



**Using Detection Dogs to Meet Conservation Objectives for Simultaneous Surveys of Northern Spotted and Barred Owls**

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Abstract:	As with many at-risk species, state and federal actions to conserve northern spotted owl ( <i>Strix occidentalis caurina</i> ) habitat are largely triggered by establishing occupancy. Northern spotted owl occupancy is typically assessed by surveys eliciting their response to simulated vocalizations. However, barred owl ( <i>Strix varia</i> ) presence—one of the most significant threats to northern spotted owls—reduces northern spotted owl responsiveness to vocalization surveys. We developed a survey method to simultaneously assess occupancy of both species that does not require <i>Strix</i> vocalization. Detection dogs ( <i>Canis lupus familiaris</i> ) located owl pellets accumulated under roost sites, guided by habitat association maps. Pellets were confirmed to species by Restriction Fragment Length Polymorphism analyses of mitochondrial DNA. After validating these methods in 2008, we applied them to project level surveys in northern California for northern spotted and barred owls in 2009. In 2010, we compared success of detection dog surveys to vocalization surveys conducted on the same sites using the U.S. Fish and Wildlife Service’s Draft 2010 Survey Protocol. Dogs located pellets at 20 out of 20 sites not known to contain owls since 1997. Fourteen sites were DNA confirmed to contain northern spotted owl and seven to contain barred owl, three of these sites contained both species. Cumulative detection probabilities for dog

	<p>teams were 70% after session 1, 95% after session 2, and 100% after session 3 (40%, 80% and 90% after DNA confirmation). Cumulative detection probabilities for vocalization survey teams were 35% after session 1, increasing to 76% by session 6 (18% to 65% after visual confirmation). Trained detection dogs provide a reliable method for establishing occupancy of both northern spotted owl and barred owl, without requiring owl vocalization, meeting objectives of Actions 23 and 24 of the 2008 Northern Spotted Owl Recovery Plan. These methods have similar applications for other vertebrates.</p>

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10 **Using Detection Dogs to Meet Conservation Objectives for Simultaneous Surveys of**

11 **Northern Spotted and Barred Owls**

12

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28 Running Head: Detection dog surveys of spotted owls

29 **Abstract:** *As with many at-risk species, state and federal actions to conserve northern spotted*  
30 *owl (*Strix occidentalis caurina*) habitat are largely triggered by establishing occupancy.*  
31 *Northern spotted owl occupancy is typically assessed by surveys eliciting their response to*  
32 *simulated vocalizations. However, barred owl (*Strix varia*) presence—one of the most*  
33 *significant threats to northern spotted owls—reduces northern spotted owl responsiveness to*  
34 *vocalization surveys. We developed a survey method to simultaneously assess occupancy of both*  
35 *species that does not require *Strix* vocalization. Detection dogs (*Canis lupus familiaris*) located*  
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37 *confirmed to species by Restriction Fragment Length Polymorphism analyses of mitochondrial*  
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39 *northern California for northern spotted and barred owls in 2009. In 2010, we compared*  
40 *success of detection dog surveys to vocalization surveys conducted on the same sites using the*  
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42 *20 sites not known to contain owls since 1997. Fourteen sites were DNA confirmed to contain*  
43 *northern spotted owl and seven to contain barred owl, three of these sites contained both*  
44 *species. Cumulative detection probabilities for dog teams were 70% after session 1, 95% after*  
45 *session 2, and 100% after session 3 (40%, 80% and 90% after DNA confirmation). Cumulative*  
46 *detection probabilities for vocalization survey teams were 35% after session 1, increasing to*  
47 *76% by session 6 (18% to 65% after visual confirmation). Trained detection dogs provide a*  
48 *reliable method for establishing occupancy of both northern spotted owl and barred owl, without*  
49 *requiring owl vocalization, meeting objectives of Actions 23 and 24 of the 2008 Northern Spotted*  
50 *Owl Recovery Plan. These methods have similar applications for other vertebrates.*

51 **Keywords:** northern spotted owl, barred owl, *Strix occidentalis caurina*, *Strix varia*, detection  
52 dogs, vocalization surveys

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53 **Introduction**

54 Many conservation actions are triggered for listed species once their occupancy is established.  
55 Problems may arise when occupancy requires a behavioral response (e.g., wildlife entering a  
56 trap, walking past a specific location, responding to play-backs), because not everyone is equally  
57 likely to respond. Use of detection dogs (*Canis lupus familiaris*) to locate DNA-confirmable  
58 wildlife sign can provide a useful alternative approach under these circumstances. Trained  
59 detection dogs can cover large landscapes over difficult terrain with a high probability of  
60 detecting sign from a wide variety of target species (Smith et al 2003; Wasser et al. 2004, 2011;  
61 Wasser 2008; Harrison 2006; MacKay et al 2008; Vynne et al. 2010). More importantly, sample  
62 detection is largely independent of the target species' behavior or other potentially biasing  
63 characteristics such as sex or reproductive condition. Sample detection occurs after the fact and  
64 is driven by the dogs' obsession to obtain a play toy reward, detaching it from species' or  
65 behavioral characteristics that might otherwise cause bias (Wasser et al. 2004).

66 The present study examines the use of detection dogs to simultaneously document  
67 occupancy of northern spotted owls, a federally threatened species, and their barred owl  
68 competitors. Many conservation actions for northern spotted owl are triggered when their  
69 occupancy is established. However, the presence of invading barred owls has suppressed  
70 northern spotted owl response rates to vocalization surveys (Olson et al. 2005, Crozier et al.  
71 2006), raising concerns regarding the effectiveness of an established survey protocol in use since  
72 1992.

