

# Complementary laboratory and field studies demonstrate synergistic fitness costs of parasitism and radiation exposure to bumblebees

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**Abstract.** There is a disparity in the scientific literature about the effects of chronic low dose radiation on wildlife. Laboratory studies predict effects to insects only occur at dose rates higher than are found in even the most contaminated areas on earth. However, some field studies in the Chernobyl Exclusion Zone (CEZ) report effects to wildlife at dose rates close to UK background levels. Two proposed explanations for this disparity are (I) that laboratory investigations generally use acute exposures and (II) radiation is studied as a single stressor without the context of the natural environment. However, co-stressors could act synergistically to exacerbate the effects of elevated radiation dose in environments like the CEZ. There have been very few studies investigating the effects of chronic low dose radiation which have taken into account co-stressors, particularly for terrestrial insects.

**KEYWORDS:** *Bumblebees, Chernobyl, Chronic low-dose radiation*

## INTRODUCTION

There has been considerable scientific debate whether, and to what degree, wildlife are affected by exposure to the elevated radiation levels in places such as the Chernobyl Exclusion Zone (CEZ) and Fukushima. The conclusions from laboratory studies which have been used to inform radiation protection do not agree with a subset of field studies primarily based in the CEZ and Fukushima as laboratory studies typically only detect effects at much higher dose rates [1]. The majority of laboratory studies have used acute radiation exposure with dose rates that are unlikely to be found in the environment and have extrapolated to predict effects at lower dose rate [2]. These dose rates are sometimes more than an order of magnitude above those present in the present-day CEZ [3]. As well as the use of acute dose rates, experiments have frequently used radiation as a single stressor and have generally been conducted under optimal conditions for the organism, e.g. ad libitum food, optimum light and temperature [1]. These laboratory studies have been criticised for having very little environmental relevance, and it is, therefore, difficult to extrapolate their results to what may be occurring in the environment.

Previous studies investigating the impacts of radiation on terrestrial invertebrates in the CEZ have focused on assessing population abundance and community diversity. Spider and insect abundance [4,5] and soil invertebrate abundance [6,7] have been shown to be negatively associated with increased radiation dose rate in the CEZ. The abundance of bumblebees and other pollinators declined in the CEZ with increasing radiation dose rates, and this negative relationship was observed from 0.01  $\mu\text{Gy h}^{-1}$  (equivalent to UK background). The decline in pollinators was associated with a measured decline in pollination of fruit trees, also associated with increased radiation dose rates [5,8]. Conversely, there are studies, some of which are directly comparable to the work described above, which have found no effect of radiation to a range of taxa in the Chernobyl Exclusion Zone. These include reproduction in aquatic invertebrates [9], abundance of invertebrates [10], abundance of large mammals [12] and genotoxic endpoints in small mammals [14].

Generally, radioecological field studies are almost always correlational and require large sample sizes and a large number of sampling sites to be able to detect the effect of radiation exposure due to the presence of other factors such as seasonality, combined with additional biotic and abiotic differences between sites [16]. The Chernobyl Exclusion Zone is of limited size (2,600km<sup>2</sup>) which can pose problems associated with replication and pseudoreplication. The radiation dose level frequently drives site selection for radioecological studies in the CEZ. The majority of the most highly contaminated areas in the Chernobyl Exclusion Zone are of pine forest habitat. Therefore, it can be challenging to establish ecologically comparable sites of the correct habitat across an appropriate radiation gradient, especially when trying to include sites with higher dose rates.

