of free-living
80 grey squirrels in two woods. The hopper was designed to overcome a number of limitations typically
81 encountered when using RFID systems to gather long-term field data. We discuss how this hopper
82 represents a significant improvement over other systems, in terms of data quality and quantity, battery
83 management and practicality; in particular, we reference a bait station previously developed to record
84 visits by PIT-tagged grey squirrels (Kenward et al., 2005). This work was carried out within the context
85 of a larger study developing a contraceptive bait for grey squirrels and optimising its delivery in the
86 field. We discuss how the device could be applied to studies with other small mammal species.

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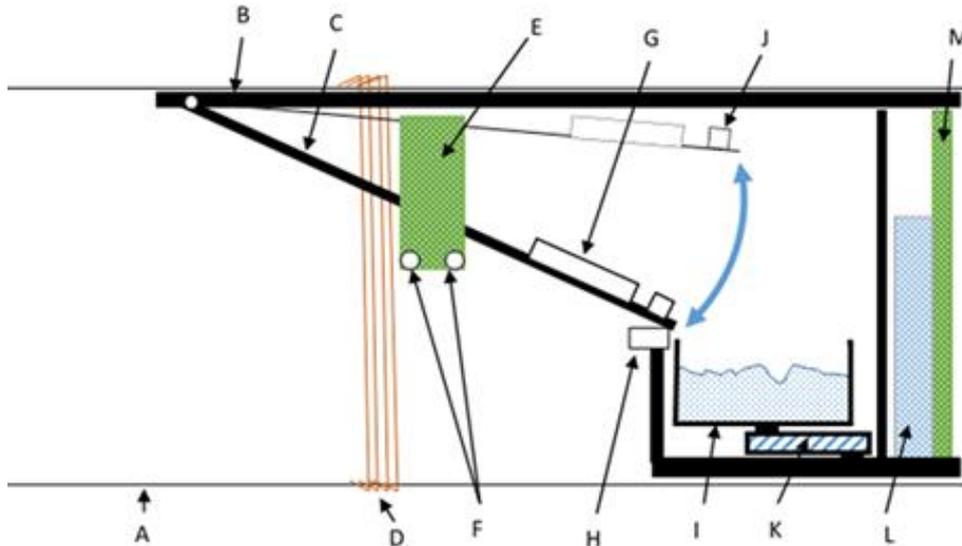
88 **Materials and Methods**

89 *Technical design*

90 The grey squirrel bait hopper was designed to record the identity of any PIT-tagged squirrel that entered
91 it and to restrict access to bait by non-target animals through a weighted door and metal exterior (Figure
92 1). The positioning of the door was based on a previous hopper design developed by the Forestry

93 Commission to deliver the rodenticide Warfarin to grey squirrels (Mayle et al., 2007). The door pivots
94 on a top hinge, angled to encourage a squirrel to push it open with its head in order to access the bait.

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97 **Figure 1.** Design of a grey squirrel bait hopper with integrated PIT-tag reader and bait weighing
98 capability. A. Body of hopper: a 10 x 10 x 55 cm length of aluminium tubing, entrance left. Includes a
99 plastic section to avoid disruption of the RFID signal; B. 3D-printed plastic insert which latches into
100 the aluminium tube; C. Pivoting plastic door; D. RFID antenna coil set to a frequency of 134 kHz; E.
101 Light beam driver/detector circuit boards; F. Infrared light beam sensors including LED emitters on one
102 side of the entrance and photodiode receptors on the other side, forming a dual light beam across the
103 entrance; G. Steel weight (70 g) attached to the door flap; H. Magnet sensitive reed switch to detect
104 when the door has been pushed open; I. Metal bait tray which can be inserted into the hopper from the
105 side. Includes plastic runners on the base to allow secure attachment; J. Magnet attached to the door;
106 operates the door reed switch; K. Strain gauge including grooves to attach the bait tray; L. Five AA
107 batteries to power the data-logger; M. Main data-logger circuit board incorporating a microcontroller
108 with non-volatile memory and clock functionality, RFID reader, LCD display, removable SD card,
109 analogue-to-digital converter (ADC), reed switch to flush data and reed switches to set date/time.