73 The U.S. Fish and Wildlife Service's Final Recovery Plan for the Northern Spotted Owl  
74 (May 2008) addressed this concern by emphasizing the need for improved survey protocols.  
75 Recovery Action 23 calls for the establishment of protocols to detect owls in a way that does not

76 increase the likelihood of aggressive interactions between barred and northern spotted owls.  
77 Recovery Action 24 specifically states the need for northern spotted owl survey methods that can  
78 account for reduced responsiveness in proximity of barred owls.

79 In 2010, the U.S. Fish and Wildlife Service (USFWS) developed a Draft Northern  
80 Spotted Owl Survey Protocol that relies on vocalization surveys. Concurrent with  
81 implementation of this new protocol, we assessed whether surveys utilizing detection dogs  
82 provide a viable alternative or complement to vocalization methods.

83 Our design was based on a pilot study in 2008, which tested whether dogs have the  
84 ability to reliably detect both barred and northern spotted owls by the scent of pellets and/or  
85 feces. A detection dog team blindly searched for northern spotted owl pellets in a 4 km<sup>2</sup> area  
86 determined by a northern spotted owl occupancy model (Zabel et al 2003) to represent the  
87 highest quality habitat encompassing each of 18 northern spotted owl roosts. Roosts had been  
88 located that same year as part of a concurrent, independent study examining impacts of off-  
89 highway vehicles on northern spotted owls (Hayard et al. 2011). The detection dog team located  
90 16 roost sites by finding pellets on 15 out of 18 occupied territories (89% of searched cells) and  
91 one detection of scat only, in a total of 20 site visits. All detections except one occurred in the  
92 first visit to the search cell. Spotted owls were visually confirmed at 15 of the 18 roosts, either by  
93 the dog handler and orienteer at the time the dog located the roost or by a separate crew using  
94 vocalization surveys methods within the next two weeks.

95 The first detailed test occurred in 2009, when USFS requested us to search a pending  
96 timber sale area for northern spotted owls. Then, in spring 2010, we conducted a double blind  
97 study comparing the cumulative detection probabilities of owls from dog surveys and  
98 vocalization surveys using the draft USFWS protocol. In both years, dog detections were DNA

99 confirmed to be northern spotted or barred owls using restriction fragment-length polymorphism  
100 (RFLP) analysis of mtDNA extracted from the pellets (see below).

101

## 102 **Methods**

### 103 **Study Area**

104 Our study was conducted in the South Fork Management Unit of Shasta-Trinity National Forest  
105 of northern California. The forest consists of mixed coniferous and deciduous trees, comprised  
106 primarily of Douglas fir (*Pseudotsuga menziesii*), Ponderosa pine (*Pinus ponderosa*) and oak  
107 (*Quercus* spp.). The topography of this area is very steep. This, coupled with high daytime  
108 temperatures sometimes exceeding 100°F in 2009, caused us to restrict searches to early morning  
109 hours that year. Barred owl were thought to be relatively uncommon in the Shasta-Trinity  
110 National Forest at the time of the study. No more than four barred owls were detected using  
111 vocalization survey methods to study 28 northern spotted owls occupying sites in an adjacent  
112 area from 2005-2008 (L. Hayward, unpublished data).

113

### 114 **Dog training and handling**

115 We trained shelter-rescued, mixed-breed dogs (a Labrador retriever mix, Australian shepherd,  
116 and an Australian cattle dog mix) to locate northern spotted owl pellets and feces by scent, using  
117 methods described in Wasser et al. (2004). Detection dogs are carefully selected for their  
118 obsessive ball drive. Full details of the training method can be found in Wasser et al (2004).  
119 Briefly, receipt of a ball reward is paired with detection of the sample odor from the target  
120 species. Previously frozen, thawed samples collected in the year prior from multiple northern  
121 spotted owls were used during training. Use of training samples from multiple individuals



122 teaches the dog to generalize to the species-specific odor found in all target-species samples. The  
123 dog is then taught to sit upon locating the sample, prior to receiving its reward, providing a  
124 clearer signal that a sample has been detected. Dog and handler teams are next taught to search  
125 the environment for hidden target species' samples, working the dog into the wind, toward the  
126 source. Handlers are taught to read wind patterns and environmental features that alter wind  
127 direction or cause scents to pool, along with the changes in the dog's behavior that indicate its  
128 detection and/or loss of the sample odor. This allows handlers to help the dog work to source, as  
129 needed, locating samples with high efficiency over complex landscapes. Dogs search off-leash to  
130 increase coverage but always remain in view of their handler.

131         The final stage of training involved transitioning dogs to the scent of undisturbed  
132 samples, naturally encountered in the field, since these may occasionally smell somewhat  
133 different than the previously-frozen samples used during training. Crews visited sites in the  
134 mornings and evenings that were previously known to have owls and elicit owl responses to  
135 simulated vocalizations. Once a response was heard, crews visually located the owl and  
136 searched the area for pellets. The dogs were then taken to the location and directed to the pellets,  
137 receiving a reward immediately upon locating the pellets. Barred owl pellets were added to the  
138 dog's repertoire by similarly rewarding the dogs as soon as they investigated them.

139         Each dog team consisted of a detection dog, a handler, and an orienteer that processed  
140 samples and kept the team within the designated survey area using a hand-held Global  
141 Positioning System (GPS) device.

