Cumulative effects on abundance and biodiversity of birds by radioactivity from Fukushima

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Abstract Species differ in their susceptibility to radiation through differences in their ability to sustain toxic and genetic effects caused by radiation. We censused breeding birds in Fukushima Prefecture, Japan, during 2011-2014 to test if the abundance and diversity of birds became increasingly negatively affected by radiation over time. The abundance of birds decreased with increasing level of background radiation, with significant interspecific variation. Even though levels of background radiation decreased over time, the relationship between abundance and radiation became more negative over time. The relationship between abundance and radiation became less negative with increasing trophic level. These findings are consistent with the hypothesis that the negative effects of radiation on abundance and species richness accumulate over time.

Keywords Birds Chernobyl Fukushima Radiation resistance

Introduction

Radiation has been demonstrated to have diverse negative effects on animals and plants under natural conditions (Møller and Mousseau 2011b). There are highly radiation-resistant bacteria and fungi that are able to cope with extreme natural levels of radiation (Dadachova et al. 2004; Daly 2009), but such organisms seem to be exceptions rather than the rule. While natural levels of radiation vary worldwide with significant effects on incidence of disease in humans (e. g. Lubin and Boice 1997; Hendry et al. 2009), and by implication also in other organisms (Møller and Mousseau 2011b), there are significant effects of radiation on DNA damage and mutations (e. g. Ghiassi-Nejad et al. 2004) since the original observation that radiation was a powerful mutagen (Nadson and Philippov 1925). There has been a recent increasing interest on the effects of low-dose radiation on public health, but also on free-living organisms following the nuclear accidents at Chernobyl and Fukushima.

Acute exposure to radiation has negative effects on a number of physiological processes such as oxidative stress and immune function, while chronic exposure across extended periods of time can result in severe accumulation of effects of mutations during the lifespan of individuals, but also across generations. Current studies generally conducted under lab conditions typically rely on acute exposure, while the chronic effects of extended exposure are rarely considered. We have previously shown that the abundance and the diversity of birds and other organisms at Chernobyl are more strongly negatively impacted by a given level of radiation than by similar levels of exposure at Fukushima (Møller et al. 2012). Thus it is not the difference in the actual levels of radiation in Chernobyl and Fukushima that is the cause since effects were quantified as effects per unit of background radiation. Some studies suggest that the negative impact of radiation may become ameliorated over time due to adaptation in terms of improved DNA repair (Boubriak et al. 2008). The stronger negative effects at Chernobyl than at Fukushima that we have previously documented (Møller et al. 2012) may be associated with a longer history of exposure, although alternative explanations such as differences in radionuclides and their toxicity may also play a role.

Here we report the results of analyses of unique data on the abundance of breeding birds in Fukushima, Japan, in relation to background level of radiation during the period 2011-2014. This period covers the radiation effects just after the Fukushima accident, but also the subsequent chronic effects accumulated during the following three years. This is the first study quantifying such cumulative temporal effects of radiation over time.

The objectives of this study were to test (1) whether the effect of radiation reduced the abundance and the species richness of birds; (2) whether this effect differed among species; and (3) whether such negative effects of radiation accumulated over time. Ionizing radiation at Chernobyl and Fukushima has negative effects on abundance and species richness of birds and other organisms (Møller and Mousseau 2007a, b; Møller et al. 2011a, b, 2012). Such effects vary considerably among species due to differences in physiology and ecology (Møller and Mousseau 2007b; Galván et al. 2011, 2014). Unfortunately, there are no population studies or studies of a broad range of species dating back to the period prior to the accidents at Chernobyl in 1986 or Fukushima in 2011. We conducted the first standardized counts of breeding birds in 2006 in Chernobyl 20 years after the accident. We started bird counts in Fukushima already in 2011 allowing for tests of effects of ionizing radiation directly from the start of radiation exposure. Here we present analyses of these unique data of the effects of radiation since 2011 at Fukushima in an attempt to determine whether such negative effects of radiation on abundance and diversity of birds accumulate over time.