110 The grey squirrel bait hopper incorporated an RFID system, produced by NatureCounters, Kent, UK.
111 To detect a PIT-tag, an RFID system must be active, which utilises a significant amount of battery
112 power. The system in this study conserves battery power through the use of infrared light beams. Each
113 visit to the hopper by an animal is designated as an “event”. An event is triggered when both infrared
114 light beams are broken, and finishes when both light beams are clear again. When an event is triggered,
115 the RFID reader is activated, creating a frequency field in the antenna coil. The field remains active
116 until the event has finished, a PIT-tag has been successfully read, or a timeout of approximately 3
117 seconds is reached. Using this system, the hopper requires only five AA batteries and can be used in
118 the field for at least four weeks before the batteries have to be replaced.

119 The RFID coil in the hopper is positioned immediately before the bait compartment. This means that it
120 is more likely that PIT-tag individual identities (IDs) will be recorded for feeding visits only, as opposed
121 to visits where an animal partly enters the hopper and does not feed. If a PIT-tag is detected but the
122 identification is not established, a ‘0’ is recorded. This offers an advantage over other RFID systems,
123 which typically only record positive identities from PIT-tagged individuals.

124 A strain gauge, upon which the bait tray is attached, weighs the amount of bait consumed by an
125 individual, identified by its PIT-tag record. The hopper records, via a magnetic door switch, every time
126 the bait compartment is accessed. When combined with remote camera monitoring, this can provide
127 valuable information on non-target animals accessing bait and an indication of the number of untagged
128 individuals in the target population. When the door magnet is disengaged, the hopper records the weight
129 of the bait tray at least 5 times per second, and calculates an average weight from these readings. The
130 PIT-tag IDs, door switch and weight data associated with each event are recorded to files on the SD
131 card, along with the date and time.

132 *Captive trial to test hopper capacity to record feeding visits by squirrels*

133 The trial was conducted using two outdoor pens (width = 2.7 metres, length = 9.7 metres, height = 2.4
134 metres), each containing one male and two female grey squirrels previously fitted with a PIT-tag
135 (Identichip®, York, UK) subcutaneously in the scruff of the neck. Throughout the trial, the squirrels

136 were provided with a varied diet including maize, peanuts, bird seed and fruit, along with environmental
137 enrichment including wool bedding, foliage, tubes, ropes, nest boxes, branches and sticks. Two hoppers
138 were placed in each pen and each hopper was positioned on top of a wooden stand (approximately 90
139 cm high) so that they were visible by closed circuit television (CCTV) cameras. During the trial, one of
140 the hoppers became obscured by a branch so was not included in the study. For four consecutive days
141 per week, for two weeks, 10 g of fresh hazelnut paste was provided daily in each hopper. Feeding visits
142 to the hoppers by individual squirrels were recorded using CCTV at peak times of feeding activity (4:00
143 to 8:00 and 16:00 to 20:00) in the second week of the trial. For the CCTV analysis, when a squirrel
144 entered a hopper, the visit was assigned to one of the following categories: 'feeding' (when it entered
145 the hopper and was subsequently observed masticating), 'full' (only the tail was visible and no evidence
146 of feeding) or 'partial' (part of its hind quarters were still visible). Only visits that could be definitively
147 assigned to one of these categories were used in the analysis.

148 The accuracy with which the hoppers recorded feeding visits was determined by checking whether a
149 PIT-tag was detected for each feeding, full or partial visit recorded on CCTV and whether the ID of the
150 squirrel was established. A Fisher's exact test was used to determine whether PIT-tag IDs were more
151 likely to be recorded for feeding visits over non-feeding visits.

152 *Field trial to test hopper capacity to record feeding visits by squirrels*

153 A field trial was conducted in December 2018 in two 8 ha woods located in Yorkshire, UK. Single-
154 catch squirrel cage traps (n=24) were deployed in each wood on one metre high wooden stands and pre-
155 baited with peanuts and whole hazelnuts every two to three days for one week. Grey squirrels were
156 trapped the following week over consecutive days. Traps were set early in the morning and checked in
157 the afternoon. Trapped squirrels were anaesthetised using isoflurane via a mask, sexed and a PIT-tag
158 (Identichip[®], York, UK) implanted subcutaneously in the scruff of the neck. Hair was clipped from the
159 end of the tail for visual identification. Once recovered from anaesthesia, squirrels were released under
160 a Natural England licence in the location at which they had been trapped.

