RESEARCH ARTICLE

Ecologically relevant radiation exposure triggers elevated metabolic rate and nectar consumption in bumblebees

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Abstract

1. Exposure to radiation is a natural part of our environment. Yet, due to nuclear accidents such as at Chernobyl, some organisms are exposed to significantly elevated dose rates. Our understanding of the effects of radiation in the environment is limited, confounded by substantial interspecific differences in radiosensitivity and conflicting findings.

2. Here we study radiation impacts on bumblebees in the laboratory using principles from life-history theory, which assume organismal investment in fitness-related traits is constrained by resource availability and resource allocation decisions. To investigate how chronic radiation might negatively affect life-history traits, we tested whether exposure affects bumblebee energy budgets by studying resource acquisition (feeding) and resource use (metabolic rate).

3. We monitored metabolic rate, movement and nectar intake of bumblebees before, during and after 10 days of radiation exposure. Subsequently, we monitored feeding and body mass across a dose rate gradient to investigate the dose rate threshold for these effects. We studied dose rates up to 200 μGy/hr: a range found today in some areas of the Chernobyl Exclusion Zone.

4. Chronic low-dose radiation affected bumblebee energy budgets. At 200 μGy/hr nectar consumption elevated by 56% relative to controls, metabolic CO_2 production increased by 18%, and time spent active rose by 30%. Once radiation exposure stopped, feeding remained elevated but CO_2 production and activity returned to baseline. Our analysis indicates that elevated metabolic rate was not driven by increased activity but was instead closely associated with feeding increases. Our data suggest bumblebee nectar consumption was affected across the 50–200 μGy/hr range.

5. We show field-realistic radiation exposure influences fundamental metabolic processes with potential to drive changes in many downstream life-history traits. We hypothesise that radiation may trigger energetically costly repair mechanisms, increasing metabolic rate and nectar requirements. This change could have significant ecological consequences in contaminated landscapes, including Chernobyl.

We demonstrate bumblebees are more sensitive to radiation than assumed by existing international frameworks for environmental radiological protection.

KEYWORDS
eco-toxicology, energy budget, insects, Ionising radiation, life history, pollinator, radiological contamination, resource allocation

1 | INTRODUCTION

Organisms are exposed to low-level ionising radiation from natural sources. Background radiation typically delivers total absorbed dose rates of ~1 µGy/hr (excluding radon), this is a normal part of organismal ecology with few fitness impacts (Beresford et al., 2008; Hosseini et al., 2008). However, accidents such as those at the nuclear power and fuel reprocessing plants of Chernobyl, Fukushima and Mayak (Russian Urals), have resulted in dramatically elevated environmental radiation exposure in localised areas (Copplestone et al., 2015). These large radionuclide releases generated novel ecological stressors against which organisms have no recent evolutionary history of adaptation. The local environmental consequences can be extreme (UNSCEAR, 2008); for example, the 1986 Chernobyl disaster initially caused a 30-fold reduction in total soil invertebrate abundance at sites close to the nuclear power plant (Geras'kin et al., 2008). Dose rates in the Chernobyl Exclusion Zone have reduced by several orders of magnitude in the decades since the accident; radiation is now spatially heterogeneous (range < 0.1–250 µGy/hr), with some areas now equivalent to uncontaminated background (Beresford, Barnett, et al., 2020). For the context of these radiation dose rates, see Figure S1. The present biological impacts of this ecologically relevant dose rate spectrum are widely debated (Beresford, Scott, et al., 2020): field studies are inconsistent as to whether they find effects of radiation and in the magnitude of these effects (Bonzom et al., 2016; Mousseau et al., 2014); there is also uncertainty as to how field measures of dose rate translate to total doses that organisms experience (Beaugelin-Seiller et al., 2020).

We know relatively little about the effects of radiation on most animals compared to humans (Basu, 2018). Many laboratory radiation effect studies, on invertebrates in particular, have been delivered at high acute dose rates (typically >60 Gy/day) (Andersson et al., 2009; ICRP, 2008). Furthermore, the wide diversity of species studied, with contrasting ecologies, varying radiation exposure pathways and differing potential life-history responses, makes general predictions about the likely effects of environmental contamination difficult. This diversity may contribute to the conflicting results from contemporary Chernobyl Exclusion Zone studies of how radiation affects animal life history and population-level metrics: findings range from no effect (Baker et al., 2001; Bonzom et al., 2016; Fuller et al., 2019) to significant negative consequences at comparatively low dose rates (Kesäniemi et al., 2019; Møller et al., 2007). Investigations include those at the population level (e.g. pollinator abundance; Møller et al., 2012), organismal physiology (e.g. sperm abnormalities in birds; Hermosell et al., 2013) and molecular effects (e.g. chromosomal aberrations in bank voles; Rykabon &

Goncharova, 2006). The mechanisms by which the dose rates currently found at Chernobyl could negatively affect animal life history are currently unclear (Smith et al., 2012). To better assess radiation effects on organismal ecology, we require understanding of biological processes that bridge the gap between the molecular signatures of exposure that are difficult to interpret, and fitness-related traits that appear to be inconsistently affected.

In this study, we use a novel experimental approach to assess the impact of ecologically relevant radiation exposure. We draw on life-history theory, which assumes an organism's investment in fitness-related traits is constrained by resource availability and by decisions on resource allocation between these traits (van Noordwijk & de Jong, 1986). Both resource acquisition and the manner in which resource trade-offs are resolved can change dramatically when organisms are exposed to stressors, potentially due to a re-allocation of resources towards traits promoting survival (Eeva et al., 2006; Fritsch et al., 2019; Hladun et al., 2012). For example, the challenges associated with responding to pathogen infection and pesticide pollution alter feeding behaviour, changing resource acquisition and metabolic rate (Baas & Kooijman, 2015; Bashir-Tanoli & Tinsley, 2014; Tyler et al., 2006). We hypothesised that studying whether resource use and acquisition are influenced by radiation would be a proximate way of assessing radiation effects that has the potential to be more generalisable across species than picking single life-history traits such as life span, fecundity or immune defence. Radiation effects on resource use might be manifested as either a decrease in energy use if radiation triggers major metabolic impairment, or as an increase in energy expenditure if radiation triggers metabolically costly repair processes or stress responses. While the effects of ionising radiation on metabolic rate have not been studied in invertebrates, ultraviolet exposure has been observed to elevate CO_2 production in mosquitos, which suggests metabolically active processes can respond to radiation exposure (Villena et al., 2018).

We studied the ecologically important bumblebee *Bombus terrestris*, a species found in the Chernobyl Exclusion Zone and in which Chernobyl-level radiation has been shown to reduce reproduction (Møller & Mousseau, 2009; Raines et al., 2020). Bumblebees are eusocial pollinators for which resources are essential for colony growth, maintenance and nest cell provisioning (Konzmann & Lunau, 2014). While their eusocial biology makes bumblebees rather unusual, many physiological responses of individual bumblebees may be generalisable to other species with solitary ecology. Floral nectar is a key energy source for bumblebees, which usually varies in sugar content between 15% and 64% (Seely, 1995); large quantities of nectar are needed to fuel a high mass specific metabolic rate and rapid colony growth trajectories (Duncan et al., 2002; Goulson, 2010). Bumblebees, like many

insects, exhibit discontinuous gas exchange, in which release of carbon dioxide and uptake of oxygen occurs cyclically (Miller, 1981).

The International Commission on Radiological Protection uses eusocial bees as a Reference Animal to generalise the likely ecological effects of radiation to all insects: this framework currently assumes insects are unaffected by radiation below 400 μGy/hr (ICRP, 2008). When this sensitivity threshold was set, there were no data on radiation effects for bumblebees below 400 μGy/hr (ICRP, 2008). Yet, some studies within the Chernobyl Exclusion Zone controversially suggest that there are significant reductions in bumblebee abundance at dose rates typical of natural background (0.01–1 μGy/hr) (Møller et al., 2012; Møller & Mousseau, 2009). Recent laboratory work demonstrates impairment of bumblebee queen production down to 50 μGy/hr (Raines et al., 2020).