It is essential to take into account habitat quality when conducting radioecological studies in the Chernobyl Exclusion Zone as habitat quality can be a significant confounding factor in studies [17]. Previous studies have used basic counts of trees [6] to assess habitat quality for birds and simplistic categorisation of ground coverage and the dominant vegetation (e.g. grassland, shrub or forest) either on a small scale or a broader scale using GIS for large mammal studies [4,12,18]. As these methods range from small to a large scale, without careful considerations as to how wildlife use the area, these studies can fail to capture the amount of food and suitable habitat available, which may ultimately drive variation in species abundance. Food resource assessments at the sites in the CEZ were conducted where and when the bumblebees were sampled. There are many ways to quantify the floral resource available to bumblebees. At a local scale, the abundance and species richness of flowering are the most important parameters that can drive an increase in pollination services [19–21]. Community composition, although varying over the course of a year, plays a vital role in driving pollinator abundance [20]. However, establishing good metrics for floral characteristics and available floral resource for bumblebees is difficult as no one metric can accurately summarise all the key metrics of community structure such as diversity and community composition, which can be disproportionately influenced by dominant species [22]. Species richness can be used to inform floral diversity and evenness [23,24]. Evenness is used to measure relative diversity measures and quantify the equality of species abundances in a community, therefore, indicating whether the community is dominated by one species [22,25]. There are a number of ways of calculating evenness and diversity [24,26] and each has their advantages and disadvantages. In this study, Pielou's measurement of evenness and the Shannon diversity index will be used as complementary species richness estimates in this field study [27].

## METHODS

### 2.1 Bumblebee Collection

Sampling was undertaken in the Chernobyl Exclusion Zone (CEZ) ( $51^{\circ}23'23.47''\text{N}$ ,  $30^{\circ}5'38.57''\text{E}$ ) in Northern Ukraine. Twenty-one sites were selected over a radiation dose rate gradient in the CEZ from  $0.1 \mu\text{Gyh}^{-1}$  (the same as background levels) to  $30 \mu\text{Gyh}^{-1}$ . All sites ranged from  $0.01 - 0.04 \text{ km}^2$  in area and were of similar habitat and floral composition as demonstrated by our habitat quality assessments. The distance between sites ranged from 2 km to 33 km with an average of 9 km. Bumblebees were sampled in July 2015, June 2016 and September 2016 using a large butterfly net and catching bumblebee workers indiscriminately. A total of 2,485 bumblebees were collected: n= 836, n= 885 and n= 764 in each sample period respectively. Collected bees were euthanised in ethanol, stored in Eppendorf tubes and returned to the UK for further analysis. Age was estimated in wild bumblebees in the CEZ by estimating the amount of wing wear on each bee wing. Wing wear has been shown to be a good proxy for establishing age in insects [28,29]. As bumblebees fly around foraging, they collide with vegetation which causes tears in the wing. Therefore, bumblebee wings accumulate non-repairable damage over their lifespan making it ideal as a proxy for age. A seven-point scale for severity of damage was established depending on the area missing from the wing area. To avoid potential bias, wings were scored double-blind using a randomly allocated code as an individual identifier, meaning that the observer was unaware of the radiation level of the site from which they had been collected. Bumblebees were removed from ethanol preservative in the laboratory, washed in clean water and dried on a paper towel to prepare samples for wing wear analysis. Small scissors were used to remove both the forewings and hind wings which were subsequently spread on scotch tape<sup>TM</sup> in a consistent left-right arrangement and analysed with a hand lens.

### 2.2 Floral resource measurements

Quantification of the available floral resource at the time of sampling was undertaken to determine whether habitat quality could be a potential confounding factor that influences bumblebee fitness and whether increased radiation dose rates influenced habitat quality. The inflorescence number and species diversity were measured to determine the quantity and quality of the floral resource. Inflorescence number across all plants and floral diversity for 21 sites were measured in July 2015, and June 2016 early in the bumblebee colony cycle (spring/early summer) because these are the critical foraging months for most bumblebee species [30]. Flowering plant species were identified, and the number of plants and their inflorescences were counted following methods outlined in [31]. Random sampling locations at each site were determined using a 50-metre transect which was laid randomly across the

site. Within a one-metre radius around six points along the transect (at 0, 10, 20, 30, 40 and 50 metres) all flowering species were identified, and the number of plants and the number of inflorescences on each plant were counted. Approximations were made when there were many inflorescences and few plants, for example, umbel type plants. Each individual inflorescence was counted on a random subsample of up to ten flower heads per site. This number was then used to calculate the total number of inflorescences based on how many umbel flower heads were available to bees.

Floral diversity was calculated using the Shannon index and evenness was calculated with Pielou's test of evenness, both from the vegan package in R [35]. The number of inflorescences were pooled for each transect, and species diversity and species evenness were averaged across the site to indicate site characteristics. The relationship between radiation dose rate and floral diversity was analysed using a generalised linear mixed effects model with a Poisson error structure. Inflorescence number was log transformed to normalise the dataset; the site was specified as a random effect.