161 Trapping was continued until it was estimated that the majority of the population had been PIT-tagged,
162 based on the ratio of new individuals to recaptured (tail clipped) individuals per day. In both woods,
163 within three weeks of PIT-tagging, each trap was replaced by a hopper, fixed to the wooden stand. To
164 simulate the deployment of a contraceptive bait, each hopper was pre-baited with 40 g of hazelnut paste
165 every two to three days for one week. The following week, each hopper was baited daily with 40 g of
166 hazelnut paste for four consecutive trial days. The trials for both woods were conducted consecutively,
167 using the same hoppers.

168 All analyses were carried out in R (R Core Team, 2019) using the MASS package (Venables & Ripley,
169 2002). A linear model (assuming a Gaussian distribution) was used to test whether there was a
170 significant relationship between the number of feeding visits (or PIT-tag records) per individual and the
171 sex of individuals or the wood where they were trapped. Significance was tested using type II analysis
172 of variance. A generalised linear model (GLM, assuming a negative binomial distribution) was used to
173 test whether there was a significant relationship between the number of hoppers visited per individual
174 and the sex of individuals or the wood where they were trapped. Significance was tested using type II
175 analysis of variance with a likelihood ratio test (LRT). Diagnostic checks of residual plots for both
176 models showed that the residuals were approximately normally distributed and model assumptions were
177 met.

178 *Laboratory trial and captive trial to test hopper capacity to measure bait uptake by individual*
179 *squirrels*

180 A trial was designed to test the accuracy of the built-in weighing scales in each hopper, using manually
181 weighed baits. To measure the accuracy of bait weight taken per visit, the first part of the trial was
182 conducted in a laboratory with nine hoppers. Approximately 70 g of 100% hazelnut paste was weighed
183 in a bait tray and placed in each hopper. To simulate different field conditions, five hoppers were placed
184 in a refrigerator and left for one hour to acclimatise to between 6°C and 8°C; the remaining four hoppers
185 were left at room temperature, between 20°C and 21°C. A small amount (0.1 to 2.2 g) of paste was then
186 removed from each hopper using a pre-weighed metal spoon, and the spoon and paste weighed (to the

187 nearest 0.1 g). After at least 10 minutes, a larger amount of paste (4.9 to 18.5 g) was removed from each
188 hopper and weighed (to the nearest 0.1 g). This was repeated until there were at least 5 weights for
189 small amounts of bait and at least 5 weights for larger amounts of bait for each hopper. To ensure the
190 strain gauge was stable, the amount of bait taken from each hopper was calculated from the weight
191 recorded by each hopper 2 minutes prior to the bait removal minus the weight taken 2 minutes after the
192 removal. These were compared with weights obtained manually using a standard balance.

193 To test whether it was possible for the hoppers to weigh bait consumed by individual squirrels, a second
194 trial was conducted using captive PIT-tagged grey squirrels. Two hoppers per pen were deployed in
195 three outdoor pens containing 2-3 squirrels per pen, 7 squirrels in total. Hoppers were placed on the
196 floor along each side of a pen, weighed down by bricks, to ensure the squirrels could not move or
197 overturn them. In week 1, the hoppers were baited on Monday, Wednesday and Friday with
198 approximately 40 g 100% hazelnut paste to encourage the squirrels to use the hoppers. On the Tuesday
199 and Wednesday of week 2, 20 ± 0.5 g of hazelnut paste was weighed into bait trays and installed in
200 each hopper at 7:15 am, immediately before the squirrel peak feeding time. After 6 hours, the bait trays
201 were removed and the remaining bait weighed (to the nearest 0.1 g). The manual weights taken for each
202 trial period were compared to those recorded by the hoppers. The weight of bait taken for each event
203 was calculated by taking the minimum weight from the most stable values recorded; those where there
204 was less than 5 units difference between the first and fifth repeat reading. The weight decrease at each
205 event was then matched with a PIT-tag record, if available, to calculate the amount of bait taken per
206 visit by individual squirrels.