142

143 **Determining search cells**

144 We previously showed that use of resource-selection models to guide sampling can  
145 markedly increase detection probabilities of target species' samples by detection dogs (Wasser et  
146 al 2011). We similarly used habitat selection models in the present study to narrow the dog's  
147 search area to the portion of the owl's home range that is most likely to contain its highly  
148 localized roost site. Thus, in all cases, a dog team searched for *Strix* pellets in a 2 km x 2 km area  
149 predicted by northern spotted owl occupancy models (Zabel et al. 2003; Carroll and Johnson  
150 2008) to have the best available habitat in cells distributed over a pending timber sale in 2009  
151 and historic nest site data in 2010. The size of our search cell was initially based on the Zabel et  
152 al. (2003) model, which predicts northern spotted owl occupancy with 94% accuracy for a 2 km<sup>2</sup>  
153 area. However, because northern spotted owl nests from the same individual could be as far as 1  
154 km apart between years (L. Hayward, unpublished data), search cell size was increased to 4 km<sup>2</sup>

155 We overlaid the habitat occupancy models of Zabel et al (2003) and Carroll and Johnson  
156 (2008) to designate search areas in 2009 and 2010. The Carroll model was added to refine  
157 placement of our 4 km<sup>2</sup> search cell within any given site since it provides much finer resolution  
158 of northern spotted owl habitat quality and was also designed to be compatible with the Zabel  
159 model. On the ground, searches within each cell were further refined by the handler focusing the  
160 dog's search on the bottom third of drainages with large trees, closed canopy and open  
161 understory, as these characteristics most strongly predict owl roost sites (Blakesley et al. 1992).

162  
163 **2009.** In 2009, the USFS requested surveys of a pending timber sale area for northern spotted  
164 owls, in addition to a suspected owl home range about 30 km to the northwest. The timber sale  
165 was comprised of two nearby areas of 160 km<sup>2</sup> and 100 km<sup>2</sup>. Two different dog teams were  
166 employed between 16 May and 3 June, 2009. The 2009 teams included an Australian shepherd

167 mix and a Labrador retriever mix. Since the area was defined by the timber sale, we did not use  
168 historic nest site data to orient our cells. Instead, we partitioned two larger areas into ten and six  
169 contiguous 16 km<sup>2</sup> cells, respectively, plus one 16 km<sup>2</sup> area over the single suspected homerange  
170 to the northwest. We then used the Zabel and Carroll models to place 4 km<sup>2</sup> cells in the highest  
171 quality habitat within each 16 km<sup>2</sup> cell. Two of these 16 km<sup>2</sup> cells had an abundance of high  
172 quality habitat remaining after placement of one 4 km<sup>2</sup> cell, so a second cell was placed within  
173 each. Each site was visited only once by a single dog team. However, a second 4 km<sup>2</sup> cell area  
174 was visited by a second dog team in two of the 17 cells. Each team covered 3-8 km in a single  
175 visit, depending on terrain, habitat available, and the dogs' abilities to cope with hot daytime  
176 temperatures. The search in any given cell was terminated if pellets were found. As indicated  
177 above, the specific search route within each 4 km<sup>2</sup> cell was focused on areas that most strongly  
178 predict owl roost sites (e.g., the bottom third of drainages with large trees, closed canopy and  
179 open understory, Blakesley et al. 1992).

180 All pellets were collected in paper bags for frozen storage, and were swabbed in the lab  
181 upon return for species identification using RFLP analyses of mtDNA.

182

183 **2010:** In 2010, we placed a 4 km<sup>2</sup> search cell within 1 km of each of 20 USFS historic nest sites  
184 recorded between 1987 and 1997. Each cell was placed to encompass as much suitable habitat as  
185 possible, as predicted by the Zabel and Carroll models. No data were available on owl presence  
186 at the 2010 sites prior to 1997 and dog crews had almost no prior experience with these areas  
187 (unintentionally, crew leaders had some familiarity with 2-3 sites from previous years).

188 We originally intended to have all twenty 4 km<sup>2</sup> cells searched by two dog teams and two  
189 vocalization survey teams between 11 May and 4 July, 2010, to compare efficacies of the two

190 survey methods. However, three cells had to be dropped after the first session due to signs of  
191 marijuana farming. An Australian cattle dog mix was used along with the Labrador retriever mix  
192 that participated in the 2009 study. All search cells were located in a late successional reserve  
193 with territories known to be historically occupied by northern spotted owls. All dog surveys  
194 were conducted independent of vocalization surveys and no team shared the results of their  
195 surveys with the other teams.

196

### 197 **2010 Detection Dog Surveys**

198 Each cell was surveyed three times (sessions 1-3) in an effort to determine how many site visits  
199 were required by a dog crew to demonstrate owl presence or infer absence. The two dog teams  
200 alternated visits on each cell so that both teams visited all cells. Dog teams focused their first two  
201 visits (Sessions 1 and 2) in the highest quality habitat indicated by the Zabel and Carroll models  
202 (Fig. 1) and surveyed all remaining habitat in session 3.

203 Unlike in 2009, dogs continued to search the area for pellet(s) after the first pellet was  
204 detected, to maximize chances of accurately characterizing the species of owls using the area. If  
205 there were more than 10 pellets in a single location the freshest five to seven were collected and  
206 swabbed. Otherwise, all pellets were collected and swabbed for DNA.