In this study, we tested whether bumblebee metabolism responds to environmentally relevant ionising radiation exposure. We investigated whether radiation exposure alters metabolic rate and nectar consumption, while also measuring bumblebee activity and body mass changes; furthermore, we tested whether radiation effects persisted once exposure stopped. We also hypothesised that altered metabolic requirements might change bumblebee nectar preferences, so we conducted experiments using nectar solutions of varying concentration. Our data demonstrate significant shifts in resource acquisition and metabolic rate caused by dose rates found at 'post-disaster' sites.

2 | MATERIALS AND METHODS

We investigated radiation effects on bumblebee energy budgets via two complimentary experiments. The first investigated the effect of three environmentally relevant dose rates on bumblebee metabolic rate, nectar consumption and activity. The second used an exposure gradient to test for a dose rate threshold in the effect of radiation on feeding.

2.1 | Study system husbandry

We purchased *Bombus terrestris audax* colonies from Biobest® for each experiment (experiment 1, $n = 10$; experiment 2, $n = 5$). To identify newly eclosed bumblebees, on arrival every colony was anaesthetised with CO_2 and all bumblebees marked using commercial bumblebee paints. Each day following marking newly eclosed (unmarked) bumblebees were removed and weighed by placing the individual into a pre-weighed tube and then subtracting the weight of that tube for final mass. Bees were then individually housed prior to experiments in clear plastic containers (55 mm (l) × 55 mm (w) × 60 mm (h)) with access to ad libitum pollen, nectar solution and cotton wool as nesting material. Bumblebees remained in these containers throughout subsequent experiments; containers were cleaned every 5–7 days. The nectar solution was 40% w/v sucrose in distilled water, provided in a 12 ml falcon tube with a hole punctured in the side for feeding. We verified bumblebees were uninfected by the common gut parasite *Crithidia bombi* by microscopically inspecting faeces from a random sample of workers per colony (minimum $n = 18$); all tested negative. This research was

conducted with the approval of the University of Stirling Animal Welfare and Ethical Review Body (AWERB, Project Number: 122 (19 20).

2.2 | Experiment 1: The effect of radiation on bumblebee metabolic rate and feeding

To test the effects of ecologically-relevant radiation dose rates on bumblebee energy budgets, we measured feeding rate (nectar volume consumed) and metabolic rate (CO_2 production). Bumblebees were placed in the University of Stirling environmentally controlled radiation facility (12 hr light:dark cycle [07:00 hr–19:00 hr]) on shelves at different distances from a ^{137}Cs source to deliver controlled doses of gamma radiation (three distances within the radiation field and one control group outside the radiation field, Figure S2). Dose rates were verified prior to the experiment by placing dosimeters at each bumblebee position on each shelf. Bumblebees were kept in containers in two adjacent rows on each shelf (one row closer to the source and one further away) and moved between rows every 2 days to ensure no systematic dose rate variation occurred. Due to the 110 mm position difference between the front of one row and the back of the other, the maximum a dose rate could vary within this space was +/− 9 μGy/hr at 200 μGy/hr, +/− 3 μGy/hr at 100 μGy/hr, then dropping to +/1 at 40 μGy/hr. Part of the same environment-controlled room with the same conditions, but not exposed to radiation, was used to house the control treatment (Figure S2). Background radiation levels at the University of Stirling are 0.11 ± 0.01 μGy/hr (Raines et al., 2020). Nine data loggers around the facility recorded temperature and humidity every 2 min; the mean of these environmental variables was calculated for each bumblebee from the nearest data logger for the 2 days before each feeding rate measurement (mean = 25.6°C, range ± 0.3) and humidity (mean = 32.1%, range ± 13.1). The mortality rate of bumblebees throughout the experiment was 7.8%, with only 5.3% mortality between days 1 and 20 ($n = 288$ bumblebees).

The experiment involved three 10-day phases. First, to verify no confounding environmental effects influenced metrics, a 'no radiation' phase in which bumblebees were placed at assigned 'dose rate' positions but the radiation source remained shielded and no radiation was delivered ($n = 148$ bumblebees). Bumblebees were assigned positions in a stratified random way so neither age (days since eclosion) nor body mass (at eclosion) differed between dose rate groups ($F_{(3,145)} = 2.84$, $p = 0.10$ and $F_{(3,145)} = 0.87$, $p = 0.36$, respectively). Then followed a 'radiation' phase with four dose rate treatments (200, 100, 40 and 0.11 μGy/hr) for 10 days; at this time, 140 more bumblebees were added ($n = 288$ in total; $n = 72$ per dose rate). Again, no differences existed between dose rates for age ($F_{[3,285]} = 0.10$, $p = 0.75$) or mass ($F_{[3,285]} = 0.16$, $p = 0.69$). The final experimental phase tested if effects on bumblebees were transient: bumblebee 'recovery' from radiation was monitored for 10 days while the source was again shielded.

To measure bumblebee feeding, we weighed nectar tubes every 2 days; feeders were re-filled when empty and changed every 4 days. From when the radiation phase started, for half of the individuals ($n = 144$), we tested whether radiation influenced bumblebee nectar preference between a high and low concentration sucrose solution

(5% vs. 40% w/v) by providing two 14 ml feeders. A nectar concentration of 5% is very low, but values below 10% can be found in some plant species (Nicolson & Thornburg, 2007).

To assess bumblebee metabolic rate and activity levels, we measured bumblebee CO_2 production while filming movement for a 60-bumblebee subset (30 from 200 μGy/hr and 30 controls) on days 7 and 9 of both 'radiation' and 'recovery' phases (days chosen to detect an effect after several days of radiation and for logistical reasons not repeated on more days). Bumblebees selected were a mixture of those that entered the experiment at the start of the 'radiation' phase and those present for all three phases. In all, 12 bumblebees died in total between the first and fourth measurement and were replaced by another randomly chosen bumblebee from that treatment to maintain sample size. CO_2 output was measured using an infrared gas analyser (IRGA: EGM-4; PP Systems). Bumblebees were taken from the radiation facility to an adjacent room and housed in transparent plastic cylinders (34.36 cm³) individually connected to the IRGA with tubing in an open flow system. This room was not temperature controlled; therefore, air was drawn through tubing from the adjacent climate-controlled facility, through the chambers containing bumblebees, and then to the IRGA using an air pump (flow rate = 0.6 L/min). Air flow temperature (mean = 25.2°C, range ± 1.3) and humidity (mean = 32.2%, range ± 9.1) were recorded and averaged for the 5-min duration of all measurements. Bumblebees were left to acclimatise for 5 min prior to recordings. CO_2 levels were measured from a single chamber at a time using batches of four chambers; a manifold was used to switch recording between chambers. CO_2 was measured every 1.6 s for 5 min; to calculate bumblebee CO_2 output we subtracted ambient CO_2 measurements recorded from air flowing through an empty reference chamber for 30 s immediately after each recording. We converted each bumblebee's mean CO_2 output to μmol/min using flow rate and the ideal gas law ($PV = nRT$) which accounts for system pressure and volume. To monitor bumblebee activity during the 5-min metabolic rate recording, a video camera (FHD camcorder, 1080p, 30MP) filmed movements. Subsequently, the video was reviewed: total time bumblebees spent inactive (standing still or only moving legs, antennae or wings) or active (walking in the tube or buzzing) was recorded. Additionally, total distance each bumblebee walked was recorded from the video based on tube length. To minimise time out of radiation exposure, bumblebees were only removed from the radiation facility for a maximum of 30 min for each measurement period. Within these 30 min, a group of four bumblebees first had a 5-min acclimatisation period, followed by sequential 5-min metabolic rate measurements on each of the bees, with 30 s background measurements taken in between.

