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Ecological differences in response of bird species to radioactivity from Chernobyl and Fukushima --Manuscript Draft--

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Abstract:	<p>Organisms differ in their susceptibility to ionizing radiation although the ecological basis for such differences remain poorly understood. We hypothesized that ecological characteristics such as body size, diet, migration and pigments of plumage would predict the impact of radiation on abundance through effects on relative food consumption rates, free radicals and antioxidants. We made 2398 breeding bird censuses and quantified the impact of radiation on abundance at Chernobyl and Fukushima providing statistical replication, but also analyses of interaction effects. The impact of radiation on abundance of birds was stronger in Fukushima than in Chernobyl. Species with small body size and hence relatively high food consumption rates were more negatively impacted. Secondary consumers showed stronger negative effects of radiation on abundance than herbivores, especially in Fukushima. There was no main effect of migration, but migrants were more negatively impacted in Chernobyl, while residents were more negatively impacted in Fukushima. Carotenoid and pheomelanin plumage pigments associated with antioxidant status showed stronger negative effects, especially in Chernobyl compared to Fukushima, while eumelanin coloration that is not related to antioxidant status did not show such an effect. These differences between Chernobyl and Fukushima may reflect differences in duration of exposure, differences in radioactive isotopes and differences in accumulation of mutations.</p>

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28 although the ecological basis for such differences remain poorly
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31 radiation on abundance through effects on relative food consumption rates,
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48

49 **Keywords** Birds Chernobyl Coloration Fukushima Pigments

50 Radiation resistance

51

52

53 **Introduction**

54 There is considerable interspecific variation among organisms in
55 susceptibility to radiation effects (e. g. Asker et al. 2011; Romanovskaya et
56 al. 2002; Dighton et al. 2008; Møller and Mousseau 2013). Why that is the
57 case remains an open question. This may arise from differences in exposure
58 and hence differences in the intensity of natural selection that have resulted
59 in the evolution of mechanisms that sustain or even nullify such effects of
60 ionizing radiation. This interesting scientific question has received little
61 attention despite the fact that many species are tolerant to extreme levels of
62 radiation (review in Møller and Mousseau 2013). A few studies that have
63 attempted to assess interspecific differences in susceptibility to radiation
64 and interspecific differences in their ecological correlates (Møller and
65 Mousseau 2007b, 2011b; Galván et al. 2011, 2014). Ionizing radiation
66 produces free radicals and thus promotes the generation of reactive
67 oxidative species that deplete antioxidant levels in animals (Riley 1994;
68 Ivaniota et al. 1998; Neyfakh et al. 1998). Not surprisingly, interspecific
69 variation in susceptibility to radiation can be linked directly to ecological
70 traits that are associated with high rates of use of antioxidants such as long
71 distance migration, dispersal, allocation of antioxidants to coloration and
72 allocation of antioxidants by mothers to their offspring (Møller and
73 Mousseau 2007b). Field studies of individual birds in the vicinity of
74 Chernobyl have shown increased rates of DNA damage at higher levels of
75 background radiation (Bonisoli-Alquati et al. 2010b), and this effect can be
76 linked directly to an increased level of oxidative stress caused by
77 background radiation (Bonisoli-Alquati et al. 2010a).

78 Among the pigments responsible for integument color, melanins
79 and carotenoids are some of the most prominent and widespread.
80 Phaeomelanin- and carotenoid-based colors share a strong dependence on
81 antioxidant status. Vertebrates synthesize two main forms of melanin:

82 eumelanin and pheomelanin, with the latter producing lighter colors than
83 the former. Glutathione (GSH) levels determine the melanin form that is
84 synthesized, as melanogenesis can either lead to the production of
85 eumelanin when the activity of tyrosinase is high and the ratio of
86 cysteine:dopaquinone is low, or to the production of pheomelanin or even
87 an absence of melanin synthesis under opposite conditions (Ozeki et al.
88 1997; Galván and Alonso-Alvarez 2009). Therefore, eumelanogenesis
89 takes place when the levels of GSH are low and pheomelanogenesis when
90 the levels of GSH are high, implying that pheomelanogenesis proceeds
91 with higher levels of endogenous oxidative stress as compared to
92 eumelanogenesis (Galván and Solano 2009). The evolutionary implication
93 of this mechanism is that species in which natural selection has favored the
94 development of pheomelanic traits may have a decreased capacity to
95 combat oxidative stress as compared to species with eumelanic traits, as the
96 maintenance of high GSH levels as required by pheomelanogenesis might
97 be metabolically costly under adverse environmental conditions that
98 generate oxidative stress and thus consume GSH resources (Galván and
99 Alonso-Alvarez 2009; Galván and Solano 2009).

