The Effects of Chronic Low-Dose Radiation on Bumblebees

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Summary abstract

The consequences to wildlife of living in contaminated areas with chronic low-dose rates of radiation are still relatively unknown. Laboratory studies using acute radiation have demonstrated that invertebrates are relatively radioresistant compared to other taxa. However, there is little scientific evidence to show how chronic low dose rates affect invertebrates. This is problematic for understanding the consequences to widllife living in highly contaminated areas and also testing assumptions made for invertebrates by the International Commission on Radiological Protection (ICRP). This thesis was designed to address a number of recommendations have been suggested to improve radioecological studies and help reduce the uncertainty as to effects at low dose rates. These include environmentally relevant laboratory studies (Chapters 2 and 4), improved dosimetry and dose assessments (Chapter 3), investgating confounding factors (Chapter 4) and continuity between laboratory experiments and field work conducted in the Chernobyl Exclusion Zone (CEZ) (Chapter4).

Chapter 2 presents an environmentally-relevant experiment testing how bumblebee reproduction and life history is affected by chronic low-dose rates. Unexpectedly, at dose rates equivalent to the CEZ, queen production declined and reproductive timing was altered. The estimation of dose rates to establish a dose-effect relationship for wild animals is difficult and a common criticism of radioecological studies, therefore, Chapter 3 tests whether the common approach to measuring only external ambient dose rates is suitable and whether the inclusion of life-history traits significantly alters the dose rate. The findings from this chapter reiterate the necessity to use dose-assessment tools to test different parameters to estimate dose rate in different scenarios to account for unknown variation. Chapter 4 demonstrates that in areas of elevated dose rates in the CEZ parasite burden was higher and bumblebees did not live as long. These results were reinforced by a laboratory study, which determined bumblebees exposed to increased radiation doses had high parasite burdens and were infected quicker, resulting in reduced longevity.

The data in this thesis detected effects below the current dose bands used in international radioprotection and therefore advocate these dose bands be re-evaluated. However, the data do not support studies which have measured adverse effects at dose rates similar to background and suggest that confounding factors such as habitat quality and co-stressors need to be included in field and laboratory studies.

Declaration of authorship

I, Katherine Elizabeth Raines, declare that I have composed this thesis and that it embodies the results of my own research. Where appropriate, I have acknowledged the nature and the extent of work carried out in collaboration with others.

Signed..

Date...

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1. Contents

Chapter 1

General Introduction

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1.1 Introduction

The discoveries of X-rays in 1895 and radioactivity in 1896 created many applications of this phenomena, firstly in medicine and subsequently in energy production (Valentin 2013). Since then, there has been significant advancement in the protection of humans from the harmful effects of radiation exposure (UNSCEAR 2008). In contrast, the consequences to the environment and non-human biota from radiation were comparatively unknown until recently (Brechignac et al. 2016). In 1991, radiological protection of the environment was based on the anthropogenic approach; if humans were protected, then it was assumed the whole environment was protected (ICRP 1991). This approach has subsequently been deemed unacceptable, and it has been acknowledged that the environment should be protected in its own right (Hinton et al. 2004; Valentin 2003). In 2007, the International Commission on Radiological Protection (ICRP) made recommendations that specified a commitment to the radiological protection of the environment, including non-human biota (ICRP 2007b).

Radioecological studies investigating the effects of radiation on non-human biota have preceded the ICRP's commitment to environmental protection. In response to the Cold War and potential nuclear Armageddon, there was an increase in experiments testing the effects of radiation on various flora and fauna (Stalter & Kincaid 2009). In the 1960s, experiments were conducted at the Brookhaven National Laboratory, USA, using large-scale irradiators to expose a forest ecosystem to chronic dose rates ranging from 8.77mGy d^{-1} to 43 mGy d^{-1} (Woodwell & Miller 1963; Woodwell & Sparrow 1963; Bostrack & Sparrow 1970). Nuclear disasters resulting in environmental releases such as Kyshtym (1957), Wind scale (1957), Three Mile Island (1979), Chernobyl (1986) and Fukushima (2011) also reiterated the importance of understanding the effects of radiation to non-human biota.

In recent years, there has been renewed interest in radioecology as understanding the consequences of radiation in the environment is critical as many countries around the world are faced with dealing with legacy radioactive wastes, restoring sites historically contaminated with radioactivity and planning for new nuclear power stations and nuclear decommissioning as well as providing a response to emergencies such as radiological accidents or terrorist events. However, despite decades of research to understand how non-human biota are affected by low-dose chronic radiation, there is still scientific debate at which chronic dose rates result in adverse effects for wildlife, and this has implications for the radiological protection of the environment.

The work presented in this thesis seeks to understand whether chronic low-dose rates are detrimental to bumblebees. This thesis presents a combination of environmentally-relevant experiments and field studies conducted in the Chernobyl Exclusion Zone with the aim to helps further the understanding of the consequences to wildlife living in areas with elevated dose rates. There has been a general lack of focus on invertebrates, in favour of mammals, as mammals have closer links to human radiological protection. Furthermore, invertebrates are considered less radiosensitive than many other taxa, and therefore are unlikely to suffer detrimental effects even in the Chernobyl Exclusion Zone. The data in this thesis will test current international radiological protection and resolve the current scientific conflict in radioecology as to the effects of chronic low-dose radiation by addressing many of the requirements which have been outlined by the radioecological community.

1.2 The approach undertaken for radiological protection of the environment

The aim for radiological protection of the environment, as derived from the International Atomic Energy Agency (IAEA) is that activities should not cause damage to the environment, observe the optimum sustainable yield of natural resources and maintain biodiversity, ecosystem and processes that are essential for the functioning of the biosphere (Larsson 2012; IAEA 2006). Environmental regulators annually monitor the releases of radionuclides from nuclear power stations, sites that produce and process nuclear fuel, research institutions, defence establishments and radiochemical producers (RIFE 2016). The monitoring of radioactivity in the environment is to fulfil requirements to demonstrate that people receive a dose of less than one mSv per year in accordance with the basic safety standards outlined by EURATOM (EC 2014). More recently, prospective dose assessments have been undertaken in the UK for sites to be licensed to released radioactivity in the environment to ensure that there will be no risk to the environment in reference to the Birds and Habitat Directive (EC 2010; EC 1992) and specifically, due to the commitments to protect Natura 2000 sites (RIFE 2016; Allott et al 2009; EC 2000).

The approach used by the International Commission on Radiological Protection (ICRP) to protect the environment is based on Reference Animals and Plants (RAPs). RAPS are hypothetical entities with known basic biological characteristics as described to taxonomic level of family (ICRP 2008). These characteristics include anatomical, physiological and life history properties which can subsequently be used to relate exposure to dose and dose to effects (ICRP 2008). The RAP approach was developed by the ICRP in 2008 to account for species diversity and provide a reference point for a range of taxa (Larsson 2016; ICRP 2008). RAPs are all considered to be wild organisms of 'typical' environments and various parameters and assumptions have been described in ICRP Publication 108; additionally, all the calculations have been conducted with best-available dose modelling tools (ICRP 2008). RAPs were chosen using taxonomic and practical criteria to emulate the "Reference person" in human radiological protection with the aim to provide baseline data and points of reference for comparisons for people undertaking wildlife dose assessments (Bradshaw et al. 2014; ICRP 2007).

Figure 1.1. presents the Derived Consideration Reference Level (DCRL) for each RAP as adapted from ICRP Publication 108. The DCRL is a band of dose rate at which anticipated deleterious effects are likely to occur (ICRP 2008). The DCRLs demonstrate different taxa's anticipated radiosensitivity, but this placement has also been constrained by the available data. For example, mammals are known to have increased radiosensitivity when compared to other taxa due to their biological complexity, however, as the DCRLs are constrained by the available data this is unequal across the different groups which can affect the placement (ICRP 2008). Therefore, the DCRLs are set on a precautionary basis and therefore not intended to be regarded as dose limits and highlight the need for a more considered level of evaluation (ICRP 2008).

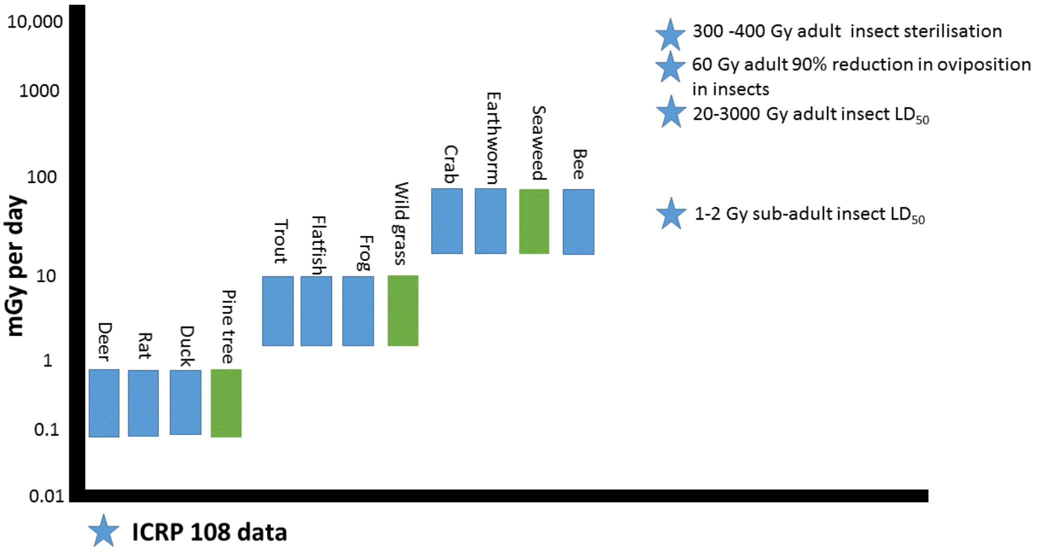

Figure 1.1 International Commission on Radiological Protection Reference Animals and Plants (RAPs) allocates a band of dose rates where adverse effects of radiation would be expected. The starred data points are the studies for the Reference bee, but these are studies are on other insects and not bees. The dose rate at which the effect happened. Blue colouring denotes animals, and green colouring denotes plant species.

There are no data in the ICRP Publication 108 below 10 mGyd^{-1} for the bee RAP and the DCRL for bees has been set at between 10 -100 mGy d^{-1} (equivalent to 400 – 4000 µGy h^{-1}) (ICRP 2008). While there is a DCRL for bees, the data provided in ICRP 108, which underpins the placement of the DCRLs presented in Figure 1, is based on experiments conducted on other insects such as wasp, weevil and fruit fly (ICRP 2008; Spirin 1996). The lack of data for the reference bee presents an opportunity to improve upon the previous laboratory studies undertaken which use acute doses and to test whether the placement of the DCRL for bumblebees is in the right place.

The bee RAP represents the Apidae family, and life history is described as semelparous (for males) with a high female: male ratio, high fecundity, population number <10,000 (ICRP 2008). These characteristics are most similar to honey bee spp., rather than other bees such as bumblebees and solitary bees (Goulson 2010). Honeybee queens mate in the autumn and establishes her colony where she lives for three years laying 200,000 eggs per year, the average lifespan of workers is 100 days. Eggs then hatch after four days; larvae pupation occurs after six days and emerge as adults at 20 days, and unfertilised eggs produce males, and some eggs develop into young queens (ICRP 2008). The life cycles for other eusocial Hymenoptera and solitary bees are very different, so this needs to be evaluated when making dose calculation assessments as, for example, lifespan could significantly influence the total dose accumulated by bees (ICRP 2008). The ICRP reports Apidae life history as being on the soil in reference to the dose conversion factors (ICRP 2008). However, many native bee species, bumblebees and solitary bees, spend most of their life below the ground in nests – including radiosensitive developmental phases (Goulson 2010; Lye 2009). The location of the nest is essential when estimating dose rate to bees as living in the soil will alter the dose rate when compared to living on top of the soil due to the inherent shielding properties from radiation of soil (IAEA 2006).

1.3 Existing literature investigating the effects of radiation on invertebrates

Radiation effects are grouped into two different types by the ICRP, stochastic and deterministic (or non-stochastic) (ICRP 2007). The aim of radioprotection for both people and wildlife is to prevent detrimental deterministic effects and to reduce the probability of stochastic effects. Stochastic effects are a function of dose and do not have a threshold (ICRP 1977) Stochastic effects are the probability of an effect is likely to occur, rather than its severity (ICRP 1977). As the deposition of energy by radiation is a random process, even at low dose rates it is possible that enough energy is deposited within a cell to result in cellular changes or cell death (ICRP 2008). Somatic effects are classed as a stochastic effect, and the main risk at low doses is carcinogenesis or increased, stochastic effects can affect individuals at the time of exposure and subsequent generations (ICRP 1977). The severity of deterministic effects is linked to the dose rate received (ICRP 1977). Deterministic effects are linked to particular tissues, in mammals, these are gonads, red bone marrow, bone, lung, thyroid and breast tissue (ICRP 1977).

However, invertebrates do not have these components and have simpler anatomy with fewer differential cells (Loeb et al. 2001). Only the gonads and the midgut epithelium have differential cells which are therefore radiosensitive (Loeb et al. 2001). For wildlife, effects which are stochastic and deterministic are of less value for biological consequence (ICRP 2008). There cannot be effects at a population level without effects occurring at an individual level, but effects in individuals will not always impact on the population (ICRP 2008). Therefore, the effects, which are of the most relevance, are the ones that inform changes at a population or ecosystem level.

The ICRP focusses on four biological endpoints mortality, morbidity, reproduction and chromosomal damage (ICRP 2008). The biological endpoints were selected as although they are most relevant to individuals, these could lead to changes in population size and structure (ICRP 2008). Mortality can lead to changes in age distribution, death rate and density and reproduction affects the birth rate, age distribution, number and density of individuals in a population. It is apparent how reductions in mortality or reproduction as caused by radiation can impact both the individual and the populations. What is unclear, however, is how reduced morbidity which reduces the fitness of individuals, therefore, resulting in difficulties surviving in the natural environment, and increased chromosomal damage impact at a population or ecosystem level.

Laboratory studies

There have been a number of laboratory studies using a range of taxa to determine how radiation affects living organisms. However, 64% of all datasets within the FREDERICA database for radiation effects studies were conducted using acute or transitory dose (Copplestone et al. 2008). Of the remaining 36% of these studies which have used chronic dose administration, 70.5% of these have been conducted on fish, mammals and terrestrial plants (Copplestone et al. 2008). There have been no previous laboratory experiments conducted on bumblebees, and there are no data on the morbidity, reproductive or morality effects on insects of dose rates below 950 µGy h^{-1} (Copplestone et al. 2008).

There are two main reasons why invertebrates have previously been overlooked in radiation effects studies. The first because insects, and invertebrates in general, are deemed to be less radiosensitive than many other taxa (ICRP 2008). (Bakri et al. 2005). Even though insects are deemed to be more radiosensitive than other taxa, radiosensitivity varies with life stage (ICRP 2008; Bakri et al. 2005; Tilton et al. 1966). Adult insects have been shown to be the most radioresistant followed by pupae, larvae and finally, the eggs are the most radiosensitive life stage (ICRP 2008; Bakri et al. 2005; Tilton et al. 1966).

Secondly, the aim of much of the research as to radiation effects on invertebrates has been associated with the sterilisation of pest insects. Many of the studies which have investigated the effects of radiation to terrestrial invertebrates have used acute dose rates. Acute dose rates in laboratory studies have commonly been used to determine the Lethal Dose (LD_{50}) values for non-human biota. LD_{50} is a common ecotoxicological term to describe the amount of something, in this case, radiation dose, that will kill 50% of a test sample (Gad 2014). LD_{50} values for large adult mammals range from 1.2 – 3.9 Gy (Bond et al. 1965) whereas LD_{50} values for adult insects are at much higher dose rates 20 – 3000 Gy (Woodhead 1998; Spirin 1996). A large proportion of the laboratory experiments investigating the effects of acute radiation on insects have been conducted for the practical application to sterilise pest insects by the International Database on Insect Disinfestation and Sterilisation (IDIDAS). Presently, there are sterility and disinfestation records for 373 different insect species as part of IDIDAS (https://nucleus.iaea.org/sites/naipc/ididas/).

