Running head: *Radioactive contamination of nest materials*

Radioactive contamination of nest materials of the Eurasian Tree Sparrow *Passer montanus* due to the Fukushima nuclear accident: the significance in the first year

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33

34 **ABSTRACT (148 words)**

35 The 2011 Fukushima nuclear accident contaminated large areas of eastern and
36 northeastern Japan, releasing vast amounts of radiation. Here we investigated
37 radioactive contamination of the nest materials of the Eurasian Tree Sparrow *Passer*
38 *montanus* from the breeding season of 2011 directly after the accident to the next
39 breeding season of 2012 at two sites. In Tokyo (222 km southwest of the plant), ambient
40 dose rates in the nestboxes were lower than those in Ibaraki (175 km southwest of the
41 plant), where the levels of 2011 were higher than those of 2012. Further, the amount of
42 radioactive Cs in each nest increased with the increase in nest weight, with a higher
43 increment at Ibaraki than at Tokyo. These data suggested higher nest contamination
44 levels in the breeding season directly after a nuclear accident than in later seasons, and
45 an increment of nest contamination levels via nest materials of birds.

46

47 “Capsule”:

48 Radioactive Cs included in the nest materials of birds increased nest contamination
49 levels.

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54 **1. Introduction**

55 On March 11, 2011, a 9.0 M earthquake struck off the east coast of Japan (38.10°N,
56 142.86°E, M 9.0 at 14:46:18 JST according to the Japan Meteorological Agency). It was
57 one of the most powerful earthquakes ever recorded with modern monitoring equipment.
58 It triggered massive tsunamis that severely damaged the Fukushima Daiichi nuclear
59 power plant and caused it to discharge vast amounts of radioactive material into the
60 atmosphere and ocean. Much of this radioactive material (e.g., radionuclides iodine-131,
61 tellurium-129m, and cesium (Cs) -134, Cs-136, and Cs-137) was deposited over large
62 areas of eastern and northeastern Japan (Kinoshita et al., 2011; Yasunari et al., 2011).

63 There is growing evidence from studies on birds at Chernobyl that even low-level
64 exposure to radiation can negatively affect partial albinism (Møller et al., 2007), blood
65 titers of carotenoids and vitamins (Møller et al., 2005a) sperm abnormalities (Møller et
66 al., 2005a), germline mutations (Ellegren et al., 1997), reproductive success (Møller et
67 al., 2005b, 2008), and survival (Ellegren et al., 1997; Møller et al., 2007; Møller and,
68 Mousseau, 2011; Møller et al., 2012). These effects have not only been recorded in birds
69 but also in other taxa (see Møller and Mousseau, 2006, 2011; Mousseau and Møller,
70 2014 for reviews). Some studies performed in the Fukushima prefecture a few years
71 after the catastrophe also reported effects of radiation on several taxa including insects,
72 birds, and mammals (Hiyama et al., 2012; Ochiai et al., 2014; Murase et al., 2015).

73 Altricial birds, which require parental care for a period of time, build nests made
74 of mud, grasses, twigs, moss, feathers, and spider webs, in which they incubate their
75 eggs and raise their offspring (Hansell, 2000). In areas with high radioactive
76 contamination, radionuclides may adhere to the surface of nest materials, elevating
77 exposures of radiation to parents and their offspring (Bonisoli-Alquati et al., 2015).

78 In this study, we describe spatial and temporal changes in radioactive contamination
79 of nest materials of the Eurasian Tree Sparrow *Passer montanus* from the breeding
80 season of 2011 directly after the Fukushima nuclear accident to the following breeding
81 season of 2012 in two regions in eastern Japan. In 2011 much of the radioactivity was
82 likely to still be adherent to the external surfaces of vegetation such as annual plants at
83 the time of nest building (i.e., at this short time after the accident the dominant
84 contamination pathway unlikely to be root uptake). Thus we made three predictions.
85 First, we predicted that levels of contamination in nestboxes would be higher in Ibaraki
86 than in Tokyo because of a greater amount of fallout from the Fukushima nuclear
87 accident on Ibaraki than on Tokyo (Yasunari et al., 2011). Second, radioactive
88 contamination of nest materials would be higher in 2011 than in 2012, because the
89 radioactive contaminants coating annual plants in 2011 would have been transferred to
90 the soil by 2012. Finally, the amount of radioactive Cs in nests would be positively
91 related to nest mass, because heavier nests are composed of more materials than light
92 nests.