207

### 208 **Pellet Swabbing, DNA extraction and RFLP species identification**

209 Latex gloves were worn whenever preparing swabs and collecting specimens. The outer surface  
210 of each pellet was swabbed twice, using buccal swabs (Epicentre Biotechnologies' Catch-All  
211 buccal swabs, catalog # QEC89100) saturated with 1X PBS buffer. The entire surface of the  
212 pellet was lightly swabbed for surface mucosal cells while rotating the swab to maximize the

213 surface area covered (Ball et al. 2007). The applicator was then placed in an empty, labeled 2 ml  
214 microcentrifuge tube, with 500uL ATL lysis buffer (Qiagen Inc., Valencia, CA). Swabbed vials  
215 were kept at room temperature until stored frozen (-20°C) that evening.

216 Each swabbed pellet was then placed in a paper bag labeled with the pellet ID, date, and  
217 UTM location. The paper bag was placed inside an identically-labelled freezer-safe plastic bag  
218 and stored in the freezer. At the end of the study, pellets were transported on dry ice to our  
219 laboratory at the University of Washington.

220 Each owl pellet swab was extracted using a modified version of Qiagen's DNeasy Tissue  
221 DNA extraction protocol (catalog # 69506) and eluted in 200uL AE buffer. Negative controls  
222 were included in every extraction to control for any laboratory contamination, and were  
223 performed in a separate room that was free of PCR products.

224 We developed a PCR-RFLP assay for species identification using mitochondrial DNA  
225 variation. We obtained numerous sequences of the control region in northern spotted owls (n=18)  
226 and barred owls (n=45) from the USFS (S. Haig, unpublished data) and GenBank. Conserved  
227 regions in both species were identified for primer development by sequence alignment using  
228 CLC DNA Workbench. The forward primer, NSO3, has the sequence  
229 CACYCTAATYCATGACA and the reverse primer, NSO2, has the sequence  
230 ACAGCTAAACTTGGGA, which together amplify a 358 bp fragment.

231 Sequence alignment also revealed an AvrII restriction enzyme cut site present in all 45  
232 barred owl sequences, while absent in all 18 northern spotted owl sequences, which cuts a 134bp  
233 fragment from the 358 bp fragment for barred owls only. Positive control tissue samples of  
234 northern spotted owl and barred owl used for assay validations had 100% consistency with  
235 expected results described above, and were included in every PCR run. All samples were

236 analyzed on an ABI3100 Genetic Analyzer using Genescan and Genotyper software (Life  
237 Technologies Applied Biosystems), with a 5' 6-FAM label attached to the forward primer NSO3.  
238

### 239 **2010 Vocalization Surveys**

240 Northern spotted owl vocalization surveys were conducted under the supervision of crew leaders  
241 with at least two years of prior experience conducting vocalization surveys for northern spotted  
242 owls, as specified by USFWS protocol. At the outset of the season, all field technicians were  
243 trained by USFWS personnel to conduct surveys in accordance with U.S. Fish and Wildlife  
244 Service's 2010 Draft Protocol for Surveying Proposed Management Activities That May Impact  
245 Northern Spotted Owls. Additionally, crews were periodically spot-checked by USFWS  
246 personnel during the study. Vocalization survey teams complied with the USFWS 2010 Draft  
247 Protocol for all visits (USFWS, 2010).

248         Vocalization survey teams were required to conduct night-time surveys in each cell six  
249 times throughout the season, with seven days between visits, with the exception of three cells  
250 dropped from the study due to drug activity (Table 3). According to protocol, historical  
251 information was used to establish call points. This information included USFS historical spotted  
252 owl sites, as well as spotted owl location data collected from the past five years from our  
253 independent ongoing study (Hayward et al. 2011), with detailed information on call points and  
254 habitat. As directed by the 2010 Draft Protocol, if the site had very recent activity from a  
255 previous season, that site would receive a daytime initial site visit prior to the night survey. Thus,  
256 per protocol, we conducted historical stand searches before night surveying of cells 7, 22, 24  
257 since owls had previously been known from our prior work to inhabit these areas.

258 Historical information, topographical maps, and aerial data were used to determine call  
259 points prior to beginning the survey period. Each cell had a different number of call points  
260 depending on road access and suitable habitat, ranging from 3 to 7 points per cell. If  
261 predetermined call points along roads did not cover all suitable habitat, continuous walking  
262 surveys were conducted for ~ 4 hrs immediately after sunrise until all suitable habitat that could  
263 not be covered by road call points had been searched and called. In such instances, calling  
264 occurred within the cell, off the road and in suitable habitat. All but two cells had excellent  
265 coverage from night vocalization call point locations.

266 We increased call times from 10 minutes to 12 minutes on sites that had no response  
267 after four visits to improve the chances that an owl would respond. No surveys were conducted  
268 in heavy wind or rain that might hinder auditory detection.

269

#### 270 **Detection Confirmation and Cumulative Detection Probability Comparisons**

271 All owl pellets located by detection dogs had to be DNA confirmed to owl species by RFLP  
272 analysis of mtDNA. The same species-specific DNA fragment had to be observed at least twice  
273 from the same sample to be listed as a species confirmation. Vocal survey confirmations  
274 required a visual identification of the northern spotted or barred owl. Cumulative detection  
275 probabilities were calculated by counting the number of cells with detections, divided by the  
276 number of cells visited. Thus, cumulative detections by the second visit would include the  
277 number of cells with detections in visit 1 or 2, divided by the number of cells twice visited. This  
278 was calculated two ways for each survey type, once for all detections and again for all confirmed  
279 detections.