Methods

Study sites

We conduced breeding bird censuses at a total of 400 sampling points (in 2011 only 300 points) in forested areas around the Fukushima Daiichi power plants in 2011-2014 totalling 1500 sampling events (Fig. 1). At least one local ornithologist (Satoe Kasahara, Shin Matsui, Isao Nishiumi or Keisuke Ueda) participated in approximately a quarter of the censuses in Japan to confirm the identity of some difficult bird species. However, all analyses presented here were based on the data recorded by APM. All sampling sites were localized using GPS coordinates and altitude was estimated to the nearest foot using a GPS.

Census methods

The point count census method provides reliable information on relative abundance of birds (Blondel et al. 1970; Møller 1983; Bibby et al. 2005; Voříšek et al. 2010). This method has provided highly repeatable results for birds and other animals at Chernobyl (Møller and Mousseau 2011a). It consists of counts lasting 5 minutes during which the number of birds seen or heard was recorded. Each census point was separated by the previous point by a minimum distance of 100 m. In Fukushima APM with the help of IN conducted these standard point counts on 11 – 15 July 2011, 14-19 2012, 14-19 2013 and 11-16 2014 in Fukushima. The fact that one person made all counts analyzed here eliminates any variance in results due to inter-observer variability.

We directly tested the reliability of our counts by letting two persons independently perform counts, and the degree of consistency was high for both species richness and abundance (details reported by Møller and Mousseau 2007a). The Pearson product–moment correlation between species richness in two series of counts conducted by two different persons was $r = 0.99$, $t = 42.06$, df = 8, $P < 0.0001$, and for abundance it was equally high at $r = 0.99$, $t = 12.47$, df = 8, $P < 0.0001$).

Abundance estimates can be affected by numerous confounding variables (Voříšek et al. 2010), and, therefore, it is important to control such variables statistically to assess the underlying relationship between radiation and species richness and abundance. We classified habitats in the field directly at the census points immediately following the five minutes count (agricultural habitats with grassland or shrub [either currently or previously cultivated], deciduous forest, or coniferous forest) and estimated to the nearest 10% ground coverage by herbs, shrub, trees, agricultural habitat, deciduous forest and coniferous forest within a distance of 50 m from the census points. Weather conditions can affect animal activity and hence census results (Voříšek et al. 2010), and we recorded cloud cover at the start of each point count (to the nearest eighth), temperature (degrees Celsius), and wind force (Beaufort). For each census point we recorded time of day when the count was started (to the nearest minute). Our bird counts were concentrated in the morning with counts extending across the entire day depending on other research activities. Because bird activity may show a curvilinear relationship with time of day, for example, with high levels of activity in the morning and to a lesser extent in the evening for bird (Voříšek et al. 2010), we also included time squared as an explanatory variable in the statistical analyses.

Background radiation

Radiation measurements at Fukushima were obtained using the same dosimeters (Model: Inspector, SE International, Inc., Summertown, TN, USA) cross-validated with readings from a dosimeter that had been calibrated and certified to be accurate by the factory during the weeks preceding the study (International Medcom, Sebastopol, CA, USA). All radiation measurements were made at the census points immediately after each bird count. We also made a cross-validation test by comparing our own measurements using the Inspector dosimeter with measurements obtained at the same locations with a TCS 171-ALOKA used by Japanese authorities. There was a very strong positive relationship (linear regression on log-log transformed data: $F = 2427.97$, $df = 1, 20$, $r^2 = 0.99$, $P < 0.0001$, slope (SE) = 1.120 (0.023)). All data are reported in Electronic Supplementary Material Table S1.

Diet

We scored the species as herbivores if they mainly fed on foliage or seeds, primary consumers if they mainly fed on insects, spiders and other invertebrates and as top consumers if they mainly fed on vertebrates, relying on information presented in del Hoyo et al. (1992-2011).

Statistical analyses

Radiation levels were $\log_{10}$-transformed and coverage with agricultural land, herbs, shrub and trees, deciduous forest, coniferous forest and cloud cover were square-root arcsine-transformed before analyses.