During these experiments, we found effects of radiation on feeding and metabolic rate; therefore, we designed a second experiment to investigate the nectar consumption effects in more detail (see below).

2.3 | Experiment 2: Dose rate threshold of the radiation effect on bumblebee nectar consumption

To further investigate the lower dose rates at which radiation effects on bumblebee feeding began to occur, we repeated our experiment using a radiation exposure gradient. Worker bumblebees ($n = 141$) of known age (1–4 days) were allocated to 19 treatments from 0.11 to 192 μGy/hr for 30 days (Figure S2). Dose rates were assigned ensuring no association between dose rate and age (Pearson correlation, $r_{[df = 140]} = −0.43$, $p = 0.67$) or mass (as recorded at eclosion) (Pearson correlation, $r_{[df = 140]} = 0.16$, $p = 0.870$). At each dose rate, bumblebee containers were kept in a single row on a shelving unit (four containers were placed at each dose rate). Bumblebees were free to move around in the 55 mm containers, meaning the maximum a dose rate could vary for a bumblebee was ± 4.5 μGy/hr at the highest dose rate of 200 μGy/hr, which dropped to ± 0.07 at the lowest exposed dose rate of 14 μGy/hr. Five data loggers recorded ambient facility temperature (mean = 25.2°C, range ± 1.8) and humidity (mean = 31.9%, range ± 15). Mean values for each bumblebee were calculated from the nearest data logger for the 2 days before each feeding measurement. Mortality rate throughout this experiment was 3.8% ($n = 144$). Experiment 1 (above) demonstrated irradiation increased nectar consumption. We predicted the magnitude of this effect would decrease at higher concentrations of nectar; therefore, in this experiment, bumblebees were randomly assigned a feeder containing 20%, 30%, 40% or 50% sucrose (w/v). Feeder weights were recorded every 2 days (± 1 day). To assess whether increased nectar consumption influenced bumblebee mass (using same protocol as above for weighing), we measured live bumblebee mass every 2 days (± 1 day), and after termination we measured cadaver dry weight. For dry weight determination, cadavers were dried for 5 days at 50°C and weighed, then reweighed after two further days to ensure that subsequent mass change was below 1 mg (following Řehoř et al., 2015). As bumblebees are partially endothermic and use considerable energy to generate heat, we also tested whether radiation exposure influenced thoracic temperature, which we measured every 2 days (± 1) using an infrared thermometer.

2.4 | Statistical analysis

We conducted analysis in R version 3.6.3 (R Core Team, 2020). All predictors except dose rate and time (days) were mean-centred and standardised to aid parameter interpretation. All analyses used a random effect for colony of origin and those involving repeated measures on bumblebees also contained individual-level random effects. Where appropriate, model simplification eliminated terms from the full model using likelihood-ratio tests, comparing models with and without the term of interest to calculate p-values. Models were validated by inspecting Q-Q plots and residual histograms. We converted nectar mass consumed to volume by dividing mass by nectar solution density. Nectar consumption and metabolic rate were square root transformed to improve model fit.

2.4.1 | Experiment 1: The effect of radiation on bumblebee metabolic rate and feeding

Radiation effects on nectar consumption and metabolic rate were analysed using linear mixed-effects models in lmer from package

LME4 (Bates et al., 2015). Models investigated radiation and recovery phases separately. Predictors included dose rate (continuous variable), time within a phase and their interaction. We included covariates for bumblebee mass and age at the start of the experiment, and assessed their interactions with dose rate to test for condition-dependent effects. Access to a second feeder was included as a factor. Environmental variables temperature, humidity (at the nearest data logger) and their interactions with each other were also included. Two-way interactions between dose rate and the environmental variables temperature and humidity were included in models analysing nectar consumed during the radiation phase from the 40% and 5% feeders to verify they did not influence dose rate effects. We tested whether radiation effects varied between radiation and recovery phases by combining data for both phases, then adding a phase term to the model alongside its interactions with dose rate and day. To investigate whether radiation dose rate influenced bumblebee activity and distance covered in the chamber during metabolic rate measurements, we constructed zero-inflated Gaussian generalised linear mixed models in GLMMTMB (Brooks et al., 2017); the response variable was number of seconds active (in 5 min), predictors were as above. A Gaussian zero-inflated model was selected as 38.3% of observations were zero movement.

We tested associations between variation in feeding, metabolic rate and activity using Bayesian multi-response mixed-effects models in MCMCGLMM (Hadfield, 2010). Response variables were as follows: total sucrose consumption in the 2 days before metabolic rate measurement; mean CO_2 output; and a two-vector response encompassing number of seconds active and inactive during metabolic rate measurements (error distributions were as follows: Gaussian, Gaussian and binomial, respectively). Fixed effects enabled independent intercepts for each response variable, and for each to be independently affected by radiation treatment (control vs. 200 μGy/hr) and bumblebee starting mass. Models had three random effects: colony, bumblebee and residual error; for each, we specified a trait interaction to estimate variances and covariances between response variables in an unstructured covariance matrix. We used parameter expanded priors for colony and bumblebee random effects. Our analysis focussed on correlations between traits in the residual error term, reflecting how between-replicate variation in the three response variables was associated. Markov chains ran for 60,000 iterations, discarding the first 10,000 interactions and sampling every 50 iterations. Parameter modes and p values were calculated from the posterior. Standard diagnostics verified low correlation between posterior samples (<0.1), chain convergence and insensitivity to prior specification.

2.4.2 | Experiment 2: Dose rate threshold of the radiation effect on bumblebee nectar consumption

To test for a lower dose rate threshold for radiation effects on nectar consumption, general linear mixed effects models (using LMER) included covariates for nectar concentration, dose rate and days

within experiment, alongside their interactions up to three ways. Additional covariates were bumblebee mass and age at the experiment start. We also tested whether consumption was influenced by the interaction between dose rate and weight at the start of the experiment. Further models investigated whether bumblebee mass, dry weight and thoracic temperature were affected by radiation exposure, with the same predictor structure (except for models with mass response variables, where start age was excluded due to variable collinearity). To test whether environmental variables influenced dose rate effects found, the two-way interactions with dose rate for humidity and temperature were included for models analysing nectar consumption and mass change.

3 | RESULTS

3.1 | Experiment 1: The effect of radiation on bumblebee nectar consumption

Before radiation exposure commenced, we verified that positional effects within the radiation facility did not influence nectar consumption. In all, 148 bumblebees were assigned positions where they would subsequently receive radiation and were then monitored for 10 days while the radiation source was shielded. Nectar consumption was not associated with future dose rate, demonstrating no confounding unmeasured environmental differences around the facility (Table S1).

During the radiation exposure phase, dose rate was significantly associated with elevated consumption of 40% nectar solution (Figure 1; Table S2; $\chi^2_{[1]} = 39.74$, $p = 2.90 \times 10^{-10}$). This effect of radiation on feeding became stronger as time exposed increased (Figure 1; Table S2; dose rate by day interaction, $\chi^2_{[1]} = 38.25$, $p = 6.22 \times 10^{-10}$). After 10 days of exposure to 200 μGy/hr, bumblebee consumption increased by 56% compared to controls. The dose rate effect remained significant when excluding data from 200 μGy/hr (Table S3; $\chi^2_{[1]} = 12.27$, $p = 1.00 \times 10^{-3}$); with a 14% feeding increase between controls and 100 μGy/hr. However, the 6% feeding elevation relative to controls at 40 μGy/hr was not significant (Table S4). Radiation effects on nectar consumption established quickly and were strongly significant after only 5 days (Day 5 data: $\chi^2_{[1]} = 16.67$, $p = 7.5 \times 10^{-5}$). However, after just 24 h of exposure, the association between dose rate and feeding was not quite significant (Day 1 data: $\chi^2_{[1]} = 3.70$, $p = 0.06$). Furthermore, for the initial 148 bumblebees, pairwise differences between nectar consumption 1 day before radiation started and 1 day after were not significant for any dose rate treatment (Table S5).