280

281

282 **Results**

283 In 2009 the two dog crews detected *Strix* on 12 of 17 sites surveyed in a single visit (Table 1).  
284 Dogs found pellets on 11 of the 12 sites and scats at eight of these 12 sites. One dog located  
285 seven roosts out of 11 sites searched. The other dog located five roosts out of six sites searched.  
286 Two sites were confirmed to be occupied by northern spotted owls by sight and/or vocalization  
287 immediately following pellet localization. DNA analyses of pellets confirmed northern spotted  
288 owls at seven of the 12 sites. A barred owl was also DNA confirmed at two of those seven sites  
289 (Table 1)

290 In 2010, dog crews found *Strix* owl pellets on all 20 sites searched. Three of these sites  
291 were subsequently determined to be too dangerous for further searching due to evidence of  
292 marijuana farming, although dogs found pellets during the first session in all three cases. A  
293 fourth site had to be similarly abandoned after the 3<sup>rd</sup> dog team visit and thus was also excluded  
294 from vocalization surveys (Table 2). DNA confirmed *Strix* from pellets at 18 of the 20 sites.  
295 Pellets from the other two sites DNA amplified for *Strix* only once and thus, by definition, were  
296 listed as an unconfirmed *Strix*. However, in one of these two sites, pellets were located in all  
297 three visits, each of which amplified only once for northern spotted owl. Pellets from fourteen of  
298 20 cells were DNA-confirmed to be northern spotted owl and seven of 20 were DNA-confirmed  
299 to be barred owl, three of which had both northern spotted owl and barred owl in the same cell  
300 (Table 2). Dog-specific detections are separately colored red and black in Table 3 to indicate  
301 between-dog differences in detection probabilities.

302 Overall success at DNA amplification and RFLP analyses to confirm species identity  
303 averaged 41% for 2009 and 48% for 2010, with the highest success (60%) for dry, intact pellets.  
304 However, our protocol of collecting numerous pellets per site generally resulted in at least one



305 DNA confirmation at any given cell (Fig 2). We believe that the vast majority of DNA  
306 confirmed pellets to the species level in our study were less than one month old based on: the  
307 overall low DNA amplification success of pellets, the tendency of pellets to disintegrate over  
308 time from rain and thawing snow, and the likelihood of pellets being eaten by ants in warmer  
309 weather.

310 Vocalization surveyors heard and/or saw *Strix* species in all but two of the 16 cells they  
311 surveyed (recall four additional cells were excluded due to suspected drug activity).

312 Vocalization survey crews located one additional owl that never responded to the simulated  
313 vocalizations and heard an owl but could not locate it in three cells. These results, plus sex and  
314 reproductive class data are also shown in Table 3. All species identified by vocalization surveys  
315 agreed with DNA results from dog-detected pellets. However, three cells also included barred  
316 owls identified from pellets that were not detected by vocalization surveys.

317 Cumulative detection probabilities for dog and vocalization survey teams are compared  
318 in Fig 2. Cumulative dog detection probabilities across sampling sessions were 70% after the  
319 first session, 95% after the second and 100% after the third session. Adjusting these to include  
320 only DNA confirmed identifications gives 40% success after the first session, 80% after the  
321 second and 90% after the third session. In comparison, cumulative detection probabilities for  
322 vocalization surveys was 35% after session 1, steadily increasing to 76% by session 5 with no  
323 new detections occurring in session 6. Visually confirmed vocal detections ranged from 18%  
324 after session 1 to 65% by session 6 (Fig. 2)

325

326 **Discussion**

327 Detection dogs provide an effective noninvasive method for determining presence of both  
328 northern spotted owl and barred owl, independent of owl responsiveness, and can be used either  
329 as an alternative or complement to vocalization surveys depending on survey objectives. While  
330 vocalization surveys are the best method for determining reproductive status of northern spotted  
331 owls, use of detection dogs may be particularly beneficial for establishing occupancy when  
332 northern spotted owl vocal responsiveness diminishes due to the presence of barred owls. This  
333 method has important implications for northern spotted owl conservation given the species'  
334 continued decline (Anthony et al. 2006) and the importance of establishing occupancy to trigger  
335 northern spotted owl conservation actions under the Northwest Forest Plan and state forest  
336 practices regulations. The low amplification success of DNA from pellets is a potential drawback  
337 of this method, but can be improved upon by focusing collections on dry, intact pellets.

338 Results indicate that: (a) dogs can locate northern spotted owl and barred owl roosts with  
339 high efficiency; (b) the Zabel and Carroll models are good predictors of northern spotted owl  
340 habitat; and (c) species identification is possible using RFLP analyses of mtDNA extracted from  
341 swabs of the owl pellets. Resource selection guided sampling has been shown to increase dog  
342 detection probabilities in other species (Wasser et al 2011). The Zabel and Carroll models may  
343 have similarly improved pellet detection by dogs, although this was not directly tested in the  
344 present study.

345 The 2010 study aimed to directly compare detection probabilities of the dog method to  
346 vocalization surveys employing the newly released draft USFWS survey protocol. Vocalization  
347 surveys were able to obtain more detailed information upon locating individual owls (e.g., sex,  
348 number of individuals, and breeding status). However, by the second visit, the DNA-confirmed  
349 cumulative detection probability of dogs was already 15% higher than the visually-confirmed

350 cumulative detection probability achieved by vocalization surveys after six visits (Fig. 2). By  
351 the third visit, dogs had detected either a barred owl or northern spotted owl at every cell (90%  
352 of which were DNA confirmed) and in no case did the vocalization surveys detect an owl species  
353 that the dogs failed to detect. It may be possible in future studies to confirm sex and individual  
354 identities from nuclear DNA analyses on a portion of these pellets.