100 GSH is one of the antioxidants most susceptible to radiation (Bump
101 and Brown 1990; Navarro et al. 1997; Vartanian et al. 2004). Therefore,
102 species that have the molecular basis to produce large amounts of
103 pheomelanin may be more limited in their use of GSH to combat oxidative
104 stress, and may thus be more susceptible to ionizing radiation than species
105 in which melanogenesis has been selected to be directed toward the
106 production of eumelanin (Galván et al. 2011). Adaptation to radiation in
107 radioactively contaminated areas and naturally contaminated areas alike
108 may result in increased glutathione levels and body condition, while
109 oxidative stress and DNA damage has decreased with increasing
110 background radiation (Galván et al. 2014). This apparent adaptation to

111 radiation appears to be linked to larger mounts of pheomelanin and smaller
112 amounts of eumelanin being costly as shown from decreased glutathione
113 levels, increased oxidative stress and DNA damage, and reduced body
114 condition (Galván et al. 2014). Finally pheomelanin has been shown to
115 induce a change towards the production of less pro-oxidant forms of
116 pheomelanin that may help acclimatize birds to radiation exposure (Galván
117 et al. 2014). Indeed levels of glutathione and pheomelanin are
118 significantly correlated (Galván et al. 2014).

119 Here we report the results of analyses of unique data on the
120 abundance of breeding birds in Fukushima, Japan and Chernobyl, Ukraine
121 in relation to background levels of radiation. There has been accumulation
122 of mutations in Chernobyl since 1986 for more than 20 generations for
123 many small birds that typically have very short lifespan (Møller and
124 Mousseau 2011b), while only a couple of generations have passed since the
125 accident in 2011 in Fukushima. Thus, we predicted significant differences
126 in the association between abundance and radiation between Chernobyl and
127 Fukushima for different species of birds implying a significant area by
128 ecological variable interaction.

129 The objectives of this study were to test (1) whether the effect of
130 radiation reduced the abundance of birds; (2) whether this effect differed
131 among species; (3) whether body mass, diet, migration and pigment-based
132 coloration affected the abundance of birds; and (4) whether the effects of
133 these predictors differed between Fukushima and Chernobyl, as predicted
134 from their different history of exposure to radioactive contaminants. Small-
135 sized species have relatively higher metabolism, higher intake rates and
136 hence higher ingestion of radionuclides. Similarly, species at higher trophic
137 levels should suffer more from radioactive contamination as a result of
138 bioaccumulation. Migratory birds through their high metabolic rate and use
139 of antioxidants should differ in impact of radiation compared to residents.

140 Indeed, negative impacts of radiation on abundance are stronger on
141 abundance of migrants than resident species (Møller and Mousseau 2007b).
142 This is contrary to expectation because migrants spend less time in the
143 contaminated breeding areas than residents. However, migrants deplete
144 their stores of antioxidants during migration resulting in very low levels
145 upon arrival (Ninni et al. 2004). This effect is detrimental because breeding
146 birds start establishing territories, nest building and egg formation upon
147 arrival causing antioxidant levels to be severely depleted (Møller et al.
148 2005). Finally, there is a trade-off between allocation of antioxidants to
149 production of feather color and self-maintenance in birds with carotenoid-
150 and pheomelanin-based plumage. Therefore, any individual that allocated a
151 large fraction of antioxidant resources to plumage coloration without
152 consideration of future uses for protection against oxidative stress caused
153 by radiation would be at a selective disadvantage. Likewise, such
154 individuals would also risk increased rates of mutations due to the lack of
155 protection against oxidative stress thus reducing survival prospects.

156

157 **Methods**

158 *Study sites*

159 The breeding bird census points were located at ca. 100 m intervals in
160 forested areas west of the exclusion zone around the Fukushima Daiichi
161 power plants in 2011-2014 (Fig. 1A) or in forested areas within the
162 Chernobyl Exclusion Zone or adjacent areas, or in areas in southern
163 Belarus around Gomel during the breeding seasons 2006-2009 (Fig. 1B).
164 At least one local ornithologist participated in the censuses in Japan to
165 confirm the identity of some difficult bird species.