We quantified the relationship between abundance of different bird species and level of radiation by estimating the slope of the relationship between abundance and $\log_{10}$-transformed radiation while including potentially confounding variables in the statistical models (coverage by herbs, shrub, trees, agricultural habitat, deciduous forest and coniferous forest, altitude, cloud cover, temperature, wind force, time of day and time of day squared). The resulting species-specific partial slopes for bird abundance with radiation were used for subsequent analyses. This approach is extremely conservative because it reduces the total counts of the entire study to a single estimate per species, or in some analyses a single estimate per species and year. We quantified species richness as the total number of species recorded at a given observation point under the assumption that such estimates will be distributed randomly across radiation levels if there were no negative effects of radiation on species richness.

A common underlying assumption of most statistical analyses is that each data point provides equally precise information about the deterministic part of total process variation, i.e. the standard deviation of the error term is constant over all values of the predictor variable(s) (Sokal and Rohlf 1995). Because estimates of slopes depend on sample sizes, and because sample sizes vary considerably among species, this can have serious consequences for conclusions (Garamzsegi and Møller 2010, 2011). The standard solution to violations of this assumption is to weigh each observation by sampling effort in order to use all data, by giving each datum a weight that reflects its degree of precision due to sampling effort (Draper and Smith 1981; Neter et al. 1996; Garamszegi and Møller 2010). Therefore, we weighted statistical models by sample size in order to use all data in relation to the precision of the estimates. Even a single observation of a species was included in the analyses because such an observation could theoretically be recorded at any of the observation points with a single point having an observation of 1 and all other points an observation of 0. The null hypothesis is that the slope in this case will be zero, while non-random locations of observations will be concentrated at low radiation levels if radiation has a negative effect on the presence and the abundance of birds.

We tested whether the abundance of species was related to level of radiation, species and the interaction between radiation and species. Next we tested if there was a temporal trend in slope of the relationship between abundance and background radiation under the prediction that the extent of negative effects would accumulate across years. Finally, we tested whether the slope of the relationship between abundance and radiation differed among categories of main diet: herbivory or carnivory, or herbivores, primary consumers or top consumers. All standard least squares analyses or in case of data that were not normally distributed non-parametric tests were made with JMP (SAS 2012).

Results

Tests for interspecific differences in effect of radiation on abundance

The number of individual birds of the 57 different species recorded at Fukushima ranged from 1 to 1715, mean (SE) = 166.7 (48.6), median = 22 individuals.

There was a significant decline in level of background radiation across years (Fig. 2; $F = 1736.70$, $df = 3, 1097$, $P < 0.0001$) with additional variation among census points ($F = 43.53$, $df = 399, 1097$, $P < 0.0001$).

The abundance of birds at Fukushima differed significantly among species, decreased with increasing level of background radiation, and this decrease differed among species as reflected by the significant species by radiation interaction (Table 1). There was a strong negative relationship between species richness and radiation level across the census points (Fig. 3A; $F = 6.73$, $df = 1, 1495$, $P < 0.0001$), and there was an equally strong negative relationship between the total number of individuals and radiation level across the census points (Fig. 3B; $F = 18.11$, $df = 1, 1495$, $P < 0.0001$).

The slope of the relationship between abundance and background radiation for 57 different species of birds ranged from -0.525 to +0.107, mean (SE) = -0.162 (0.023) (Table 2). The mean slope differed significantly from zero in an analysis weighted by sample size (one-sample t-test, $t = -7.16$, $df = 56$, $P < 0.0001$). This implies that species were on average less abundant at high levels of radiation.

Differences in slopes among years

Slopes of the relationship between abundance and radiation differed among years in an analysis weighted by sample size (Fig. 4; $F = 5.25$, $df = 3, 107$, $r^2 = 0.05$, $P = 0.0020$) in a model that accounted for species ($F = 73.17$, $df = 56, 107$, $P < 0.0001$).

We compared the slope of the relationship between abundance and radiation for the same species in different years under the assumption that the relationship for slopes of different species in subsequent years should be positive with a slope of one. Any deviation from this null expectation would imply that factors other than statistical dependence were involved. There was a significant change in slope across years with 57 species for which there were data for at least three years (Kendall $\tau = -0.192$, SE = 0.074, $P = 0.012$). While the relationship between abundance and background radiation was weakly negative in 2011, it became strongly negative in 2012 (Fig. 5A) and it became even more strongly negative in 2013 compared to 2012 (Fig. 5B). Thus species tended to become more negatively affected by radiation over time. In contrast, there was no evidence that the relationship between abundance and background radiation changed with sample size in the species for which there were data for at least three years (Kendall $\tau = -0.060$, SE = 0.098, $P = 0.542$).