93

94 **2. Methods**

95 ***2.1. Study site and data collection***

96 This study was conducted in Ibaraki Prefecture (175 km southwest of the Fukushima
97 Daiichi nuclear power plant) from January to October 2011 and from January to May
98 2012, and in Tokyo (222 km from the power plant) from January to October 2011 and
99 from January to August 2012.

100 We erected different numbers of wooden nestboxes at each site and in each year
101 (Ibaraki: $n = 70$ in 2011, $n = 61$ in 2012; Tokyo: $n = 36$ in 2011, $n = 36$ in 2012). The
102 nestboxes measured $19 \times 20 \times 30$ cm (width $\times$ depth $\times$ height) with an entrance hole
103 measuring 3.5 cm in diameter. The nestboxes at Ibaraki were placed on safety rails on
104 the roofs of buildings or on tree trunks 2.5–3.5 m off the ground. In Tokyo, they were
105 attached to tree trunks 2.5–3.5 m off the ground.

106 We measured γ radiation levels ($\mu\text{Gy/h}$) 19 times at 10-s intervals over a 3-min
107 period inside each nestbox and immediately repeated the process outside the nestbox at
108 the same height using a GAMMA-SCOUT dosimeter (GAMMA-SCOUT GmbH & Co.
109 KG, Schriesheim, Germany). We measured the radiation levels inside and outside 10
110 nestboxes on October 14, 2011, in Ibaraki; 15 nestboxes on 13–26 May, 2012 in Ibaraki;
111 7 nestboxes on October 1, 2011, in Tokyo; and 12 nestboxes on August 31, 2012, in
112 Tokyo. All nestboxes contained active nests defined as nests containing eggs or

113 nestlings. Subsequently, we weighed the nest materials of each nest (g-wet). Nest
114 materials of 5 nestboxes were dried for 48 h at approximately 70°C; the relationship
115 between wet weight (g-wet) and dry weight (g-dry) of nest materials was strongly
116 positively correlated (Spearman's $r = 1.0$, $P < 0.05$).

117 Nest materials at Ibaraki (n = 10) and Tokyo (n = 9) in 2012 were sampled
118 homogenized, and analyzed by a germanium detector to obtain a γ -ray spectrum and to
119 measure Cs activity concentrations (Bq/kg). Before analysis, nest materials were dried
120 in the sun during the daytime, followed by drying with a warm current of air for 6 h
121 using a drying machine, after which they were weighed (g-dry). The amount of Cs in the
122 nestboxes was calculated using the formula:

123 Amount of Cs in a nestbox (Bq/nest) = Cs activity concentration (Bq/g) * nest dry
124 weight (g-dry).

125 All data are expressed as Mean $\pm$ SD unless otherwise stated.

126

127 **2.2. Statistics**

128 We used paired t -test to compare ambient dose rate between the inside and the outside
129 of the nestboxes in Tokyo and Ibaraki in 2011 and 2012. To compare the difference
130 between 2011 and 2012 within a site, we used student t -test. Comparison in nest weight
131 between the two sites in the two years was performed with the use of two-factor
132 ANOVA based on Type II sums of squares (Bartlett's test: $df = 3$, $P = 0.75$). Nest weight

133 or the difference of ambient dose rate between the inside and the outside of the
134 nestboxes of the four groups (Tokyo in 2011, Tokyo in 2012, Ibaraki in 2011, and
135 Ibaraki in 2012) were tested and confirmed for normality (One-sample Kolmogorov–
136 Smirnov test).