This study also investigated if species composition at each site varied with the radiation dose rate, or if some species were more or less dominant in the community depending on the radiation dose rate. A principal components analysis was conducted to test whether floral species composition depended on the site, sampling year and radiation dose rate. The principal components analysis (PCA: vegan package [32] was conducted based on the plant species counted and identified at each site over the two sampling periods. For the PCA analysis and visualisation, sites were grouped depending on a categorical classification of radiation contamination: control (background (up to  $0.2 \mu\text{Gy h}^{-1}$ ), low ( $0.5 - 6 \mu\text{Gy h}^{-1}$ ) and high ( $6.1 - 30 \mu\text{Gy h}^{-1}$ ). Non-metric multidimensional scaling (NDMS) was used to test the effects of radiation dose rate and the year in which the transect was undertaken. NDMS was chosen as it has been shown to be more robust to for taking into account the occurrence of rare species than other ordination methods [33]. Vectors were fitted onto the NDMS ordination to visualise the direction and strength of the environmental effects.

### **2.3 Testing the relationship between increasing radiation dose rate and age in wild bumblebees**

Models were constructed to test the relationship between radiation dose rate and age (wing wear) in wild bumblebees. A linear mixed effects model (lme4 [34]) tested how wing wear score was affected by fixed factors: radiation dose rate, sample period, bumblebee species and floral characteristics at the site (inflorescence number, evenness and species diversity). Two-way interactions were allowed for radiation dose rate, sample period and species diversity; site was used as a random effect. To normalise the wing wear score, one was added to the score, and this number was subsequently square root transformed.

## **RESULTS**

### **3.1 Increased radiation dose rates not associated with a reduction in floral resource quality**

We tested for an association between floral community characteristics at sites across the Chernobyl Exclusion zone and radiation dose rate; an association would suggest an impact of radiation exposure on plant growth. Furthermore, if floral habitat quality covaried with radiation dose rate, this could represent a confounding factor in our field study measuring how radiation exposure influences bumblebee fitness. Habitat quality for bumblebees was inferred from measures of floral community diversity, floral community evenness and the sum of inflorescence numbers per plant species across the whole area. Floral diversity and inflorescence counts were conducted in July 2015 (sites  $n = 14$ ) and June 2016 (sites  $n = 13$ ). The Shannon index of plant species diversity varied from 0.3 to 2.08, with a mean of  $1.19 \pm 0.09$ . Pielou's measure of community composition evenness determined how similar the numbers of each floral species are at each site. Pielou's measure of evenness ranged from 0.02 to 0.85 with a mean of  $0.54 \pm 0.037$ . The total number of inflorescences across each site, indicating the amount of food resource available, varied from 26 to 33986 inflorescences per  $\text{m}^2$  with a mean of 1137 inflorescences per  $\text{m}^2$  ( $\pm 2361 \text{ SE}$ ) across sites. The data suggested there was a non-significant positive relationship between radiation dose rate and floral species diversity as measured by Shannon's index (slope =  $0.02 \pm 0.04$ ,  $\chi^2 = 1.65$ ,  $p = 0.20$ ). To detect whether the radiation dose rate affected floral community similarity for each site, the floral species abundance scores for all species were ordinated using a principal components analysis. The PCA performed on all species gave no significant result and explained little variation, due to the presence of many species with a frequency lower than 20%. The most common species present were *Myositis* sp. (*Myosotis stricta* and *Myosotis ramosissima*), *Vicia*

*spp.* (*Vicia cracca* and *Vicia tetrasperma*), *Centaurea nigra* and *Trifolium dubium*. Non-metric multidimensional scaling was used to assess compositional changes between the two sampling years, across different sites and taking into account the radiation dose rate. Radiation dose rate did not affect species composition in sites across the Chernobyl Exclusion Zone. When the data were subset only to include these common species as there was not enough statistical power to perform a PCA analysis otherwise (Figure 1).