There was a significant difference in slope between species with a herbivorous and a carnivorous diet ($F = 25.20$, df = 1, 55, $r^2 = 0.30$, $P <$

0.0001). Surprisingly herbivorous species had a more strongly negative slope (-0.32 (SE = 0.04), $N = 10$) than carnivorous species (-0.10 (0.02), $N = 47$). There was also a significant positive association between slope of the relationship between abundance and radiation and trophic level ($F = 12.52$, df = 2, 54, $r^2 = 0.29$, $P < 0.0001$). The mean slope was the steepest for primary consumers (-0.32 (SE = 0.04, $N = 10$) over low level predators (-0.10 (0.02), $N = 38$) to top predators (-0.04 (0.13), $N = 9$).

Discussion

The main results of this study of radiation and species richness and abundance of birds at Fukushima, Japan, during 2011-2014 were that (1) overall abundance and diversity of species on average decreased with increasing level of background radiation, (2) the relationship differed among species, with most species decreasing, but some species increasing in abundance with increasing level of radiation, and (3) the relationship became more strongly negative across years, while there was no effect of change in abundance.

The overall negative relationship between abundance and level of background radiation differed among species. This result parallels previous findings from Chernobyl (Møller and Mousseau 2007a). We conducted our censuses under the assumption that there would be no significant difference in abundance because radiation should be distributed randomly across habitats that were censused. Therefore, we should expect the effect of radiation to remain after controlling statistically for the confounding effects of habitat, weather and time of day. We suggest that the difference in effect of radiation on abundance between Fukushima and Chernobyl could be ascribed to differences in duration of exposure to radiation with (1) mutations accumulating for longer time in Chernobyl, and (2) selection due to radiation not having acted for equally long time in the two areas.

We had expected an effect of bioaccumulation of radionuclides in the food web because bioaccumulation is common (Voitovich and Afonin 2002; Yakushev et al. 1999), and animals at higher trophic levels generally have higher levels of radionuclide concentrations than animals at lower levels (e. g. Kryshev and Ryabov 1990; Kryshev et al. 1992; Smith et al. 2002). However, the evidence suggested an opposite effect, with more strongly negative effects of radiation on abundance at low trophic levels. Our findings are therefore possibly more consistent with the hypothesis that oxidative stress in contaminated areas and reduced antioxidant levels (Møller et al. 2005) are a consequence of chronic radiation exposure (Møller and Mousseau 2007b), with negative effects on reproduction and survival and ultimately population trends.

This study has implications for the assessment of biological effects of ionizing radiation on free-living organisms. We have shown that the negative impact of radiation on abundance and species richness change over time, and this effect varies among species. If we had not conducted the first breeding bird censes in 2011 immediately after the Fukushima accident, we would not have been able to document a temporal change in abundance as we have reported here. Although bird species on average declined in abundance with increasing background radiation, there were several species that clearly increased in abundance. The reason for such changes can be changes in land-use or release from competition due to reductions in abundance of other species. The patterns of change in abundance with radiation level were only weakly species-specific, and even closely related species such as barn swallow *Hirundo rustica* and house martin *Delichon urbica*, carrion crow *Corvus corone* and jungle crow *Corvus macrorhynchos*, and great tit *Parus major* and varied tit *Parus varius* varied significantly in impact of radiation on abundance (Table 2). This makes it unlikely that competitive release is an important factor.