355         The detection dog surveys also detected barred owls and northern spotted owls that were  
356 missed by vocalization surveys. These included several cases where barred owls occurred on  
357 sites already occupied by northern spotted owls and one case where a northern spotted owl was  
358 present at a site occupied by a nesting barred owl pair. This suggests that detection dogs may be  
359 able to provide more thorough information about *Strix* presence than can be obtained from  
360 vocalization surveys and observation alone. They could facilitate early detection of barred owl  
361 invasion as well as determine whether northern spotted owls are still present in an area  
362 dominated by barred owl. However, true joint surveys of northern spotted and barred owls  
363 would require expanding habitat selection models to also include habitat features uniquely  
364 selected by barred owls given differences in home range attributes and habitat selection between  
365 these two species (Hamer et al 2007). Carroll and Johnson (2008) made similar  
366 recommendations for expanding their habitat selection models to include barred owls.

367         Where noninvasive survey techniques are desired or where both northern spotted owls  
368 and barred owls are present, detection dogs can provide an attractive alternative to vocalization  
369 surveys that does not rely on altering the behavior of the target species. Combining detection  
370 dog and vocalization survey methods could provide additional biological and ecological insights  
371 into the consequences of competitive interaction between these two owl species. For example,  
372 the three northern spotted owl pairs found in cells that were sympatric with barred owls were

373 non-reproductive and no barred owls were documented in the three cells where northern spotted  
374 owl pairs were nesting (Table 3). These observations suggest that successful northern spotted  
375 owl reproduction may be influenced by the presence of barred owls. Models of empirical data  
376 support this observation, showing a negative correlation between barred owl presence and  
377 northern spotted owl fecundity (Olsen et al. 2004) and are consistent with the aggressive,  
378 territorial behavior widely reported for barred owl (Mazur and James 2000, Gutierrez et al. 2007,  
379 Singleton et al. 2010).

380 Radio-tracking both species simultaneously would provide similar information, but over  
381 a continuous, much longer timeframe. The combination of vocalization and canine surveys,  
382 however, provides a snapshot in time of the same information.

383 It is possible that the dog's presence on territories could be a source of disturbance to the  
384 owl, although our dogs were trained to ignore the presence of owls when in view. Future studies  
385 could evaluate these impacts by comparing corticosterone levels (Wasser et al. 1997; Wasser and  
386 Hunt 2005; Hayward et al. 2011) in fecal samples collected from owls 2-24 hrs after detection by  
387 detection dog versus vocalization surveys.

388

### 389 **Management Implications**

390 Many conservation and management issues need to be addressed on a large geographic scale,  
391 requiring cost-effective, reliable methods of monitoring wildlife over large landscapes.  
392 Moreover, establishment of occupancy on these landscapes is often required before conservation  
393 actions are triggered. Northern spotted owl conservation actions at state and federal levels  
394 provide a case in point. Using detection dogs to simultaneously search for both northern spotted  
395 owls and barred owls can help these efforts, particularly since detection dogs have repeatedly

396 proven effective at searching large geographic areas for multiple species (Wasser et al. 2004,  
397 2011; MacKay et al 2008; Vynne et al. 2010). This method also addresses Recovery Actions 23  
398 and 24 of the 2008 Recovery Plan for the federally threatened northern spotted owl by providing  
399 cost-effective, reliable methods to simultaneously determine the presence of northern spotted  
400 owls, as well as barred owls on northern spotted owl territories. The ability of detection dogs to  
401 simultaneously locate both species may be especially valuable when: 1) Barred owls have not yet  
402 established territories but may be in the early stages of range expansion, 2) barred owls may be  
403 reaching a threshold level where they will soon become the dominant owl on the landscape, 3)  
404 northern spotted owls are only present on the landscape in very small numbers, and 4) when  
405 northern spotted owls are no longer present. Each of these scenarios has very different  
406 management implications and probabilities of success when implementing northern spotted owl  
407 conservation actions. More broadly, we hope that this survey technique will continue to provide  
408 an efficient, unbiased, and non-invasive alternative for determining presence or absence of a  
409 variety of species of concern.

410

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419

420 **Disclaimer**

421 "The findings and conclusions in this article are those of the author(s) and do not necessarily  
422 represent the views of the U.S. Fish and Wildlife Service."  
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499

500 **Figure Legends:**

501 **Figure 1.** Sampling cells, survey routes, pellet locations, and northern spotted owl and barred  
 502 owl locations confirmed by mtDNA analyses of pellets detected by dog surveys and  
 503 visual location by vocalization surveys, overlaid on habitat association maps from the  
 504 Zabel et al and Carroll and Johnson habitat models. Red squares correspond to northern  
 505 spotted owls and yellow squares correspond to barred owls. An owl inside the square  
 506 indicates a dog detection, sound wave illustration inside the square indicates a  
 507 vocalization survey detection. A ? inside the square indicates a one-time DNA  
 508 amplification from a pellet, which is insufficient to confirm a species. Blue circles  
 509 represent pellets located by dogs that did not amplify for mtDNA. The cell number is  
 510 indicated in white inside the black square outlining the cell. The thin black lines indicate  
 511 dog survey routes. Habitat quality ranges from high (green) to intermediate (yellow) to  
 512 (low) brown.