Figure 1. No relationship between radiation dose rate and floral species composition. Principal components analysis for ordination using the first two principal components (PCs) based on floral species composition. The data points represent each site, and the ovals are grouped by the radiation dose rate as the environmental variable. Control (background (up to 0.2  $\mu\text{Gy h}^{-1}$ ), low (0.5 - 6  $\mu\text{Gy h}^{-1}$ ) and high (6.1 – 30  $\mu\text{Gy h}^{-1}$ ). The blue arrow represents the year of sampling.

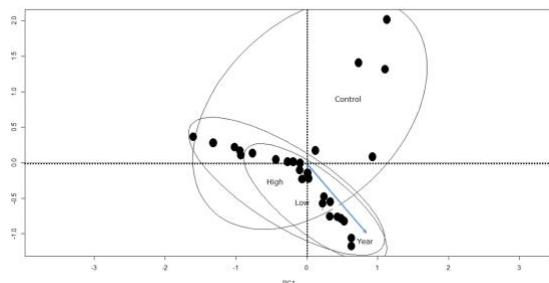


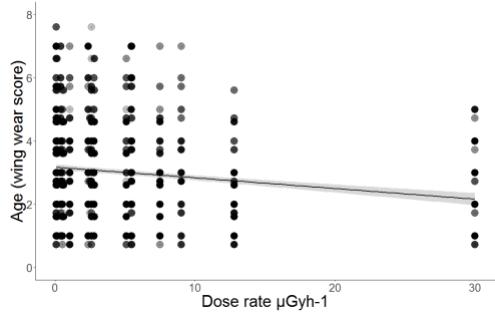
Table 1. Testing the influence of environmental variables: site, year and radiation dose rate. Environmental variables were fitted to the non-metric multidimensional scaling (NDMS) using regression in Vegan [32]

Predictors	PC1	PC2	R <sup>2</sup>	P
Site	-0.00695	0.999	0.33	0.008 **
Year	0.986	-0.166	0.684	0.001***
Radiation dose rate	-0.409	-0.912	0.0645	0.438

#### Lower mean wing wear score at higher radiation sites

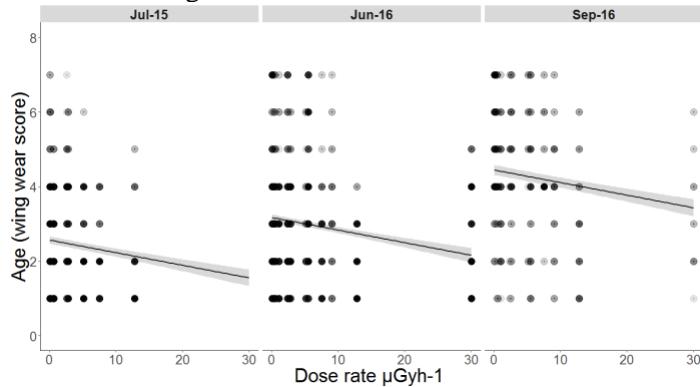
To test for evidence that bumblebee lifespan was affected by radiation exposure, age was estimated from the wing wear score of worker bumblebees ( $n = 2021$ ) sampled across sites with varying radiation levels (up to 30  $\mu\text{Gy h}^{-1}$ ). Wing wear score ranged from 1-7; bumblebees from highly contaminated areas of the Chernobyl Exclusion Zone had a lower mean wing wear score than bumblebees from sites with lower contamination (slope =  $-0.04 \pm 0.01$ ,  $\chi^2_{(1)} = 7.00$ ,  $p = 0.008$ , Figure 2). The model predicted that a 5  $\mu\text{Gy h}^{-1}$  increase in radiation dose rates led to a 4% reduction in the amount of wing wear accumulated.

Figure 2. The negative association between radiation dose rate and age. Wing wear as a proxy for age measured in worker bumblebees caught in different sites across a gradient of radiation in the Chernobyl Exclusion Zone. The points are the raw data, and the trend line is from the model fit accounting for the site and sample period, with 95% confidence intervals. The shading of the points indicates how many data points were collected at each radiation dose and for each wing wear score (darker coloured points indicate more data).