166

167 *Census methods*

168 We adopted the point count census method, which provides reliable
169 information on relative abundance of birds (Blondel et al. 1970; Møller
170 1983; Bibby et al. 2005; Voříšek et al. 2010). The method is based on an
171 observer recording for a period of five minutes all birds seen and heard.
172 Extensive national monitoring programs for breeding and wintering birds
173 based on point counts occur in many different countries, and this effort is
174 part of environmental monitoring by the European Union (Voříšek et al.
175 2010). This method has provided highly repeatable results for birds and
176 other animals at Chernobyl (Møller and Mousseau 2011a). It consists of
177 counts lasting 5 minutes during which the number of birds seen or heard is
178 recorded. APM conducted these standard point counts during 29 May – 9
179 June 2006, 1 – 11 June 2007, 29 May – 5 June 2008, 1 – 6 June 2009 in the
180 surroundings of Chernobyl (898 census points) and during 11 – 15 July
181 2011, 14-19 2012, 14-19 2013 and 11-16 2014 in Fukushima (1500 census
182 points). Thus one single five minutes count was recorded for each point in
183 each of the study years. The fact that one person made all counts eliminates
184 any variance in results due to inter-observer variability. There are no bird
185 census data from Chernobyl or Fukushima before the accidents, nor have
186 other scientists to the best of our knowledge conducted bird censuses
187 comparable to ours in the years following the accidents.

188 We directly tested the reliability of our counts by letting two persons
189 independently perform counts, and the degree of consistency was high for
190 both species richness, total abundance and abundance of individual species
191 (details reported by Møller and Mousseau 2007a for Chernobyl; similar
192 results exist for Fukushima (A. P. Møller, I. Nishiumi and T. A. Mousseau
193 unpublished data)).

194 Abundance estimates can be affected by numerous confounding
195 variables (Voříšek et al. 2010), and, therefore, it is important to control
196 such variables statistically to assess the underlying relationship between

197 radiation and species richness and abundance. We classified habitats
198 (agricultural habitats with grassland or shrub, deciduous forest, or
199 coniferous forest) and estimated to the nearest 10% ground cover by herbs,
200 shrub, trees, agricultural habitat, deciduous forest and coniferous forest
201 within a distance of 50 m from the census points. We recorded altitude to
202 the nearest foot, using GPS. Weather conditions can affect animal activity
203 and hence census results (Voříšek et al. 2010), and we recorded cloud
204 cover at the start of each point count (to the nearest eighth), temperature
205 (degrees Celsius), and wind force (Beaufort). For each census point we
206 recorded time of day when the count was started (to the nearest minute).
207 Because activity may show a curvilinear relationship with time of day, with
208 high levels of bird activity in the morning and to a lesser extent in the
209 evening (Voříšek et al. 2010), we also included time squared as an
210 explanatory variable.

211

212 *Background radiation*

213 We measured radiation in the field and cross-validated these measurements
214 with those reported by the Ukrainian Ministry of Emergencies. Once
215 having finished the 5 minutes census we measured α , β , and γ radiation
216 levels at ground level directly in the field at each point where were
217 censused invertebrates using a hand-held dosimeter (Model: Inspector, SE
218 International, Inc., Summertown, TN, USA). We measured levels two-three
219 times at each site and averaged the results. We cross-validated our
220 measurements in Ukraine against data from the governmental
221 measurements published by Shestopalov (1996), estimated as the mid-point
222 of the ranges published. This analysis revealed a very strong positive
223 relationship (linear regression on log-log transformed data: $F = 1546.49$, df
224 $= 1, 252$, $r^2 = 0.86$, $P < 0.0001$, slope (SE) = 1.28 (0.10)), suggesting that

225 our field estimates of radiation provided reliable measurements of levels of
226 radiation among sites.

227 In Fukushima we used the same dosimeters, and the measurements
228 were cross-validated with readings with a dosimeter that had been recently
229 calibrated and certified to be accurate by the factory (International
230 Medcom, Sebastopol, CA, USA). We cross-validated tests at Fukushima by
231 comparing our own measurements with measurements obtained at the same
232 locations with a TCS 171-ALOKA used by Japanese authorities. Again,
233 there was a very strong positive relationship (linear regression on log-log
234 transformed data: $F = 2427.97$, $df = 1, 20$, $r^2 = 0.99$, $P < 0.0001$, slope (SE)
235 = 1.120 (0.023)). We have made extensive measurements of internal dose
236 in hundreds of birds in Chernobyl and found very strong positive
237 correlations between internal dose and background radiation level (A. P.
238 Møller and T. A. Mousseau unpublished manuscript).

239

240 *Ecological variables*

241 Body mass

242 We extracted mean body mass of males and females during the breeding
243 season from Cramp and Simmons (1977-1994), Lislevand et al. (2007) and
244 del Hoyo et al. (1995-2011). Body mass was estimated as the mean value
245 of the means for males and females.

246

247 Diet

248 We scored the diet of different species on a two level classification based
249 on information in Cramp and Simmons (1977-1994) and del Hoyo et al.
250 (1995-2011): (0) mainly plant material, especially outside the breeding
251 season; and (1) primary predators eating herbivores such as insects and
252 spiders, but also vertebrates.