Bumblebees with higher body mass consumed more nectar (Table S2; $\chi^2_{[1]} = 16.94$, $p = 3.85 \times 10^{-5}$), but mass did not affect response to radiation (Table S2; dose rate by bumblebee mass, $\chi^2_{[1]} = 0.14$, $p = 0.70$). Slight temperature and humidity variation in the controlled environment facility affected feeding; both variables (which were standardised in analyses) were positively associated with nectar volume consumed; however, their effects were independent
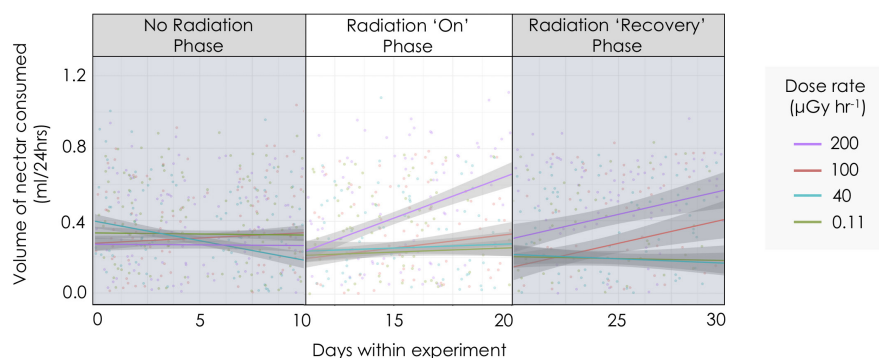
FIGURE 1 Exposure to radiation dose rates elevated bumblebee nectar consumption (40% sucrose), both during a 10-day irradiation phase and throughout the subsequent 10-day recovery. Data are presented for consumption during the no radiation phase (graph on the left), radiation 'on' phase (graph in Centre) and 'recovery' phase (graph on the right). The trend lines and shaded 95% confidence intervals were calculated from a mixed effects model with the same terms as shown in Table S2. The figure was generated from an analysis on each phase to provide an independent estimate of the dose rate effect. Plotted points represent raw data values and were jittered. For pre-radiation phase: $N = 444$ observations on $n = 148$ bumblebees. For radiation 'on' and 'recovery' phases: $N = 864$ observations on $n = 288$ bumblebees.

of radiation dose rate (Table S2). We tested whether radiation affected bumblebee preference for nectar sucrose concentration: half the bumblebees had a second feeder containing 5% nectar in addition to the 40% nectar feeder (which the analyses above focussed on). When comparing how much a bumblebee consumed from both the 40% and 5% feeders, across all feeding records ($n = 2,275$) bumblebees consumed 52.1% (± 2.1 SE) from the 40% feeder. There was no significant effect of radiation on the volume of nectar consumed from the 5% feeder (Table S6; $\chi^2_{[1]} = 0.37$, $p = 0.54$).

We assessed 40% nectar consumption during a 10-day recovery phase after radiation exposure stopped: the effect of the previously delivered dose rate persisted (Table S7; $\chi^2_{[1]} = 21.35$, $p = 3.84 \times 10^{-6}$). Similar to the radiation phase, effects of prior dose rate on appetite continued to increase with time for the higher dose rates, despite bumblebees no longer being exposed (Figure 1; dose rate by day interaction, $\chi^2_{[1]} = 12.48$, $p = 4.11 \times 10^{-4}$). As further evidence that elevated nectar consumption persisted once radiation exposure stopped, we pooled radiation and recovery phase data and found, after accounting for temporal changes, no difference in the effect of radiation between phases (Table S8).

3.2 | Experiment 1: The effect of radiation on bumblebee metabolic rate and activity

We assessed metabolic rate by measuring CO_2 production in a subset of 60 bumblebees split equally between 0.11 and 200 μGy/hr. During the exposure phase, CO_2 production was 18% higher in bumblebees receiving 200 μGy/hr than in controls (Table S9; $\chi^2_{[1]} = 4.80$, $p = 0.03$). The strength of this effect was consistent on both days 7 and 10 of exposure (Table S9; radiation exposure by day interaction, $\chi^2_{[1]} = 0.11$, $p = 0.75$). Across both treatments, CO_2 production fell significantly between days 7 and 10; it was also affected by small variations in air temperature, though this temperature effect was independent of radiation exposure (Table S9). During the recovery phase (when radiation ceased),

the difference in CO_2 production between the control and 200 μGy/hr treatments was no longer significant (Table S10; $\chi^2_{[1]} = 1.66$, $p = 0.20$). Indeed, there was a significant change in the effect of radiation between the exposure and recovery phases (Table S11; radiation exposure by phase interaction, $\chi^2_{[1]} = 5.54$, $p = 0.02$) (Figure 2).

Bumblebees exposed to radiation moved more during the metabolic rate assays. Across all activity observations, bumblebees were inactive for 28% of the time. Our zero-inflated analysis demonstrated no effect of radiation on the probability of bees moving (Table S12). However, for those bees that did move, time active was 30% higher following exposure to 200 μGy/hr compared to controls (Table S12; $\chi^2_{[1]} = 2.10$, $p = 0.04$). This difference disappeared by 7 days into the 'recovery' phase (Figure 3; Table S13). We found quantitatively the same results when considering the distance a bumblebee travelled as a metric of movement (Tables S14 and S15).

To investigate links between radiation-induced changes in bumblebee metabolic rate, nectar consumption and activity, we assessed the extent that variation in these measures was correlated during the radiation phase. Across all bumblebees, nectar consumption in the 2 days prior to metabolic rate measurements was significantly positively associated with CO_2 output (correlation $+0.31$, 95% HPD 1.36×10^{-3}–0.47; $p_{MCMC} = 0.03$); however, metabolic rate was not correlated with bumblebee activity levels during those measurements (correlation $+0.01$, 95% HPD -0.21–0.23; $p_{MCMC} = 0.49$). There was a weak but non-significant positive association between nectar consumption leading up to measurement and bumblebee activity levels (correlation $+0.22$, 95% HPD -0.12–0.40; $p_{MCMC} = 0.12$). Recovery phase results were qualitatively similar, though associations were weaker.

3.3 | Experiment 2: Dose rate threshold of the radiation effect on bumblebee nectar consumption