These experiments have predominantly been focussed on the establishment of LD_{50} values and testing at which dose rates sterility occurs in insects (Dyck et al. 2005; Bakri et al. 2005). The work conducted by IDIDAS has shown that radiosensitivity of sterility in terrestrial insects varies between families and among orders by up to two orders of magnitude. Arctiidae and Pyralidae (Lepidoptera) were the most radioresistant (100 -300 Gy for sterilisation) (Chakroun et al. 2017; Rahman et al. 2002) and Acrididae (Orthoptera) (Dushimirimana et al. 2012) and Blaberidae (Dictyoptera) were the least radioresistant (<5 Gy) (Bakri et al. 2005).

The Honeybee (*Apis mellifera)* has been studied as part of IDIDAS. At 75 Gy, 99% of eggs failed to hatch and, at 35 Gy, 40% of the eggs laid were inviable (Lee 1957). At 4 Gy, the average percentage of non-hatching eggs laid by queens that had received irradiated sperm was 16% and 55% at 18 Gy (Lee 1957). The dose-response curve of these experiments demonstrated an overall significant departure from linearity but, the relationship was close to linear at low dose rates (Lee 1957). *Apis mellifera adansonii* drone pupae were irradiated with acute radiation doses (10 -30 Gy) and subsequently crossed with non-irradiated queens but there was no detectable effect on the viability of progeny (Sakamato & Takahashi 1981).

There are a large number of studies both in the FREDERICA effects database (Copplestone et al. 2008) and IDIDAS database investigating the effects of acute radiation dose to invertebrates. However, these studies present challenges when trying to extrapolate to wildlife living in radiologically contaminated environments as the dose rates in these areas are significantly lower than have been used in all experiments mentioned above (Pentreath et al. 2013; Larsson 2012). Consensus statements from radioecologists have identified the need for laboratory studies to have increased environmental relevance; this can be achieved by using environmentally relevant dose rates, linking laboratory and field studies and increasing the realism of laboratory studies by investigating the consequences of multiple stressors (Mothersill et al. 2018; Brechignac et al. 2016). These consensus statements have been used to design the laboratory studies in this thesis, therefore increasing the environmental relevance and helping to improve our understanding of the effects of chronic low-dose radiation to insects.

One of the reasons that laboratory studies need to have increased environmental relevance is due to nonconsensus amongst the academic community as to the effects of chronic low-dose radiation in contaminated environments. Laboratory studies have predicted effects to wildlife at dose rates eight times higher than those detected in field studies (Garnier-Laplace et al. 2013), but this is in part due to the lack of environmental relevance in existing laboratory-based studies. Based on the existing laboratory studies, dose rates in current-day Chernobyl Exclusion Zone are not high enough to result in detrimental effects to invertebrates. Yet, some field studies have detected effects to a range of invertebrate species as low as 0.03 µGy h^{-1} (Møller et al. 2012), which raises concerns for the regulation of radiological protection of the environment.

Field studies in the Chernobyl Exclusion Zone

Field studies are important to be able to understand realistic exposure scenarios beyond individual effects and to improve our knowledge of how exposure to radionuclides interacts with other environmental factors and determine how populations could be affected by this exposure (Brechignac et al. 2016). Existing radioecological field studies have predominantly been conducted in the Chernobyl Exclusion Zone (CEZ) since the accident in 1986. However, recently there have been a number of emerging field studies examining the effects to wildlife in Fukushima Daichii since the accident in 2011. The work in this thesis has been conducted in the CEZ and therefore the focus of this review of effects to invertebrates will be focussed on field studies in the CEZ. The CEZ offers a unique opportunity as a living laboratory to study the effects of low dose chronic ionising radiation on wildlife. Specifically, this thesis will help by improving our understanding of the effects to bumblebees living in areas of low dose chronic radiation and inform radiological protection and address a lack of data.

The Chernobyl Accident occurred on 26th April of 1986 in Ukraine when reactor four of the nuclear power station caught fire and subsequently exploded. The total release of fission products (excluding inert radioactive gases) has been estimated to be 1.85x10^{18} Bq (IAEA 2006). The contamination was heterogeneous across northern and western Europe in particular, but the most affected areas were natural and agricultural regions within 30km of the Nuclear Power Plant (NPP) which later became the exclusion zone (Beresford et al. 2016; Alkhomashi & Monged 2015). There were three contamination periods during the Chernobyl Accident. The first contamination period caused the maximum acute exposure to biota lasted 10-20 days. After this period, dose rates rapidly decreased due to the presence of short-lived radionuclides, which decayed rapidly. The radiation exposure rates were mainly due to gamma radiation from deposited radionuclides and ranged up to 20 Gyd^{-1} (IAEA 2006; Geras'kin et al. 2008).

The second phase of the accident occurred during the summer and autumn of 1986. After the decay of short-lived radionuclides, the longer-lived radionuclides became the contributor to dose rate and were transported by biological, physical and chemical processes into the environment. This transportation, occurring within the first three months caused 80% of the total radiation dose accumulated by plants and animals. The contribution from beta radiation made up 95% of the total dose rate (IAEA 2006; Geras'kin et al. 2008). At the time of the accident, and shortly after, acute effects to flora and fauna were observed within the CEZ; the dose rates at this time were up to 20 Gy d^{-1} (Geras'kin et al. 2008). Therefore, severe fitness consequences to non-human biota at high dose rates were consistent with the data obtained from laboratory studies (Geras'kin et al. 2008). The Chernobyl NPP accident occurred during the spring-time (April) when many flora and fauna are in the early stages of development and therefore, the most radiosensitive (Geras'kin et al. 2008; ICRP 2008).

Present-day is considered the third stage of radiation exposure after the Chernobyl accident. Radiation dose rates are chronic, but the dose rates are now less than 1% of initial values at the time of the accident and derived mainly from ^{137}Cs and ^{90}Sr contamination with smaller contributions from ^{126}I, $^{238,\ 238,\ 240}$Pu and ^{241}Am (Bonzom et al. 2016; Geras'kin 2016). The CEZ is the area of approximately 2600km^2, which had the highest contamination after the accident. Over 30 years later, it remains one the most highly radioactively contaminated areas in the world (Bonzom et al. 2016). The evacuation of approximately 116,000 people took place within the first few months of the accident, and much of the CEZ remains abandoned today (Beresford et al. 2016).

The dose rates in the present-day CEZ are much lower than they were at the time of the accident, and most areas of the exclusion zone are between background and 100μGy h^{-1}, however, some sites in the CEZ have been measured at 400 μGy h^{-1} and greater (Beresford et al. 2018). Even though the dose rates in the CEZ are much lower now than at the time of the accident, recent studies in the CEZ have detected adverse effects to a range of taxa at low dose rates. Although the work presented in this thesis focusses on bumblebees, over 70% of radioecological studies have been carried out on birds, mammals and terrestrial plants. Therefore, to demonstrate the types of effects which have been observed in the CEZ the following summary of the effects which on birds which are one of the most widely studied wildlife group in the CEZ.

Studies have shown that abundance and species richness of forest birds declined from dose rates of 4 μGy h^{-1}, which was associated with either a direct effect of radiation; reduction in survival rates and fecundity, or, indirectly by reducing habitat quality in areas of high radiation dose rates (Møller & Mousseau 2008). Similarly, raptor abundance was reduced from dose rates of 8 μGy h^{-1} (Møller & Mousseau 2008). Galván et al. demonstrated that reduced abundance of birds in the CEZ was associated with increased pheomelanic plumage as pheomelanic plumage relies on high levels of glutathione (GSH) which is associated with oxidative stress (Galván et al. 2011). Therefore, it is hypothesised that as ionising radiation increases oxidative stress, GSH is susceptible to radiation and species with large proportions of pheomelanic pelt or feathers may suffer more from radiation effects (Galván et al. 2011). Increased oxidative stress was further supported in studies examining antioxidant levels in *Hirundo rustica,* and a negative association between radiation levels and antioxidant levels was found (Møller et al. 2005).

Studies using *Hirundo rustica* (barn swallow) have demonstrated adverse effects to condition, reproduction and survival in the CEZ compared to other areas of Europe (Moller et al. 2005). Changes were detected to reproductive parameters; the proportion of non-reproducing adults was 23% higher in the CEZ than other European populations and, in the CEZ the percentage was greater with increasing radiation dose rate (Moller et al. 2005). Clutch and brood size were reduced by 7% and 14% respectively and hatching success was reduced by 5% relative to the control area, however, laying date was not significantly altered (Moller et al. 2005). Annual adult survival was 12% lower in CEZ when compared to other European regions (Moller et al. 2005).

Physiological changes were also measured in *Hirundo rustica* in the CEZ. Sperm abnormality was an order of magnitude higher in contaminated areas compared to control areas (Møller et al. 2005) and, sperm swimming behaviour was impaired from 0.093 µGy h^{-1} (Møller et al. 2008). Other extreme morphological abnormalities have been reported in *Hirundo rustica* as birds in areas of the CEZ with elevated radiation dose rates had a 5% reduction in brain size (Møller et al. 2011). Increased radiation dose rates in CEZ were also associated with depressed lymphocyte and immunoglobin concentrations (Camplani et al. 1999), increased albinism (Møller & Mousseau 2001), increased oxidative stress (Bonisoli-Alquati et al. 2010), higher levels of DNA damage (Bonisoli-Alquati et al. 2010) mutation rates in birds (Møller & Mousseau 2015).

All the above studies were conducted within the same research group, and to date, there are no studies which have independently investigated the effects of radiation on birds. But there is a disparity between these radioecological field studies based in the CEZ as studies investigating other taxa with similar radiosensitivity as birds have found no effects of radiation. There was no demonstrated effect of radiation on bank voles in regards to micronucleus frequency up to 930 µGy h^{-1} (Baker et al. 1996), genotoxicity up to 2000 µGy h^{-1} and (Wickliffe et al. 2002; Rodgers et al. 2001) and no alteration population structure 800 µGy h^{-1} (Baker et al. 2001).

The disparity between these field studies is further illustrated as some studies in the CEZ directly contradict one another. For example, Bonzom et al. (2016) found a positive relationship between radiation levels and leaf litter soil biota community composition at dose rates up to 150 µGy h^{-1}, whereas, Møller & Mousseau (2018) & Mousseau et al. (2014) found a negative relationship up 189 µGy h^{-1} and 100 µGy h^{-1} respectively. Both Møller (2002) and Fuller et al. (2017) investigated fluctuating asymmetry in invertebrates Møller studied stag beetles and Fuller et al. studied the aquatic invertebrate *Asellus aquaticus,* but these studies found contradictory results. Mammal abundance was determined by tracks in the snow throughout the exclusion zone by three different studies. Two of these studies determined no relationship with radiation dose (Webster et al. 2016; Deryabina et al. 2015). Whereas, conversely, Moller & Mousseau found a significant decrease in mammal abundance with increasing radiation in the Exclusion Zone.

Studies on invertebrate populations in the Chernobyl Exclusion Zone

Since the Chernobyl Accident, there have been a number of studies investigating the effects on invertebrates in the CEZ. There have been fewer studies conducted on invertebrates than mammals and birds, and even fewer of these have studied insects. Immediately after the Chernobyl Nuclear Power Plant accident in 1986, a decrease in the density and reproductive fitness of soil invertebrates was measured (1986 -1988). Just after the accident, the absorbed dose was as high as 100 Gy in the most affected areas, however, as dose rates declined, the population density recovered by 1990 (Krivolutzkii & Pokarzhevskii 1992).

Research investigating invertebrate abundance and diversity was undertaken in the CEZ in 2002 (Jackson et al. 2005). Sub-surface bait lamina, pitfall traps and sweep net sampling were used to estimate invertebrate abundance (Jackson et al. 2005). There was a decline in the number of penetrations on the bait-lamina sticks which indicates reduced abundance, with increasing radiation dose rate and at the highest radiation dose rates, there was also a loss of diversity of soil macrofauna observed but no change to biomass (Jackson et al. 2005). More recently, a study, which experimentally manipulated wood quality in the CEZ, found a decline in soil invertebrate abundance with increasing radiation dose rates, from dose rates of 1 μGyh^{-1} (Møller & Mousseau 2018).

There have been very few studies focussed on insect pollinators such as bumblebees in the CEZ. At least 41 species of bumblebees have been recorded in Ukraine; *B. pascorum, B. humilis, B. muscorum, B. sylvarum* are widely distributed over the country with *B. pascorum* being the most eurytopic species (Konovalova 2010). *B. terrestris* is considered ubiquitous and accounted for approximately 70% of observations made within the CEZ (Møller & Mousseau 2009). In 2006-2009 a census of all birds, bumblebees and butterflies was conducted at 898 points across the CEZ at dose rates ranging from 0.01 to 379 μGy h^{-1} (Møller et al. 2012). Møller et al. (2012) aimed to investigate if the abundance of pollinators was altered in areas of increased radiation dose, and if so, how would this affect fruit set in apple, pear, rowan, wild rose and cranberry trees and bushes. Møller et al. (2012) reported an unprecedented scarcity of pollinators in the CEZ from dose rates of 0.1 μGy h^{-1}, with knock-on adverse effects for fruit trees and subsequently the birds that feed on them.

This suppressed pollination effect was measured from 0.1 µGy h^{-1}, the abundance of bumblebees, butterflies and the number of apples were negatively associated with radiation dose (Møller et al. 2012). Furthermore, in the same sampling period, transects were conducted to estimate the abundance of a range of insects and spiders in the CEZ; bumblebees, butterflies, grasshoppers, dragonflies and spider webs (Møller & Mousseau 2009). These abundance studies of invertebrates reinforced the findings in the study conducted on bumblebees and butterflies, as all invertebrate abundance declined with increasing radiation dose rate from 0.1 µGy h^{-1} (Møller & Mousseau 2009).

The research on insects and spiders in the CEZ mirrors the research that has been presented previously on birds. These dramatic declines in both pollinating insects and pollination services in the CEZ raise many issues for radiological protection of the environment. To contextualise the dose rates at which these studies are finding severe negative effects, it should be noted that UK background dose rates are around 0.1 µGy h^{-1} with the most contaminated areas in the UK (Sellafield nuclear fuel reprocessing site) reaching up to 0.2 µGy h^{-1} (RIFE 2015). The disparity between the reported field studies highlights the need to further understand the effects of low dose chronic radiation on wildlife as this has implications for radiological protection at both a national and international level. The CEZ offers a unique opportunity to improve the understanding of the effects to wildlife living for multiple generations in this contaminated landscape.

Generally, in any ecological field-based study, inconsistencies may arise due to an array of issues: methodological, biotic/abiotic contexts such as habitat quality and temporal variation (Lemoine et al. 2016). Estimating the dose rates to biota is a well-acknowledged limitation of field studies in the CEZ, as poor dosimetry can result in establishing an inaccurate dose-response relationship (Aramrun et al. 2018; Beresford et al. 2016). There are further complications when estimating dose rates to non-human biota due to the consequences of radiation type (alpha, beta and gamma) to the internal and external dose rate. Furthermore, temporal and spatial variations in radiation contamination and varying radiosensitivities for different taxa create increased uncertainty as to the actual dose rates organisms receive in the wild (Geras'kin et al. 2008; Brèchignac et al. 2009). Another critical issue associated with radioecological field studies in the CEZ is that many lack statistical power due to lack of replication and the limited number of sites that have been sampled. The CEZ is a limited size of 2,600km^{2,} and when conducting field studies, there are often problems finding the study species and the right habitat at the higher dose rates (over 50 µGyh^{-1}) as this occurs in predominantly areas of pine forest. The problem of replication of study sites intensifies when sampling aquatic organisms residing in ponds or lakes, as these are of limited availability across the CEZ. The lower statistical power of limited replication, therefore, increases the probability of committing a type II error (Lemoine et al. 2016). The problem of lack of replication will be explored further in chapter four.

The lack of a clear consensus as to the effects of chronic low-dose radiation in the natural environment has implications for radiological protection of the environment. In recent years, to address the uncertainty as to the effects of radiation to wildlife, international research consortiums have been established to increase the knowledge surrounding the effects to wildlife at low doses and improve our ability to measure dose rates in the field to inform radiological protection. For example, Coordination and implementation of a pan-Europe instrument for radioecology (COMET: http://www.radioecology-exchange.org/content/comet), Strategy for Allied Radioecology (STAR: http://www.radioecology-exchange.org/content/star) and Radioactivity and the Environment (RATE: https://www.bgs.ac.uk/rate), of which this PhD is part.