253

254 Migration distance

255 We estimated migration distance as the difference in latitude between the
256 mean of the northernmost and the southernmost breeding distribution and
257 the mean of the northernmost and the southernmost winter distribution,
258 relying on information in Cramp and Perrins (1977-1994) and del Hoyo et
259 al. (1995-2011). A strict classification of species as either residents or
260 migrants resulted in similar conclusions (unpubl. data)).

261

262 Carotenoid-based coloration

263 We only considered pigment-based coloration in this study because we did
264 not have information on structural color for the species considered. The
265 extent of plumage patches colored by carotenoids was determined by
266 examining illustrations of adult breeding birds in Cramp and Simmons
267 (1977-1994) and Wild Bird Society of Japan (1982). We considered colors
268 that were yellow, orange and red to be caused by carotenoids (Tella et al.
269 2004; Olson and Owens 2005). We assigned scores that ranged from 0
270 (total lack of carotenoid-based color) to 5 (all carotenoid-based).

271

272 Melanin-based coloration

273 We used a method for assigning coloration to melanin developed by
274 (Galván et al. 2011). Information on melanin-based plumage coloration
275 was obtained by examining illustrations of bird species censused in
276 Chernobyl and Fukushima in Cramp and Simmons (1977-1994) and Wild
277 Bird Society of Japan (1982). Eumelanic and pheomelanic traits are
278 generally of distinctive colors, the former being responsible for black and
279 grey colors and the latter for yellowish, reddish, chestnut and brown colors
280 (Toral et al. 2008). Eumelanin and pheomelanin normally occur
281 simultaneously in tissues (Ozeki et al. 1997), but the darker colors
282 conferred by eumelanin (Toral et al. 2008) make evident the lower content

283 of this pigment in chestnut and brown as compared to black and grey colors
284 (Galván and Alonso-Alvarez 2009). Furthermore, many bird species have
285 feather melanin contents of high purity ($> 90\%$ of either eumelanin or
286 pheomelanin; McGraw and Wakamatsu 2004; J. J. Negro pers. com.).
287 Therefore, we considered that black and grey plumage colors were
288 predominantly generated by eumelanin, while chestnut and brown colors
289 were predominantly generated by pheomelanin. We did not consider
290 conspicuous yellow or red colorations assumed to be generated by other
291 pigments (i.e. carotenoids), unless chemically identified as melanin-based
292 by Toral et al. (2008). Although a rough approximation to the real
293 proportion of eumelanic and pheomelanic plumage, the assumption that
294 black-grey colors are eumelanic and brown-chestnut colors are
295 pheomelanic should be adequate for comparative purposes (Owens and
296 Hartley 1998). Thus, we quantified the proportion of melanic plumage
297 parts by examining illustrations in Cramp and Simmons (1977-1994) and
298 and Wild Bird Society of Japan (1982). Illustrations of both resting and
299 flying adult birds in breeding plumage were examined. The method used by
300 Beauchamp and Heeb (2001) and Galván (2008) was followed to obtain
301 estimates of the proportion of eu- and pheomelanic color present in the
302 plumage of each species, assigning scores that ranged from 0 (total lack of
303 melanic color) to 5 (all melanic). When a species was sexually dichromatic
304 regarding melanin-based coloration, eumelanic and pheomelanic scores
305 were the average obtained for males and females. When a species had
306 different subspecies or color morphs differing in extent or type of melanin-
307 based coloration, we used the nominate subspecies or the most common
308 morph, respectively. It must be noted that eu- and pheomelanic color
309 patches can coexist in the same feathers, and thus the sum of both color
310 scores, in a species that presents both color types, is not always necessarily
311 5, but higher values are also possible.

312 All interspecific data are reported in Appendix 1 in the electronic
313 supplementary material.

314

315 *Statistical analyses*

316 Body mass, migration distance and radiation level were \log_{10} -transformed
317 and coverage with agricultural land, herbs, shrub and trees, deciduous
318 forest, coniferous forest and cloud cover were square-root arcsine-
319 transformed before analyses.

320 We quantified the relationship between abundance of different
321 species and level of radiation by estimating the Kendall rank-order
322 correlation for the relationship between abundance and \log_{10} -transformed
323 radiation while including all potentially confounding variables in the
324 statistical models (coverage by herbs, shrub, trees, agricultural habitat,
325 deciduous forest and coniferous forest, altitude, cloud cover, temperature,
326 wind force, time of day and time of day squared). We used non-parametric
327 rank order correlations because the assumptions of parametric analyses
328 were violated. We have previously shown that statistical control for the
329 confounding variables listed above did not affect the conclusions of
330 analyses (Møller and Mousseau 2011a). Therefore, we used the Kendall
331 rank-order correlations from analyses of abundance in relation to radiation
332 rather than partial rank-order correlations in the subsequent analysis. In this
333 model we included slope as the response variable and body mass, diet,
334 migration distance and area and the two-way interactions between body
335 mass, diet, migration distance and area as predictors. We did not include
336 other interactions because there were no a priori predictions.