We then undertook a completely new experiment in which we investigated whether we could determine if a detectable dose rate
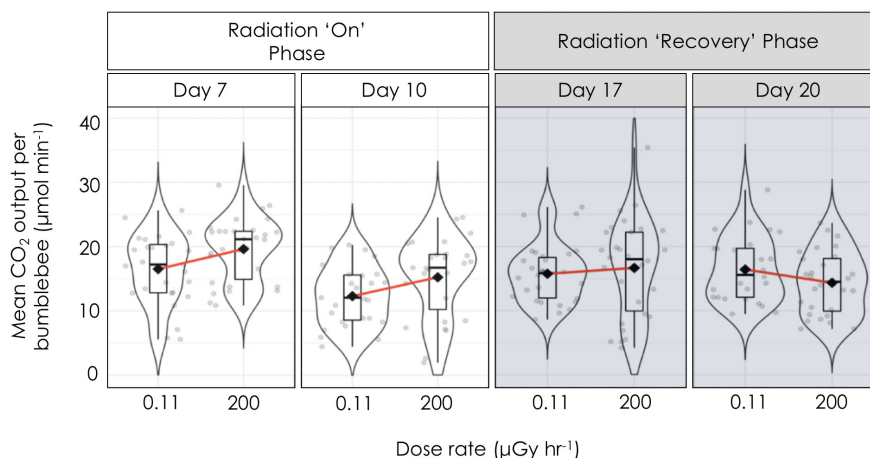
FIGURE 2 Bumblebee metabolic rate elevated during radiation exposure, a difference that disappeared when exposure stopped during the 'recovery' phase. Graphs show differences in mean carbon dioxide output for bumblebees on days 7 and 10 of the radiation phase (left) and recovery phase (right). Points on each graph show mean carbon dioxide output per bumblebee. The model analysing these data is shown in Table S11, which combines both the radiation and recovery phase data; the fit is represented by the red line and black diamonds, highlighting the differences between dose rates in mean CO_2 output. $N = 240$ observations, $n = 60$ bumblebees.
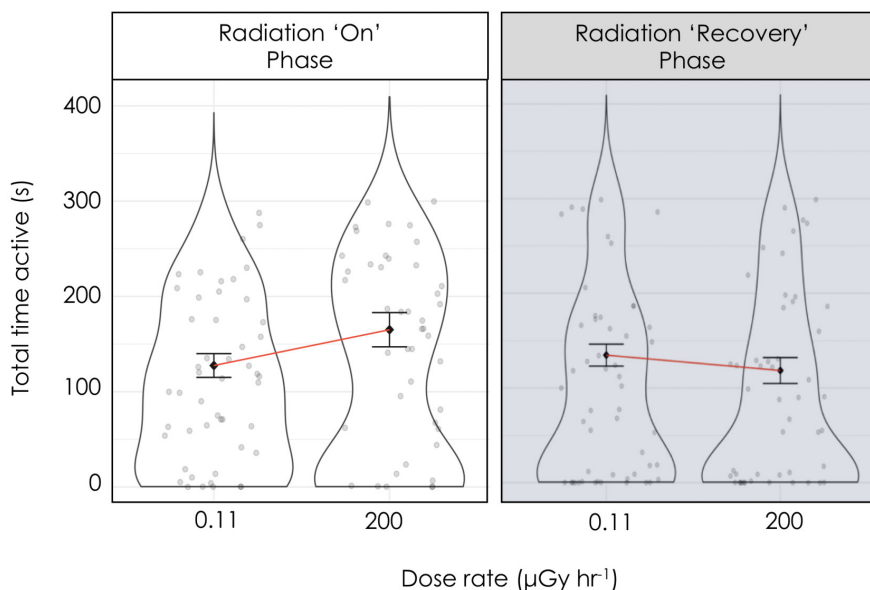

FIGURE 3 The time bumblebees spent active increased under radiation exposure. The left panel shows the 'on' phase when bumblebees were exposed to radiation and the right panel shows the 'recovery' phase when radiation exposure stopped. The single black point and whiskers represents mean time a bumblebee spent moving and the standard error calculated from the Gaussian part of the zero-inflated model. The red line denotes differences in mean time a bee was active between control bumblebees and irradiated bumblebees. Grey points represent raw data. The model from which this was calculated is the minimal model presented in Tables S12 and S13 combining both the radiation recovery data. $N = 240$ observations, $n = 60$ bumblebees.

threshold existed for the effect of radiation on nectar consumption that we found above. We conducted an independent experiment on 141 bumblebees to investigate appetite effects along a dose rate gradient (192–0.11 µGy/hr). We also tested whether radiation effects changed in response to increased sugar availability, by giving bumblebees one of four different nectar concentrations. The methodology for this was the same as above in terms of weighing of feeders and bumblebees to monitor consumption.

Increasing dose rate was again associated with increased nectar consumption during 30 days of exposure (Figure 4; Table S16;

$\chi^2_{[1]} = 4.89$, $p = 0.03$). While there was an overall trend that higher dose rates were associated with greater feeding, in this experiment the magnitude of this response varied with time and for different nectar concentrations (Table S16; Figure S3; concentration of nectar by dose rate by days within experiment, $\chi^2_{[1]} = 6.03$, $p = 0.01$). After 10 days of exposure, the dose rate effect was only evident for bumblebees consuming 50% nectar, whereas after 20 days it was the 40% nectar group that showed a compelling trend (Figure S3).

Clearly, the major driver of the increase in Figure 4 is because there was a substantial effect of radiation on feeding at higher dose
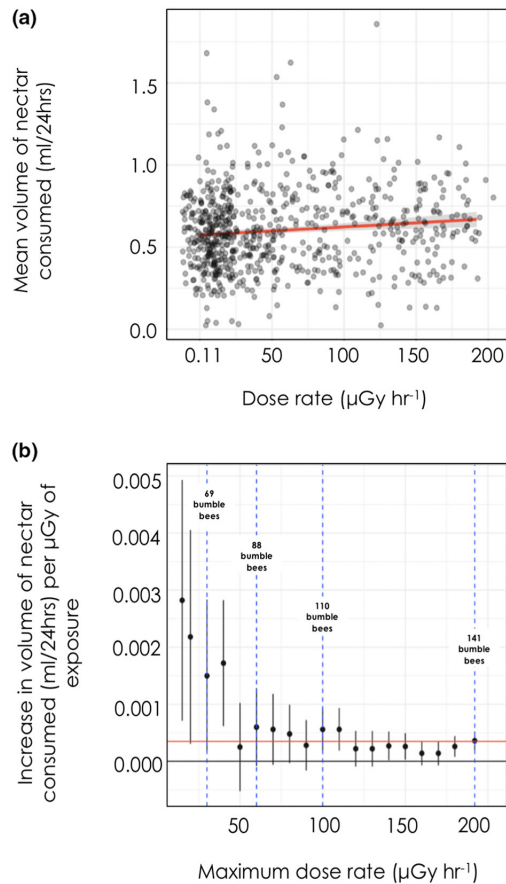
(a)

(b)

FIGURE 4 (a) The mean volume of nectar consumed by bumblebees increased with radiation dose rate. Data are pooled across the 30-day experimental period. Plotted points represent raw data values and were jittered. The red line represents model fit from Table S16. (b) the increase in nectar consumed per µGy of exposure. Parameter estimates were calculated by progressively omitting the highest doses of radiation from the model presented in Table S16. The red line denotes mean increase in volume consumed, calculated from all data up to 192 µGy for reference. Blue dashed lines denote the number of bumblebees remaining in the analysis when the doses above were removed. Error bars denote standard error. $N = 847$ observations, $n = 141$ bumblebees.

rates. However, we investigated this dataset to determine whether the lower and intermediate dose rates also generated a statistically significant uplift in feeding. To do this, we systematically removed data points from the analysis in increments of 10 µGy/hr, starting with the highest dose rates, thereby restricting our analysis to progressively lower dose rates. This process inevitably reduced our sample size and statistical power. For three of the truncated datasets between 192 and 100 µGy/hr, the radiation effect remained significant (Table S17). To compare the effect-size at different dose rates, we calculated the increase in nectar consumption per unit of exposure (µGy/hr) for each truncated dataset; this parameter remained approximately consistent down to dose rates of 50 µGy/hr (Figure 4b). This does not show that the total radiation effect on appetite at 50 µGy/hr was the same as at 192 µGy/hr but instead that the effect of increasing dose rate was broadly linear between 50

and 192 µGy/hr. At the lowest exposed dose rates we studied (14–30 µGy/hr), our estimates of feeding elevation per µGy/hr became substantially larger, though sample sizes for these analyses were small and confidence intervals much broader (Figure 4b).