207

208 **Results**

209 *Captive trial to test hopper capacity to record feeding visits by squirrels*

210 In total, 97 feeding visits, 47 full visits and 102 partial visits to the three hoppers by grey squirrels were
211 recorded on CCTV during the trial. Feeding visits were recorded for five of the six squirrels and full

212 and partial visits for all six squirrels. An average of 27 (range = 9 to 48) PIT-tag records were obtained
213 for each squirrel. A PIT-tag was detected for 100% of feeding visits, 96% of full visits and 64% of
214 partial visits; a PIT-tag ID was established for 95% of feeding visits, 77% of full visits and 25% of
215 partial visits. The percentage of PIT-tag IDs recorded was significantly higher for visits where bait was
216 taken (95%), compared with visits where no bait was taken (41%, $p < 0.001$). The ratio of feeding visits
217 recorded by CCTV to PIT-tag IDs was 1:1.6, as the hoppers would sometimes record multiple IDs for
218 animal that spent more time inside them. On 28 occasions, it was observed that the squirrel visiting a
219 hopper was displaced by another squirrel.

220 *Field trial to test hopper capacity to record feeding visits by squirrels*

221 In wood 1, on three trap days, 21 adult squirrels (13 females and 8 males) were trapped, PIT-tagged and
222 released; the percentage of the total number of squirrels PIT-tagged each day was 86%, 14% and 0%.
223 In wood 2, on four trap days, 30 adult squirrels (16 females and 14 males) were trapped, PIT-tagged
224 and released; the percentage of the total number of squirrels PIT-tagged for each trap day was 40%,
225 40%, 10% and 10%.

226 In wood 1, on the four trial days 24 hoppers logged 1,582 PIT-tag records and each hopper recorded
227 visits from 2 to 8 squirrels, with 17 hoppers visited by least 4 individuals. In wood 2, on the four trial
228 days, 24 hoppers logged 2,844 PIT-tag records and each hopper recorded visits from 1 to 8 squirrels,
229 with 19 hoppers recording visits from at least 4 individuals.

230 Overall, 19 of the 21 (90.5%) PIT-tagged squirrels in wood 1 visited at least one hopper, all within the
231 first 24 hours of pre-bait and 18 squirrels visited during the four trial days. In wood 2, 28 of the 30
232 (93%) PIT-tagged squirrels entered hoppers, all within the first 72 hours of prebait and 26 squirrels
233 visited during the four trial days. The number of PIT-tag records per squirrel did not differ significantly
234 between woods ($F=0.96$, $df=1$, 50, $p=0.33$); however, on average, more PIT-tag records were recorded
235 for males than females ($F=4.6$, $df=1$, 50, $p=0.04$; Fig. 2 a). The median number of PIT-tag records was
236 77 for females and 107 for males.

237 The number of hoppers visited by individual PIT-tagged squirrels did not differ significantly between
238 woods (LRT= 1.51, df=1, p=0.22); however, males visited a higher number of hoppers than females
239 (LRT=5.56, df=1, p=0.02; Fig. 2 b). The median number of hoppers visited was 4 for females and 6 for
240 males.

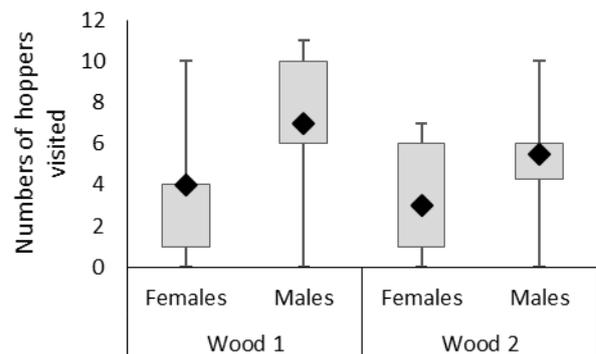
241 During the field study, temperatures ranged from -4 °C to 14 °C. Low temperatures did not affect hopper
242 function and no hopper faults were recorded for wood 1. In wood 2, 3 of the 24 hoppers deployed
243 stopped recording PIT-tags on the final two trial days, due to a migration in the RFID frequency range,
244 likely caused by wet weather conditions affecting the electronics. Each hopper functioned throughout
245 the two trials conducted over four weeks on the same set of batteries.