Although there has been great public interest concerning the ecological, genetic and potential health consequences of the Fukushima radiological disaster, basic research to date has been surprisingly limited with only a handful of studies published since the disaster. A recent study of bull sperm and testis from the Fukushima region found no evidence for significant histological changes in the testes or sperm morphology (Yamashiro et al. 2013) although this study was very preliminary with only two bulls from a relatively uncontaminated part of Fukushima represented for the analysis of sperm. A study of aphids revealed large effects of radiation on morphology although aberrant forms were only reported for one location in an area of relatively low contamination (Akimoto 2014). Similarly, a recent study of Japanese macaques found evidence for radiation effects on various characteristics of their blood but individuals used in this study were obtained from areas surrounding Fukushima City where contamination levels, though measurable, are low relative to other parts of Fukushima Prefecture (Ochiai et al 2014). Ishida (2013) reported on surveys of some bird populations living in more heavily contaminated areas of the region and suggested that there was no evidence of significant declines resulting from the disaster. However, the number of sites surveyed was relatively few (56 in May and 38 in June 2012) and the analyses did not control for the many other potentially confounding factors that influence bird abundance and distribution making this study very preliminary. Recent seminal studies of butterflies exposed to radioactive contaminants associated with the Fukushima disaster found strong evidence for increased mutation rates, developmental abnormalities, and population effects as a direct consequence of exposure to radionuclides (Hiyama et al. 2012, 2013). These studies by Hiyama et al. (2012, 2013) were greatly strengthened by laboratory experiments that used both internal and external radiation sources, and these unambiguously supported observations of the elevated mutation rates and phenotypic effects observed in the field (Møller and Mousseau 2013), although, as with other studies, the number of populations studied, and hence the level of replication of observations, was very limited. Murase et al. (2015) made an equally compelling case for radiation having a negative impact on reproductive performance in Japanese goshawks *Accipiter gentilis fujijamae* declining compared to the pre-accident years progressively declining over time directly linked to air dose rate.

In conclusion, we have shown substantial evidence based on rigorous and highly replicated observations across space and time that is consistent with the hypothesis that the species richness and the abundance of different species of birds were suppressed at high levels of background radiation in Fukushima. The relationship between abundance and radiation differed significantly among species, with most species decreasing, but some species increasing in abundance with increasing level of radiation. Importantly, the relationship between abundance and radiation became more strongly negative over the four years studied, while there was no change in effect of radiation on abundance with change in abundance over years.

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Legends to figures

Fig. 1 Location of census areas around Fukushima, Japan indicated by lines of dark blue dots in relation to background radiation level. Circles show distances of 5 and 50 km from the reactors. Radiation level increases from the lowest level for light blue to the highest level for the darkest shade of red. Adapted from http://www.nnistar.com/gmap/fukushima, generated by the Japanese Ministry of Education, Culture, Sports, Science and Technology (MEXT) and local government.

Fig. 2 Box plots of background radiation (μSv/h) at census points during the years 2011-2014. The box plots show median, quartiles, 5- and 95-percentiles and extreme observations.

Fig. 3 (A) Number of bird species and (B) number of bird individuals at census points in relation to background radiation (μSv/h). The lines are the linear regression lines.

Fig. 4 Box plots of the correlation coefficients between abundance and background radiation during the years 2011-2014. The box plots show median, quartiles, 5- and 95-percentiles and extreme observations.

Fig. 5 Relationship between correlation coefficients for abundance of different bird species and background radiation (μSv/h) at census points during (A) 2011 and 2012 and (B) 2012 and 2013.