In general, bumblebees lost mass during the experiment (Table S18; $\chi^2_{[1]} = 18.09$, $p = 2.17 \times 10^{-5}$), but dose rate did not influence this mass loss (dose rate by days interaction, $\chi^2_{[1]} = 0.53$, $p = 0.47$). In contrast, when we assessed the effect of radiation on bumblebee dry weight at the end of the experiment, there was a significant effect of dose rate mediated by the effect of bumblebee starting mass (wet weight). Higher dose rates were associated with greater dry mass for bumblebees that started the experiment at mid and heavy mass, but there was little effect of dose rate for light bumblebees (Figure 5; Table S19; dose rate by starting mass interaction; $\chi^2_{[1]} = 18.71$, $p = 1.76 \times 10^{-5}$). Bumblebees regulate body temperature partly by endothermic heat generation; while there was a marginal trend for bumblebees at higher dose rates to be warmer, this was not significant (Table S20; $\chi^2_{[1]} = 2.54$, $p = 0.11$).

4 | DISCUSSION

We present evidence that ionising radiation significantly affects insect metabolism and energy budgets, demonstrating that field-realistic radiation exposure influences fundamental metabolic processes in an ecologically important species. Substantial increases in bumblebee nectar consumption occurred during irradiation and remained even after exposure. Radiation-induced increases in food intake and metabolism might potentially influence many life-history traits through changes in resource budgets. We observed significant energy budget changes at 100–200 µGy/hr; dose rates found today in more highly contaminated areas of the Chernobyl Exclusion Zone (Beresford, Scott, et al., 2020). These effects occurred at dose rates below those currently considered safe for bumblebees by the International Commission on Radiological Protection (ICRP, 2008).

Bumblebee nectar consumption increased by 52% at 200 µGy/hr compared to controls following 10 days of irradiation. Bumblebees must have stored, metabolised or excreted this additional nectar. While our ability to detect resource storage was limited, radiation dose rate did drive an increase in dry weight (at least for larger bees) during 30 days of exposure. However, we detected no radiation effect on wet mass of live bumblebees during the experiment. This change in dry weight and not the wet mass of a live bumblebee suggests some material is accumulating within the bumblebees during radiation exposure; one potential explanation is that a stress response is occurring that has led to some excess nectar being stored as fat. Indeed, fat storage occurs in bumblebees under other stresses such as parasite infection (Vesterlund & Sorvari, 2014); however, further work is required to test whether this is the case for radiation exposure. We did not assess excretion; therefore, we cannot rule out that some of the additional nectar passed straight through the gut. We found no evidence that bumblebees used additional nectar for endothermic heat generation
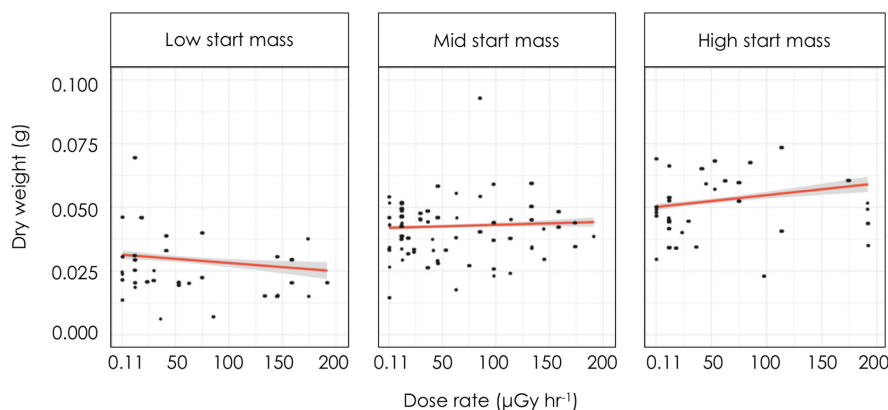
FIGURE 5 Exposure to radiation elevated dry weight of bumblebees depending on their mass when they entered the experiment. Data are presented for the mass of bees as they entered the experiment and categorised into low (0–0.12 g), mid (0.12–0.2 g) and high (>0.2 g) start weights. The trend lines and shaded 95% confidence intervals were calculated from a mixed-effects model with the same terms as shown in Table S19. The figure was generated by fitting a categorical factor for weight of a bumblebee when it entered the experiment, alongside a start weight by dose rate interaction, to provide an independent estimate of the dose rate effect for each weight category, $n = 121$ bumblebees.

because body temperature was not significantly influenced by radiation. However, metabolic rate increased by 18% at 200 µGy/hr; because nectar consumption increased by 52%, this suggests that only about one-third of the additional sucrose eaten contributed to metabolic rate elevation. While bumblebee activity increased by 30% at 200 µGy/hr, residual variance in activity was not associated with between-individual metabolic rate variation, indicating that increased movement was not the main driver of elevated metabolism. Instead, between-bumblebee variation in metabolic rate was significantly correlated with nectar consumption, suggesting radiation-induced feeding elevation may be a response to fuel unmeasured energetically costly radiation responses. Bumblebees do indeed suffer adverse fitness effects from radiation at these dose rates, such as impaired reproduction (Raines et al., 2020). We speculate that the increased bumblebee movement we observed happened either because increased appetite triggered food searching or as a direct effect of radiation on behaviour.

To further investigate whether radiation-induced nectar consumption occurred to support elevated energetic demands, we tested how feeding responses were influenced by artificial nectar sucrose content. Bumblebees naturally forage on nectar of widely varying sugar concentrations (Seely, 1995). During experiment one, we offered bees high and low sucrose nectar to test whether radiation-associated feeding increases were to acquire more sugar or more water. Bumblebees fed almost equally from the 40% and 5% nectar feeders but the significant effect of radiation on feeding occurred for the 40% sucrose, not the 5% sucrose feeder, suggesting that the radiation-induced response was to acquire additional sugar resources. During our second experiment, bumblebees received one of four nectar concentrations: we predicted that if bumblebees optimally balanced feeding and energy use, the radiation feeding increase might be smaller when consuming high sugar concentrations. Feeding elevations triggered by radiation were indeed sensitive to sucrose concentration; however, contrary to our hypothesis, feeding

increases again tended to be quicker and larger for higher concentration nectar.

Radiation effects on nectar consumption began rapidly within a few days of exposure, became stronger during 10 days of irradiation, and continued to develop even after exposure ceased. While metabolic rate elevation similarly established relatively rapidly (by our first measurement on exposure day 7), it had dropped again by 7 days post-exposure. Thus, bumblebee nectar consumption continued increasing after metabolic rate returned to baseline. Bumblebees may have entered metabolic deficit during irradiation, then continued elevated feeding after exposure to recoup lost resources. Alternatively, if radiation impaired the gut or feeding physiology, this continued elevated feeding could be non-adaptive.

The post-exposure period during which radiation effects on feeding persisted is a substantial proportion of a worker bumblebee's 22–69 days life span (Smeets & Duchateau, 2003). Bumblebees in radiologically contaminated landscapes such as Chernobyl may spend their entire life exposed to the dose rates we studied; therefore, radiation-induced feeding increases might escalate over their lifecycle. Increased nectar consumption would require more and longer foraging flights. Increased foraging might invoke other costs for bumblebees, such as elevating parasite transmission, which generally occurs on flowers during feeding (Shykoff & Schmid-Hempel, 1991). Worker foraging efficiency would be reduced by higher metabolic rates, which might also increase resource requirements of larvae in developing broods. Radiation-induced metabolic rate elevation could directly impact bumblebee life expectancy, as workers with higher resting metabolic rates die sooner (Kelemen et al., 2019). Radiation effects like these may well impair bumblebee colony reproduction, as has been shown in the laboratory (Raines et al., 2020). Nevertheless, our current study did not address colony-level fitness; therefore, it is not possible to extrapolate directly from the physiological and behavioural effects of radiation we observed on workers to the potential consequences for bumblebee

populations in the field. It remains possible that the cumulative impacts of the effects we detected could have wider detrimental impacts on pollination ecosystem services in radiologically contaminated environments.

