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1 Profiling in wildlife crime: recovery of human DNA deposited outside

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4 Abstract

5 Incidents of bird of prey persecution receive a lot of media coverage in the UK, with
6 investigations rarely recovering sufficient evidence to proceed to prosecution. One of
7 the main challenges is to identify a suspect, as these offences are carried out in
8 remote locations without witnesses, and crime scenes may not be found for days.
9 However, traps, poisoned baits and bird of prey carcasses can be recovered from
10 these crime scenes. This study aimed to determine whether reportable human DNA
11 profiles could be recovered from any of these substrates after periods of time
12 outside.

13 Experiments depositing human touch DNA on duplicate substrates (traps, rabbit
14 baits and corvid carcasses) set for 0, 1, 2, 4, 7 and 10 days outside were carried out,
15 with DNA recovery and profiling following standard operating procedures for Scottish
16 Police Authority Forensic Services. Weather conditions varied among experiments,
17 including some heavy rainfall. Results demonstrated that it was possible to obtain
18 reportable DNA profiles from all substrates after at least 1 day outside. Most
19 promisingly, the traps showed no drop-off in DNA persistence over the experiments
20 as complete DNA profiles were obtained after the full 10 days outside. A further
21 experiment using 4 bird of prey carcasses confirmed that it is possible to obtain
22 reportable human DNA profiles from them after 1 day outside (n=2 reportable
23 profiles). These results show that touch DNA can persist in an outdoor environment,
24 and provide a tantalising avenue for inquiry in bird of prey persecution investigations.

25

26 1. Introduction

27 Despite birds of prey having legal protection in Scotland since the 1950s,
28 persecution is still a prominent issue. The principle reasons for this are conflicts
29 between some gamebird shooting interests and a wide range of raptor species [Ref
30 A, Ref B], conflict between some elements of the livestock industry, in particular low-

31 intensity sheep-farming, and eagles, and the targeting of hawks and falcons by some
32 individuals or groups who seek to protect their racing pigeons from predation [1-3
33 Ref C]. The persecution of raptors can occur at any time of year either in response to
34 particular seasonal issues such as lambing or the release of pheasant poults, or it
35 may be undertaken opportunistically at any time..

36 There were 280 recorded bird-related offences in Scotland from 2010-2015 with just
37 16% of these cases resulting in convictions [4]. Many of these crimes take place in
38 remote or hard to access locations making it difficult to gather sufficient evidence to
39 identify and prosecute the perpetrators , forensic evidence in particular can often be
40 lacking. Persecution of birds of prey can have a substantial impact on populations at
41 the local, regional and even national level [4-5], as some of the species involved are
42 rare and endangered. This can lead to substantial pressure to increase convictions
43 and reduce offending [3-4]. The most common methods for killing birds of prey are
44 shooting, poisoning, trapping and nest destruction [4]. In many of these instances the
45 offender will have to either handle a trap, a bait or the bird itself, potentially
46 depositing touch DNA in the process. The recovery of touch DNA from many
47 substrates during forensic investigations is routine and widely accepted around the
48 world [6-9]. However, it is rarely applied to evidence recovered outside, and there
49 are no reports of these techniques being used successfully to recover human touch
50 DNA from bird of prey crime scenes.

51 As with other crime types, human DNA could be a key tool to link individuals to
52 scenes, items to scenes and items together [10-13]. The discovery of human DNA
53 on a bird of prey carcass would be particularly significant because there are few
54 explanations for its presence. Two published studies are available investigating the
55 recovery of human DNA from animal carcasses [14, 15]. Here, human touch DNA
56 was recovered from the legs of deer immediately following slaughter. Samples from
57 5 out of 10 deer produced reportable results. However, this study did not investigate
58 whether recovery of DNA would be affected by the length of time a substrate spent
59 outside. Due to the remote location of these crimes, scenes may not be discovered
60 for many days after the crime took place, if indeed they are found at all. There is
61 therefore a need to investigate the persistence of human DNA on animal carcasses

62 and crime items that are recovered outside, because weather conditions can vary
63 considerably in Scotland and exposure to inclement conditions might be encountered
64 in casework at any time of year.