137 We used generalized linear models (GLMs) with an identity link and a Gaussian
138 error distribution to elucidate the factors affecting the dose rate ($\mu\text{Gy/h}$) in nestboxes in
139 the breeding seasons. We conducted model selection on the basis of Akaike's
140 information criteria (AIC). Model comparisons were based on AIC differences (ΔAIC).
141 We included six explanatory variables (year, site, nest weight, and these three
142 interactions) in the initial model for the ambient dose rate in the nestboxes. We
143 compared 17 models (generated from all combinations among the six explanatory
144 variables and the null model) by ΔAIC . The dose rate in the nestboxes of the four
145 groups (Tokyo in 2011, Tokyo in 2012, Ibaraki in 2011, and Ibaraki in 2012) were tested
146 and confirmed for normality (One-sample Kolmogorov–Smirnov test).

147 We also used GLM with an identity link and Gaussian error distribution to
148 examine the relationship between nest weight (g-dry) and the amounts of Cs in the
149 nestboxes (Bq/nest) in 2012 in Tokyo and Ibaraki. We compared five models generated
150 from combinations among the three explanatory variables (nest weight, site, and their
151 interaction) in the initial model, and a null model based on ΔAIC (Burnham and
152 Anderson, 2002). The amounts of Cs in nestboxes of the two groups at Tokyo and

153 Ibaraki were tested and confirmed for normality (One-sample Kolmogorov–Smirnov
154 test).

155 We used a computer application “R, version 3.1.2” (R Foundation for Statistical
156 Computing, Vienna, Austria)

157

158 **3. Results**

159 Materials of Eurasian Tree Sparrows’ nests, which were randomly selected in Tokyo (n
160 = 3) and Ibaraki (n = 3), consisted of dead herbaceous plants ($89 \pm 10\%$), twigs ($5 \pm 6\%$),
161 dead leaves of trees ($1 \pm 1\%$), and other materials ($5 \pm 8\%$) – percentages by weight.

162 Nest building in 2011 started earlier in Tokyo (March 18) than in Ibaraki (April 12). The
163 mean date for the start of nest building in 2011 was April 21 (range April 13–April 25, n
164 = 10) in Ibaraki and April 12 (range March 30–April 21, n = 7) in Tokyo. Therefore, all
165 nests were started after the accident at the Fukushima Daiichi nuclear power plant and
166 may have been made with materials contaminated by radionuclides (Kinoshita et al.,
167 2011).

168 The best GLM model for the ambient dose rate in nestboxes, selected by model
169 selection, included three explanatory variables: site, year, and their interaction. The dose
170 rate was higher in Ibaraki than in Tokyo, and in Ibaraki, it was higher in 2011 than it
171 was in 2012 (**Table 1, Fig. 1**, GLM; $F_{3,40} = 52.79$, $P < 0.001$). In addition, a second
172 model with $\Delta AIC = 1.39$ included four explanatory variables: site ($0.068 \pm 0.011^*$), year

173 (0.005 $\pm$ 0.014), nest weight (0.00008 $\pm$ 0.0001), and the interaction between year and
174 site (0.058 $\pm$ 0.018*) has substantial support because of $\Delta\text{AIC} < 2$ (Estimate $\pm$ SE, * $P <$
175 0.05; Burnham & Anderson 2002); namely, the ambient dose rate in nests slightly
176 increased with increment of nest weight.

177 Ambient dose rates inside nestboxes were significantly higher than those outside
178 nestboxes in Ibaraki in either 2011 or 2012, although there were no significant
179 differences in Tokyo in both 2011 and 2012 (**Table 2**). The differences between ambient
180 dose rates both inside and outside the nestboxes in 2011 was significantly larger than
181 those in 2012 in Ibaraki (Student t -test; $t_{23} = 4.52$, $P < 0.001$). There was no significant
182 difference between nest weight in Tokyo and in Ibaraki (LM; $F_{1,41} = 2.80$, $P = 0.10$), in
183 either 2011 or 2012 (LM; $F_{1,41} = 0.367$, $P = 0.55$).