1.4 Existing literature about how bumblebees respond to stress

Bumblebees were selected as a model organism for the work conducted in this thesis for several reasons, they are one of the least studied ICRP Reference animals and therefore, gaining information to inform the placement of the DCRL is important for international radiological protection. Bumblebees are ubiquitous in temperate zones and important pollinators, intrinsic to eco-system function. They were selected over honeybees as they are wild pollinators in contrast to honeybees which are generally domesticated. Bumblebees have also been used in a range of ecotoxicological studies investigating the effects of different natural and anthropogenic stressors and as a result, have a well-characterised life history.

Bumblebee (*Bombus)* species have an annual lifecycle; new queens emerge at the end of the summer, mate and then dig a hibernaculum, approximately 10 -15cm long in which overwintering occurs (Goulson 2010). Inseminated new queens emerge from overwintering from February to June. Some bumblebees prefer to establish colonies in disused nests of other animals such as wood mice or shrews (Lye et al. 2012). In common European species such as *B. terrestris, B. lucorum* and *B. lapidarius* nests are subterranean, whereas carder bees such as *B. pascorum* tend to be on the surface, amongst tussocks of vegetation and *B. hypnorum* utilises bird nests in shrubs and trees (O'connor et al. 2017; Lye et al. 2012).

In early springtime, pollen and nectar consumption enables the emerging queen to replenish her depleted fat reserves from overwintering and then spend a period of time from several days to a week, nest searching (Goulson 2010). Once a suitable nest area has been found a ball of pollen is used, a single wax cell is filled with nectar, and the first cohort of eggs is laid in the pollen ball (Sladen, 1912; Goulson 2010). The eggs are then incubated using the queen's body heat and develop into larvae, pupating after 3-5 weeks (Sladen, 1912; Goulson 2010). Newly eclosed workers being to assist the queen with foraging and nest care, e.g. incubating and feeding the brood, removing dead or defective individuals (Sladen, 1912; Free and Butler, 1959; Goulson, 2010). Nest weight can increase tenfold within 3 - 4 weeks with some bees remain in nest their entire life undertaking in-nest duties whereas other will venture out to forage (Sladen, 1912; Goulson 2010). Worker longevity is highly dependent on allocated tasks, varies between species and cast but ranges from 13 to 41 days (Goulson 2010). After the colony's growth spell, the queen switches to producing reproductives, new queens (gynes) and males, and worker production ceases. Males leave and do not return, but gynes (new queens) frequent the nest for several days to increase fat stores and then leave to mate before over-wintering just below the soil surface (Goulson 2010).

Worker bumblebees do not mate. However, they are capable of laying unfertilised eggs which can develop into males (Holland et al. 2013). Unfertilised egg laying occurs when the dominance of the queen is reduced, or the queen dies (Duchateau, 1989; Cnaani et al., 2000; Alaux, 2004). Queens dominate their nests through physical contact and a pheromone called "queen substance" which suppresses the workers' ovarian development (Duchateau, 1989; Cnaani et al., 2000; Alaux, 2004). When the queen switches to production of reproductives, the level of "queen substances" starts to decrease enabling gynes to develop fully. Physical colony dominance also decreases, and some workers may attempt to lay their eggs (Alaux et al. 2004; Bloch & Hefetz 1999). Breakdown of social order ensues, with more workers trying to reproduce and egg policing occurs whereby the queen and workers eat each other's eggs to reduce competition. Conflict may increase until the queen is killed or driven out by the workers (Yagound et al. 2012; Bloch & Hefetz 1999).

Bees are an ideal model system to study the fitness consequences of environmental stressors due to extensive previous research and well-studied life history. Bees (mainly bumblebees, honeybees and some solitary bee species) have been used as bioindicators of environmental pollution (Kevan 1999) for various contaminants: pesticides (Baron et al. 2017; Dos Santos et al. 2016; Stanley et al. 2016; Whitehorn et al. 2012), polycyclic aromatic hydrocarbons (Lambert, Veyrand, et al. 2012; Perugini et al. 2009), radionuclides (Barišić et al. 1999) and heavy metals (Moroń et al. 2012; Van Der Steen et al. 2012; Perugini et al. 2011). Bees are ideal as a sentinel species as they are exposed to many contaminants during their foraging activities, such as pollutants in the atmosphere, soil, vegetation and water. For example, the hairs on their bodies can easily hold atmospheric residue, and there is a range of different contamination pathways through food resources (nectar/pollen) (Lambert et al. 2012; Negri et al. 2015). The impact of heavy metals (cadmium, lead and zinc) has been tested by assessing species number, diversity, abundance and natural mortality in solitary bees (Lambert et al. 2012; Moroń et al. 2012). Lead was shown to cause physiological, behavioural damages, and potential death (Lambert et al. 2012; Moroń et al. 2012).

In recent years, there has been an increase in research investigating the adverse effects of pesticides used in farming on wild pollinators, specifically bumblebees. This research has demonstrated how behaviour such as foraging (Gill et al. 2012; Feltham et al. 2014; Gill and Raine, 2014; Kessler *et al.*, 2015; Stanley and Raine, 2016; Stanley *et al.*, 2016) and homing ability can be negatively impacted by pesticides (Stanley et al. 2016). Research investigating how life history is affected by pesticide exposure has shown that colony growth (Stanley et al. 2016; Whitehorn et al. 2012), colony initiation (Baron et al. 2017), queen production (Whitehorn et al. 2012) and mortality (Gill et al. 2012) are all negatively impacted by pesticide exposure.

Experiments testing the consequence of a single stressor are much more straightforward than investigating the interactions of multiple stressors. However, recent studies have emerged using bumblebees demonstrating that pesticides as a stressor do not act in isolation but the effects are exacerbated by other stressors wild bees encounter. For example, diet and pesticides (Dance et al. 2017) and pesticides and parasites - in particular *Crithidia bombi* (Baron et al. 2014; Fauser-Misslin et al. 2014; Goulson et al. 2015; Piiroinen & Goulson 2016a; Piiroinen & Goulson 2016b; Fauser et al. 2017; Pettis et al. 2012).

Stressors can interact in three ways: synergistically, antagonistically and additively. Synergism is defined as a cumulative effect of multiple stressors that is greater than the additive sum of effects by the stressors acting in isolation. Antagonism is used to define a cumulative effect that is less than additive and additive where effects are thought to accumulate additively (Piggott et al. 2015; Folt & Chen 1999). Synergistic behaviour has been demonstrated for non-radioactive contaminants resulting in the effects for two stressors differing from the effect of a single stressor and effects at concentrations which a single contaminant does not show effects (Vanhoudt et al. 2012).

Overall in a review of experiments investigating the synergistic interactions between radiation and other environmental stressors found that 58% of interactions were positive (additive, synergistic, increased effects), 26% were negative (antagonism), and 16% found no interactions (Vanhoudt et al. 2012). There has been little research done as to the interactions between radiation and parasite exposure, particularly in field-based studies (Morley 2012). It is not known whether these stressors would interact, but parasites are ubiquitous in the environment and therefore, could synergistically interact with radiation. In work presented in this thesis, the interaction between radiation dose rate and the common bumblebee gut parasite, *Crithidia bombi* will be tested to determine whether the inclusion of a co-stressor in the natural environment exacerbates the fitness consequences of radiation as a single stressor.

There has been a significant amount of research conducted on how environmental stressors interact with parasites in bumblebees. Insect immune defences consist of two branches – cellular and humoral immunity (Schmid-Hempel 2003). Cellular immunity is driven by circulating cells called haemocytes which can attack a range of pathogens through phagocytosis, nodulation and encapsulation (Miller & Cotter 2018). An example of the humoral immune response is the release of antimicrobial peptides and activation of the phenoloxidase enzyme cascade and during infection, resulting in the immune responses are upregulated (Miller & Cotter 2018; Rauw 2012). Mounting an immune response after exposure to a pathogen can be costly (Miller & Cotter 2018; Kutzer & Armitage 2016). First there is the cost of having an immune system, and second, there is the cost of using it (Tyler et al. 2006). The cost of immunity has trade-offs, for example, increased metabolic rate or food consumption (Tyler et al. 2006).

Bumblebees and *C. bombi* are a model system for investigating host-parasite dynamic and the extensive previous knowledge will help gain understanding about the effects of radiation in the context of other co-stressors (Logan et al. 2005). After oral intake, *Crithidia bombi* settles in the hindgut of the host, 2-3 days post-infection transmission stages are released into the faeces (Cisarovsky & Schmid-Hempel 2014). Infection by *Crithidia bombi* is extracellular, developing within a few days and results in large numbers of parasite cells lining the walls of the midgut and rectum (Riddell et al. 2011). Parasitemia peaks and plateaus around 7-10 days into the infection (Cisarovsky & Schmid-Hempel 2014). Bumblebee colonies and individuals vary in their susceptibility to *C. bombi*, this has been attributed to a number of reasons including differences in immune gene expression profiles (Brunner et al. 2013; Barribeau & Schmid-Hempel 2013; Riddell et al. 2011) , levels of homozygosity (Whitehorn et al. 2014) and the gut microbiome (Mockler et al. 2018; Koch et al. 2017).

Using the extensive information that is already known about bumblebee life history in the context of stressors and the well-studied host-parasite dynamics of *C. bombi,* this thesis will explore if and how chronic low dose radiation affects bumblebee life history traits and fitness. Combining complementary laboratory and field studies will further investigate how these co-stressors interact and help resolve whether the consequences of synergistically interacting co-stressors could be responsible for the disparity between radioecological field and laboratory studies.

1.5 Necessary issues to improve the understanding of how chronic low-dose rates affect wildlife

In recent years, there have been a number of consensus statements identifying the need for a different approach to radioecology in order to understand how chronic low-dose radiation affects wildlife and whether existing contaminated sites, such as Chernobyl, have the potential to cause significant damage to flora and fauna (Mothersill et al. 2018; Brechignac et al. 2016; Hinton et al. 2013; Beresford & Copplestone 2011; Beresford et al. 2016). This thesis aims to investigate several suggestions made by these consensus statements and reflective articles. Firstly, improving dosimetry to wildlife by including contributions from beta and differences across life history, which is conducted in chapter three of this thesis to support the dose rates measured in chapter four. Secondly, to account for habitat quality to test whether floral resources co-vary with increasing radiation dose rate across the Chernobyl Exclusion Zone. Finally, the work conducted in this thesis investigates alterations to bumblebee life history in response to chronic low-dose radiation, both at an individual and colony level. This approach aligns with the proposed eco-system level approach to radiation protection, which has been proposed to address some of the issues associated with RAPs and to undertake a more holistic approach.

Estimating dose rates to wildlife living in contaminated areas

There are many proposed reasons as to why there is a disparity in the scientific literature surrounding the effects of radiation on wildlife. As well as measuring sensitive endpoints, complementary laboratory and field studies and including measures of habitat quality in field studies and co-stressors in a laboratory experiment, the measurement of dose rate to wild animals results in complications in forming dose-response relationships (Garnier-Laplace et al. 2013). The CEZ is a unique system; the radioactive contamination is very heterogeneous, and dose rates can be difficult to establish for animals which move around and cover wide-ranging distances as the dose rate varies on a small spatial scale (Stark et al. 2017).

The most common way of measuring the radiation dose wildlife receive is to measure ambient dose rates. However, measuring ambient dose rates is not sufficient to establish total dose rates and can lead to under predicting of the dose rate, therefore, contradicting the effects data used to determine threshold levels for effects (Beresford et al. 2018). The absorbed dose rate needs to be calculated for wildlife before examining effects on biota and forming a dose-response relationship to relate effects to the ICRP benchmarks (Beresford et al. 2018; Beresford & Copplestone 2011; Lecomte-Pradines et al. 2014; Brèchignac & Doi 2009).

The fundamental quantity for calculating exposures to ionising radiation is the absorbed dose. The activity is the expected value of the number of nuclear transformations in a given material per unit of time, the unit is Becquerel (Bq) and is usually expressed as Bq per unit weight (ICRP 2007). Activity concentration can be converted from Bq per unit weight into an absorbed dose rate Gy per unit time using radionuclide specific conversion values (ICRP 2007). Absorbed dose is defined as the amount of energy absorbed per unit mass of tissue of an organ or organism; it is given in units of Gray (Gy) (ICRP 2008). There are three different types of ionising radiation, alpha, beta and gamma, and these different types of radiation are known to produce effects to different degrees in the same biological tissue (ICRP 2008). Internal exposure in animals results from ingestion of contaminated food, water or air whereas external exposure results from contaminated environment or habitat.

Internal exposure depends on the activity concentration, distribution and biological half-life of the radionuclide and the physiology of the affected tissue. Internal exposure depends on physical decay properties, characteristics and the biological half-life of the radionuclide at a whole body or organ-specific level (Figure 1.3) (Ulanovsky & Pröhl 2012, ICRP 2008). External exposure depends on the distribution and activity concentrations in the environment, shielding properties of the medium such as soil or air, surrounding the organism, physical properties of the radionuclides, and species-specific habitat preferences (Stark et al. 2017). The key factors affecting radiation exposure and the dose to animals and plants are; (I) the internal distribution of the source, (II) the external distribution of the source, (III) the characteristics of the organism and, (IIV) the location of the organism in regards to exposure sources (Figure 1.3; Stark et al. 2017).

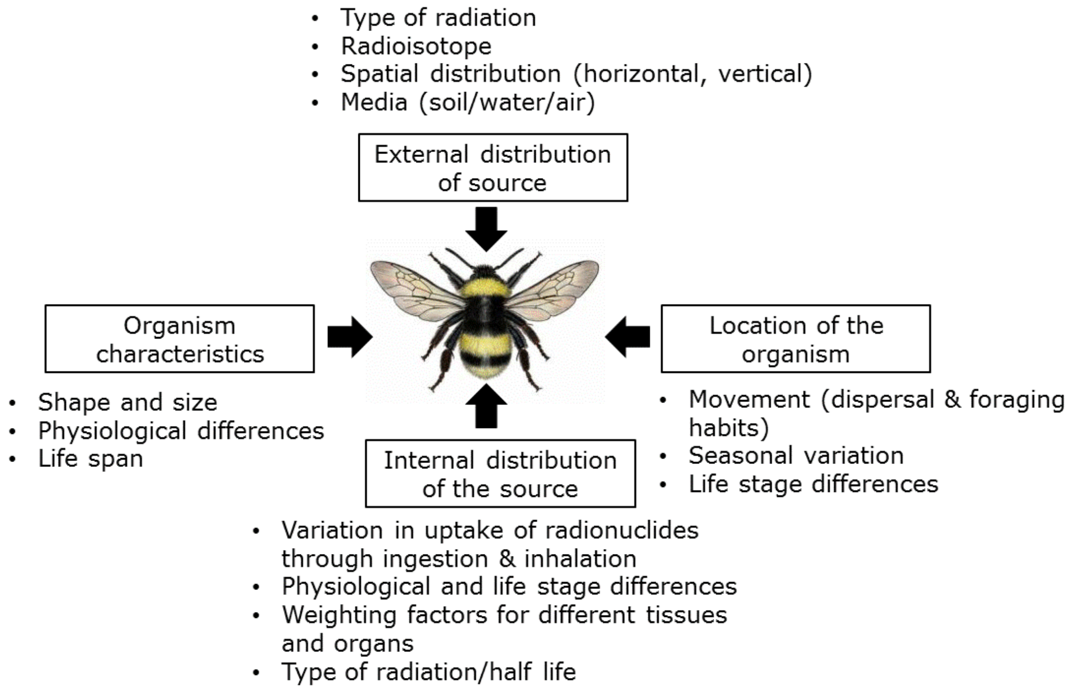

Figure 1.2. The parameters affecting radiation dose to wildlife in a radionuclide contaminated site (adapted from (Stark et al. 2017)

Applying dosimetric models to flora and fauna is problematic due to diversity and variation in habitat occupation, lifestyles, body shape, feeding, metabolism and exposure conditions, all of which affect the dose that an individual receives (Figure 1.2). These factors cannot be explicitly calculated for each individual, and therefore assumptions must be made (Ulanovsky & Pröhl 2012). The models used in radiation protection to assess dose rates are simplified. For example, using assumed simple body shapes (usually spheres or ellipsoids) of uniform composition and density, homogenous internal contamination and uniform radionuclide distribution in the surrounding environment. Internal structures, metabolic and biokinetic properties are not generally accounted for, and assumptions as to how flora and fauna are exposed to external radiation sources (Ulanovsky & Pröhl 2012). Furthermore, contributions from alpha particles to external doses are generally excluded as the radiation emissions are unable to penetrate long distances and exposure can be prevented from affecting radiosensitive tissues by layers of dead skin and fur (Ulanovsky & Pröhl 2012).