65 While research has been carried out on DNA persistence indoors [16], significantly
66 less research is available concerning the persistence of DNA on objects and
67 surfaces in outdoor conditions. A study by Raymond et al. [17] found that the
68 concentration of DNA solutions left exposed halved after 2 weeks, but there was no
69 clear trend in DNA loss over the time period, suggesting that multiple factors were
70 involved.

71 This study aims to determine whether existing human DNA profiling techniques can
72 be utilised to recover offender touch DNA from bird of prey carcasses, poisoned bait
73 carcasses and traps that have been left outside for up to 10 days. This key
74 identification tool would offer a significant advancement in the investigation of wildlife
75 crimes and indeed any crime where DNA evidence has been exposed to the
76 elements.

77

78 **2. Materials and Methods**

79 **2.1 Substrates**

80 Springer Mark 6 traps were used for the trap experiments (n=12). All the traps
81 showed a similar degree of mild corrosion. The rabbits, which are often used as
82 poisoned baits, and corvids, as a proxy for a bird of prey carcass, were legally killed
83 as part of normal land management activity. Four bird of prey carcasses (buzzard,
84 sparrowhawk, kestrel and tawny owl) were donated to the project having died of
85 natural causes.

86 **2.2 DNA Transfer**

87 The individual shedder status of 3 participants was assessed using the methods
88 described in Lowe et al. [18] (minitapes and 5 minute holding time) to ensure that a
89 poor DNA shedder was not selected for the experiments (S1). An initial proof of
90 concept experiment demonstrated that full STR profiles could be obtained from all
91 substrates immediately after handling (S2). Prior to handling the items, the

92 participant refrained from hand washing activities for 1 hour, but continued daily
 93 tasks. A 10-minute interval was taken between handling each item. For the traps the
 94 participant used both hands to set the trap and hold it until 1 minute had elapsed. For
 95 the rabbit carcasses the participant was asked to carry the rabbit by holding the back
 96 legs in one hand for a period of 1 minute. The corvid and bird of prey carcasses were
 97 held in one hand for a period of 1 minute. The palm was placed on the birds' back,
 98 with fingers wrapped around the neck and upper body area, enclosing the folded
 99 wings.

100 2.3 Experimental Set-up

101 DNA was deposited by a single participant onto the different substrates and these
 102 were left either outside or inside for 0 to 10 days, as detailed in Table 1. Duplicate
 103 substrates for each time period within an experiment were run wherever possible.
 104 Experimental substrates left indoors were placed on a sterile plastic tray at room
 105 temperature, whereas those left outside were placed on the ground in a flat grassy
 106 compound. A wood and chicken wire cage structure was placed over the
 107 experimental substrates left outside to prevent access by wildlife without sheltering
 108 the items from rainfall or sunlight.

109

Location of substrate / exposure period	Experiment				
	Trap Expt1	Trap Expt2	Rabbit Expt3	Corvid Expt4	Corvid Expt5
Inside / 0 days (control)	2	2	2	2	0
Outside / 1 day	2	2	2	2	2
Outside / 2 days	2	0	0	2	2
Outside / 4 days	2	2	2	2	2
Outside / 7 days	2	2	2	2	2
Outside / 10 days	2	2	2	2	0
Inside / 10 days (control)	2	0	1	2	0

110

111 **Table 1:** Number of substrates used, and different treatments involved in
112 experiments 1 to 5.

113 Due to limited availability of traps and carcasses, not all time periods were
114 completed for each experiment, and the two trap experiments and corvid
115 experiments are not exact replicates (Table 1). A final small experiment, Experiment
116 6, was carried out using the 4 bird of prey carcasses which were handled as
117 described for the corvids and left for 1 day outdoors.