246

247 a)



b)



248

249 **Figure 2.** The number of PIT-tag records (a) and the number of hoppers visited (b) logged for 22 male
250 and 29 female PIT-tagged grey squirrels in two woods during 4 consecutive trial days. Median values
251 are shown by diamonds, 50% of the records for each group are shown by boxes and minimum and
252 maximum values are shown by whiskers.

253

254 *Laboratory trial and captive squirrel trial to test hopper capacity to measure bait uptake by*
255 *individual squirrels*

256 Over 90 occasions of bait removal, the average difference between the manually weighed bait removed
257 and the weights recorded by the hoppers was 0.3 g (range = 0.0 to 5.6). The seven highest differences
258 (all greater than 1 g) were recorded by one hopper. When the data from this hopper were removed from
259 the analysis, the average weight difference was 0.1 g (range = 0.0 to 0.9).

260 The average amount of bait taken by squirrels during the captive trial was 11.5 g (range = 0.0 to 23.5)
261 per hopper per day. The average difference between the manually weighed bait weights and the weights
262 recorded by the six hoppers on both trial days was 0.5 g (range = 0.1 to 1.2). All seven PIT-tagged
263 squirrels were recorded feeding from the hoppers. In total, on the two trial days, 138 g of bait was
264 removed from the six hoppers. All of the bait consumed could be attributed to an unconfirmed PIT-tag,
265 while 122 g could be attributed to a positive PIT-tag ID. On trial day 2, 12 g were removed from one
266 hopper that later stopped recording PIT-tag IDs, with the fault likely caused by wet weather conditions.
267 The average amount of bait taken by a squirrel on each visit was 1.2 g (range = -0.4 to 6.9). On one
268 occasion there was a mis-read PIT-tag but the error concerned the last two digits of the thirteen digit ID
269 only and was easily corrected.

270

271 **Discussion**

272 The squirrel hopper with integrated PIT-tag reader proved effective for measuring patterns and
273 quantities of bait uptake by individual grey squirrels and the results in this study represent a significant
274 advancement when measuring the dose rates of rodenticides, oral vaccines and contraceptives and other
275 bait-delivered drugs. A PIT-tag was detected for every feeding visit made to a hopper by a grey squirrel
276 in captivity, a PIT-tag ID was recorded for 95% of individuals that fed from a hopper and 97% of the
277 bait taken from functioning hoppers could be attributed to a PIT-tag ID. A PIT-tag ID was more likely
278 to be recorded for visits where bait was taken than those where it was not. In other bait station designs,
279 the RFID reader has been positioned at the entrance of the feeder, rather than immediately before the
280 bait compartment (Kenward et al., 2005). This will increase the likelihood that PIT-tags will be recorded
281 for squirrels who visit or pass the feeder but do not take any bait.

282 A novel component of the hopper design was the addition of a strain gauge to weigh the bait taken on
283 each visit. When combined with the PIT-tag data using numbered events, the amount of bait taken on
284 each visit by individual squirrels was measured to an accuracy of 0.1 g. Remote devices with strain
285 gauges have been used to measure the body weights of animals (Bosch et al., 2015) but none have
286 demonstrated the accuracy of weighing necessary to calculate the dose rates of contraceptives or other
287 drugs.

288 The hoppers proved to be highly effective at delivering bait to grey squirrels in two woods, with the
289 squirrels showing little neophobia towards these devices. This can be partly attributed to the design of
290 the door used to access the bait. Other studies have found some types of door designs can deter squirrels
291 through the way they move or the noise that they make (Kenward et al., 2005). Overall, 47 of the 51
292 squirrels PIT-tagged were subsequently recorded by the hoppers within 72 hours of bait deployment. It
293 is unknown whether the remaining four individuals had lost their tags, left the study area before the trial
294 or whether they were present but would not enter the hoppers. Low rates of tag loss and mortality have
295 been found when PIT-tagging bats (van Harten et al., 2019) and ground squirrels (Schooley et al., 1993)
296 and this study provides further evidence that PIT-tagging is a reliable method for gathering behavioural
297 data on small mammals in their natural environment.