Dosimetry for wildlife, therefore, represents a wide range of exposure conditions, as well as the inevitable variability of species and habitats. A number of modelling approaches have been developed to estimate absorbed doses received by wildlife which have drastically increased capability to calculate dose rates for wildlife (EMRAS 2012; Vives I Batlle et al. 2011; Beresford et al. 2008) including commonly used screening tools such as the ERICA Tool (Brown et al. 2016; Brown et al. 2008). The ERICA tool is a software system based on the ERICA Integrated Approach which assesses the radiological risk to non-human biota (Brown et al. 2016; Brown et al. 2008; Larsson 2008). Data from the environmental transfer of radionuclides and dosimetry allow the dose rate to an organism to be calculated. The tool uses calculation steps to estimate activity concentrations in biota and environmental media and then estimate the dose rate to biota.

Stark et al. (2017) have made recommendations to improve dosimetry for wildlife living in contaminated environments: i) it is necessary to have knowledge about the life-cycle of the study organism, work out what are the most sensitive life stages and take into account all essential exposure pathways 2) it is also necessary to understand how the animal moves in the environment and the seasonal habitat preferences, 3) take into account the external and internal radiation source and the type of radiation emitted depending on where the organism lives and considering temporal and spatial heterogeneity.

The importance of habitat quality for radioecological studies

Habitat quality has frequently been overlooked in radioecological studies in the CEZ. Before the accident in 1986, much of the land in the CEZ was used for either agriculture or forestry - similar to many other areas in Central/Eastern Europe. Farming practices were non-intensive and of a more traditional style; using low levels of mechanisation, pesticides and artificial fertilisers and dependant on manual labour (Queiroz et al. 2014). This arable land has been abandoned for 30 years, and regardless of the radioactive contamination, the consequences to the biodiversity of abandoning arable land have been much debated in the ecological literature. Abandonment leads to spontaneous succession which can increase variation between arable fields, but also may result in them becoming more similar to the native surrounding habitat such as forests and grasslands through succession (Sojneková & Chytrý 2015; Queiroz et al. 2014). Oak-hornbeam or thermophilous oak forest is the expected climax community of the CEZ from areas of arable grassland in approximately 200 years (Laćan et al. 2015), but presently, there is abundant habitat for bumblebees due to the abundance of wildflowers which are now growing on the abandoned land. The other main habitat of the CEZ is Scots Pine forest found in the large areas of former plantations. The Scots Pine plantations, as they are not managed as much as they were before the accident, are expected to expand over time (Evangeliou et al. 2014). Within the CEZ forests covered 54% of the area before 1986, and 15 years later this coverage had increased to 70% (Evangeliou et al. 2014; Jírová et al. 2012; Sojneková & Chytrý 2015).

Quantification of habitat quality is important because the quality, area, quality of resources and spatial arrangement of a given habitat can drive population viability (Hodgson et al. 2011). Habitat quality has been shown to be an essential factor for increased abundance of wildlife in the natural environment, potentially more so than area and spatial arrangement (Hodgson et al. 2011; Öckinger et al. 2009). Increased habitat quality improves population size by increasing population growth and the likelihood of colonisation. However, measuring habitat quality has frequently been overlooked in radioecological studies in the CEZ. Although a highly replicated and well-powered study should be able to detect the effects of an environmental stressor such as radiation, this is more difficult in a limited area such as the CEZ, particularly for larger mammals, which will have a large territory. In Deryabina et al. (2015) and Webster et al. (2016) habitat was quantified by type (e.g. pine forest, deciduous woodland, wetlands). However, as these studies measured the abundance of a range of large mammal species across a wide area, generalised characterisation of habitat type will not greatly inform about the resources available to specific species of interest.

The work described in this thesis quantified floral resource and floral community characteristics to determine whether habitat quality co-varied with radiation dose rate. For bumblebees, habitat quality relies primarily on the floral resource being available throughout the colony’s season and tussock grass for nesting depending upon the species. The floral resource is most likely to be either wildflowers or tree blossom. In meadow plant communities in the CEZ, there have been plant community shifts as radioresistant species increased significantly, while the total number of plants and species diversity decreased with the increased level of radiation exposure (Geras’kin 2016b). For example, studies conducted over ten years after the accident have found the proportion of sexually reproducing plants increased, and the role of asexual reproduction decreased in populations of *Hypericum perforatum* (St John’s Wort) within the CEZ, therefore, increasing the chance of gene combinations, which can enhance a population’s tolerance to radiation exposure (Geras’kin 2016b). It may be possible that the quality and number of floral resources may also be affected by increased radiation dose rates, and therefore co-vary with radiation dose. Radiosensitivity varies among plants which means that species within a community may respond differently to radiation, depending on their radiosensitivity. At some dose rates, the productivity of a community may increase but with an altered species composition due to radiation contamination (Geras’kin 2016b).

Ecosystem-level approach to radiological protection of the environment

The ICRP system for radiological protection of the environment focuses on individuals and works on a "bottom-up approach" (Bréchignac 2003). The bottom-up approach focuses on the individual and experiments in artificial ecosystems and subsequently extrapolates these results to the natural environment and entire landscapes (Beketov & Liess 2012). There has been recent discourse in the radioecology scientific community advocating a "top-down" approach to radiological protection of the environment (Brechignac et al. 2016; Brechignac 2016; Brèchignac & Doi 2009; Bréchignac 2003). The "top-down" approach aims to develop an understanding of whole ecological systems by investigating the properties of such systems as each ecological system has emergent proprieties which are difficult to determine from their constituent parts (Beketov & Liess 2012; Figure 1.3).

The debate as to whether top-down or bottom-up is the best approach for protecting the environment is not confined to radioecology. In other eco-toxicological fields, there is significant discourse as to which approach is best suited to protect the environment (Beaumelle et al. 2016; Dyer et al. 2000). The ecosystem-level approach to radiation protection helps to focus the protection on populations and the ecosystem rather than the individual, as shown by the RAPs (Brechignac 2016; Bradshaw et al. 2014; Figure 1.3). Figure 1.3 compares the current approach using RAPs to the proposed ecosystem approach. It is acknowledged that the 12 designated RAPs provide a beneficial contribution to the radiological protection of the environment, but gaining knowledge as to the interactions between species in an ecosystem is crucial to understanding any subsequent secondary and tertiary changes, such as reduction in pollination or decomposition services (Mothersill et al. 2018). Figure 1.3 demonstrates that the ecosystem approach would focus on endpoints associated with populations and structure and function at the community level. In recent years, there has been research examining the effects of pesticides on bumblebees which examine some of these endpoints, for example, the likelihood of population extinction calculated from the reduction of queens founding nests in the spring-time (Baron et al. 2017) and the alteration of interactions between bumblebees and flowers (Stanley & Raine 2016).

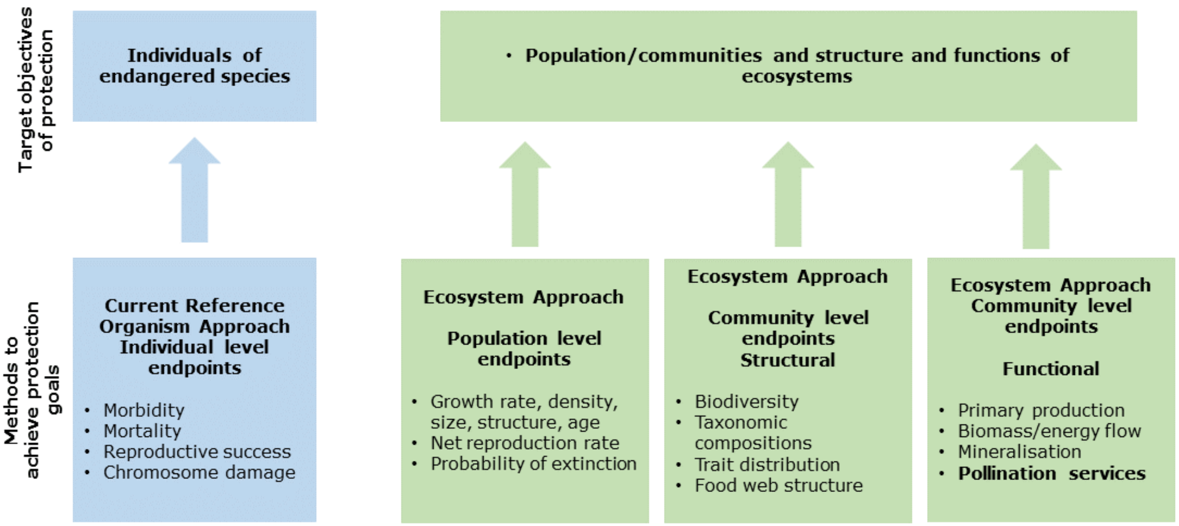

Figure 1.3 The ecosystem level approach to radiation protection. The target objectives of environmental protection versus methods to achieve them.

Adapted from Bradshaw et al 2014

The ecosystem approach to radiological protection of the environment has limited pragmatic, practical use at the current time due to the lack of data and development of a regulatory approach. However, it has been acknowledged that the ecosystem approach could evolve to become a more conservative approach to ensure the environment is protected. In a recent consensus statement from radioecologists (Mothersill et al. 2018), to improve the understanding of how radiation affects the ecosystem, there should be a focus on life history traits, dose and dose rates to specific organs, the presence of other stressors and population and functional endpoints.

The work in this thesis aims to address some of these issues by investigating whether floral resources for bumblebees is affected by increased radiation dose rate and therefore could be a confounding factor in the field study. Furthermore, as well as investigating the consequences of radiation exposure to individuals, effects will also be measured at a colony level and the introduction of the common bumblebee parasite *C. bombi* will help further understand how co-stressors could interact in a natural environment such as the CEZ.

1.6 Aims and objectives

The overall aim of this thesis is to provide data to help resolve the disparity amongst the scientific community as to the effects of low dose chronic ionising radiation on wildlife. The literature investigating radiation effects suggests that there is a lack of laboratory studies with environmental relevance for a number of key wildlife groups resulting in difficulty extrapolating these results to inform radiation protection and infer what may be happening in the CEZ. The field studies which have been conducted in the CEZ, have frequently had been poor replication which is essential as these studies are nearly all correlational, lack consideration of the whole ecosystem and how confounding factors and co-stressors interact and the approaches to dosimetry are often simplistic which are all reasons for conflicting results. A taxonomic bias exists towards mammals and birds both in laboratory studies and in field studies from contaminated landscapes and therefore this study will use bumblebees, therefore providing information that will test the DCRLs used by the ICRP.

Therefore, I hypothesise that:

(I) Bumblebee life history traits will be altered by exposure to chronic low-dose radiation equivalent to dose rates found in the Chernobyl Exclusion Zone (Chapter 2)

(II) The use of a handheld device which measures external radiation is suitable to estimate total radiation dose rate to bumblebees living in different regions of the CEZ (Chapter 3)

(III) The ERICA tool can be used to take into account different bumblebee castes and life stages to provide an accurate estimation of dose rate (Chapter 3)

(IV) Wild bumblebee age will be adversely affected by increased dose rates in the CEZ (Chapter 4)

(V) Bumblebee parasite *Crithidia bombi* burden will be increased in wild bees exposed to elevated radiation dose rates in CEZ (Chapter 4)

(VI) *Crithidia bombi* and radiation dose interact to the detriment of bumblebee fitness under laboratory conditions (Chapter 4)

Chapter 2

Chernobyl-level radiation exposure damages bumblebee reproduction: an experimental test

2. Chernobyl-level radiation exposure damages bumblebee reproduction: an experimental test

2.1 Abstract

The long-term consequences to wildlife of living in radiologically contaminated environments are uncertain. Substantial scientific debate exists as laboratory studies indicate insects are relatively radiation-resistant, information that has shaped environmental protection policy. However, field studies from the Chernobyl Exclusion Zone (CEZ) suggest invertebrates suffer adverse effects of radiation at substantially lower dose rates than predicted. The controversy in radioecology was addressed through a laboratory study which investigated how chronic low-dose radiation exposure affects bumblebee reproduction.

At dose rates of 100 μGy h^{-1} (comparable to dose rates found in the CEZ), bumblebee queen production declined by 6%. There was a 28% reduction in the number of queens produced from colonies exposed to 400 μGy h^{-1} which is the lowest dose rate that negative effects are expected to affect insects as designated by the International Commission on Radiological Protection. At 2800 μGy h^{-1}, which is close to the highest dose rate in the dose band predicted to have negative effects to insects, queen production was almost nil. Radiation exposure also significantly delayed colony growth but did not affect colony weight or longevity. This is the first laboratory study to experimentally test how environmentally relevant radiation exposure affects insect life history. The data presented in this thesis demonstrates significant negative consequences at previously unprecedented dose rates. As a result of these data, I advocate a reduction in the dose band allocated by the International Commission on Radiological Protection for the reference bee.

2.2 Introduction

Ionising radiation damages biological molecules and cells (Azzam et al. 2012; Brown & Rzucidlo 2011); when delivered at high dose rates it can cause death or significant fitness-loss to organisms (McLean et al. 2017; Geras'kin et al. 2008). The majority of ionising radiation in the environment comes from natural sources, but some geographic regions have elevated radionuclide levels due to anthropogenic actions. A small proportion of these contaminated landscapes deliver considerable radiation exposure to wildlife (Copplestone et al. 2015; UNSCEAR 2008). Major debate exists as to the consequences for wildlife of living in radiologically contaminated areas such as the Chernobyl Exclusion Zone (CEZ) (Ukraine) and Fukushima Daiichi (Japan) (Fuller et al. 2015; Copplestone et al. 2017; Beresford et al. 2016; Møller & Mousseau 2016; Møller & Mousseau 2013). Contamination across the CEZ is highly heterogeneous and estimated total dose rates to wildlife and plants in the highest areas are 400 μGyh^{-1} but are typically lower, between background and 100 μGyh^{-1} (Geras'kin et al. 2008). Some controversial studies conducted in the CEZ suggest radiation is very damaging and report reduced abundance of birds (Møller & Mousseau 2008; Moller & Mousseau 2007), insects and spiders (Møller et al. 2012; Møller & Mousseau 2009) at little greater than typical background dose rates. However, other research suggests radiation has either positive effects (Bonzom et al. 2016; Bezrukov et al. 2015) or no effects (Fuller et al. 2017; Fuller et al. 2018; Deryabina et al. 2015) on wildlife.

The disparity between laboratory and field-based studies

Most laboratory studies investigating radiation effects to insects use acute exposures (high dose rates over a short time) and assess metrics such as death or sterility, which have relevance for acute exposure but little relevance at low doses (Dyck et al. 2005; Bakri et al. 2005). It is unclear how well these experimental conditions mimic the effects of chronic exposure in contaminated field environments (Brechignac et al. 2016; ICRP 2008). Field studies in unique and heterogeneous environments such as the CEZ or Fukushima Daiichi also face multiple challenges and are generally correlative and therefore need to be supported by laboratory studies. Determining that radiation is the sole cause of negative effects is difficult as multiple potential stressors and confounding factors may mask or amplify effects of radiation (Geras'kin 2016a; Bradshaw et al. 2014). Complementary laboratory experiments are therefore needed to validate field studies (Brechignac et al. 2016). The work in this chapter presents an environmentally relevant laboratory experiment exposing bumblebee colonies to a radiation gradient which tests whether life history traits were altered.

This study not only offers insight into the ecological impacts of radiological contamination to wildlife, but it also provides an imperative test of the international framework for radiation protection of the environment that has mostly been derived from acute exposure high dose rate laboratory studies There is a strong taxonomic bias across these studies towards mammals; insects represent only 7% of these studies (Copplestone et al. 2008), therefore to address the paucity in data for invertebrates, the work in this chapter will be conducted on bumblebees.