118 Experiments were not run concurrently, but began on different days over a one
119 month period (June/July 2017). Data on rainfall during the experiments was
120 obtained from the Met Office Monthly Climate Report for Edinburgh Gogarbank (S3).

121 **2.4 DNA Recovery**

122 Experimental substrates were collected and left indoors on a sterile plastic tray to dry
123 (30 mins for traps and 1 hr for carcasses). These durations were chosen as it is
124 unlikely that DNA recovery would be attempted at the scene in wet conditions. A
125 proof of concept study suggested the minitaping method was better for DNA
126 recovery from these substrates than the double-swab method (S2). All experiments
127 used minitapes for DNA recovery, as per the standard operating procedure used by
128 the Scottish Police Authority (SPA) Forensic Services. The minitape (Scenesafe, WA
129 Products) was applied to the sample area with pressure multiple times until
130 adhesiveness was lost. The tapes were transported to the SPA laboratory and the
131 adhesive section of the tape was removed and placed into an Autolys tube (Hamilton
132 Robotics) before submission for DNA profiling.

133

134 **2.5 DNA Profiling**

135 Samples were processed on the AutoLys STAR and ID STARlet automated
136 platforms (Hamilton Robotics). DNA was extracted using the PrepFiler DNA
137 extraction kit, and amplified using the GlobalFiler PCR kit on the Veriti thermal cycler
138 (AppliedBiosystems™). PCR product was run on the 3500xL Genetic Analyzer and

139 the profiles analysed using GeneMapper ID-X software, version 1.5
140 (AppliedBiosystems™).

141 **2.6 Data Interpretation**

142 The resulting GlobalFiler STR profiles were initially examined on GeneMapper ID-X
143 for any profile artefacts. The presence or absence of DNA from a contributor other
144 than the donor was assessed and the number and type of alleles at each locus was
145 noted. The following criteria, derived by SPA Forensic Services during validation,
146 were used for all loci excluding Yindel and DYS391 for reporting of profiles:

- 147 - Two peaks at a locus = heterozygous.
- 148 - 1 Peak >400rfu peak height = homozygous.
- 149 - 1 Peak <400rfu peak height = potentially heterozygous with allele drop out.
- 150 - 0 Peaks = No result.

151 DNA profiles were then compared to the participant's reference profile and the
152 number of alleles present out of 46 was counted and converted to a percentage.
153 Profiles in this study that were reviewed and considered to be reportable were more
154 than 50% complete, i.e. at least 23 of a possible 46 alleles present.