337 It is a common underlying assumption of most statistical analyses
338 that each data point provides equally precise information about the
339 deterministic part of total process variation, i.e. the standard deviation of
340 the error term is constant over all values of the predictor variable(s) (Sokal

341 and Rohlf 1995). Because estimates of slopes depend on sample sizes, and
342 because sample sizes vary considerably among bird species, such variation
343 can have serious consequences for conclusions (Garamszegi and Møller
344 2010, 2011). The standard solution to this problem is to weight each
345 observation by sampling effort in order to use all data relative to their
346 importance, by giving each datum a weight that reflects its degree of
347 precision due to sampling effort (Draper and Smith 1981; Neter et al. 1996;
348 Garamszegi and Møller 2010). Therefore, we weighted the statistical model
349 by sample size. All analyses were made with JMP (SAS 2012).

350

351 **Results**

352 We investigated the impact of low-dose radiation on the abundance of 154
353 species of birds using 2398 standardized point counts, which resulted in
354 records of 15,811 individual birds. A total of 14 species are common to
355 Chernobyl and Fukushima allowing for comparison of the effect of
356 radiation on abundance of the same species in two different environments
357 (Møller et al. 2012a). The three species showing the strongest negative
358 correlated with level of background radiation level in Chernobyl were
359 yellowhammer *Emberiza citrinella*, chaffinch *Fringilla coelebs* and
360 blackcap *Sylvia atricapilla*. In Fukushima they were tree sparrow *Passer*
361 *montanus*, carrion crow *Corvus corone* and barn swallow *Hirundo rustica*.
362 The mean Kendall rank-order correlation between abundance and radiation
363 was -0.109 (SE = 0.012), $N = 154$ species, differing significantly from the
364 expected value zero in the absence of any effect of radiation ($t = 9.18$, $df =$
365 153 , $P < 0.0001$). The mean correlation was -0.047 (0.008) in Chernobyl,
366 but almost three-fold stronger at -0.150 (SE = 0.022) in Fukushima, with
367 both the mean and the variance being significantly larger in Fukushima
368 than in Chernobyl (Welch ANOVA: $F = 19.04$, $df = 1$, 11735, $P < 0.0001$;
369 Levene's test, $F = 21.73$, $df = 1$, 152, $P < 0.0001$).

370 The statistical model explaining variation in the correlations
371 between abundance and background radiation for different species
372 accounted for 64% of the variance (Table 1). Five out of seven main effects
373 were significant as were five out of six interaction effects between area and
374 ecological variables (Table 1). The correlations between abundance and
375 radiation level were more negative in species with smaller body mass, and
376 this effect differed significantly between Chernobyl and Fukushima as
377 shown by the significant interaction (Fig. 2; Table 1). Carnivorous species
378 showed more strongly negative correlations than herbivorous species and
379 this effect differed between areas with a larger difference in Fukushima
380 than in Chernobyl (Fig. 3; Table 1). Migration distance did not show a
381 significant main effect, but the migration effect differed between
382 Chernobyl and Fukushima with a stronger negative effect in migrants in
383 Chernobyl, and a stronger negative effect in residents in Fukushima (Fig. 4;
384 Table 1).

385 Plumage color pigments showed different relationships with impact
386 of radiation on abundance. Species with carotenoid-based coloration
387 showed a weaker negative effect than species without carotenoid-based
388 coloration (Table 1). The interaction between carotenoids and area was
389 significant with a stronger negative effect in species with than without
390 carotenoid-based plumage in Chernobyl, and a weaker negative effect in
391 species with than without such plumage in Fukushima (Fig. 5A). There was
392 only a weak and non-significant main effect of eumelanin and no
393 significant effect of the interaction (Fig. 5B, Table 1). There was no
394 significant main effect of pheomelanin (Table 1). A strong interaction
395 between pheomelanin and area implies that in Chernobyl species with
396 pheomelanin-based coloration experienced stronger negative effects of
397 radiation than species without such coloration, while in Fukushima species

398 with pheomelanin showed weaker negative effects than species without
399 pheomelanin in their plumage (Fig. 5C).

400

401 **Discussion**

402 The main findings of this extensive study of breeding birds at Chernobyl
403 and Fukushima were a negative impact of ionizing radiation on abundance
404 and a number of ecological characteristics explaining variation in
405 heterogeneity of this effect. We found significant main effects of body
406 mass, diet, migration and feather pigments on the impact of radiation on
407 abundance. Importantly, there were significant interactions between area
408 and ecological characteristics, implying that the effects of radiation were
409 not the same at Chernobyl and Fukushima. We will briefly discuss these
410 findings.