513

514

515 Figure 2. Comparison of cumulative detection probabilities across  
 516 sampling sessions for all cells sampled by dog versus vocalization  
 517 surveys. Circles indicate dog surveys and squares indicate  
 518 vocalization surveys. Dashed lines with open symbols indicate all  
 519 detections (confirmed and unconfirmed). Solid lines and symbols  
 520 indicate confirmed detections only. Dog detections are confirmed by  
 521 DNA, vocalization surveys are confirmed by visually locating the owl.

**Deleted: Figure 2.** Comparison of cumulative detection probabilities across sampling sessions for all cells sampled by dog versus vocalization surveys. Solid black line and circle indicates dog detection of pellets prior to DNA confirmation. Solid black line and open circle indicates dog detection of pellets that were DNA confirmed. Dashed black line and open square indicates detections by vocalization surveys.¶

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**Table 1: Summary of outcome for each site surveyed with detection dogs in 2009.**

**Auditory (\*) and visual (°) confirmations were accomplished by either the dog handler and orienteer at the time the dog located the roost.**

<i>Cell #</i>	<i>FS</i>	<i>Dog</i>	<i>Visits/</i>	<i>Pellet</i>	<i>Scat</i>	<i>mtDNA</i>	<i>Dog</i>
	<i>Site #</i>	<i>Detection</i>	<i>Cell</i>			<i>Species ID</i>	
4	674, 698	Yes	1	Yes	Yes		Shrek
5	695	Yes	1	Yes	Yes	NSO	Shrek
6		Yes	1	Yes	No		Lulu
7		Yes	1	Yes	Yes	NSO	Lulu
9	752	Yes	1	No	Yes		Shrek
11	801	Yes	1	Yes	No	NSO	Shrek
12	811, 756	Yes*°	1	Yes	No		Shrek
13	826	Yes	1	Yes	Yes	NSO	Lulu
14	802	Yes	1	Yes	Yes	NSO & BO	Lulu
17	848	Yes*	1	Yes	Yes	NSO & BO	Lulu
18		No	1	No	No		Shrek
20	819	No	1	No	No		Shrek
21	803, 845	Yes*?	1	Yes	No		Shrek
23		No	1	No	No		Shrek
24	825	No	1	No	No		Lulu
25		Yes	1	Yes	Yes	NSO	Shrek
26		No	1	No	No		Shrek
TOTAL		12 Y: 5 N	17	11 Y: 6 N	8 Y: 9 N		

**Table 2. Northern spotted owl (NSO) and barred owl (BO) roosts located by detection dog versus vocalization surveys. Species confirmation required that mtDNA be amplified at least twice from same pellet. Dog-specific detections are also listed.**

Cell	FS Site	Dog Survey Results			Vocalization Survey Results			
		Session 1	Session 2	Session 3	Species ID	# Visits	# Visits	# and type
5	413			NSO	NSO Singleton	NR	5	6 NS; 1 CWS
6	n/a	NSO	(BO)	NSO	NSO Pair	5	5	6 NS; 1 FU
7	412		BO	NSO	NSO Pair & BO	1	2	5 NS; 5 FU
8	412, 512	NSO, (BO)	NSO		NSO Pair	4	4	6 NS; 1 FU
9	440			NSO	NSO Nesting Pair	1	1	6 NS; 1 FU
10	605		NSO	NSO	NR	NR	n/a	6 NS; 1 CWS
11	506			NSO	Unk Ad	5	No Loc	6 NS; 1 CWS; 1 FU
12	416, 438	(BO)			NR	NR	n/a	6 NS; 3 CWS
14	428	BO			1 visit only	NR	n/a	1 NS
15	431	NSO	NSO		NSO Nesting Pair	3	3	6 NS; 1 FU

16	n/a	BO	BO	BO Pair	2	No Loc	6 NS; 3 CWS
19	430	NSO		No visit	n/a	n/a	0
20	410		NSO BO	Unk Ad	2	No Loc	3 NS; 1 FU
21	510	BO, (NSO)		Unk Ad	1	No Loc	1 NS; 1 FU
22	711, 712		NSO NSO	NSO Pair	1	2(F), 3(M & 2 juv)	6 NS; 1 CWS; 4 FU
23	714		BO	BO Pair	3	5	6 NS; 1 CWS
24	702		NSO	NSO Singleton	1	1	6 NS; 1 CWS
25	717	(NSO)	(NSO) (NSO)	NSO Nesting Pair w/ 2 Juv and NSO Singleton	1	1	3 NS; 1 FU
27	700	NSO	NSO	Unk Ad	1	No Loc	6 NS; 1 CWS
28	434		NSO NSO, BO	NSO Singleton	2	2	6 NS; 1 FU

<i>Dog</i>	<i>Session 1</i>	<i>Session 2</i>	<i>Session 3</i>
Max	17	31	23
Shrek	29	17	15