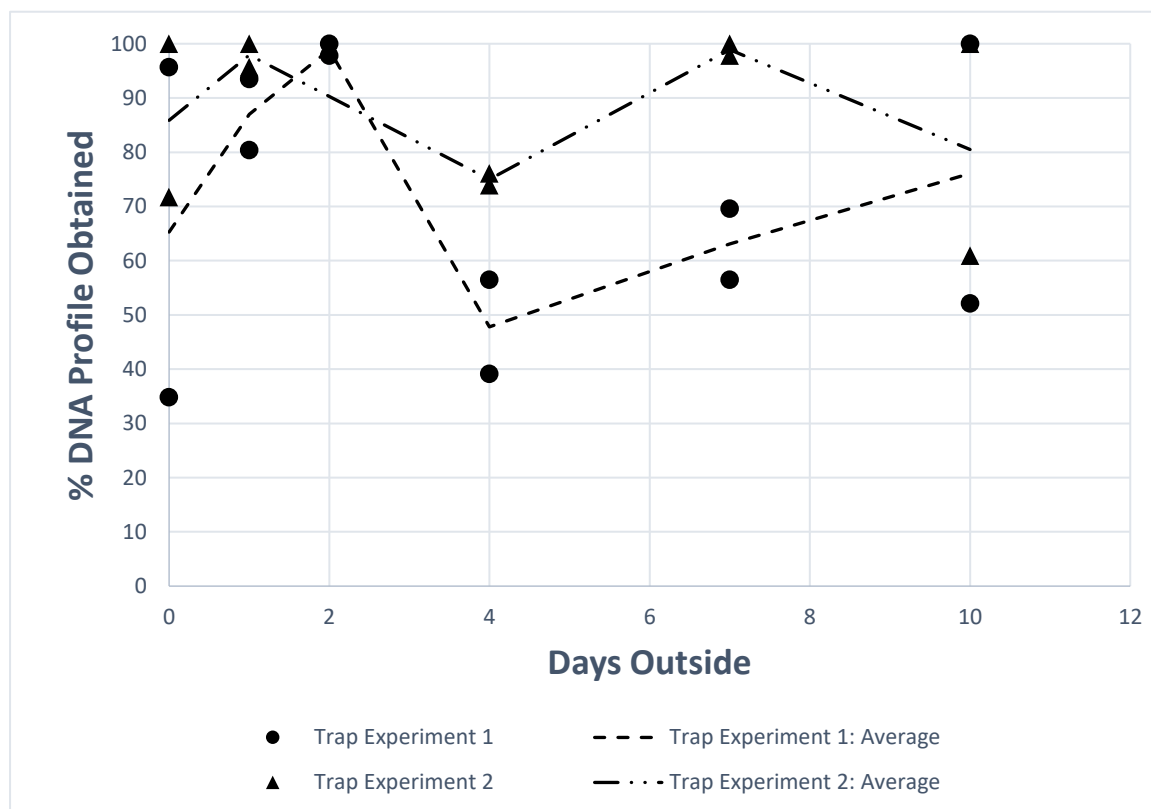
155 To statistically assess the effect of experimental treatment (i.e. time outside) on the
156 percentage of complete STR profile produced, analysis of variance (ANOVA) was
157 performed for each experiment, carrying out data transformation where necessary.

158

159 **3. Results and Discussion**

160 **3.1 Trap experiments**

161 Good quality DNA profiles were recovered from almost all of the traps in experiment
162 1 (Figure 1). Those from all but 2 traps, one of which had been left indoors for 10
163 days, were at least 50% complete. However, there was great variation between the
164 duplicates indicating that time outside was not the only major factor influencing the
165 recovery of DNA (Figure 1). There was less variance in the percentage of DNA
166 profile obtained in experiment 2 with most duplicates collected on the same day
167 producing similar qualities of profiles, and all samples producing reportable profiles
168 of at least 50% complete (Figure 1). Treatment did not affect DNA profile recovery
169 over the course of these experiments (Expt 1:F(6,7)=464, p=0.49;
170 Expt2:F(4,5)=274.9, p=0.572).



171

172 **Figure 1:** Percentage of DNA profile obtained (%) from samples collected from
173 traps left outside in experiment 1 and 2. The two points at each time period represent
174 two separate trap samples on the same day at the same time. The line links the

175 *average percentage DNA profiles from one time period to the next to assess for a*
176 *trend in the results.*