Fig. 1

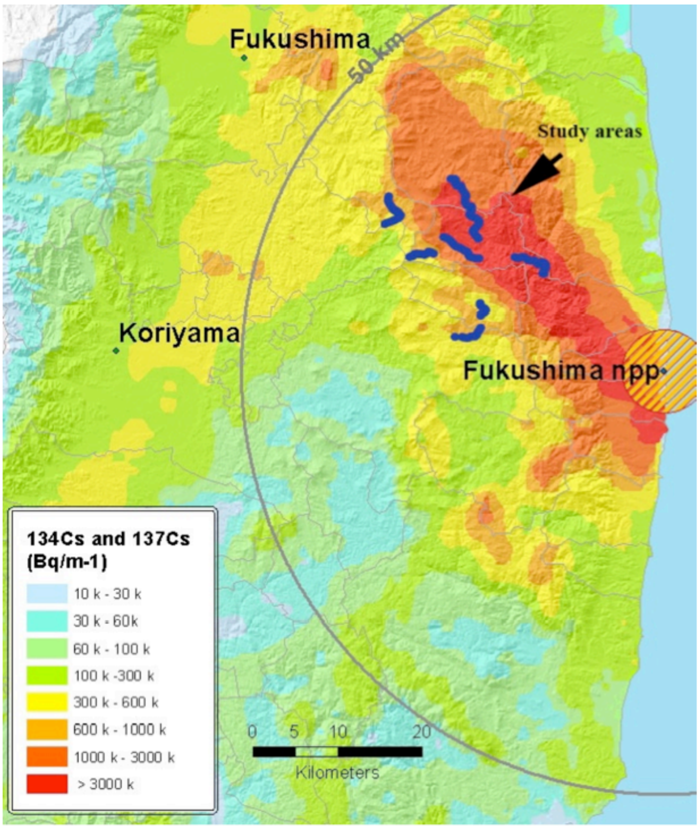

Fukushima
50 km
Study areas
Koriyama
Fukushima npp
134Cs and 137Cs
(Bq/m-1)
10 k - 30 k
30 k - 60k
60 k - 100 k
100 k -300 k
300 k - 600 k