411 Body size is an important correlate of life history, physiology and
412 ecology (Bennett and Owens 2002). Small species eat disproportionately
413 more for their body size than large species due to their surface to volume
414 ratio. Therefore, we should expect small species to ingest
415 disproportionately more radionuclides than large species. Among bird
416 species in Fukushima there was a stronger negative relationship between
417 abundance and radiation in small-bodied species than among large species,
418 and a similar relationship was found in Chernobyl with a significant
419 interaction between radiation and area. Higher metabolism and higher rates
420 of ingestion by small species should also result in greater production of free
421 radicals and hence greater competition for anti-oxidants between use for
422 metabolism and use due to radiation exposure.

423 Diet and its interaction with study area was a significant predictor of
424 impact of radiation on abundance. We predicted and found an effect of
425 bioaccumulation of radionuclides in the food web (Voitovich and Afonin
426 2002; Yakushev et al. 1999). Animals at higher trophic levels generally

427 have higher levels of radionuclide concentrations than animals at lower
428 levels (e. g. Kryshev and Ryabov 1990; Kryshev et al. 1992; Smith et al.
429 2002). Here we found a stronger negative effect in secondary consumers
430 compared to herbivores, and the interaction between diet and area was
431 statistically significant, with a stronger effect in Fukushima compared to
432 Chernobyl.

433 Long-distance migration involves high metabolic effort and
434 extensive use of antioxidants. Møller and Mousseau (2007b) have
435 previously reported for birds from Chernobyl that species with longer
436 migrations had lower abundance at high radiation levels. Here we were
437 unable to document a significant main effect of migration on the impact of
438 radiation on abundance. However, we did show a stronger negative impact
439 of radiation on migrants in Chernobyl, but on residents in Fukushima. The
440 Japanese and the European avifaunas differ in their migration distance
441 because more than twice as many species of birds at Fukushima were
442 residents (54% of 57 species) as the species at Chernobyl (21% of 97
443 species). Furthermore, Western Palearctic species migrate longer distances
444 and cross more inhospitable deserts than Eastern Palearctic migrants, while
445 Japanese migrants have the possibility to stop at any of the many
446 archipelagoes during migration. Thus it appears that it is whether a species
447 is migrating or not rather than the actual migration distance that is the
448 important predictor of the effects of radiation on abundance.

449 The slope of the relationship between abundance and radiation was
450 significantly related to whether plumage color was based on carotenoids
451 and pheomelanin. In Fukushima, species without carotenoids in their
452 plumage had lower abundance at high radiation levels, while that was not
453 the case for species with carotenoid-based plumage color. We have
454 previously reported for Chernobyl that species with carotenoid-based
455 plumage are more strongly negatively impacted (Møller and Mousseau

2007b) as we report here. The slope of the relationship between abundance and background radiation differed between species with and without plumage containing carotenoids and phaeomelanin, but not eumelanin, as we a priori had predicted. The two former kinds of pigment-based coloration impose a trade-off between antioxidant use for maintenance caused by radiation and use of antioxidant pigments allocated to plumage (Møller and Mousseau 2007b; Galván et al. 2011). Interestingly, these effects differed between Fukushima and Chernobyl for carotenoid- and phaeomelanin-based coloration, but not for eumelanin coloration. The main difference between Fukushima and Chernobyl is the duration of chronic exposure to radiation (3 versus 28 years), the duration of accumulation of deleterious mutations (Møller and Mousseau 2011b) and differences in composition of radionuclides (cesium in Fukushima and strontium, cesium and plutonium in Chernobyl). Galván et al. (2014) have previously suggested and provided evidence for pheomelanin playing an active role in resistance to radiation.

We have previously investigated the importance of similarity in effect of ionizing radiation on abundance due to common phylogenetic descent, showing that conclusions are independent of phylogeny (Møller and Mousseau 2007b; Galván et al. 2011, 2014). Hence this justifies the absence of phylogenetic analyses in the present study.

There may be many causes for differences in population trends of birds. Here we have attempted to control statistically for a number of such factors. We have previously shown that a habitat preference for human proximity and other factors did not account for differences in population trends among species (Møller et al. 2012a, b).

In conclusion, we have provided extensive information on the impact of radiation on the abundance of breeding birds at Chernobyl and Fukushima, and the ecological characteristics associated with such impacts

485 generally confirmed findings from previous studies at Chernobyl, although
486 most characteristics showed significant differences between Chernobyl and
487 Fukushima. This level of replication suggests that birds may constitute a
488 useful model system for investigating the impact of radiation and the
489 evolution of radiation-resistance in vertebrates.