We tested for a lower dose rate threshold at which increased nectar consumption disappeared. Feeding increases were significant between 100 and 200 µGy/hr. Below 100 µGy/hr, with smaller sample sizes, feeding elevation was not statistically significant, but the effect per unit of radiation exposure stayed relatively constant down to 50 µGy/hr, indicating radiation effects may persist into this dose rate range. Our findings have policy implications for the International Commission on Radiological Protection's environmental protection framework, which classifies dose rates below 400 µGy/hr as safe for bumblebees and other insects. Our data corroborate recommendations of Raines et al. (2020) that this threshold should be lowered.

The Chernobyl Exclusion Zone landscape is heterogeneously contaminated with ambient external dose rates ranging from typical background levels up to 250 µGy/hr (Beresford, Scott, et al., 2020). In contaminated environments, radiation exposure occurs both externally from gamma radiation, but also from other routes including internal accumulation of radionuclides. However, 95% of dose exposure to bumblebees at Chernobyl is from external gamma, indicating that our experimental design mimics natural exposure effectively (Beresford, Barnett, et al., 2020). Many studies from Chernobyl report negative radiation effects on organisms (Hermosell et al., 2013; Kesäniemi et al., 2019; Møller et al., 2007; Møller et al., 2012; Møller & Mousseau, 2009). Yet, the mechanisms driving these effects generally remain unclear. Our study identifies a process that may underpin some of these radiation impacts in contaminated environments. Resource availability is a dominant constraint on life-history trait investment (van Noordwijk & de Jong, 1986). We have discovered that radiation increases bumblebee resource requirements, elevating metabolism and feeding. The extent to which these effects can indeed be generalised to other insects and animals more widely will depend on whether they are specific to bumblebees (with their eusocial biology). While eusociality does make bumblebees atypical, the fact that we studied individual workers (rather than colony-level traits) means that our results may well be relevant to other organisms with solitary ecology.

We provide experimental evidence that ecologically relevant ionising radiation exposure leads to increased metabolic rate, feeding and activity. This could begin to explain some of the negative effects of radiation previously reported in bumblebees (Møller & Mousseau, 2009; Raines et al., 2020). The fundamental importance of resource acquisition and metabolic efficiency for animal life history means that studying these metrics may provide a novel unifying method to detect and explain radiation effects in a wide range of species.

AUTHORS' CONTRIBUTIONS

J.E.B., M.C.T. and D.C. conceived the ideas and designed the methodology; D.C. provided the training; N.A.B. and K.E.R. assisted the project planning; J.E.B. collected the data; J.E.B. and M.C.T. analysed the data; J.E.B. and M.C.T. led the manuscript writing. All authors contributed critically to drafts and gave final approval for publication.

CONFLICT OF INTEREST

The authors declare they have no competing interests.

DATA AVAILABILITY STATEMENT

Data are available from the NERC Environmental Data Centre https://doi.org/10.5285/0da32d7f-eea1-4200-8fde-3a32d0d9ed05 (Burrows et al., 2021).

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REFERENCES

Andersson, P., Garnier-Laplace, J., Beresford, N. A., Copplestone, D., Howard, B. J., Howe, P., Oughton, D., & Whitehouse, P. (2009). Protection of the environment from ionising radiation in a regulatory context (protect): Proposed numerical benchmark values. *Journal of Environmental Radioactivity*, 100, 1100–1108. https://doi.org/10.1016/j.jenvrad.2009.05.010

Baas, J., & Kooijman, S. A. L. M. (2015). Sensitivity of animals to chemical compounds links to metabolic rate. *Ecotoxicology*, 24, 657–663. https://doi.org/10.1007/s10646-014-1413-5

Baker, R. J., Bickham, A. M., Bondarkov, M., Gaschak, S. P., Matson, C. W., Rodgers, B. E., Wickliffe, J. K., & Chesser, R. K. (2001). Consequences of polluted environments on population structure: The bank vole (*Clethrionomys glareolus*) at Chernobyl. *Ecotoxicology*, 10, 211–216. https://doi.org/10.1023/a:1016665226716

Bashir-Tanoli, S., & Tinsley, M. C. (2014). Immune response costs are associated with changes in resource acquisition and not resource reallocation. *Functional Ecology*, 28, 1011–1019. https://doi.org/10.1111/1365-2435.12236

Basu, A. K. (2018). DNA damage, mutagenesis and cancer. *International Journal of Molecular Sciences*, 19, 1–13. https://doi.org/10.3390/ijms19040970

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, 67, 1–48. https://doi.org/10.18637/jss.v067.i01

Beaugelin-Seiller, K., Garnier-Laplace, J., Della-Vedova, C., Métivier, J. M., Lepage, H., Mousseau, T. A., & Møller, A. P. (2020). Dose reconstruction supports the interpretation of decreased abundance of mammals in the Chernobyl exclusion zone. *Scientific Reports*, 10, 1–14. https://doi.org/10.1038/s41598-020-70699-3

Beresford, N. A., Barnett, C. L., Gashchak, S., Maksimenko, A., Guliaichenko, E., Wood, M. D., & Izquierdo, M. (2020). Radionuclide transfer to wildlife at a "reference site" in the Chernobyl exclusion zone and resultant radiation exposures. *Journal of Environmental*

Radioactivity, *211*, 105661. https://doi.org/10.1016/j.jenvrad.2018.02.007

Beresford, N. A., Barnett, C. L., Jones, D. G., Wood, M. D., Appleton, J. D., Breward, N., & Copplestone, D. (2008). Background exposure rates of terrestrial wildlife in England and Wales. *Journal of Environmental Radioactivity*, *99*, 1430–1439. https://doi.org/10.1016/j.jenvrad.2008.03.003

Beresford, N. A., Scott, E. M., & Copplestone, D. (2020). Field effects studies in the Chernobyl exclusion zone: Lessons to be learnt. *Journal of Environmental Radioactivity*, *211*, 105893. https://doi.org/10.1016/j.jenvrad.2019.01.005

Bonzom, J.-M., Hättenschwiler, S., Lecomte-Pradines, C., Chauvet, E., Gaschak, S., Beaugelin-Seiller, K., Della-Vedova, C., Dubourg, N., Maksimenko, A., Garnier-Laplace, J., & Adam-Guillermin, C. (2016). Effects of radionuclide contamination on leaf litter decomposition in the Chernobyl exclusion zone. *Science of the Total Environment*, *562*, 596–603. https://doi.org/10.1016/j.scitotenv.2016.04.006

Brooks, M. E., Kristensen, K., van Benthem, K. J., Magnusson, A., Berg, C. W., Nielsen, A., Skaug, H. J., Mächler, M., & Bolker, B. M. (2017). glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modelling. *The R Journal*, *9*, 378–400. https://doi.org/10.32614/RJ-2017-066

Burrows, J., Copplestone, D., Beresford, N. A., Raines, K., & Tinsley, M. (2021). *Nectar consumption, metabolic rate and activity datasets for bumblebees exposed to ecologically relevant radiation dose rates*. NERC Environmental Information Data Centre. (Dataset). https://doi.org/10.5285/0da32d7f-eea1-4200-8fde-3a32d0d9ed05

Copplestone, D., Larsson, C. M., Strand, P., & Sneve, M. K. (2015). Protection of the environment in existing exposure situations. *Annals of the ICRP*, *45*, 91–105. https://doi.org/10.1177/0146645316630167

Duncan, F. D., Krasnov, B., & McMaster, M. (2002). Metabolic rate and respiratory gas-exchange patterns in tenebrionid beetles from the Negev highlands, Israel. *The Journal of Experimental Biology*, *798*, 791–798. https://doi.org/10.1006/jare.1994.1070

Eeva, T., Hakkarainen, H., & Laaksonen, T. (2006). Environmental pollution has sex-dependent effects on local survival. *Biology Letters*, *2*, 298–300. https://doi.org/10.1098/rsbl.2006.0443

Fritsch, C., Jankowiak, Ł., & Wysocki, D. (2019). Exposure to pb impairs breeding success and is associated with longer lifespan in urban European blackbirds. *Scientific Reports*, *9*, 1–11. https://doi.org/10.1038/s41598-018-36463-4

Fuller, N., Ford, A. T., Lerebours, A., Gudkov, D. I., Nagorskaya, L. L., & Smith, J. T. (2019). Chronic radiation exposure at Chernobyl shows no effect on genetic diversity in the freshwater crustacean, *Asellus aquaticus* thirty years on. *Ecology and Evolution*, *9*, 10135–10144. https://doi.org/10.1002/ece3.5478

Geras'kin, S. A., Fesenko, S. V., & Alexakhin, R. M. (2008). Effects of non-human species irradiation after the Chernobyl NPP accident. *Environment International*, *34*, 880–897. https://doi.org/10.1016/j.envint.2007.12.012

Goulson, D. (2010). *Bumblebees: Their behaviour and ecology* (4th ed.). OUP Oxford.