Consequences for the System of International Radiological Protection

The International Commission on Radiological Protection (ICRP) provides global recommendations and guidance on radiation protection for both humans and wildlife. The ICRP radiological protection framework for wildlife uses Reference Animals and Plants (RAPs) across taxa groups to identify a range of doses where radiation is likely to have a deleterious effect on an individual organism. These dose ranges are called Derived Consideration Reference Levels (DCRLs) (ICRP 2008). The RAP for insects is a 'eusocial bee', and the DCRL is currently set at 400 - 4000 μGyh^{-1}; but below this dose band, there are no published data investigating radiation effects (Larsson 2012; ICRP 2008). This experiment provides the first evidence allowing us to test whether this bee DCRL is in the correct place.

Bumblebee life-history

Bumblebee queens found new colonies in spring that grow by the cumulative production of workers; later in the season males and new queens are produced. The production of new queens is the most important metric to assess colony fitness because new queens mate in autumn and are the only individuals to overwinter (Goulson 2010). Reduced queen production can negatively affect natural populations as it may reduce bumblebee colony abundance in future generations (Baron et al. 2017), with potential ecosystem-wide impacts due to impaired pollination services (Winfree et al. 2015).

The work presented in this chapter is the first experiment to bridge the gap between laboratory and field radioecological studies, clarifying our understanding of the consequences for insects of chronic low dose ionising radiation. This laboratory experiment exposed individual bumblebee colonies along a radiation gradient for a substantial proportion of colony lifespan, followed by a period foraging under unexposed field conditions. Sensitive and ecologically important life history traits were studied: reproductive output, colony growth and longevity. The data demonstrated substantial negative impacts of radiation exposure at dose rates found in contaminated environments such as the CEZ.

Hypotheses

This chapter will test the following hypotheses:

1) Bumblebee colony queen production will be reduced by exposure to radiation dose rates equivalent to those found in the Chernobyl Exclusion Zone and the dose rates of the bee RAP DCRL used by the ICRP
2) The peak timing of colony growth will be postponed by exposure to ionising radiation compared to colonies which have been exposed to elevated radiation dose rates
3) Bumblebee colony production of workers and males will be reduced by exposure to radiation dose rates equivalent to those found in the Chernobyl Exclusion Zone and the dose rates of the bee RAP DCRL used by the ICRP
4) Colony, queen and worker longevity will be reduced by exposure to ionising radiation equivalent to the CEZ and the dose rates used to derive the DCRL used by the ICRP
5) Colony weight will be reduced by exposure to chronic low-dose ionising radiation
6) Colonies will require a greater food resource and consume more food when exposed to ionising radiation dose rates compared to colonies which are unexposed

2.3 Method

Radiation exposure under controlled conditions

Fifty-nine commercial *Bombus terrestris audax* colonies (comprising a queen, some workers and the brood) were purchased from Biobest© in June 2016. Colonies were positioned in a radiation facility at Stirling University for four weeks. The facility contains a gamma emitting caesium-137 source, and a control area shielded from this radiation. Varying colony distance from the source achieved dose rates ranging from 20 - 3000 μGyh^{-1}. Environmental conditions were controlled: 25°C and a 12 hr L:D light cycle (07h – 19h); colonies received *ad libitum* dried pollen and artificial nectar (Bioglauc©). Monitoring, maintenance and marking were conducted in the control area under red light.

Colonies were weighed before the experiment, then distributed across the radiation exposure gradient ensuring weight variation was not associated with radiation dose rate ($F_{(1,\ 56)} = 0.071$, $p = 0.791$). To identify workers already present in the colony prior to radiation exposure, all workers from nine boxes across the gradient were marked on the thorax with bee marking paint. To protect against wax moth infestations, anti-wax moth concentrate (Certan/B401 from Dragonfli ©, 1 in 20 dilution in water) was applied to each colony's cardboard container at week two and week five of the experiment.

Colonies were rotated 180° every two days (to reduce within colony radiation exposure heterogeneity) and monitored every 1-2 days for colony death (defined as fewer than five workers observed in a colony). After four weeks, colonies were removed from radiation exposure and placed outdoors to enable natural foraging. Total colony weight was measured weekly during both the radiation exposure and the field phases, until colony death. Bioglauc© (food) reservoir weight was measured weekly during the exposure phase.

Natural foraging

During week four, before colonies were placed outdoors, a subset of 30 workers from half the colonies (n = 29) were marked using coloured number tags attached to the thorax with superglue to enable worker longevity monitoring (Blacher et al. 2017). Two days prior to outdoor placement pollen supply was reduced; pollen and nectar feeding stopped on the release day, and a door on each colony was opened to encourage natural foraging. Colonies were randomly assigned outdoor locations in the University of Stirling campus gardens and sheltered from the elements in plastic boxes. The gardens of the University of Stirling (56.149759, -3.910263) are close to ornamental gardens, deciduous woodland and mixed farmland.

Once outside, colonies were checked after sunset every 1-2 days for colony and queen death; marked workers were recorded as alive or dead when seen. Worker longevity was expressed as the number of days between the marking date and the estimated day of worker death (the last day a worker was seen alive). Queen longevity was defined as the time in days from the start of the experiment to the date at which the queen was observed dead (Holland & Bourke 2015). Upon colony death the entire colony was euthanised at -80°C for 45 minutes. Colonies are constructed of cells in which pupae develop; males and workers develop in similarly sized cells whereas queen cells are much larger. Posthumous colony dissection allowed colony counts of the combined worker and male population, as well as queen production (Goulson et al. 2002).

Statistical Analysis

Analyses were run in R (version 3.4.3; R Core Team, 2017). Maximal models were established, then each predictor was evaluated using a likelihood ratio test comparing the goodness of fit between models with and without that term. Non-significant predictor variables were removed from models. Model diagnostics were checked to validate fit to assumptions. Descriptive results are summarised as mean ± standard error; slope estimates were calculated from scaled predictor models which calculated the mean and the standard deviation from the data set, the mean was subtracted and divided by the standard deviation to enable slope comparison.

Negative binomial generalised models were constructed to investigate associations between colony queen production and dose rate using glm.nb from the MASS package (Venables & Ripley 2002). The maximal model included continuous predictor variables: dose rate, colony starting weight, the combined number of worker and male pupal cells, as well as a categorical variable for colony worker tagging status. To test whether the effect of dose rate on queen production was influenced by the size of the male-worker population the interaction between the combined male-worker cell count and dose rate was included. The fit of the polynomial terms for dose rate were assessed, but the best fit models involved linear dose rate covariates.

Factors influencing the combined count of workers and male pupal cells were investigated using linear models. Maximal models included colony starting weight, colony worker tagging status and dose rate as main effects, as well as the interaction between colony, start weight and dose rate.

The week in which colonies reached their peak weight occurred at either week four or week five of the experiment. A GLM with a binomial distribution (after checking for overdispersion) was used to fit a maximal model containing tagging status, dose rate, peak colony weight and a dose rate by peak colony weight interaction.

Changes in colony weight throughout the experiment were investigated in two sections: the exposure phase (weeks 1 – 4; increasing weight) and the field release phase (week 5 onwards; declining weight). Linear mixed effects models were fitted using lmer from "lme4" (Bates et al. 2015) with colony identity as a random effect to account for repeated measures. Fixed covariates in these models were dose rate, colony starting weight and the week number at which weight was recorded. To test whether an effect of dose rate on colony weight was influenced by either colony starting weight or week number the relevant interaction terms were included. The same model structure was used to assess causes of variation in food consumption (bioglauc© food reservoir mass).

The factors influencing colony and queen longevity were examined separately using Cox's proportional hazards models (coxph from the package "survival") (Therneau & Grambsch 2002); dose rate, start colony weight and tagging status were fitted as predictor variables. Worker longevity variation was analysed using mixed effects Cox models ("coxme") (Therneau & Grambsch 2002): models included a dose rate and start weight as fixed effects and colony identity as a random effect.

2.4 Results

Radiation exposure impairs colony reproductive output

Bumblebee colonies exposed to radiation produced fewer queens than unexposed colonies. Queen production per colony ranged from 0 to 58 (mean = 16.1 ± 1.67, n = 59). Queen production was highest in unexposed colonies and declined to near-zero at the highest dose rates ($F_{1, 56}$ = 9.80, p = 0.0017, Figure 2.1). The model predicted that, compared to unexposed controls, the reduction in colony queen production was 6% at 100 μGyh^{-1}, 28% at 400 μGyh^{-1} and 84% in colonies exposed to 2800 μGyh^{-1}.

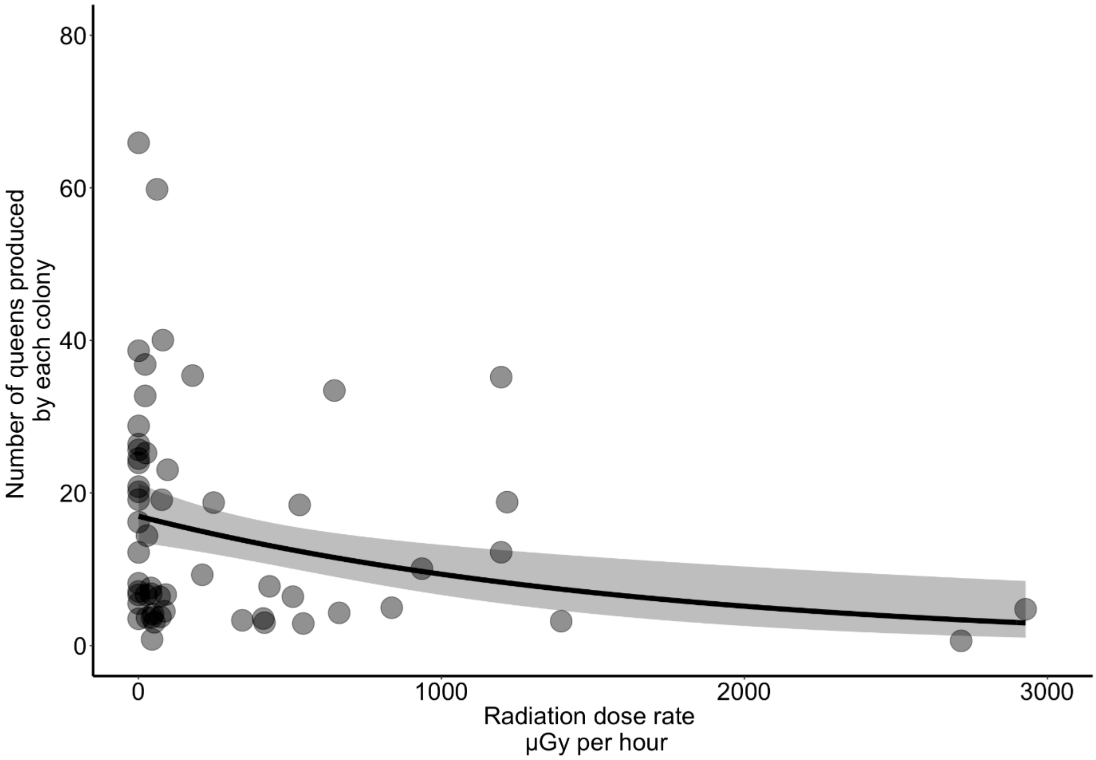

Figure 2.1 The impact of ionising radiation on bumblebee colony reproduction. The negative relationship between external radiation dose rate and the number of new queens produced by each colony was significant both for the full dataset (n = 59). Data points are predicted values from a negative binomial model controlling for variation in total colony size (number of workers and males produced). Fitted lines are derived from the models with their 95% confidence intervals and X-axes are offset from zero to effectively show control colony data.

A series of analyses were conducted using subsets of the overall dataset to assess how robustly the data support this negative effect of radiation exposure. These analyses also tested whether there was a lower dose threshold below which the negative effect was undetectable. The two highest queen producing colonies were notable outliers in the data set (producing >50% more queens than the other colonies), and the colonies at the two highest dose rates were potentially influential observations (Figure 2.1). To test the influence of these points on the relationship between dose rate and queen production, models were run excluding these four data points (Table 2.1). The slope parameter deviated little between models, providing confidence that these data points did not drive the negative relationship between queen production and dose rate. This conservative approach was continued by reducing the dose range and re-analysing the subset data to test whether this result was not simply driven by the higher dose rates and whether there is a lowest dose threshold at which the negative relationship between radiation dose rate and queen production is maintained (Table 2.1).

Table 2.1. Sensitivity analysis for the relationship between radiation dose rate and the number of new queens produced from colonies by the removal of influential data values. This sensitivity analysis also detects whether there is a clear low dose threshold below which the effect is not detectable. *The negative binomial generalised linear model including the number of males produced from colonies. Slope estimates are as stated by the model are scaled mean centred model outputs*.

Description of the data set	N	Intercept	Slope	Standard error	χ^2	P value
Full data set	59	4.19	-0.00059	0.0001	9.80	0.0017*
Queen production < 50	57	3.97	-0.00052	0.00017	8.37	0.0037*
Queen production < 50 Dose rate < 1000 μGyh^{-1}	51	3.87	-0.00380	0.00024	5.69	0.017*
Dose rate < 50 μGyh-1	28	4.21	-0.018	0.0063	6.82	0.008*
Queen production < 50 Dose rate < 100 μGyh^{-1}	30	3.39	-0.013	0.0058	4.49	0.033*

The total number of worker and male cells produced from each colony was counted. Workers and males are indistinguishable due to the lack of cell size difference. Increasing radiation dose rates were associated with a significant reduction in workers and males ($F_{1,56}$ = 4.37, p = 0.04). A significant colony start weight by dose rate interaction was detected: the positive influence of colony start weight on the total number of males and workers produced became stronger at higher dose rates (start weight interaction by dose $F_{4, 56}$ = 5.41, p = 0.001, Figure 2.2).

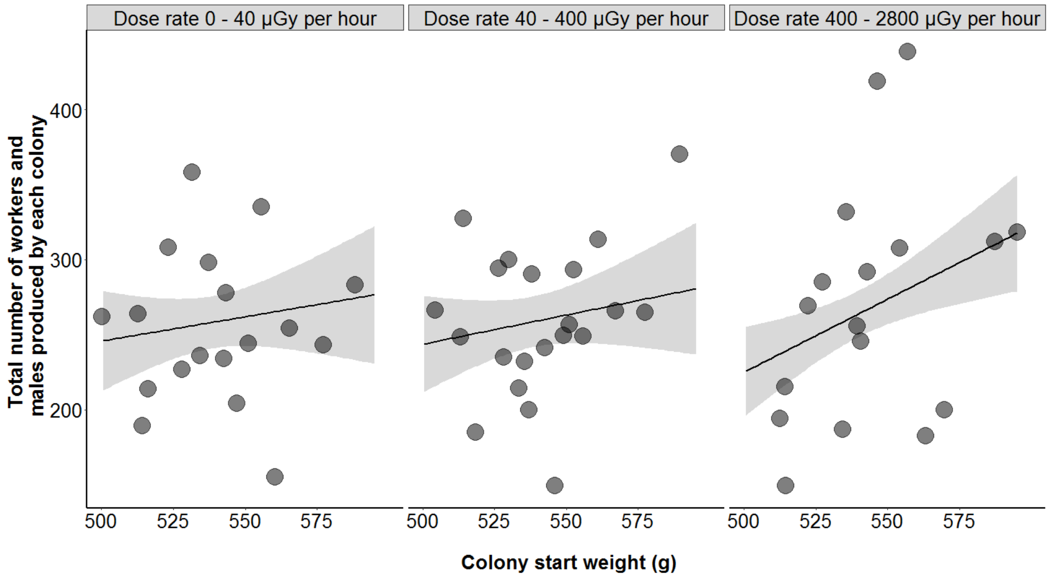

Figure 2.2 Radiation exposure was negatively associated with the combined number of males and workers produced from each colony; this effect was most pronounced in colonies with low starting weight. The statistical interaction between colony start weight and radiation exposure was significant (see text). Fitted lines are derived from the models and the shaded area around the fitted line represents the 95% confidence intervals. Data are presented as subset by the three ICRP dose bands where adverse effects are expected to the organism. 0 – 40 µGyh-1 is the value used for environmental monitoring of radiation in the environment, 40 – 400 µGyh-1 is the dose band below the current DCRL and 400 - 2800 µGyh-1 is within the DCRL currently designated for the ICRP reference bee.