184 The best GLM model for the amount of radioactive Cs in each nest included
185 three explanatory variables: nest weight, site, and their interaction. The amount of
186 radioactive Cs in each nest increased with the increase in nest weight, and the increment
187 was higher in Ibaraki than in Tokyo (**Table 3, Fig. 2**, GLM; $F_{3,15} = 41.87$, $P < 0.001$).
188 There were no other models with $\Delta\text{AIC} < 2$ that indicated a substantial support
189 (Burnham & Anderson, 2002).

190

191

192

193 **Table 1**

194 Parameter estimates of the selected best GLM model according to ΔAIC (see Results).
 195 The model explaining the variation in ambient dose rate in nestboxes of Eurasian Tree
 196 Sparrows included three explanatory variables: year (2011, 2012), site (Ibaraki, Tokyo),
 197 and their interaction. Other variables (nest weight and other interactions) were not
 198 included in the best model.

199	Explanatory Variables	Estimate	SE	<i>t</i> -value	<i>P</i>
200	Intercept	0.102	0.008	12.96	<0.001
201	Site_Ibaraki	0.071	0.011	6.75	<0.001
202	Year_2011	0.008	0.013	0.62	0.54
203	Site:Year	0.055	0.017	3.19	0.003

204

205 **Table 2**

206 Ambient dose rate ($\mu\text{Gy/h}$) inside and outside the nestboxes where Eurasian Tree
 207 Sparrows laid eggs in Ibaraki and Tokyo in 2011 and 2012.

		Ambient dose rate ($\mu\text{Gy/h}$)										
		Nest mass (g wet)			Inside		Outside		Differences		Paired <i>t</i> -test	
		Mean	SD	n	Mean	SD	Mean	SD	Mean	SD	<i>t</i>	<i>P</i>
Ibaraki	2011	122	46	10	0.24	0.03	0.16	0.02	0.07	0.03	6.98	< 0.001
	2012	133	36	15	0.17	0.03	0.16	0.02	0.02	0.03	2.21	< 0.05
Tokyo	2011	129	46	7	0.11	0.01	0.13	0.02	-0.02	0.02	-1.59	0.16
	2012	96	35	12	0.10	0.02	0.09	0.01	0.02	0.03	2.18	0.05

208

209 **Table 3**

210 Parameter estimates of the selected best GLM model according to ΔAIC (see Results).

211 The model explaining the variation in the amount of Cesium in the nestboxes (Bq/nest)

212 of Eurasian Tree Sparrows included three explanatory variables: nest weight (g-dry),

213 site (Ibaraki, Tokyo), and their interaction.

214	Explanatory Variables	Estimate	SE	<i>t</i> -value	<i>P</i>
215	Intercept	−204.48	148.91	−1.37	0.19
216	Nest weight	3.64	1.54	2.36	0.032
217	Site_Ibaraki	−238.98	219.20	−1.09	0.29
218	Nest weight:Site	4.86	1.92	2.53	0.023

219

220

221 **Fig. 1.** Median, interquartile interval of ambient dose rate in the nestboxes of Eurasian
 222 Tree Sparrows in 2011 and 2012 in Ibaraki and Tokyo, 175 km and 222 km southwest of
 223 the Fukushima Daiichi nuclear power plant, respectively. Outliers, which lie more than
 224 1.5 times the interquartile range above the third quartile and less than 1.5 times the
 225 interquartile range below the first quartile, are indicated by open dots. Different letters
 226 above box plots indicate a
 227 significant difference
 228 according to GLM analysis
 229 (GLM; $F_{3,40} = 52.79$, $P <$
 230 0.001, see Table 1).

232 **Fig. 2.** The relationship between the
 233 weight of nest materials and the
 234 amount of Cesium in the nestboxes
 235 in Ibaraki and Tokyo in 2012. The
 236 data from Ibaraki are indicated by
 237 filled circles and those from Tokyo
 238 by open circles. Regression lines

239 were fitted to the data from Ibaraki (solid line: $y = 8.50 * \text{nest weight} - 443.46$) and
 240 from Tokyo (broken line: $y = 3.64 * \text{nest weight} - 204.48$) according to GLM analysis

241 (GLM; $F_{3,15} = 41.87$, $P < 0.001$, see Table 3).