490

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500

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651
652

653 **Legends to figures**

654

655 **Fig. 1** Approximate location of census areas around Fukushima,
656 Japan indicated by dark blue dots (A) and Chernobyl, Ukraine and Belarus
657 indicated by light blue dots (B). Note that levels of radiation have only
658 been recorded at a limited number of sites

659

660 **Fig. 2** Slope of the relationship between abundance and background
661 radiation in relation to body mass (g) in different species of birds

662

663 **Fig. 3** Slope of the relationship between abundance and background
664 radiation in relation to diet in different species of birds

665

666 **Fig. 4** Slope of the relationship between abundance and background
667 radiation in relation to migration in different species of birds

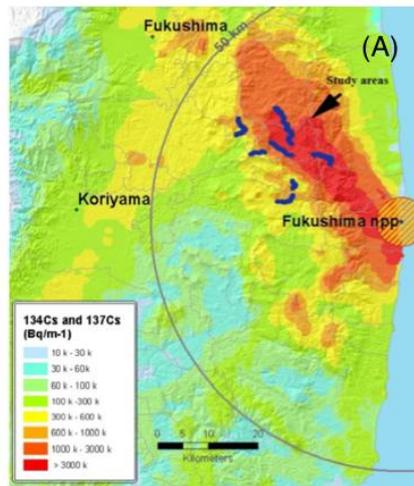
668

669 **Fig. 5** Slope of the relationship between abundance and background
670 radiation in relation to plumage color based on (A) carotenoids, (B)
671 eumelanin and (C) pheomelanin in different species of birds

672

673

674 Fig. 1



675

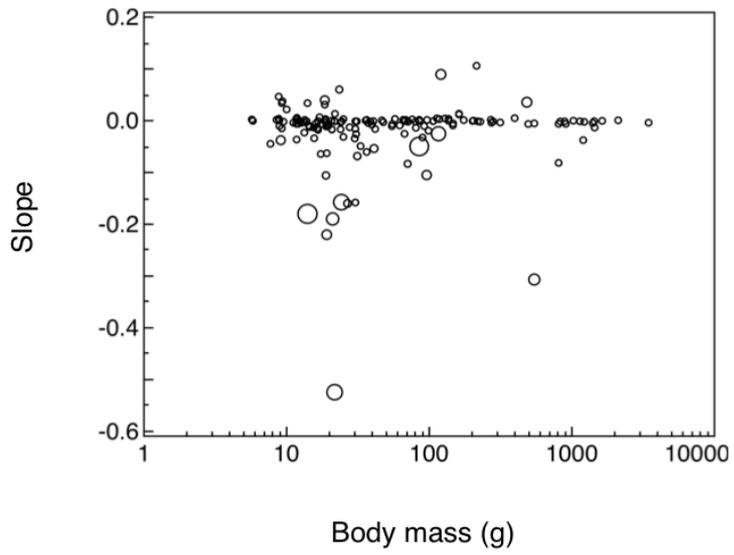


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678 Fig. 2

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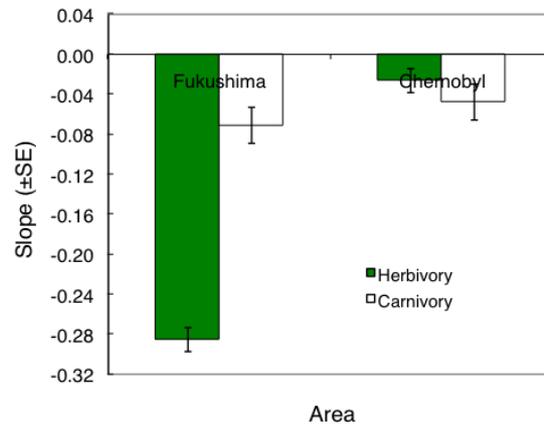


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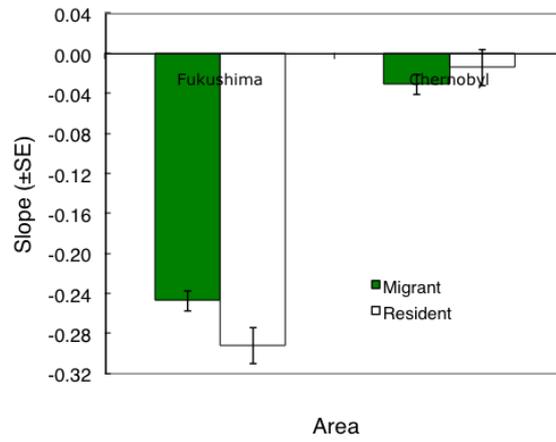
683 Fig. 3