To test whether relationship between radiation dose rate and start weight was being driven by colonies with small start weights, the intercept deviation was tested. The smallest colonies were used as the model intercept instead of colonies of a mean weight. The negative relationship between radiation dose rate was maintained ($F_{1, 56}$ = 4.5, p = 0.047).

Bumblebee colonies typically undergo a growth phase as workers are produced and then peak in weight as they start to generate reproductive towards the end of the colony life cycle. The time taken for colonies to reach their peak weight became longer with increasing radiation dose rate (F=$_{1,\ 56}$ = 6.71, p = 0.01). All colonies reached their peak weight at either week four or week five of the experiment. Only colonies exposed to dose rates less than 200 μGyh^{-1} (n = 40) peaked during week four (45%), whereas 85% of colonies at and above 200 μGyh^{-1} (n = 19) peaked during week five (Figure 2.3).

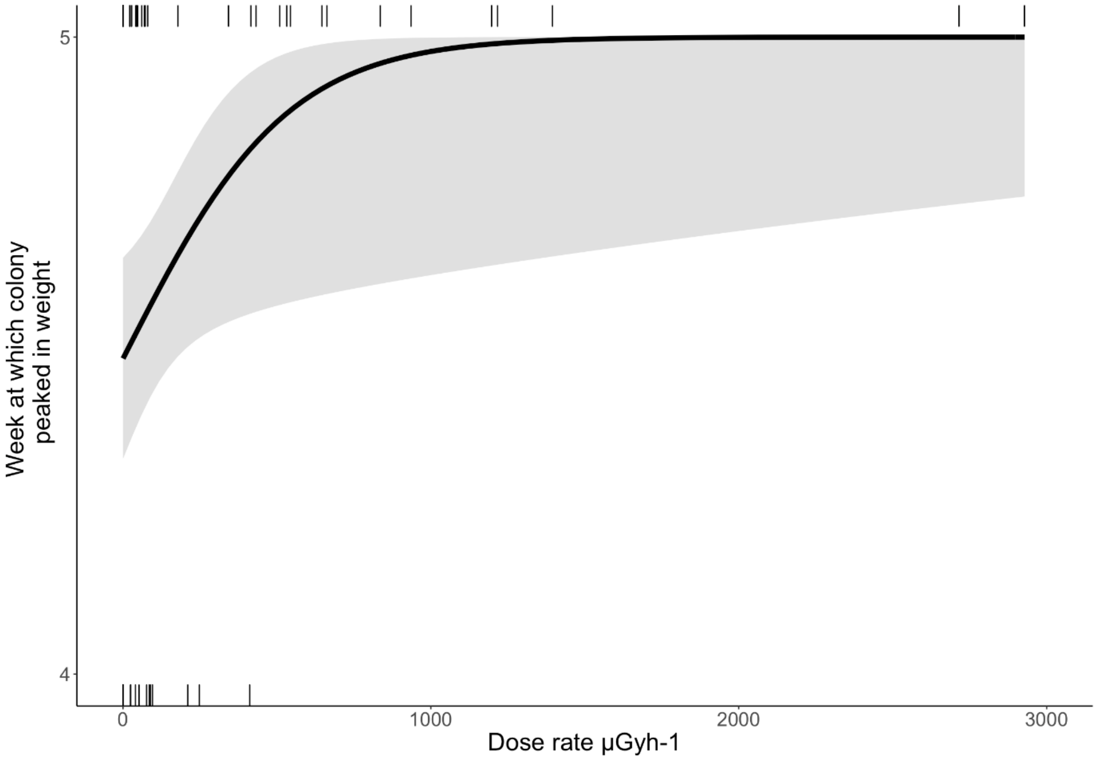

Figure 2.3. Bumblebee colonies exposed to radiation reach their peak weight later than unexposed colonies. Fitted line is the predicted values from the binomial generalised linear model, 95% confidence intervals and the rug demonstrates where the data falls along the x-axis

No relationship between weight change and radiation dose

All bumblebee colonies gained weight during the four weeks of the laboratory exposure phase; then after release into the field, all colony weights declined until colony death due to loss of individuals and resources. Colony weights at the start of the study ranged from 485 g to 595g. The data were analysed in two separate parts to investigate colonies gaining weight and after reaching peak weight, the decline in weight. For both datasets, start weight and experiment week resulted in either a decline or increase in weight, depending on whether before or after peak weight (Table 2.2). There was no relationship between the radiation dose rate and colony weight change (Table 2.2).

No relationship between food consumption and radiation dose

Food consumption by colonies during the exposure phase of the experiment was measured by the weight of the sugar reservoir in each colony. Food consumption was not affected by radiation dose. Colony consumption increased over time as larger colonies had a greater rate of food consumption (Table 2.2).

No effect of radiation on longevity

Colony lifespan was assessed as the start of the experiment and when fewer than five workers remained alive. All colonies died during the field phase of the experiment with longevity ranging between 38 and 70 days (mean = 49.5 ± 1.16 days). Cox survival analysis demonstrated no significant effect of dose rate on colony longevity and showed that colonies with the largest start weight had greater longevity (Table 2.2). During the experiment, queen death was observed in 33% (n = 59) of colonies, but no association with dose rate and the date the queen died was detected ($\chi^2_1 = 0.179$, p = 0.16).

To enable estimation of the lifespan of individual workers, 920 workers spread across 9 colonies were labelled with numbered bee tags. After release into the field, 23% of these workers were re-sighted and included in the analysis. Radiation dose rate was non-significantly negatively associated with worker longevity (Table 2.2).

No relationship between tagging on colony queen or male production

Colonies tagged during week three had greater longevity than untagged colonies (Table 2.2), but these were evenly distributed across the radiation gradient. The process of tagging the worker bees did not affect colony queen number ($F_{1, 55}$ = 1.85, p = 0.17), nor the production of workers and males ($F_{1, 55}$ = 0.05, p = 0.81).

Table 2.2 Parameter estimates from all models referenced in this study. B-coefficient is the mean- centred scaled parameter estimate.

Bumblebee colony life history traits							
Response variable	**Predictor**	**Estimate**	**SE**	**β**	**SE**	**χ^2**	**P**
Number of queens	Radiation dose	-0.0006	0.0001	-0.35	0.10	9.80	0.0017*
Number of queens	Workers and males	-0.005	0.0001	-0.31	0.09	8.72	0.0031*
Number of workers and males	Radiation dose	-0.86	0.4132	7.27	7.73	4.378	0.041*
Number of workers and males	Colony start weight	0.324	0.3726	21.0	7.70	0.755	0.388
Number of workers and males	Colony start weight: radiation dose	0.0016	0.0007	24.29	11.45	4.502	0.038*
Changes to bumblebee colony weight							
Response variable	**Predictor**	**Estimate**	**SE**	**β**	**SE**	**χ^2**	**P**
Peak weight	Radiation dose	0.00025	0.00001	0.152	0.060	6.716	0.012*
Colony weight weeks 1 - 4	Start weight	1.15	0.2350	33.87	4.28	43.67	<0.0001*
Colony weight weeks 1 - 4	Experiment week	65.85	2.000	53.92	1.64	277.5	<0.0001*
Colony weight weeks 1 - 4	Radiation dose	0.0002	0.0007	1.58	4.23	0.141	0.707
Colony weight weeks 5- 8	Start weight	0.61	0.289	15.01	7.054	37.62	<0.0001*
Colony weight weeks 5- 8	Experiment week	-27.44	3.464	-21.65	2.733	4.50	0.0338*

<table>
<tr><th colspan="8">Changes to bumblebee colony weight (continued)</th></tr>
<tr><th>Response variable</th><th>Predictor</th><th>Estimate</th><th>SE</th><th>β</th><th>SE</th><th>χ^2</th><th>P</th></tr>
<tr><td>Colony weight weeks 5- 8</td><td>Radiation dose</td><td>-0.007</td><td>0.121</td><td>-4.37</td><td>7.03</td><td>0.41</td><td>0.518</td></tr>
<tr><td>Food consumption</td><td>Colony start weight</td><td>0.48</td><td>0.402</td><td>-313.6</td><td>-98.81</td><td>1.46</td><td>0.226</td></tr>
<tr><td>Food consumption</td><td>Experiment week</td><td>209.2</td><td>48.38</td><td>-47.90</td><td>-6.37</td><td>18.14</td><td><0.0001*</td></tr>
<tr><td>Food consumption</td><td>Colony start weight: Experiment weeks</td><td>-0.80</td><td>0.089</td><td>-28.33</td><td>-8.91</td><td>68.95</td><td><0.0001*</td></tr>
<tr><td>Food consumption</td><td>Radiation dose rate</td><td>-0.0004</td><td>0.0012</td><td>-2.51</td><td>7.58</td><td>0.115</td><td>0.734</td></tr>
<tr><th colspan="8">Longevity</th></tr>
<tr><th>Response variable</th><th>Predictor</th><th>Estimate</th><th>SE</th><th>β</th><th>SE</th><th>χ^2</th><th>P</th></tr>
<tr><td>Colony longevity</td><td>Start weight</td><td>-0.004</td><td>0.001</td><td></td><td></td><td>5.98</td><td>0.014*</td></tr>
<tr><td>Colony longevity</td><td>Tagged</td><td>0.62</td><td>0.270</td><td></td><td></td><td>6.37</td><td>0.020*</td></tr>
<tr><td>Colony longevity</td><td>Radiation dose</td><td>0.0002</td><td>0.0002</td><td></td><td></td><td>0.659</td><td>0.416</td></tr>
<tr><td>Queen longevity</td><td>Start weight</td><td>0.018</td><td>0.010</td><td></td><td></td><td>2.92</td><td>0.087</td></tr>
<tr><td>Queen longevity</td><td>Radiation dose</td><td>0.00085</td><td>0.0006</td><td></td><td></td><td>1.79</td><td>0.179</td></tr>
<tr><td>Worker longevity</td><td>Radiation dose</td><td>-0.0027</td><td>0.0019</td><td></td><td></td><td>1.2</td><td>0.15</td></tr>
</table>

2.5 Discussion

This chapter presents the first study to experimentally demonstrate that bumblebees are negatively affected by exposure to ionising radiation at dose rates well below those previously thought likely to impact insects. Radiation impaired colony reproduction and delayed colony growth. This study helps resolve the ongoing controversy of whether radiation dose rates at contaminated sites like the CEZ are damaging to invertebrates (Garnier-laplace et al. 2013; Moller & Mousseau 2013; Smith et al. 2012; Beresford & Copplestone 2011). It shows the clear potential for the highest contaminated areas within the CEZ to negatively impact insect reproduction.

Previous laboratory studies have suggested that invertebrates are relatively resistant to ionising radiation. The data from this experiment challenge this conclusion, which has been a central pillar informing current environmental radiation protection policy. Other laboratory studies have typically used high dose rates to deliver short-term acute exposures (Larsson 2012; Garnier-Laplace et al. 2013; Mothersill et al. 2018). Instead, this experiment mimicked field conditions by delivering chronic radiation dose rates ranging from those found in the CEZ to the highest dose rates considered within the international system of radiological protection of the environment.

The results presented in this chapter indicate that exposure to even 100 μGyh^{-1} decreased bumblebee queen production by 6%. Queens are demographically limiting, so this small decrease in queen production could substantially impact bumblebee populations (Crone & Williams 2016; Bréchignac 2003). The international system of radiological protection of the environment for assessing radiation risks to wildlife has been based on the assumption that invertebrates do not suffer significant adverse effects from dose rates below 400 μGyh^{-1}. Dose rates in the environment are unlikely to be as high as 100-400 μGyh^{-1} except in areas contaminated by major accidents or incidents: at 400 μGyh^{-1}, the data suggested a 28% loss of bumblebee reproductive output. A recent study on the effects of pesticide exposure indicated that a 26% reduction in bumblebee colony founding success dramatically increased the likelihood of local population extinction (Baron et al. 2017). This chapter suggests that chronic exposure to a 400 μGyh^{-1} radiation dose could have similar dramatic effects; if this occurred significant impairment of the pollination services bumblebees provide could result in consequences to the whole ecosystem (Winfree et al. 2015).

The estimates provided in this chapter demonstrate that the negative consequences of radiation exposure are likely to be conservative compared to those experienced by wild populations because the bumblebees in this experiment were housed in optimum conditions with *ad libitum* feeding (Crone & Williams 2016; Holland & Bourke 2015). Furthermore, although colonies were exposed for a large proportion of their lifespan, queens can live up to a year and therefore total accumulated dose throughout the lifetime of queens in the field would be much larger than experienced in this experiment. Also, wild colonies are exposed to multiple other stressors including the costs of foraging, limited food, climatic factors, parasites and pathogens, which could all exacerbate the negative effects of radiation observed in this experiment (Goulson et al. 2015).

Some studies have previously reported negative impacts of radiation on a range of organisms including bumblebees (Møller et al. 2012; Møller & Mousseau 2009). These studies have been received sceptically by some researchers as the dose rates stated in some of these papers at which severe negative effects have been reported are incongruous as they equate to typical background radiation levels. The findings of this chapter support the conclusion that the exposure levels in some areas of the CEZ may result in significant harm to bumblebees, however, the severe declines in bumblebee abundance at dose rates equivalent to background (Møller et al. 2012; Møller & Mousseau 2009; Møller et al. 2012) are not supported by the data provided in this chapter.

The sensitivity analysis conducted to test whether the influential data at high radiation dose rates and the colonies which produced the greatest number of queens also aimed to detect whether there was a low dose threshold below which an effect of radiation on queen production was not detectable. The sensitivity analysis demonstrated that the effect size was not reduced when the data were subset and increased when compared the complete data set. However, these smaller effect sizes, although consistently negative, were not significant with the inclusion of the two highest queen producing colonies. When the high queen producing colonies were included and the data analysed as subset below a dose rate of 50 μGyh^{-1}, the relationship was statistically significant. Although the effect size changes throughout the sensitivity analysis, the negative relationship continued to dose rates as low as 50 μGyh^{-1}; this study found no evidence therefore that the negative effects stop below 100 μGyh^{-1}.

The accumulated dose rates from this experiment were calculated to provide context for the studies undertaken in this chapter in comparison with previous studies which have used acute dose rates. Colonies exposed to chronic exposure to 100 μGyh^{-1} received an accumulated dose of 0.07 Gy over the colony lifespan, while the most exposed colonies received 2 Gy over the four-week course of the experiment. Previous research has found that exposure to 1-2 Gy of acute radiation resulted in LD$_{50}$ for sub-adult Hymenoptera (ICRP 108, Bakri 2005). In this study, bumblebee reproduction failed almost entirely at dose rates around 2800 μGyh^{-1} (2 Gy), where there was an 82% reduction in queen production. Previous data suggest that more damage occurs from acute radiation than chronic because acute exposure overwhelms repair mechanisms; however, the responses to populations to low-level chronic exposure are poorly understood (Geras’kin 2016b).

Increased radiation exposure also reduced the total number of workers and males produced by colonies. This evidence of a slightly complicated effect of radiation dose rate on worker/male production suggests an additional route to colony fitness impairment: the workforce may be smaller and in some conditions colony reproduction through the male route may be reduced. However, colonies with a small starting weight that were exposed to radiation suffered impaired production of males and workers; whereas larger colonies appeared to be buffered from this effect.

As well as affecting bumblebee reproduction, the data show that radiation exposure delayed colony growth. 45% of colonies exposed to less than 200 μGyh^{-1} reached their peak weight at the earliest time point in the experiment (week four) whereas only 5% of colonies exposed to radiation doses greater than 200 μGyh^{-1} peaked at this early time point. The timing of peak weight is a proxy for the timing of the production of reproductives. Colonies have a period of exponential growth when workers are produced before a switch point occurs around the time of colony peak weight when queens stop laying worker eggs and start to lay reproductive eggs (Mitesser et al. 2007; Holland & Bourke 2015; Shykoff & Mullier 1995). The timing of the switch from worker to sexual production is important: it is hypothesised this switch should occur as early as possible to maximise reproductive fitness (Klok & Thissen 2009; Beekman & van Stratum 1998). Colonies switching earlier tend to produce more queens and the males that are produced have a greater number of mating opportunities than later males (Duchateau et al. 2004; Beekman & van Stratum 1998; Beekman et al. 1998). Delay to the timing of reproduction is a common phenotypic response to stress in bumblebees and relies on cues provided by the colony's growth stage and resource availability (Holland et al. 2013; Badyaev 2005).