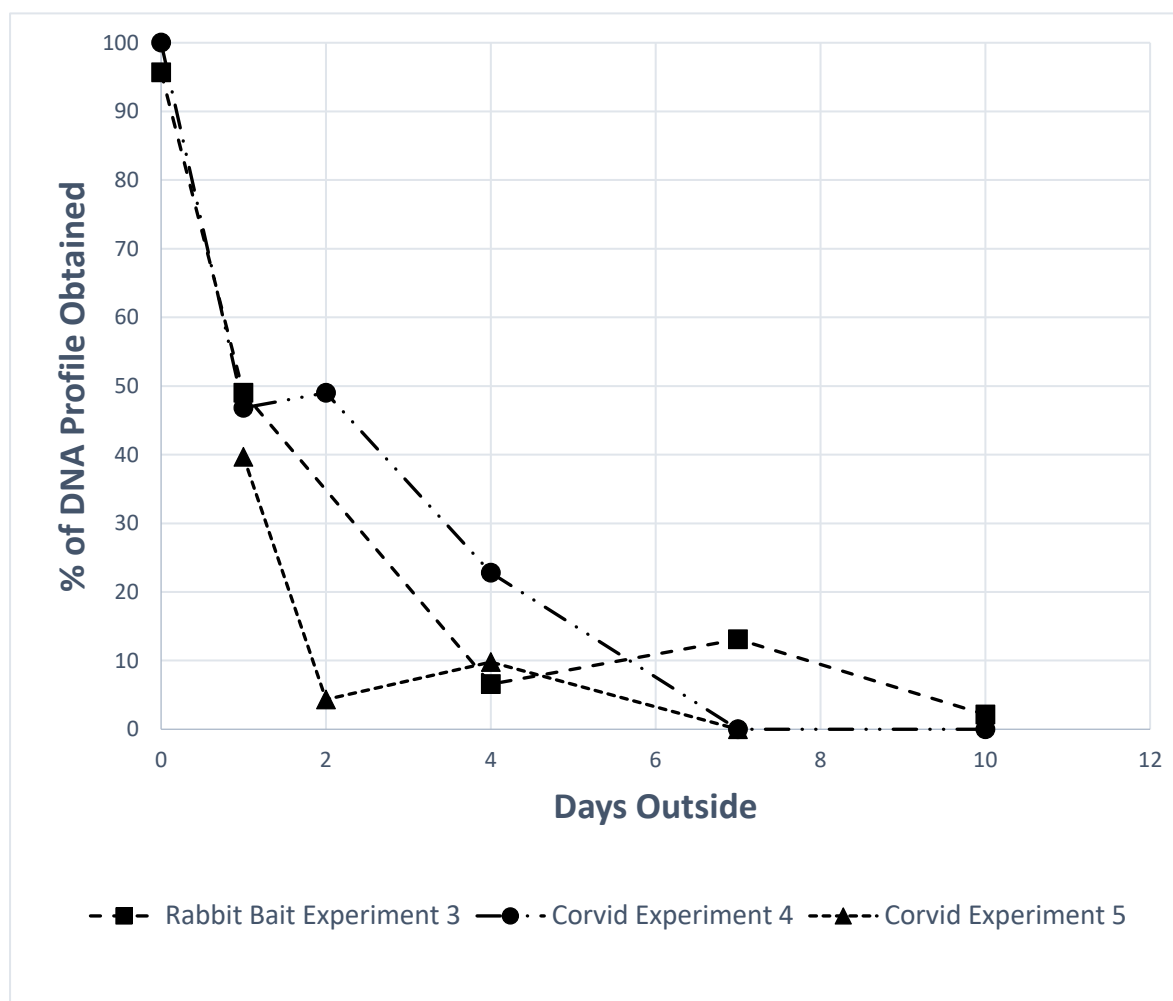
177

178 Importantly, most of the profiles obtained from both experiments were of sufficient
179 quality to be reported in a police investigation. Although all traps picked for this
180 experiment exhibited a similar degree of corrosion, the microscopic surface texture
181 of each trap could be completely different. This may have caused the differences
182 observed between the duplicates and the variance in quality of profiles over time.
183 Indeed, the two traps left indoors for 10 days for experiment 1 gave very different
184 results (10.9% and 87% complete STR profiles respectively). New corrosion-free
185 traps could have been used but they would not represent the common condition of
186 traps used in real crimes. Most of the contact points sampled existed on the
187 underside of the trap as these are the areas touched during trap set up. These areas
188 are protected from direct rainfall, which may account for the persistence of DNA on
189 the traps after heavy rain.