Hadfield, J. D. (2010). MCMC methods for multi-response generalized linear mixed models: The MCMCglmm R package. *Journal of Statistical Software*, *33*, 1–22. https://doi.org/10.18637/jss.v033.i02

Hermosell, I. G., Laskemoen, T., Rowe, M., Møller, A. P., Mousseau, T. A., Albrecht, T., & Lifjeld, J. T. (2013). Patterns of sperm damage in Chernobyl passerine birds suggest a trade-off between sperm length and integrity. *Biology Letters*, *10*, 8–11. https://doi.org/10.1098/rsbl.2013.0530

Hladun, K. R., Smith, B. H., Mustard, J. A., Morton, R. R., & Trumble, J. T. (2012). Selenium toxicity to honey bee (*Apis mellifera* L.) pollinators: Effects on behaviours and survival. *PLoS ONE*, *7*, 1–10. https://doi.org/10.1371/journal.pone.0034137

Hosseini, A., Thørring, H., Brown, J. E., Saxén, R., & Ilus, E. (2008). Transfer of radionuclides in aquatic ecosystems—Default concentration ratios for aquatic biota in the Erica tool. *Journal of Environmental Radioactivity*, *99*, 1408–1429. https://doi.org/10.1016/j.jenvrad.2008.01.012

ICRP. (2008). Environmental protection: The concept and use of reference animals and plants. *ICRP publication 108*. *Annals of the ICRP*, *38*, 4–6. https://doi.org/10.1016/j.icrp.2006.06.001

Kelemen, E. P., Cao, N., Cao, T., Davidowitz, G., & Dornhaus, A. (2019). Metabolic rate predicts the lifespan of workers in the bumblebee *Bombus impatiens*. *Apidologie*, *50*, 195–203. https://doi.org/10.1007/s13592-018-0630-y

Kesäniemi, J., Jernfors, T., Lavrinienko, A., Kivisaari, K., Kiljunen, M., Mappes, T., & Watts, P. C. (2019). Exposure to environmental radionuclides is associated with altered metabolic and immunity pathways in a wild rodent. *Molecular Ecology*, *28*, 4620–4635. https://doi.org/10.1111/mec.15241

Konzmann, S., & Lunau, K. (2014). Divergent rules for pollen and nectar foraging bumblebees - a laboratory study with artificial flowers offering diluted nectar substitute and pollen surrogate. *PLoS ONE*, *9*, e91900. https://doi.org/10.1371/journal.pone.0091900

Miller, P. (1981). *Locomotion and energetics in arthropods* (1st ed.). Edited by C. F. Herreid. Plenum Press.

Møller, A. P., Barnier, F., & Mousseau, T. A. (2012). Ecosystems effects 25 years after Chernobyl: Pollinators, fruit set and recruitment. *Oecologia*, *170*, 1155–1165. https://doi.org/10.1007/s00442-012-2374-0

Møller, A. P., & Mousseau, T. A. (2009). Reduced abundance of insects and spiders linked to radiation at Chernobyl 20 years after the accident. *Biology Letters*, *5*, 356–359. https://doi.org/10.1098/rsbl.2008.0778

Møller, A. P., Mousseau, T. A., de Lope, F., & Saino, N. (2007). Elevated frequency of abnormalities in barn swallows from Chernobyl. *Biology Letters*, *3*, 414–417. https://doi.org/10.1098/rsbl.2007.0136

Mousseau, T. A., Milinevsky, G., Kenney-Hunt, J., & Møller, A. P. (2014). Highly reduced mass loss rates and increased litter layer in radioactively contaminated areas. *Oecologia*, *175*, 429–437. https://doi.org/10.1007/s00442-014-2908-8

Nicolson, S. W., & Thornburg, R. W. (2007). Nectar chemistry. In *Nectaries and nectar* (pp. 215–264). Springer.

R Core Team. (2020). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. https://www.R-project.org/

Raines, K. E., Whitehorn, P. R., Copplestone, D., & Tinsley, M. C. (2020). Chernobyl-level radiation exposure damages bumblebee reproduction: A laboratory experiment. *Proceedings of the Royal Society: Biological Sciences*, *287*, 20201638. https://doi.org/10.1098/rspb.2020.1638

Řehoř, I., Macháčková, L., Bučánková, A., Matějková, S., Černá, K., & Straka, J. (2015). Measuring the sugar consumption of larvae in bumblebee microcolonies: A promising new method for tracking food economics in bees. *Apidologie*, *45*, 116–128. https://doi.org/10.1007/s13592-013-0233-6

Rykabon, N., & Goncharova, R. (2006). Transgenerational accumulation of radiation damage in small mammals chronically exposed to Chernobyl fallout. *Radiation Environmental Biophysics*, *45*, 167–177. https://doi.org/10.1007/s00411-006-0054-3

Seely, T. (1995). *The wisdom of the hive* (1st ed.). Harvard University Press.

Shykoff, J., & Schmid-Hempel, P. (1991). Incidence and effects of four parasites in natural populations of bumblebees in Switzerland. *Apidologie*, *22*, 117–125. https://doi.org/10.1051/apido:19910204

Smeets, P. A. M., & Duchateau, M. (2003). Longevity of *Bombus terrestris* workers (Hymenoptera: Apidae) in relation to pollen availability, in the absence of foraging. *Apidologie*, *34*, 333–337. https://doi.org/10.1051/apido:2003026

Smith, J. T., Willey, N. J., & Hancock, J. T. (2012). Low dose ionizing radiation produces too few reactive oxygen species to directly affect antioxidant concentrations in cells. *Biology Letters*, *8*, 594–597. https://doi.org/10.1098/rsbl.2012.0150

Tyler, E., Adams, S., & Mallon, E. B. (2006). An immune response in the bumblebee, *Bombus terrestris* leads to increased food consumption. *BMC Physiology*, *6*, 1–4. https://doi.org/10.1186/1472-6793-6-6

UNSCEAR. (2008). *Sources and effects of Ionizing radiation. Volume I: SOURCES*. Report to the General Assembly Scientific Annexes A and B. Retrieved from https://www.unscear.org/docs/publications/2008/UNSCEAR_2008_Report_Vol.I.pdf

van Noordwijk, A. J., & de Jong, G. (1986). Acquisition and allocation of resources: Their influence on variation in life history tactics. *The American Naturalist*, *128*, 137–142.

Vesterlund, S., & Sorvari, J. (2014). Longevity of starved bumblebee queens (Hymenoptera: Apidae) is shorter at high than low temperatures. *European Journal of Entomology*, *111*, 217–220. https://doi.org/10.14411/eje.2014.035

Villena, O. C., Momen, B., Sullivan, J., & Leisnham, P. T. (2018). Effects of ultraviolet radiation on metabolic rate and fitness of *Aedes albopictus* and *Culex pipiens* mosquitoes. *PeerJ*, *8*, 1–20. https://doi.org/10.7717/peerj.6133

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