This experiment did not investigate the mechanism for these effects of low dose radiation on bumblebee life history, but one of the key questions resulting from this study is whether this is only the case for bumblebees or can be generalised for all insects. Eusocial insects are known to be less susceptible to stressors compared to other species as they can buffer against stress (Straub et al. 2015). If this observed decrease in reproductive success and alteration to life history traits can be attributed to molecular damage, resource trade-offs or worker impairment, the data suggest these effects may not be confined to bumblebees.

The work in this chapter has significant implications for the ICRP international radiological protection framework used to make recommendations for global radiation protection guidelines. This framework is mostly underpinned by data from laboratory studies which may have limited environmental applicability. These laboratory studies have been extrapolated to estimate bands of dose rate called Derived Consideration Reference Levels (DCRL) within which negative effects to a particular taxon may be expected (ICRP 2008). The DCRL for insects is 400 – 4000 μGyh^{-1}. The data from this chapter suggest a 28% reduction in bumblebee queen production at the lowest end of this dose band with a smaller, but still significant, reduction at 100 μGyh^{-1}. These findings provide the first data to contribute towards an accurate evaluation of this DCRL for the Reference Bee and suggests it is wrong. Based on the findings from this study, I strongly recommend to the International Commission on Radiological Protection that this dose band should be reduced to 40 - 400 μGyh^{-1} for the bee RAP· The reduction of this dose band places insects within the same dose rate range as for fish, amphibians and plants within the international system of radiological protection of the environment.

The findings from this study contribute towards resolving the ongoing controversy in radioecology being the first study to experimentally test how environmentally relevant radiation exposure affects bumblebee life history. This experiment has demonstrated significant negative consequences at dose rates previously not predicted to cause adverse effects, and which are equivalent to some dose rates found presently in the CEZ. The effects measured in this experiment could have significant implications for wild populations; further research should determine the mechanism for this fitness loss and establish how relevant these findings are to other invertebrates. The data produced from this experiment strongly advocate that the current international system for radiological protection of the environment is re-evaluated to incorporate this new information.

Chapter 3

A case study comparing techniques for measuring radiation dose rates to bumblebees in the Chernobyl Exclusion Zone

3. A case study comparing techniques for measuring radiation dose rates to bumblebees in the Chernobyl Exclusion Zone

3.1 Abstract

The Chernobyl accident happened over 30 years ago and created widespread contamination across north-west Europe. Although radiation dose rates have decreased since the accident, the area surrounding the reactor is still among the most radioactively contaminated on earth. In recent years, there have been studies conducted in the Chernobyl Exclusion Zone which have found severe impacts on different wildlife groups at dose rates that are below levels considered to be safe for wildlife. One of the key challenges to these studies is the claim of an inadequate dose assessment methodology being used that could result in an incorrect dose-response relationship.

This chapter seeks to improve the estimation of dose rates to bumblebees by comparing different techniques to measure radiation exposure or levels in a heterogeneously contaminated area like the Chernobyl Exclusion Zone (CEZ). This study measured the contributions to dose rate from gamma and beta radiation; beta is not frequently measured in field-based radioecological studies. Dose assessments were conducted by estimating the internal and external dose rates while taking into account the ecological and life history parameters of bumblebees. The ERICA tool, which is frequently used to make dose assessments, was used to estimate internal and external dose rates to bumblebees using the activity concentration of ^{137}Cs. The data collected demonstrate that the ambient beta radiation dose rate in many sites was higher than the gamma dose rate, but this did not significantly increase the total dose rate or the accumulated dose as the contribution to beta for internal dose rate is relatively small in bumblebees, compared to other wildlife. The dose assessments support the use of an external handheld monitor as providing a good estimate of dose rates for bumblebees due to the low internal dose contribution. Both dose assessment methods, the ERICA tool and the calculations using the different measured dose rates suggest that the position of the nest, whether below or above ground is one of most significant predictors as to the dose rate bumblebees are likely to receive.

3.2 Introduction

The Chernobyl Exclusion Zone is a unique, complicated and heterogeneous environment. As a result, there are challenges when trying to establish the likely dose rates an organism receives and the accumulated lifetime dose when living in this environment. The Chernobyl accident occurred in April 1986 and released large scale radioactive fallout across Northern Europe. The total release of fission products (excluding noble gases) was estimated to be 1.85 EBq (1 EBq = 10^{18} Bq) (IAEA 2006), including 1.8 EBq of ^{131}I, 0.085 EBq of ^{137}Cs and other caesium radioisotopes, 0.01 EBq of ^{90}Sr and 0.003 EBq of plutonium radioisotopes. The location of radionuclide deposition was wide-ranging and heterogeneous following the trajectories of the radioactive plumes to the north and the west (Bonzom et al. 2016). The Chernobyl Exclusion Zone is an area of approximately 2,600km^2 which had the highest contamination after the accident and remains the most affected area over 30 years later (Bonzom et al. 2016; Geras'kin et al. 2013).

The highest exposure to biota occurred within the first 10 -20 days after the accident due to the release of short and long-lived radionuclides into the atmosphere (Geras'kin et al. 2008). Presently, ^{137}Cs and ^{90}Sr are the most significant contributors to the dose rate for organisms living in the Chernobyl Exclusion Zone, and there are also contributions from iodine and alpha-emitting plutonium and americium (Bonzom et al. 2016). ^{137}Cs and ^{90}Sr have relatively long half-lives (30, and 28 years respectively) and are homologues for potassium (^{137}Cs) and calcium (^{90}Sr) therefore are easily incorporated into the body of many animals (Chesser et al. 2000). Most strontium and plutonium radioisotopes were deposited within 100 km of the reactor as they were contained within sizeable particulate matter (IAEA 2006).

Further heterogeneity in the Chernobyl Exclusion zone is due to the presence of radioactive particles or "hot" particles which are typically present in the upper few centimetres of the soil (Kashparov et al. 2003). The composition of these particles are similar to the fuel from the reactor, and the deposition of hot particles has been shown to decrease with increased distance from the reactor (Salbu et al. 2017; Beresford et al. 2016). However, research has demonstrated the importance of radioactive particles in the environment but so far, how they interact with wildlife is not fully understood (Salbu et al. 2018), therefore, although hot particles are likely to contribute to the dose rate to bumblebees, there will not be a focus on hot particles in this study.

In this chapter, total external dose rate accounts for external dose rate comprised of gamma radiation from both ambient measurements and using the ERICA Tool. Total dose rates include the contribution from internal as well as external dose rates (gamma and beta). The accumulated dose is the radiation dose an organism receives over its lifetime, taking into account ecological and life history parameters. Ambient dose rates are made using a handheld meter and only refer to external dose rates.

Improved dosimetry is imperative for radioecology and radiation protection

The Chernobyl Exclusion Zone (CEZ) enables further research and the development of techniques to measure radiation dose rates in a heterogeneous environment and better understand the effects to non-human biota to test the dose-response relationship and verify the parameters that are used in the radiological protection of the environment. In recent years, there has been controversy in the scientific community surrounding the effects studies predominantly undertaken in the Chernobyl Exclusion Zone. Dosimetry has been a central point of criticism and discussion, and the lack of robust dosimetry is a well-acknowledged problem in radioecological studies (Bonzom et al. 2016; Brechignac et al. 2016; Garnier-Laplace et al. 2013).

There have been some recent studies investigating the effects of radiation on wildlife. A subset of these studies have found severe consequences to a range of different taxa in the Chernobyl Exclusion Zone (Lehmann et al. 2016; Møller et al. 2011; Bonisoli-Alquati et al. 2010; Moller & Mousseau 2007). These studies have detected severe adverse effects to invertebrates, which are generally considered more radioresistant than other taxa such as mammals, at levels close to background (Møller et al. 2012; Møller & Mousseau 2009). Effects to biota have been recorded in Chernobyl at dose rates up to eight times lower than controlled experiments (Garnier-Laplace et al. 2013). Conversely, there have been many other studies also on a range of taxa and using the same dose rates, and often the same sites in the CEZ, which have found no effects of radiation, including invertebrates (Fuller et al. 2018; Bonzom et al. 2016; Deryabina et al. 2015; Lecomte-Pradines et al. 2014; Rodgers & Baker 2000).

Establishing an accurate dose-response relationship relies on reasonable estimates of dose rates to the study organism. Measuring total dose rates for wildlife is much more complicated than calculating the dose rate to organisms exposed under controlled conditions (Garnier-Laplace et al. 2013). Presently, most radioecological studies rely on taking measurements of the ambient dose rate across the site where the organism has been sampled. These measurements are most commonly conducted using a handheld meter to measure external gamma radiation (Lehmann et al. 2016; Bezrukov et al. 2015; Baker et al. 1996). The use of a handheld meter to measure ambient dose rates is common practice to estimate dose rates to humans in radiological monitoring. However, these dose rate measurements are usually combined with habits surveys which work out what food is eaten and the likely radionuclide concentrations, therefore, enabling best estimates for internal dose as well as the external dose (RIFE 2015). Habits surveys for food consumption are not appropriate for wildlife, but researching and adapting information about different species diet can give insight into the contribution to internal dose rate (Beresford et al. 2000). Wildlife eat different food and occupy different niches at different times resulting in different exposures at different points in their life cycle. Different stages of the life cycle have varying radiosensitivity, to account for this in humans there is the "Reference Person" but also infants and children (ICRP 2007a). For wildlife, these parameters are highly variable, even between closely related species and broad generalisations are not appropriate.

It has been shown that the frequent use of ambient dose rate in radioecological studies may underestimate the absorbed dose rate to organisms by over an order of magnitude (Beresford et al. 2018). Generally, Beresford et al. (2018) found that ambient dose rates taken from across a sampling site were 3-13 times lower than the total absorbed dose rate estimates, but this was not the case for A*pidae* spp (Beresford et al. 2018).

Measuring total dose to an organism

The key factors affecting radiation exposure and the dose to animals and plants are; (I) the internal distribution of the source, (II) the external distribution of the source, (III) the characteristics of the organism, and (iv) the location of the organism in regards to external exposure sources (Figure 3.1) (Stark et al. 2017).

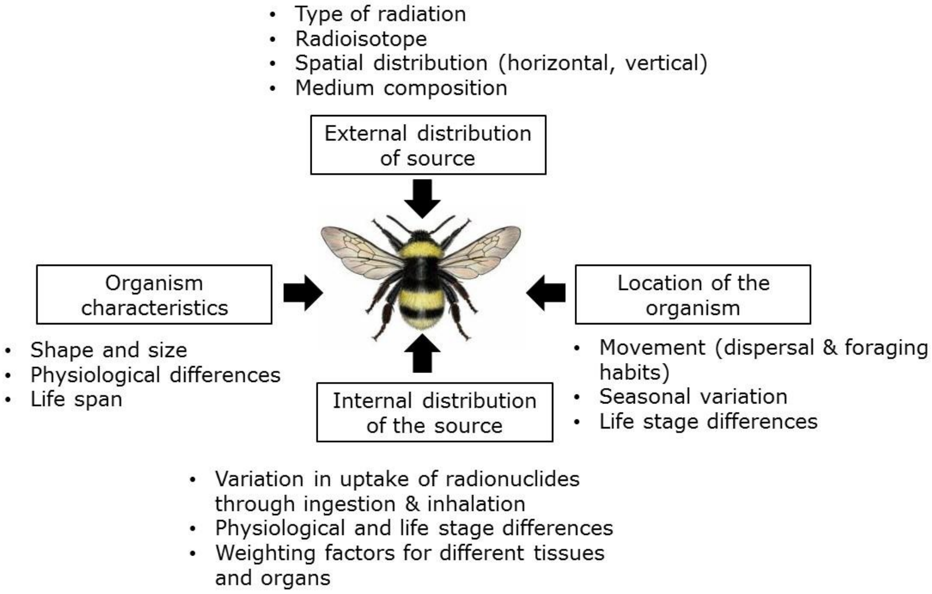

Figure 3.1 The parameters affecting radiation dose to wildlife in a site contaminated with radionuclides (adapted from (Stark et al. 2017))

Alpha (α), beta (β) and gamma (γ) radiation are the contributors to dose rate in environmental conditions. However, contributions from alpha and beta particles to external doses are excluded as low energy betas have low penetration and are prevented from affecting radiosensitive tissues by layers of dead skin and fur. Beta dose rates are important for calculating internal dose rates (Ulanovsky & Pröhl 2012).

Gray (Gy) is the SI unit of absorbed dose (J kg^{-1}) and measures the mean energy imparted into matter of mass by ionising radiation (ICRP 2007). The Effective dose, measured in Sievert (Sv), is the tissue-weighted sum of equivalent dose (ICRP 2007). When measuring dose rate to wildlife, the absorbed dose is used (Gy) because the aim is to estimate the dose rate to the whole organism and not specific organs (Stark et al. 2017). Wildlife are exposed externally and internally to ionising radiation from radionuclides in the environment. External exposure (µGy h^{-1}) comes from the surrounding media. Internal exposure occurs when radionuclides may have been ingested, inhaled or otherwise temporarily enter cavities within an organism and results from accumulation in tissue and organs over time (Ulanovsky et al. 2008). Internal and external exposure depends on physical decay properties and characteristics of the radionuclide and the biological half-life of the radionuclide at a whole body or organ-specific level (Ulanovsky & Pröhl 2012; ICRP 2008, Figure 3.1). Dose rate contributions from internal and external absorbed dose rates combine to give a total dose rate.

The process of only measuring ambient dose rates to infer absorbed total dose rates can lead to drastic under-predictions which can confuse when trying to contextualise field effects to the established benchmarks of the ICRP (Stark et al. 2017). Preferably, the total dose rate (TDR) absorbed by wildlife which is composed of external and internal dose rate needs to be established. However, the contribution from internal dose rate to the overall dose rate varies depending on the life history traits of the study organism (Figure 3.1) (Beresford et al. 2018; Copplestone et al. 2017; Beresford & Copplestone 2011; Lecomte-Pradines et al. 2014; Brèchignac et al. 2009).

The use of models to estimate dose rates to biota

Dosimetric models can be used to estimate the total dose rate to biota. However, dosimetric models are not frequently used in radioecological studies, and principally, the ambient dose rate it used to infer the dose rate to wildlife. Dosimetric models are useful but have to make assumptions due to diversity and variation in habitat occupation, lifestyles, and body shape, feeding, metabolism and exposure conditions (Figure 3.1). These factors affect the internal and external doses an individual receives but cannot be explicitly calculated for each individual (Ulanovsky & Pröhl 2012). Models used for radiological protection are simplified using assumed simple body shapes with uniform composition and density, homogenous internal contamination and uniform radionuclide distribution in the surrounding environment. Models need to be validated using direct dosimetric measurements of wild animals, and this has been greatly improved over the last few years (EMRAS 2012; Stark et al. 2017; Beresford et al. 2010; Thiessen et al. 2008). There is a need to accurately measure exposure for in situ organisms and increase capabilities of direct practical dose measurement technologies (Ulanovsky et al. 2017; Ulanovsky et al. 2008).

The ERICA Integrated Approach enables the quantification of environmental risk by using data on environmental transfer and dosimetry to provide a measure of exposure to different wildlife types. The radionuclide specific parameters used in deriving dose rates from measured activity concentrations in soil are called concentration ratios. Concentration ratios are the activity concentration in the whole body of an organism to that in the media (Brown et al. 2016; Brown et al. 2008). The ERICA tool contains reference organisms, similarly to the ICRP reference animals and plants, including a flying insect to represent the bee as described in the introduction of this thesis, but also allow the creation of wildlife to specific parameters such as size and occupancy (Larsson 2008).