The average wing wear score for bumblebee workers differed between sample periods: June 2015 (intercept =  $2.11 \pm 0.23$ ), July 2016 ( $2.65 \pm 0.19$ ) and September 2016 (intercept =  $4.61 \pm 0.24$ ), with significantly higher wing wear in June 2015 than July 2016 ( $\chi^2_{(2)} = 32$ ,  $p = <0.001$ , Figure 3). The impact of radiation dose rate on mean age (wing wear) did not differ between sampling periods (radiation dose rate by sampling period interaction:  $\chi^2_{(2)} = 2.35$ ,  $p = 0.30$ , Figure 3).

Figure 3. The association between average age for worker bumblebees and radiation dose rate broken down by sampling period. Sampling periods were July 2015, June 2016 and September 2016. The interaction between sample period and radiation dose was included in the model, but the interaction term was not significant.



The dataset was reanalysed removing bees of higher wing wear. This was to test whether the negative relationship between age and dose rate was driven by an overall reduction in wing wear score across all bumblebees (consistent with an increased risk of death across the entire age spectrum). Or whether the effect of radiation on wing wear was driven only by the absence of older age classes at highly contaminated sites as a result of premature death in the terminal phase of life. The relationship between radiation dose rate and age remains significant when excluding wing wear scores 7 – 3. However, the effect size is larger for the data set that includes all bumblebees when compared to the data set excluding the highest age class. This analysis indicates that the radiation-age trend is not simply driven by an absence of aged bumblebees at the highest radiation dose rate sites, but that the negative impacts of radiation probably begin in middle age.

In total 928 males were caught during sampling (alongside the workers described above); no males were caught in June 2015, 40 males were caught in July 2016, and 888 were caught in September 2016. Overall, mean wing wear score for workers ( $6.17 \pm 0.07$ ) was significantly higher than in males ( $4.91 \pm 0.12$ ). Males were found at all sites in September 2016: there was no detectable effect of radiation dose rate on the number of males caught in the sample at each site (slope =  $0.03 \pm 0.02$ ,  $\chi^2_{(1)} = 1.06$ ,  $p = 0.30$ ). There was no relationship between site radiation dose rate and wing wear for males (slope =  $-0.002 \pm 0.01$ ,  $\chi^2_{(1)} = 0.015$ ,  $p = 0.900$ ).

Out of the 2021 worker bumblebees collected over the 3 sampling periods, 49% were *Bombus terrestris*. *Bombus lucorum*, *Bombus pascorum* and *Bombus lapidarius* were also frequently sampled (17%, 10.5% and 8.5% respectively). Wing wear varied across species ( $\chi^2_{(13)} = 83.31$ ,  $p = <0.01$ ). Intercepts were compared to the *B. terrestris*, and wing wear scores for *B. hortorum*, *B. lapidarius*, *B. lucorum*, *B.*

*muscorum*, *B. ruderarius*, *B. subterraneus* and *B. sylvarum* were found to have significantly different intercepts from *B. terrestris*. The differences across intercepts are possibly associated with the different timings of the colony cycle. There was no evidence that the impact of radiation dose rate on mean worker age varied between bumblebee species (dose rate by species interaction:  $\chi^2_{(13)} = 2.31$ ,  $p = 0.85$ ).

## DISCUSSION

Although we detected effects to bumblebees at lower dose rates than predicted from previous laboratory studies, there was no effect detected either in the field or in the laboratory study as low as reported in some studies at dose rates of  $0.1 \mu\text{Gy h}^{-1}$  (Møller et al. 2012; Møller & Mousseau 2009). There have been some arguments that suggest correlative radioecological field studies may be undermined if the correlations between organism fitness and radiation exposure are driven by an unrelated unmeasured covariate [35,36]. This argument can be countered by appropriately powered experiments with sufficient site replication. Also, this study assessed a form of ecological variation which is probably the most significant ecological factor that influences bumblebee fitness: forage plant community composition. This study conclusively demonstrated that floral community parameters were not correlated with radiation exposure at the field sites

There were no detected differences in habitat quality across the different sites. The community level metrics of radiation effects in plants are likely to be less sensitive to radiation dose rate than the individual-level measures that were conducted on bumblebees. Some plant species were severely affected in the first few days after the Chernobyl accident, with most affected being *Pinus sylvestris* (Geras'kin et al. 2013; Geras'kin et al. 2008; Yeamans et al. 2014). Studies at Semipalatinsk test site demonstrated that cytogenetic changes occurred in plant populations *Vicia cracca* at doses of  $10 \mu\text{Gy h}^{-1}$  but did not detect an effect on plant populations [40]. More recently, studies have shown that the timing and rates of seed germination in *Daucus carota* were negatively associated with increased radiation dose rates in the CEZ [41].