600 k - 1000 k
1000 k - 3000 k
> 3000 k
0 5 10 20
Kilometers

Fig. 2

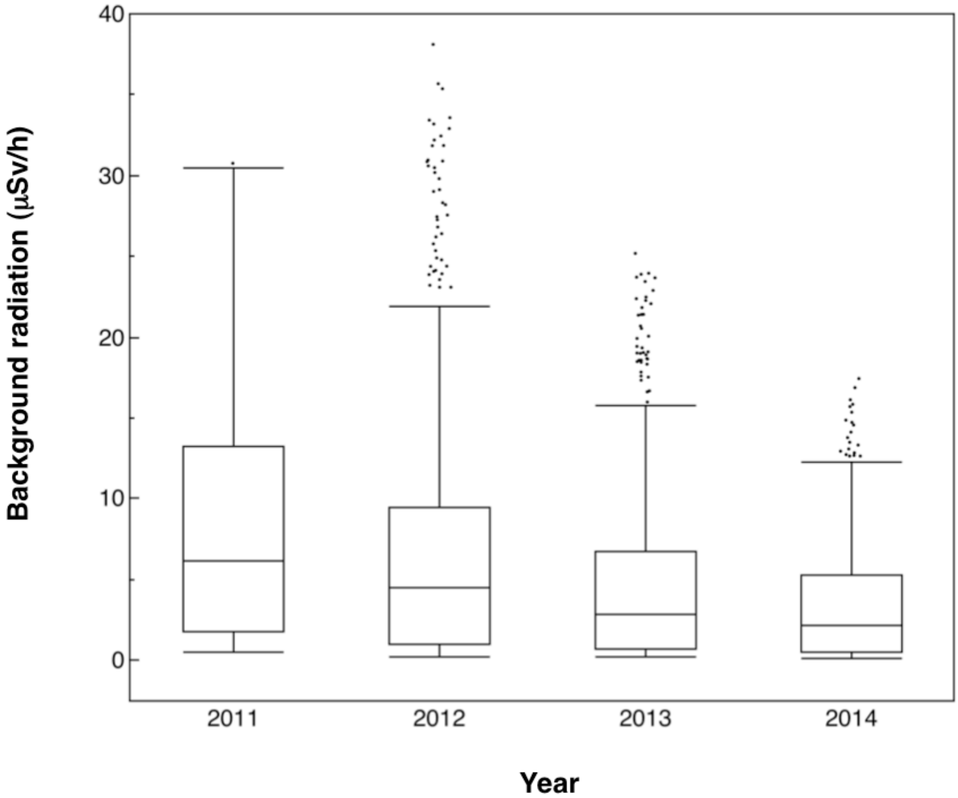
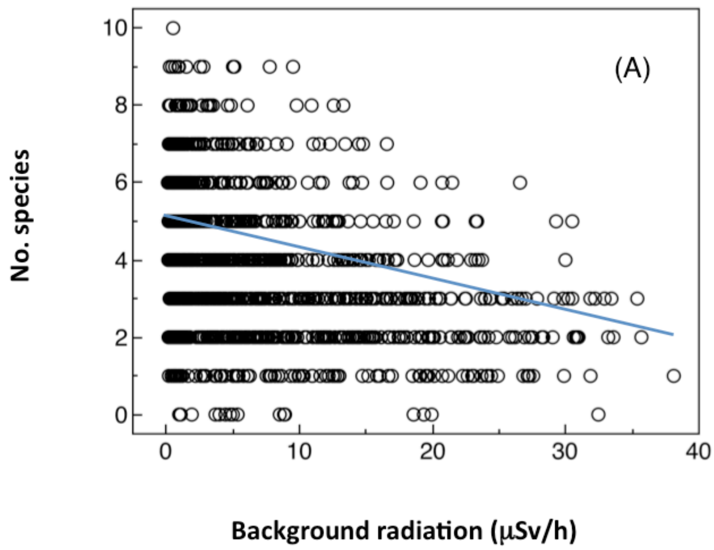
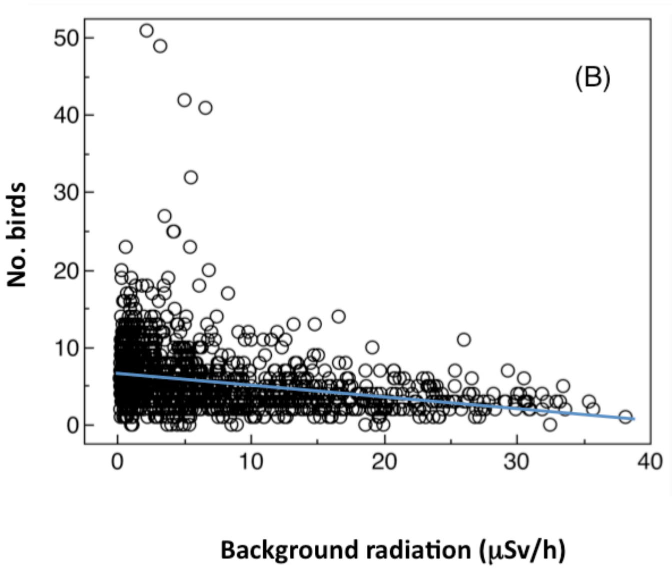