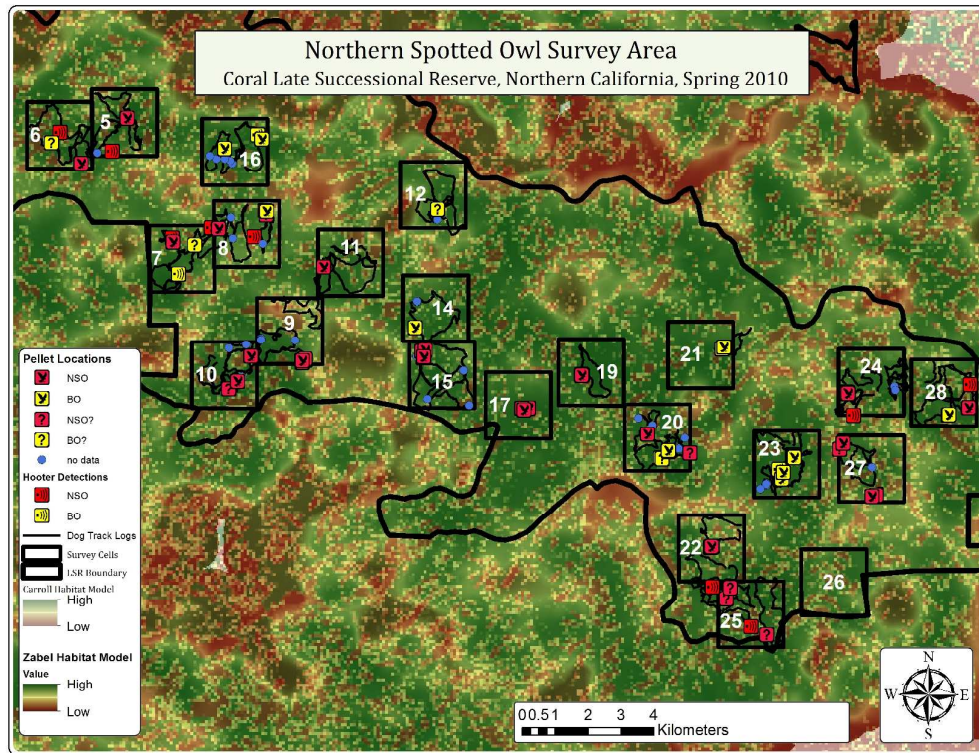
( ) = Unconfirmed species, shown in parentheses, are from pellets that DNA amplified only once.

NR=No Response; NS=Night survey; CWS=Continuous walking survey;

FU=Follow-up, Unk Ad= Unknown Adult, No Loc = No Location

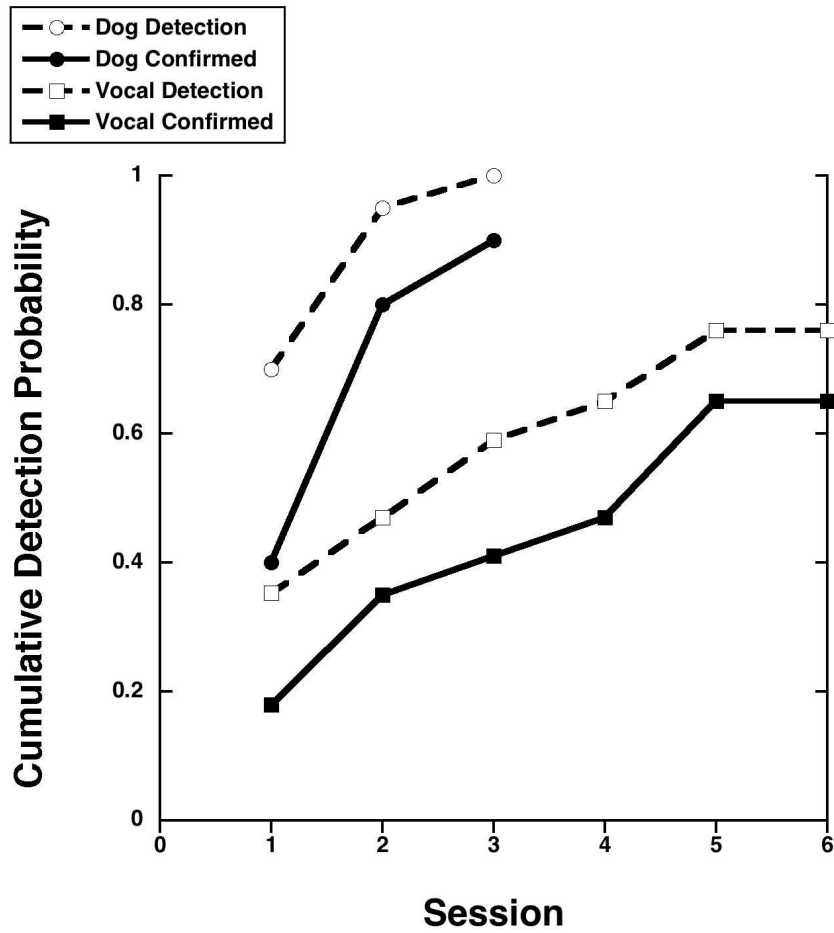
■ = Closed due to drug activity.

For review only



Sampling cells, survey routes, pellet locations, and northern spotted owl and barred owl locations confirmed by mtDNA analyses of pellets detected by dog surveys and visual location by vocalization surveys, overlaid on habitat association maps from the Zabel et al and Carroll and Johnson habitat models. Red squares correspond to northern spotted owls and yellow squares correspond to barred owls. An owl inside the square indicates a dog detection, sound wave illustration inside the square indicates a vocalization survey detection. A ? inside the square indicates a one-time DNA amplification from a pellet, which is insufficient to confirm a species. Blue circles represent pellets located by dogs that did not amplify for mtDNA. The cell number is indicated in white inside the black square outlining the cell. The thin black lines indicate dog survey routes. Habitat quality ranges from high (green) to intermediate (yellow) to (low) brown.

279x215mm (300 x 300 DPI)



Comparison of cumulative detection probabilities across sampling sessions for all cells sampled by dog versus vocalization surveys. Solid black line and circle indicates dog detection of pellets prior to DNA confirmation. Solid black line and open circle indicates dog detection of pellets that were DNA confirmed. Dashed black line and open square indicates detections by vocalization surveys.

133x128mm (288 x 288 DPI)