Other studies in the CEZ have shown that chronic low dose radiation can affect pollen viability and increase the frequency of morphological and cytogenic abnormalities [42,43]. A reduction in pollen viability and quality has been shown to impact the amount of reward available for wild pollinators such as bumblebees. Although there was no relationship detected in this study between floral species diversity, number or composition, to investigate the quality of available pollen in flowering plants, further work is proposed. Furthermore, establishing pollinator networks including all wild pollinators by mapping interactions to understand if there is an ecosystem-level consequence to living in contaminated areas in the CEZ is recommended.

## FUNDING

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## REFERENCES

1. Garnier-Laplace J, Geras'kin S, Della-Vedova C, Beaugelin-Seiller K, Hinton TG, Real A, Oudalova A. 2013 Are radiosensitivity data derived from natural field conditions consistent with data from controlled exposures? A case study of Chernobyl wildlife chronically exposed to low dose rates. *J. Environ. Radioact.* **121**, 12–21. (doi:10.1016/j.jenvrad.2012.01.013)
2. Andersson P, Garnier-Laplace J, Beresford NA, Copplestone D, Howard BJ, Howe P, Oughton D, Whitehouse P. 2009 Protection of the environment from ionising radiation in a regulatory context (protect): proposed numerical benchmark values. *J. Environ. Radioact.* **100**, 1100–1108. (doi:10.1016/j.jenvrad.2009.05.010)
3. ICRP. 2008 Environmental protection: the concept and use of reference animals and plants. *Ann. ICRP* **108**. (doi:10.1016/j.icrp.2006.06.001)
4. Bezrukov V, Møller AP, Milinevsky G, Rushkovsky S, Sobol M, Mousseau T a. 2015 Heterogeneous relationships between abundance of soil surface invertebrates and radiation from Chernobyl. *Ecol. Indic.* **52**, 128–133. (doi:10.1016/j.ecolind.2014.11.014)