40
30
20
10
0
Background radiation (μSv/h)
2011
2012
2013
2014
Year

Fig. 3


(A)
No. species
Background radiation (μSv/h)
0
2
4
6
8
10
0
10
20
30
40


(B)
No. birds
Background radiation (μSv/h)
0
10
20
30
40
50
0
10
20
30
40

552

Fig. 4

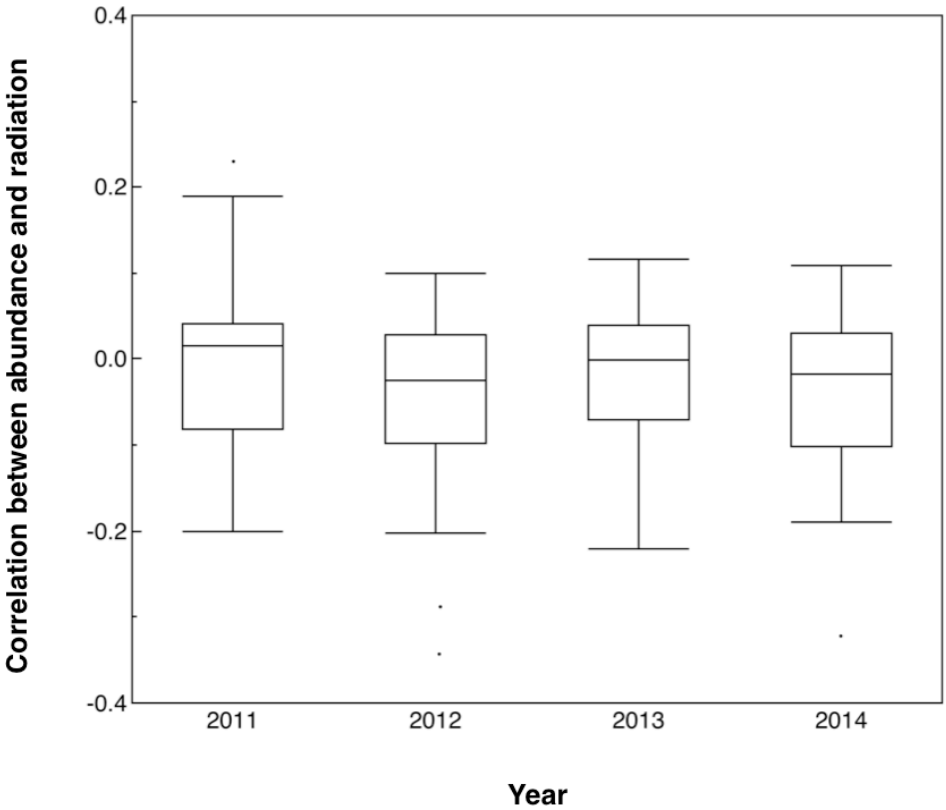
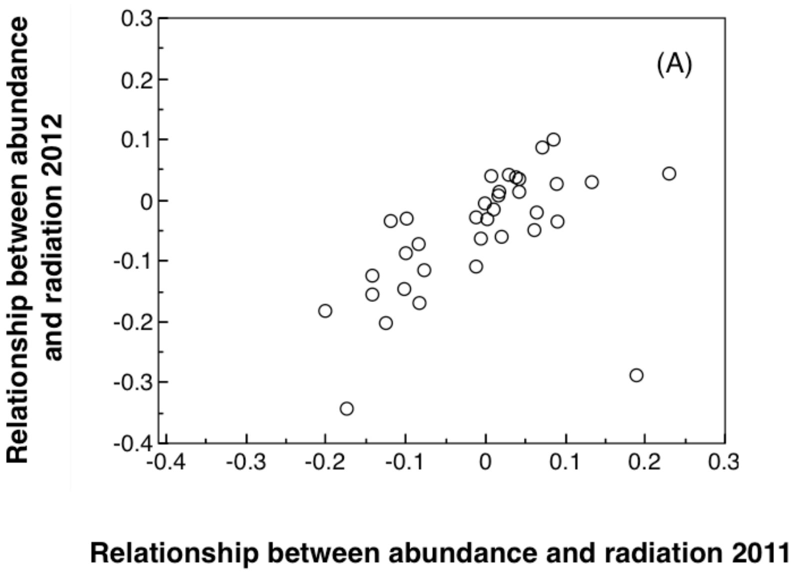
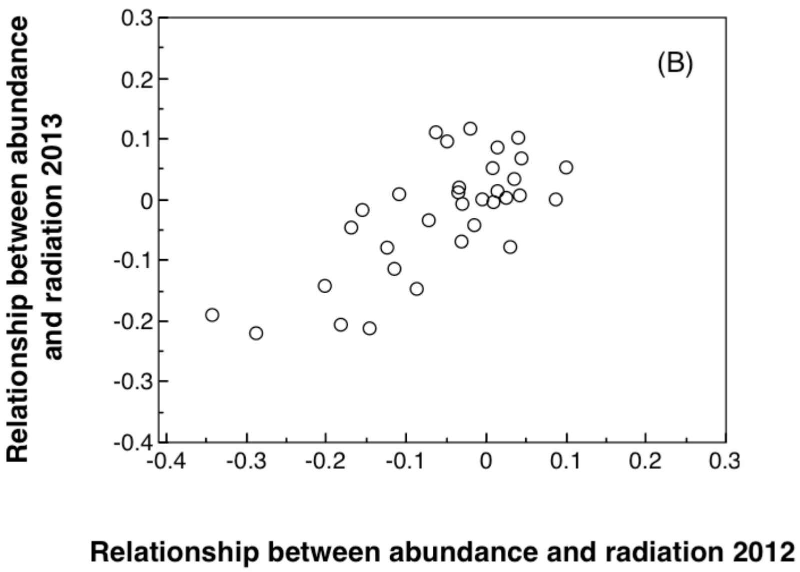

0.4
0.2
0.0
-0.2
-0.4
Correlation between abundance and radiation
2011
2012
2013
2014
Year

Fig. 5


(A)
Relationship between abundance and radiation 2012
Relationship between abundance and radiation 2011
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
-0.4
-0.4
-0.3
-0.2
-0.1
0
0.1
0.2
0.3


(B)
Relationship between abundance and radiation 2013
Relationship between abundance and radiation 2012
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
-0.4
-0.4
-0.3
-0.2
-0.1
0
0.1
0.2
0.3