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686 Fig. 4



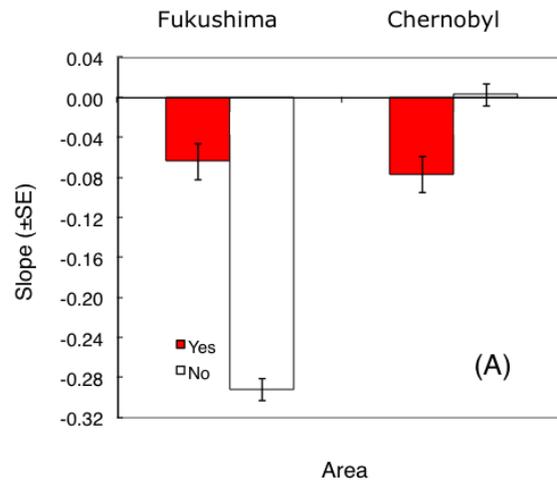
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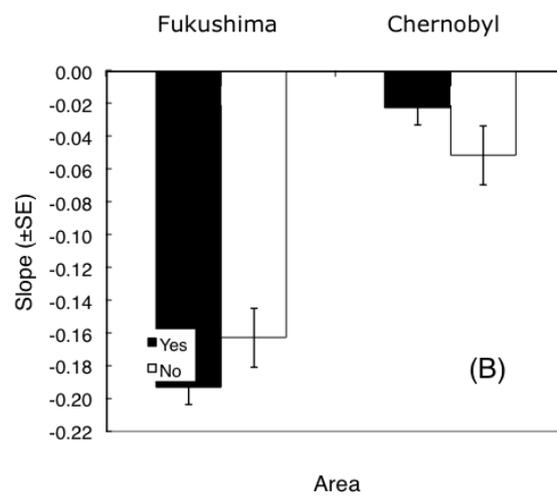
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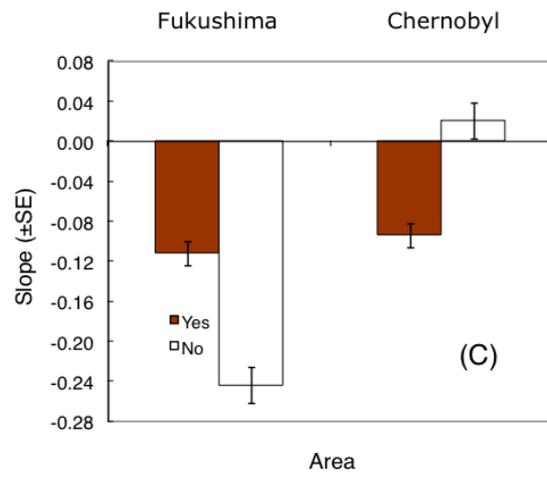
691 Fig. 5



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693



695 **Table 1** Model of slope of the relationship between abundance and level of background radiation in relation to
 696 body mass (g), diet (0 – herbivory, 1 – carnivory), migration distance (° latitude), carotenoid score, eumelanin
 697 score, pheomelanin score, area (Chernobyl, Fukushima), area x body mass, area x diet, area x migration distance,
 698 area x carotenoids, area x eumelanin, area x pheomelanin in a model weighted by sample size. The model had the
 699 statistics $F = 22.16$, $df = 13, 140$, $r^2 = 0.64$, $P < 0.0001$

Source	<i>df</i>	Sum of squares	<i>F</i>	<i>P</i>	Estimate	Error
Intercept				< 0.0001	-0.267	0.050
Body mass	1	17.330	21.85	< 0.0001	0.099	0.021
Diet	1	10.303	10.30	0.0004	-0.045	0.012
Migration distance	1	0.237	0.30	0.59	-0.008	0.014
Carotenoids	1	10.821	13.65	0.0003	0.034	0.009
Eumelanin	1	3.019	3.81	0.053	-0.016	0.008
Pheomelanin	1	0.201	0.25	0.62	0.004	0.007
Area	1	22.620	22.62	< 0.0001	0.073	0.014
Area x Body mass	1	10.361	13.07	0.0004	-0.076	0.021
Area x Diet	1	15.959	20.12	< 0.0001	0.056	0.012

Area x Migration distance	1	12.148	15.31	< 0.0001	0.055	0.014
Area x Carotenoids	1	25.771	32.50	< 0.0001	-0.052	0.009
Area x Eumelanin	1	0.302	0.38	0.54	-0.016	0.008
Area x Pheomelanin	1	22.583	28.48	< 0.0001	-0.038	0.007
Error	153	111.023				

Electronic Supplementary Material

[Click here to download Electronic Supplementary Material: ESM.doc](#)

Dear Erik,

Thank you for your email. We have made all the changes that you suggested. Please find enclosed this revised version.

All the best wishes,

Anders