242

243 **4. Discussion**

244 In this study, we made three predictions and found support for all of them. First, we
245 observed that the levels of radioactive contamination inside the nestboxes were higher
246 in Ibaraki than in Tokyo. Greater amounts of radioactive Cs have been deposited on the
247 ground surface of Ibaraki than that of Tokyo after the nuclear disaster (Yasunari et al.,
248 2011). Second, we observed that the level of radioactive contamination inside the
249 nestboxes was higher in 2011 directly after the Fukushima nuclear accident than in 2012,
250 but this difference was significant only in Ibaraki. This suggested that in the
251 contaminated area, external doses in the nests were higher in the first breeding season,
252 directly after the Fukushima nuclear accident, than in the later breeding season. Third,
253 we observed a relationship between the amount of radioactive Cs inside the nestboxes
254 and the weight of the nests that had been built inside them during the breeding season
255 with a higher increment at Ibaraki than at Tokyo

256 The effects of elevated levels of radioactivity in nests could be particularly
257 important for offspring raised in contaminated nests. This is because embryos and
258 young animals have a greater number of undifferentiated cells that are more sensitive to
259 the mutagenic effects of radiation, which could cause detrimental phenotypic effects
260 (Jacquet, 1995; ICRP, 2003). The negative effects of radiation on adults may also be

261 exacerbated during reproduction because the immune system of adults may be
262 suppressed during reproduction (Deerenberg et al., 1997; Nordling et al., 1998; Moreno
263 et al., 1999). Therefore, this route of exposure needs to be taken into account in future
264 when considering the effects of chronic exposure to radiation in both parents and their
265 offspring.

266 Whereas we did not estimate exposure rate to the birds and eggs, in our study sites,
267 at least 170 km from the Fukushima Daiichi nuclear power plant, the ambient dose rate
268 inside nests (average 0.10–0.24 $\mu\text{Gy/h}$) is much less than the screening criterion
269 suggested by the ERICA Integrated Approach, namely, a dose rate of 10 $\mu\text{Gy/h}$ above
270 the background radiation level for all ecosystems and organisms (Larsson, 2008).

271 The radiation sensitivity observed in field data on wildlife chronically exposed to
272 ionizing radiation appears to be higher than that of their relatives tested under laboratory
273 or controlled field conditions (Garnier-Laplace et al., 2013). For example, growth rate
274 and asymptotic body mass of nestling Tree Swallows *Iridoprocne bicolor*, which were
275 acutely exposed to 2.7 and 4.5 Gy gamma radiation, were significantly lower than that
276 of the control group, whereas doses of 0.9 Gy had little effect on their growth in the
277 irradiation experiment (Zach and Mayoh, 1984). On the contrary, several researches
278 found negative effects of phenotypes and fitness of Barn Swallows at Chernobyl on
279 comparatively low-level ambient dose rates (c. 3.4 $\mu\text{Gy/h}$) (Møller et al., 2005; Møller
280 and Mousseau, 2006). Although mechanisms to account for this discrepancy remains

281 obscure, in some cases that exposure rate to each individual is substantially higher than
282 ambient dose rate in the habitat, the risk due to radiation exposure of wildlife regarding
283 the ambient dose rate as the exposure rate would be underestimated. Vigilance is
284 necessary around the evacuation zone where a dose rate is more than 10 $\mu\text{Gy/h}$ in the
285 Fukushima Prefecture to ascertain the effects of additional external exposure of ionizing
286 radiation via nest materials on wildlife.

287

288 ***4.1. Conclusion***

289 We have provided the evidence that nesting materials can expose birds and potentially
290 other nesting animals, and that contamination increases with the quantity of nest
291 materials. Further research is required to understand the effects of exposure to elevated
292 radiation levels from nests on animals. While the circumstances of the multiple core
293 meltdowns at the Fukushima Daiichi nuclear power plant are tragic, they also offer a
294 second opportunity (after Chernobyl) to study the effects of ionizing radiation on
295 wildlife.

296

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