5. Møller AP, Mousseau TA. 2009 Reduced abundance of insects and spiders linked to radiation at Chernobyl 20 years after the accident. *Biol. Lett.* **5**, 356–9. (doi:10.1098/rsbl.2008.0778)
6. Møller AP, Mousseau TA. 2018 Reduced colonization by soil invertebrates to irradiated decomposing wood in Chernobyl. *Sci. Total Environ.* **645**, 773–779. (doi:10.1016/j.scitotenv.2018.07.195)
7. Mousseau TA, Milinevsky G, Kenney-Hunt J, Møller AP. 2014 Highly reduced mass loss rates and increased litter layer in radioactively contaminated areas. *Oecologia* **175**, 429–437. (doi:10.1007/s00442-014-2908-8)
8. Møller AP, Barnier F, Mousseau TA. 2012 Ecosystems effects 25 years after Chernobyl: pollinators, fruit set and recruitment. *Oecologia* **170**, 1155–65. (doi:10.1007/s00442-012-2374-0)
9. Fuller N, Ford AT, Nagorskaya LL, Gudkov DI, Smith JT. 2018 Reproduction in the freshwater crustacean *Asellus aquaticus* along a gradient of radionuclide contamination at Chernobyl. *Sci. Total Environ.* **628–629**, 11–17. (doi:10.1016/j.scitotenv.2018.01.309)
10. Bonzom J *et al.* 2016 Effects of radionuclide contamination on leaf litter decomposition in the Chernobyl exclusion zone. *Sci. Total Environ.* **562**, 596–603. (doi:10.1016/j.scitotenv.2016.04.006)
11. Jackson D, Copplestone D, Stone D., Smith G. 2005 Terrestrial invertebrate population studies in the Chernobyl exclusion zone, Ukraine. *Radioprotection* **40**, 527–532. (doi:10.1051/radiopro)
12. Deryabina TG, Kuchmel SV, Nagorskaya LL, Hinton TG, Beasley JC, Lerebours A, Smith JT. 2015 Long-term census data reveal abundant wildlife populations at Chernobyl. *Curr. Biol.* **25**, 824–826. (doi:10.1016/j.cub.2015.08.017)
13. Gashchak S, Gulyaichenko Y, Beresford NA, Wood MD. 2016 BROWN BEAR ( URSUS ARCTOS L.) IN THE CHORNOBYL EXCLUSION ZONE Introduction A recent analysis of data on large carnivores in Europe demonstrated that the brown bear is the most numerous and in most areas there are no concerns with respect to the future of. *Proc. Theriol. Sch.* **14**, 71–84.
14. Wickliffe JK, Chesser RK, Rodgers BE, Baker RJ. 2002 Assessing the genotoxicity of chronic environmental irradiation by using mitochondrial DNA heteroplasmy in the bank vole (*Clethrionomys glareolus*) at Chornobyl, Ukraine. *Environ. Toxicol. Chem.* **21**, 1249–1254. (doi:Doi 10.1897/1551-5028(2002)021<1249:Atgoce>2.0.Co;2)
15. Rodgers BRER, Baker ROJB. 2000 Frequencies of Micronuclei in Bank Voles From Zones of High Radiation At Chornobyl , Ukraine. *Environ. Toxicol. Chem.* **19**, 1644–1648.
16. Steel EA, Kennedy MC, Cunningham PG, Stanovick JS. 2013 Applied statistics in ecology: Common pitfalls and simple solutions. *Ecosphere* **4**, 1–13. (doi:10.1890/ES13-00160.1)
17. Baker RJ, Bickham AM, Bondarkov M, Gaschak SP, Matson CW, Rodgers BE, Wickliffe JK, Chesser RK. 2001 Consequences of polluted environments on population structure: The bank vole (*Clethrionomys glareolus*) at Chornobyl. In *Ecotoxicology*, pp. 211–216. (doi:10.1023/A:1016665226716)
18. Møller AP, Mousseau T a. 2015 Strong effects of ionizing radiation from Chernobyl on mutation rates. *Sci. Rep.* **5**, 8363. (doi:10.1038/srep08363)
19. Venjakob C, Klein AM, Ebeling A, Tscharntke T, Scherber C. 2016 Plant diversity increases spatio-temporal niche complementarity in plant-pollinator interactions. *Ecol. Evol.* **6**, 2249–2261. (doi:10.1002/ece3.2026)
20. Grass I, Albrecht J, Jauker F, Diekötter T, Warzecha D, Wolters V, Farwig N. 2016 Much more than bees—Wildflower plantings support highly diverse flower-visitor communities from complex to structurally simple agricultural landscapes. *Agric. Ecosyst. Environ.* **225**, 45–53. (doi:<http://dx.doi.org/10.1016/j.agee.2016.04.001>)
21. Fowler RE, Rotheray EL, Goulson D. 2016 Floral abundance and resource quality influence pollinator choice. *Insect Conserv. Divers.* **9**, 481–494. (doi:10.1111/icad.12197)
22. Ricotta C. 2003 On parametric evenness measures. *J. Theor. Biol.* **222**, 189–197. (doi:10.1016/S0022-5193(03)00026-2)
23. Jost L. 2010 The relation between evenness and diversity. *Diversity* **2**, 207–232. (doi:10.3390/d2020207)
24. Alatalo R V. 1981 Problems in the Measurement of Evenness in Ecology. *Oikos* **37**, 199.