298 For maximum efficacy, oral contraceptives should primarily target the females in a population (Massei
299 & Cowan, 2014). The PIT-tag data gathered in the field in this study showed large variations in the
300 numbers of visits to hoppers by individual squirrels and, on average, males visited more hoppers and
301 made more feeding visits than females. These data indicate that some social factors, such as dominance,
302 may influence feeding behaviour in grey squirrel populations. This is supported by the findings of other
303 studies (Lawton et al., 2016) and in this study the displacement of squirrels from hoppers by other
304 squirrels was observed. However, most hoppers were used by multiple individuals and the majority of
305 females fed multiple times and from more than one hopper. These results suggest that social factors
306 should not significantly reduce bait delivery effectiveness, providing the ratio of hoppers to squirrels is
307 adequate.

308 Research requiring marked individuals has traditionally relied on external tags, which have the potential
309 to be lost, damaged or mis-read, and often require some kind of human intervention, through recapture
310 or tracking, to gather data (Gibbons & Andrews, 2004). This study further demonstrated that the
311 detection of PIT-tags using hoppers can be achieved remotely, with minimal human interference and a
312 high identification accuracy. This method therefore represents an improvement in welfare standards and
313 behavioural data quality for animal research and provides a practical and accurate method to determine
314 individual bait uptake in field conditions where otherwise multiple captures of animals would be
315 required.

316 The hoppers were relatively robust, with few failures, despite wet and cold weather conditions during
317 the trials. The few hopper faults that were recorded were likely caused by wet conditions affecting the
318 electrical circuits and can be easily mitigated against with improved weather-proofing. The battery
319 management system meant there were no issues with battery life during the captive or field trials, with
320 each hopper functioning for four weeks on the same set of five AA batteries. Other studies have not
321 been able to demonstrate the same degree of longevity, despite using larger batteries such as car batteries
322 to power bait stations (Kenward et al., 2005). Larger batteries reduce the portability and practicality of
323 devices for use in the field and the regular monitoring and changing of batteries can be labour-intensive
324 and could potentially disrupt the focal species' natural behaviour. Alternatively, to reduce power
325 consumption, RFID systems can be programmed to activate at intervals when PIT-tagged individuals
326 are more likely to be present (Bridge et al., 2019), but this will often result in some loss of data and is
327 unsuitable if 24 hour monitoring is required.

328 The 3D-printed insert upon which the electronics are fixed allows the hoppers to be easily modified;
329 therefore, the hoppers can be adapted to record other types of data from different animal species.
330 Adaptations of the RFID system described in this study are currently being used in studies on other
331 small mammals (NatureCounters, 2020) including the monitoring of burrow use by European hamsters
332 and a weighing device for measuring the body weights of a captive colony of fruit bats. By assessing
333 patterns of bait consumption in representative population samples, adapted hoppers could be used to
334 model the efficacy of bait delivery campaigns and inform bait delivery strategies at a larger scale. This

335 could include vaccines to control wildlife disease such as an oral rabies vaccine for the Small Indian
336 Mongoose *Herpestes auropunctatus* (Vos et al., 2013) or oral contraceptives for the control of other
337 overabundant wildlife, such as the Norway rat *Rattus norvegicus* (Pyzyna et al., 2016).

338

339 **Conclusion**

340 The novel design of hopper with integrated PIT-tag reader and bait weighing capability proved highly
341 effective at measuring patterns and quantities of bait uptake by individual grey squirrels. The unique
342 modifications to the hopper design in this study resulted in improved data collection and battery life in
343 the field when compared to other similar devices. The adaptability of the hopper design means that it
344 has wider applications for wildlife management; in particular, efficacy studies for bait-delivered drugs
345 in the context of wildlife disease control and/or population reduction.

346

347 **Ethical statement**

348 This study was approved by APHA's Animal Welfare and Ethical Review Body (AWERB) and
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350

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356

357 **Author's contributions**

358 Sarah Beatham, Giovanna Massei, Philip Stephens and Dominic Goodwin conceived the ideas and
359 designed methodology; Sarah Beatham and Julia Coats collected the data; Sarah Beatham analysed

360 the data; Sarah Beatham led the writing of the manuscript with input from Dominic Goodwin,
361 Giovanna Massei and Philip Stephens. All authors contributed critically to the drafts and gave final
362 approval for publication.