190 Although efforts were made to standardise both trap experiments, the weather
191 conditions were substantially different (S3). By day ten the experiment 1 traps had
192 been exposed to exceptional precipitation, with 101.8mm of rain falling, but
193 experiment 2 traps had only received a total of 9mm over the same interval. Notably,
194 full profiles were obtained from traps exposed to the extraordinary weather in
195 experiment 1. Therefore, illegally-set traps discovered in wildlife crime investigations
196 should be sampled, even if they have been subject to exceptional wet weather
197 conditions, as there is a good chance that human DNA may have persisted.

198 **3.2 Carcass experiments**

199 Full DNA profiles were obtained from the control rabbit and corvid carcasses that
200 had been left indoors for 10 days in experiments 3 and 4 (Table 1). However, a rapid
201 decline in recovery of DNA over time was observed from carcasses left outside.



202

203 **Figure 2:** Average percentage of DNA profile obtained (%) from samples collected
204 from different carcass types left outside for different periods of time after DNA
205 transfer in experiments 3, 4 and 5. The average percentage DNA profile is calculated
206 from two separate trap samples on the same day at the same time.

207

208

209

210 The results of the rabbit bait experiment show a clear drop in percentage of DNA
211 profile obtained over time (Figure 2), and the treatment effect is significant
212 ($F(5,5)=69.94$, $p<0.01$). The percentage of DNA profiles obtained at time 0 were
213 100% and 91.3% dropping to 45.7% and 52.2% after 1 day outside. From 4 days, all
214 profiles were less than 20% complete, dropping to less than 5% complete at 10
215 days.

216 A major issue with sampling the rabbit baits was how quickly the carcasses
217 decomposed. Seven days after handling, the rabbit carcasses had severely
218 decomposed making sampling very difficult. Consequently, for the last two samples
219 DNA collection was undertaken at the scene. The lack of DNA recovered from either
220 of the 10 days samples suggests that DNA is unlikely to be recovered from severely
221 decomposed remains (Figure 2). DNA persistence drops dramatically between the
222 day 1 and day 4 samples, with no reportable profiles obtained at 4 days. We are
223 unable to comment on the persistence of DNA up to 3 days as no samples were
224 taken at points between this range. For casework, provided the carcass appears
225 reasonably fresh, DNA profiling could be successful.

226 The corvid experiments, showed a similar decline in the quality of STR profiles over
227 time (Figure 2). The effect of treatment was significant for experiment 4
228 ($F(6,7)=57.10$, $p<0.01$) but not for experiment 5 ($F(3,4)=586.3$, $p=0.66$), although this
229 could be due to a lack of controls for this experiment (Table 1). The highest quality
230 profiles were recovered from the immediate samples (experiment 4 only, Figure 2).
231 This was followed by a significant decrease in percentage of DNA profile obtained
232 after 1-2 days in the outdoor environment (Figure 2). No DNA profiles were
233 recovered from treatments involving 7 or 10 days outside. There are numerous
234 factors that could have affected the quantity of DNA transferred and the quality and
235 quantity of the DNA collected between duplicates and after different time periods.
236 The corvid carcasses were relatively decomposed after 7 days and at 10 days a
237 number of feathers had fallen away. This meant that a large proportion of the DNA
238 transfer area had been lost. The actions of flies and other invertebrate scavengers
239 may reduce the amount of DNA on the surface. In general, higher quality profiles
240 were recovered from corvids in experiment 4 than experiment 5. One major variable

241 between the two corvid experiments was precipitation. The day 7 samples from
242 experiment 4 had only been exposed to 8.4 mm of rain, whereas the day 7 samples
243 from experiment 5 were exposed to 46.8mm. This difference in precipitation
244 between the two experiments could account for the lower quantities and poorer
245 profiles in the second experiment. Although this variation in rainfall is typical in
246 Scotland, DNA transferred onto substrates in dryer cooler conditions may persist for
247 much longer periods of time. Results from both experiments show that even after
248 rain (S3), reportable DNA samples can be recovered from corvid carcasses after a 2
249 day period.