- (doi:10.2307/3544465)
25. Kvålseth TO. 2015 Evenness indices once again: critical analysis of properties. *Springerplus* **4**. (doi:10.1186/s40064-015-0944-4)
  26. Hill MO. 1973 Diversity and Evenness : A Unifying Notation and Its Consequences. *Ecology* **54**, 427–432. (doi:10.2307/1934352)
  27. Ricotta C, Avena G. 2003 On the relationship between Pielou's evenness and landscape dominance within the context of Hill's diversity profiles. *Ecol. Indic.* **2**, 361–365. (doi:10.1016/S1470-160X(03)00005-0)
  28. Foster DJ, Cartar R V. 2011 What causes wing wear in foraging bumble bees? *J. Exp. Biol.* **214**, 1896–1901. (doi:10.1242/jeb.051730)
  29. Cartar R V. 1992 Morphological Senescence and Longevity : An Experiment Relating Wing Wear and Life Span in Foraging Wild Bumble Bees Authors ( s ): Ralph V . Cartar Source : Journal of Animal Ecology , Vol . 61 , No . 1 ( Feb . , 1992 ), pp . 225-231 Published by : Britis. *J. Anim. Ecol.* **61**, 225–231.
  30. Wray JC, Elle E. 2014 Flowering phenology and nesting resources influence pollinator community composition in a fragmented ecosystem. *Landsc. Ecol.* **30**, 261–272. (doi:10.1007/s10980-014-0121-0)
  31. Baldock KCR *et al.* 2015 Where is the UK ' s pollinator biodiversity ? The importance of urban areas for flower- visiting insects. *Proc. Biol. Sci.*
  32. Oksanen J. 2015 Multivariate analysis of ecological communities in R: vegan tutorial. *R Doc.* , 43. (doi:10.1016/0169-5347(88)90124-3)
  33. Cao Y, Larsen DP, Throne R. 2001 Rare species in multivariate analysis for bioassessment : some considerations. *J. N. Am. Benthol. Soc* **20**, 144–153.
  34. Bates D, Mächler M, Bolker B, Walker S. 2014 Fitting Linear Mixed-Effects Models using lme4. *eprint arXiv:1406.5823* **67**, 51. (doi:10.18637/jss.v067.i01)
  35. Brechignac F. 2016 The need to integrate laboratory-and ecosystem-level research for assessment of the ecological impact of radiation. *Integr. Environ. Assess. Manag.* **12**, 673–676. (doi:10.1002/ieam.1830)
  36. Brechignac F *et al.* 2016 Addressing ecological effects of radiation on populations and ecosystems to improve protection of the environment against radiation: agreed statements from a Consensus Symposium. *J. Environ. Radioact.* **159**, 21–29. (doi:10.1016/j.jenvrad.2016.03.021)
  37. Geras'kin S, Evseeva T, Oudalova A. 2013 Effects of long-term chronic exposure to radionuclides in plant populations. *J. Environ. Radioact.* **121**, 22–32. (doi:10.1016/j.jenvrad.2012.03.007)
  38. Geras'kin SA, Fesenko S V, Alexakhin RM. 2008 Effects of non-human species irradiation after the Chernobyl NPP accident. *Environ. Int.* **34**, 880–97. (doi:10.1016/j.envint.2007.12.012)
  39. Yeaman RL, Roulston TH, Carr DE. 2014 Pollen quality for pollinators tracks pollen quality for plants in *Mimulus guttatus*. *Ecosphere* **5**. (doi:10.1890/ES14-00099.1)
  40. Evseeva T, Majstrenko T, Geras'kin S, Brown JE, Belykh E. 2009 Estimation of ionizing radiation impact on natural *Vicia cracca* populations inhabiting areas contaminated with uranium mill tailings and radium production wastes. *Sci. Total Environ.* **407**, 5335–43. (doi:10.1016/j.scitotenv.2009.06.037)
  41. Boratyski Z, Arias JM, Garcia C, Mappes T, Mousseau TA, Møller AP, Pajares AJM, Piwczyński M, Tukalenko E. 2016 Ionizing radiation from Chernobyl affects development of wild carrot plants. *Sci. Rep.* **6**, 1–8. (doi:10.1038/srep39282)
  42. Møller AP, Shyu JC, Mousseau TA. 2016 Ionizing Radiation from Chernobyl and the Fraction of Viable Pollen. *Int. J. Plant Sci.* **177**, 727–735. (doi:10.1086/688873)
  43. Geras'kin S a, Oudalova a a, Dikarev VG, Dikareva NS, Mozolin EM, Hinton T, Spiridonov SI, Copplestone D, Garnier-laplace J. 2012 Effects of chronic exposure in populations of *Koeleria gracilis* Pers . from the Semipalatinsk nuclear test site , Kazakhstan. *J. Environ. Radioact.* **104**, 55–63. (doi:10.1016/j.jenvrad.2011.09.015)