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364 **Data availability statement**

365 Data available from the Dryad Digital Repository doi:10.5061/dryad.jq2bvq888 (Beatham, 2021).

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367 **References**

368 Algeo, T. P., Norhenberg, G., Hale, R., Montoney, A., Chipman, R. B., & Slate, D. (2013). Oral rabies
369 vaccination variation in tetracycline biomarking among Ohio raccoons. *Journal of wildlife*
370 *diseases*, 49(2), 332-337.

371 Beatham, S (2021) Trials testing a bait hopper equipped with a PIT-tag reader and bait weighing device,
372 to record bait uptake by individual grey squirrels. *Dryad Digital Repository*.
373 doi:10.5061/dryad.jq2bvq888

374 Bosch, S., Spiessl, M., Müller, M., Lurz, P. W. W., & Haalboom, T. (2015). Mechatronics meets
375 biology: experiences and first results with a multipurpose small mammal monitoring unit used
376 in red squirrel habitats. *Hystrix, the Italian Journal of Mammalogy*, 26(2), 169-172.
377 doi:10.4404/hystrix-26.2-11475

378 Bridge, E. S., Wilhelm, J., Pandit, M. M., Moreno, A., Curry, C. M., Pearson, T. D., Ruyle, J. E. (2019).
379 An Arduino-Based RFID Platform for Animal Research. *Frontiers in Ecology and Evolution*,
380 7(257). doi:10.3389/fevo.2019.00257

381 Derbridge, J. J., Pepper, H. W., & Koprowski, J. L. (2016). Economic damage by invasive grey squirrels
382 in Europe. In C. M. Shuttleworth, P. W. W. Lurz, & J. Gurnell (Eds.), *The Grey Squirrel:*
383 *Ecology and management of an invasive species in Europe* (pp. 393-406): European Squirrel
384 Initiative.

385 Dunn, M., Marzano, M., Forster, J., & Gill, R. M. A. (2018). Public attitudes towards “pest”
386 management: Perceptions on squirrel management strategies in the UK. *Biological*
387 *Conservation*, 222, 52-63. doi:10.1016/j.biocon.2018.03.020

388 Forestry Commission. (2014). Grey squirrels and England's woodland. Retrieved from
389 [https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/](https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/700022/Grey-squirrels-policy-and-action-plan.pdf)
390 [file/700022/Grey-squirrels-policy-and-action-plan.pdf](https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/700022/Grey-squirrels-policy-and-action-plan.pdf)

391 Gibbons, W. J., & Andrews, K. M. (2004). PIT Tagging: Simple Technology at Its Best. *BioScience*,
392 54(5), 447-454. doi:10.1641/0006-3568(2004)054[0447:ptstai]2.0.co;2

393 Godsall, B., Coulson, T., & Malo, A. F. (2014). From physiology to space use: energy reserves and
394 androgenization explain home-range size variation in a woodland rodent. *Journal of Animal*
395 *Ecology*, 83(1), 126-135. doi:10.1111/1365-2656.12116