250 **3.3 Bird of prey experiment**

251 A further, small experiment was carried out on 4 bird of prey carcasses left outside
252 for 1 day. The highest quality profile was recovered from the buzzard with 78.3%
253 profile obtained, followed by the sparrowhawk (67.4%), and both of these profiles
254 were considered to be reportable (i.e. > 50% complete). The profile results recovered
255 from the tawny owl (0%) or kestrel (17.4%) were not reportable.

256 The lack of DNA recovered from the owl may be due to unique feather structures
257 found in this order of birds [19]. Furthermore, the owl was much wetter than the other
258 birds during DNA collection and this may have impaired DNA recovery. The kestrel
259 had a much lower quality profile than the other raptors, which may be due to the loss
260 of feathers on its back. Overall, this small experiment has demonstrated that human
261 touch DNA can be recovered from bird of prey carcasses after 1 day outside. It had
262 rained during the experiment (0.8mm precipitation), so bird of prey carcasses
263 discovered in wildlife crime investigation should be sampled, even if they have been
264 subject to wet weather conditions. As no carcasses were left for longer, it cannot be
265 demonstrated whether DNA would persist for longer than 1 day on raptor carcasses.
266 However, the results from the corvid experiment illustrate where recovery of human
267 DNA may be possible; as with the rabbit carcasses, provided no significant
268 decomposition is observed, human DNA recovery should be attempted from bird
269 carcasses in criminal casework.

270

271 **3.4 Comparison of all substrates.**

272 When comparing the results of all substrate types a clear difference is seen between
273 the results from the traps and all carcasses. Figure 1 highlights that the average
274 percentage of DNA profile obtained in both trap experiments did not drop over time,
275 as might be expected, but instead remained fairly consistent over the 10 day period.
276 Contrastingly, the carcass experiments showed a rapid decline in DNA profile
277 recovery over time, with only one reportable profile recovered at 2 days outside and
278 no profiles recovered after this time.

279 There are a number of possible reasons why more touch DNA was recovered from
280 traps than the bird and bait carcasses. Firstly, the initial amount of DNA transferred
281 to the trap may have been higher than the DNA transferred to the carcasses. This
282 was indeed found to be the case in the proof of concept experiment after immediate
283 sampling (S2). The carcasses were only gripped tight enough to allow for holding
284 but the traps needed sufficient force to set the device. In addition, the decomposition
285 process on the carcasses meant that some of the sampling areas were
286 compromised, but in the trap experiment no sampling areas were lost. Another
287 significant difference between the sampling of traps and bait/corvid carcasses was
288 the condition of the samples during DNA collection. All trap samples were dry during
289 the minitaping procedure but some of the carcasses were damp or very wet.
290 Interestingly, trap experiment 1 received the worst weather conditions and still
291 produced higher quality profiles than all carcass samples. It must also be noted that
292 evidence such as bird of prey carcasses are often disposed of in sheltered areas,
293 such as in stone walls or rabbit burrows. Any protection from the elements may
294 increase the chance of recovering DNA from carcasses.

295 **4. Conclusion.**

296 These experiments have demonstrated that reportable human DNA profiles can be
297 recovered from traps, baits and birds that have been left exposed outside, even after
298 exceptional rainfall. The time of exposure where reportable profiles can be recovered
299 is variable, being at least 10 days for traps and at least 1 day for carcasses. For
300 criminal casework, it is unlikely that the time interval between a crime and the

301 location of evidence will be known. Our results suggest that recovery of DNA from
302 illegally-set traps should always be attempted, and recovery from carcasses should
303 be attempted providing only limited decomposition is observed, irrespective of rainfall
304 levels.

305 The recovery of human touch DNA from evidence retrieved from outdoor locations
306 during bird of prey crime investigations, or indeed in other crimes, may provide a key
307 identification tool for linking or excluding suspects. The recovery of human DNA from
308 traps, bird of prey carcasses and bait carcasses should be applied as soon as
309 possible to assist with raptor persecution investigations.

310

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