- 396 Gurnell, J., Lurz, P. W. W., & Shuttleworth, C. M. (2016). Ecosystem impacts of an alien invader in
 397 Europe, the grey squirrel *Sciurus carolinensis*. In C. M. Shuttleworth, P. W. W. Lurz, & J.
 398 Gurnell (Eds.), *The Grey Squirrel: Ecology and management of an invasive species in Europe*
 399 (pp. 307-328): European Squirrel Initiative.
- 400 Kenward, B., Kenward, R., & Kacelnik, A. (2005). An automatic technique for selective feeding and
 401 logging of individual wild squirrels. *Ethology Ecology & Evolution*, *17*(3), 271-277.
- 402 Kukalová, M., Gazárková, A., & Adamík, P. (2013). Should I stay or should I go? The influence of
 403 handling by researchers on den use in an arboreal nocturnal rodent. *Ethology*, *119*(10), 848-
 404 859.
- 405 Lawton, C., Shuttleworth, C. M., & Kenward, R. E. (2016). Ranging behaviour, density and social
 406 structure in grey squirrels. In C. M. Shuttleworth, P. W. W. Lurz, & J. Gurnell (Eds.), *The Grey*
 407 *Squirrel: Ecology and management of an invasive species in Europe* (pp. 133-152): European
 408 Squirrel Initiative.
- 409 Marks, C. A., & Bloomfield, T. E. (1999). Bait uptake by foxes (*Vulpes vulpes*) in urban Melbourne:
 410 the potential of oral vaccination for rabies control. *Wildlife Research*, *26*(6), 777-787.
 411 doi:<https://doi.org/10.1071/WR98063>
- 412 Massei, G., & Cowan, D. (2014). Fertility control to mitigate human-wildlife conflicts: a review.
 413 *Wildlife Research*, *41*(1), 1-21. doi:10.1071/wr13141
- 414 Mayle, B., Ferryman, M., & Pepper, H. (2007). *Controlling grey squirrel damage to woodlands*:
 415 Forestry Commission, Edinburgh.
- 416 Messmer, T. A. (2019). Mice and Rats: Perceptions, Realities, and Impacts on Humankind. *Human–*
 417 *Wildlife Interactions*, *13*(2), 3.
- 418 NatureCounters (2021). NatureCounters. Retrieved from <https://naturecounters.com>
- 419 Pimentel, D., Lach, L., Zuniga, R., & Morrison, D. (2000). Environmental and economic costs of
 420 nonindigenous species in the United States. *BioScience*, *50*(1), 53-66.
- 421 Pyzyna, B., Whish, S., Dyer, C. A., Mayer, L. P., Witmer, G., & Moulton, R. (2016). *Free Ranging*
 422 *Wild-Caught Norway Rats Have Reduced Fecundity after Consuming Liquid Oral Fertility Bait*
 423 *Containing 4-Vinylcyclohexene Diepoxide and Triptolide*. Paper presented at the Proceedings
 424 of the Vertebrate Pest Conference.
- 425 R Core Team. (2019). A language and environment for statistical computing. R Foundation for
 426 Statistical Computing, Vienna, Austria. Retrieved from <https://www.R-project.org>
- 427 Rushton, S. P., Lurz, P. W. W., Gurnell, J., Nettleton, P., Bruemmer, C., Shirley, M. D. F., & Sainsbury,
 428 A. W. (2006). Disease threats posed by alien species: the role of a poxvirus in the decline of
 429 the native red squirrel in Britain. *Epidemiology and Infection*, *134*(3), 521-533.
 430 doi:10.1017/S0950268805005303
- 431 Schooley, R. L., Van Horne, B., & Burnham, K. P. (1993). Passive Integrated Transponders for Marking
 432 Free-Ranging Townsend's Ground Squirrels. *Journal of Mammalogy*, *74*(2), 480-484.
 433 doi:10.2307/1382406
- 434 Slate, D., Rupprecht, C. E., Rooney, J. A., Donovan, D., Lein, D. H., & Chipman, R. B. (2005). Status
 435 of oral rabies vaccination in wild carnivores in the United States. *Virus research*, *111*(1), 68-
 436 76.

- 437 Smyth, B., & Nebel, S. (2013). Passive integrated transponder (PIT) tags in the study of animal
438 movement. *Nature Education Knowledge*, 4(3), 3.
- 439 Towns, D. R., & Broome, K. G. (2003). From small Maria to massive Campbell: forty years of rat
440 eradications from New Zealand islands. *New Zealand Journal of Zoology*, 30(4), 377-398.
- 441 van Harten, E., Reardon, T., Lumsden, L. F., Meyers, N., Prowse, T. A., Weyland, J., & Lawrence, R.
442 (2019). High detectability with low impact: Optimizing large PIT tracking systems for cave-
443 dwelling bats. *Ecology and Evolution*, 10916-10928.
- 444 Venables, W., & Ripley, B. (2002). Random and mixed effects *Modern applied statistics with S* (pp.
445 271-300): Springer.
- 446 Vos, A., Kretzschmar, A., Ortmann, S., Lojkic, I., Habla, C., Müller, T., Schuster, P. (2013). Oral
447 Vaccination of Captive Small Indian Mongoose (*Herpestes auropunctatus*) against Rabies.
448 *Journal of wildlife diseases*, 49(4), 1033-1036. doi:10.7589/2013-02-035