Different approaches to estimate dose rate in the field

Areas around nuclear sites are routinely monitored for radiation contamination (RIFE 2016; Mudge et al. 1996). Routine monitoring consists of considering the likely sources of radiation, define pathways by which people can be exposed, collect monitoring data, for example, activity concentration or dose rates and calculate the doses (RIFE 2016). Some measurements of activity concentrations are undertaken using the extraction of discrete samples and/or soil cores from a site and subsequently measuring the activity in the whole sample or slicing it at different depths and measuring these individually (Varley et al. 2016). However, this is a time intensive and can fail to capture the heterogeneity at a large scale contaminated site such as the Chernobyl Exclusion Zone. The use of air kerma, gamma spectroscopy and beta scintillators improve the ability to measure heterogeneous environments up to 10m) but, in an area such as the CEZ, this can still be insufficient. There are well-known issues as to how to best estimate dose rates across a large spatial scale with heterogeneous contamination – the options are to either intensively sample a smaller area or use a sampling unit of larger dimensions to gain more information on the heterogeneity present (Scott & Dixon 2008).

In situ measurements can be conducted using a scintillator which offers the added advantage of activity estimation and the burial depth of ^{137}Cs. Sodium iodide detectors are ideal for this approach as they are relatively cheap, robust, lightweight, do not need a cooling system and provide acceptable energy resolution to detect peaks of interest. Sodium iodide detectors, when combined with GPS such as the Mobile Gamma Spectrometry System (MoGSS), can effectively inform about spatial heterogeneity measuring per second to enable mapping of contamination in an area, estimate the activity concentration, identify radionuclides and inform depth distribution (Varley et al. 2017).

Dosimeters can be used in many different ways to calculate the dose rate for wildlife. There are three main kinds; thermoluminescence dosimeter (TLD), optically stimulated luminescence dosimeter (OSLD) and radiophotoluminesence dosimeter (RPLD) and there are different advantages and disadvantages to each (Aramrun et al. 2018; Olko 2010). Radiophotoluminesence dosimeter (RPLD) have been used to establish the total dose rate to nematodes in the soil (Lecomte-Pradines et al. 2014). The nematode total dose rate was conducted by estimating soil radionuclide concentrations and applying equilibrium-based concentration ratios for the soil detrivorous invertebrate values from ERICA tool. Total dose rate varied from 0.7 to 222 μGy h^{-1} with alpha and beta majorly contributing to the internal dose rate, up to 15 μGy h^{-1}. However, the internal dose rate (11 μGy h^{-1}) was negligible when compared to the external dose rate (183 μGy h^{-1}). The underestimation measured dose rates using RPLDs (up to 22 μGy h^{-1}) may be explained by parameters used for invertebrate buried in the soil. In-soil calculations will give a larger dose rate compared to an RPLD which has been placed on the soil surface (Lecomte-Pradines et al. 2014). RPLDs have also been used in conjunction with soil cores to measure radiation dose to soil fauna associated with leaf litter decomposition. Total dose rates to soil fauna were found to be up to three times higher than measured by the average ambient dose rates (Bonzom et al. 2016). This further demonstrates the need for the dosimetric approach to measuring dose rates to in-soil organisms to include dose assessments and not solely use measures of ambient dose rates from a handheld monitor at one metre from the ground.

Affixing dosimeters to wildlife to provide *in situ* total dose rate measurements over the organism’s lifetime have also been used in the Chernobyl Exclusion Zone. The use of dosimeters has demonstrated effectiveness when measuring medium to large mammals; for example, mice and voles through the use of a TLD mounted on collars and combined with live monitoring to establish whole body activity concentrations (EMRAS 2012; Beresford et al. 2008). TLDs have been used to monitor radiation dose for lizards, salamanders, plaice and Chironomidae larvae (Aramrun et al. 2018). TLDs will not be fitted to wild bumblebees due to the logistical difficulties in attaching TLDs to wild bumblebees and ensuring collection at the end of the measurement period, but they will be placed at likely depths of a bumblebee nest in the soil column.

This chapter presents bumblebees as a case study to test different techniques to measure the dose rate to bumblebees in contaminated areas such as the Chernobyl Exclusion Zone. Four different dose measurement methods were used in the field and two dose assessments, using the ERICA tool and the measured dose rates, were conducted for each different caste (with differing life history traits taken into account). The different techniques are compared and evaluated to determine the best method to estimate the dose rate to bumblebees, and these dose rates are used in the data analysis in Chapter 4.

Hypotheses

1) The current approach of using only ambient dose rates to estimate dose rates to widllife in the Chernobyl Exclusion Zone is inadequate when compared to other methods as internal dose rate, not only external dose rate needs to be considered.
2) Radiation dose rates will be higher in the ground when compared to above the ground, especially for beta dose rates
3) Spatial variability of radiological contamination will be high and therefore it is important to estimate dose rates across a range of scenarios e.g. worst case and average exposure
4) Sites located close to the Chernobyl Nuclear Power Plant will have higher contamination compared to sites further away
5) Bumblebees which nest underground will have receive a higher dose rate compared to bumblebees nesting on top of the ground

3.3 Method

Site description

21 sites were sampled in the Chernobyl Exclusion Zone, Ukraine 51.389853°N, 30.094047° E. All sites used in this study were meadow areas acros the Chernobyl Exclusion Zone which were also used for the field work in Chapter 4. Meadow sites were chosen as it is the most likely habitat for bumblebees to forage and nest. Generally across the CEZ the soil is very sandy almost without stones and the clay and humus contents are low, therefore, this type of soil is best described as soddy-podzolic sand (Bossew et al. 2004). As sandy soils allow particulate matter to easily percolate downwards with rainwater, the location of the majority of the ^{137}Cs in sandy and soddy-podzolic soils has been shown to be between 0-15cm (Varley et al. 2017; Evangeliou et al. 2014).

Site 1 in this study was also used in a recent study (Beresford et al. 2018a) to investigate radionuclide transfer to wildlife. Using the activity concentration in the soil sampled at 15 locations across the site as provided in Beresford et al., and the ERICA tool, the mean total absorbed dose rate for bee species was calculated at 19 μGyh^{-1} with the 95th quartile 37 μGyh^{-1}. However, ^{137}Cs contributed 50% of the internal dose rate for Apidae species with a further 37%, 10% and 3% contributed by ^{90}Sr, ^{241}Am and Pu isotopes respectively. Furthermore, the internal exposure only accounted for 6% of the total exposure to Apidae in Chernobyl (Beresford et al. 2018).

Later in this study, sites were grouped into three principal groups depending distance and direction from the Chernobyl nuclear power plant (NPP) to assess similarity in dose rates depending on geographic locations. Sites 1-4 are all located close to the reactor (within 8 km). Sites 5-10 are all located towards the Belarusian border on the North trace. Sites which were more than 8 km along the west trace were groups: 11-15 and sites located south of the Chernobyl NPP were grouped: 16-21 (Figure 3.2).

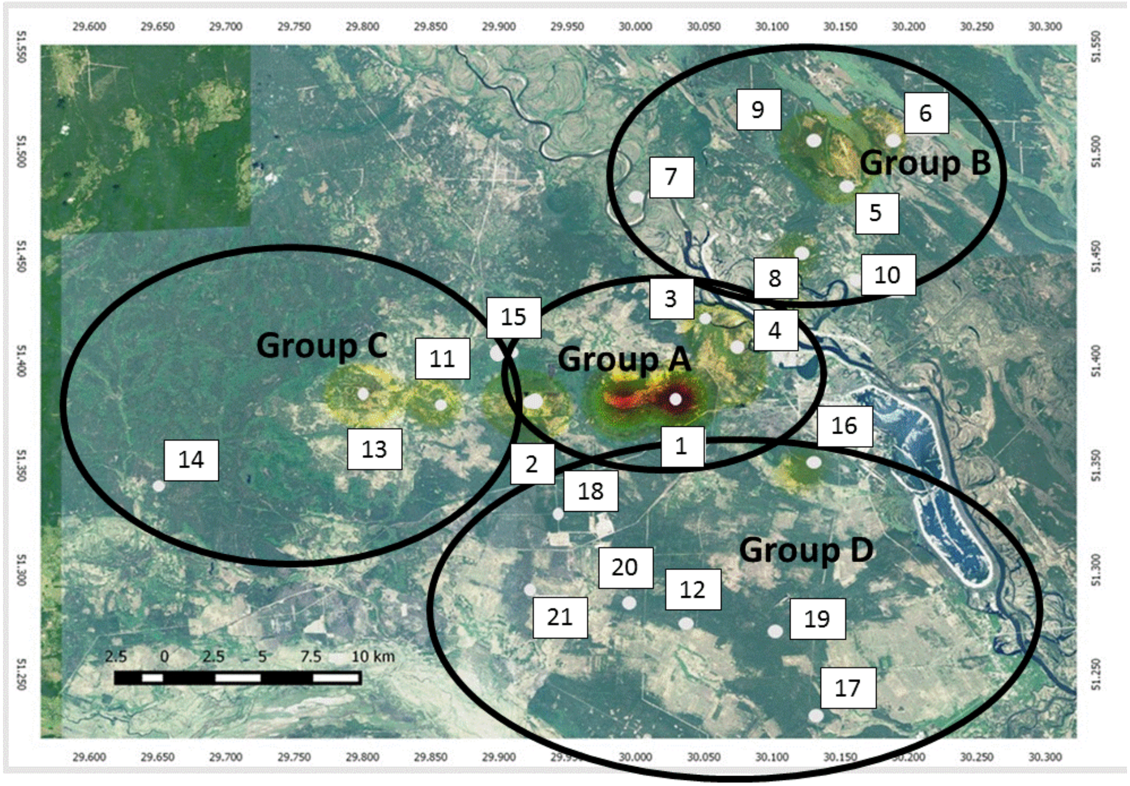

Figure 3.2 A map of sites where bumblebees were sampled across Chernobyl with a gradient of radiation taken from sampling using MoGSS data. The grouping of the sites are allocated based on distance and direction from the Chernobyl Nuclear Power Plant

Below-ground measurements

The measurement of below-ground beta and gamma dose rates were obtained using thermoluminescent dosimeters at five sites of different radiological contamination. Thermoluminescent dosimeters (TLDs) were used to enable the measurement of beta and gamma radiation at two different depths within the soil column.

Sub-surface dose rate estimates were obtained at 10cm and 30cm to estimate the dose rates to bumblebee nests. 50 thermoluminescent dosimeters (TLD) doped lithium fluoride (LiF: Mg, Cu, P) were obtained from Public Health England. TLDs were removed from their stalls and placed into housing which allowed below-ground estimates for beta and gamma radiation. Separate beta and gamma dose rate estimates were measured by using different shielding. 1 cm of Perspex shields beta radiation, therefore, only allowing contribution from gamma. TLDs were either embedded into one centimetre thick Perspex pole with a Perspex plug to protect the TLD and to shield from beta radiation (Figure 3.3). Alternatively, the TLDs were inserted into Perspex rulers and covered in thin polypropylene-based tape to protect from moisture and dirt but allow both beta and gamma radiation to penetrate (Figure 3.4). The poles and rulers were designed to be driven into the ground and left for a time period equivalent to a bumblebee colony lifespan (87 days) to measure below ground dose rates (Goulson 2010).

TLDs in their housing were inserted into the ground to measure dose rates at 10cm and 30 cm in the soil column. Two pairs of TLDs in their housing were placed at five sites across the Chernobyl Exclusion Zone. The TLDs were transported by plane from the United Kingdom to Ukraine and control TLDs residing in the laboratory outside of Chernobyl Exclusion Zone in Slavutych allowed measurement of general background exposure. In Public Health England (PHE) performance tests, the overall relative standard deviation and overall bias was typically 5% (PHE 1999).

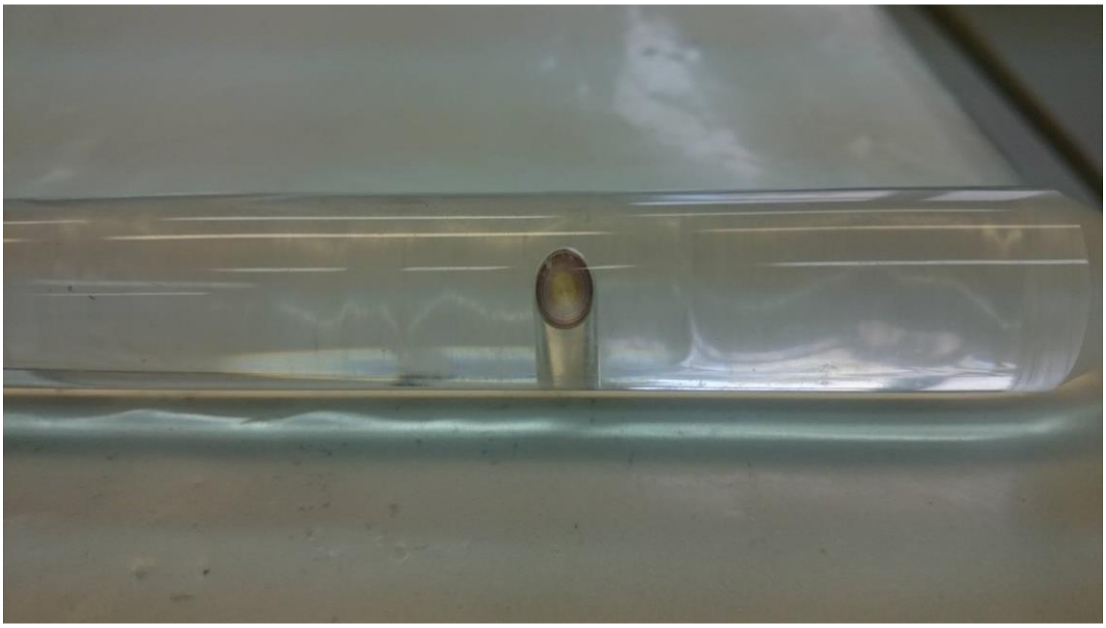

Figure 3.3 One centimetre Perspex pole with inserted TLD to shield from beta and alpha radiation to be buried in the ground. TLD mounted at 10 and 30 cm.

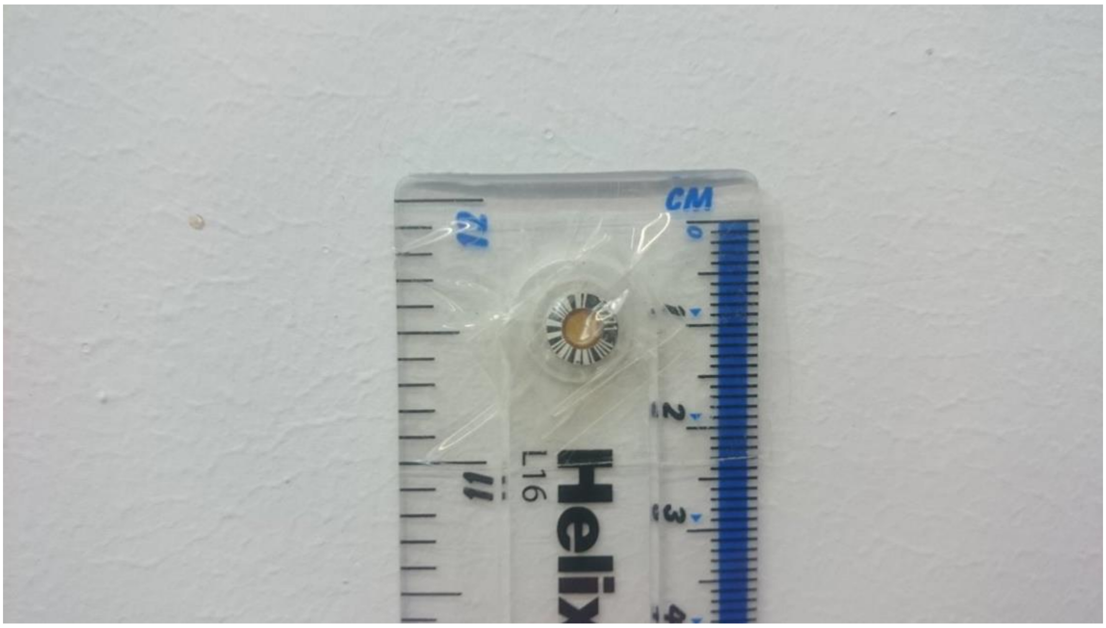

Figure 3.4 Perspex ruler with inserted TLD to shield to allow contribution from beta and gamma radiation to be buried into the ground. TLD mounted at 10 and 30 cm.

Above ground measurements: Mobile Gamma Spectrometry System (MoGSS)

In order to map the ^{137}Cs activity concentration and dose rates across all 21 sites in the Chernobyl Exclusion Zone, a mobile gamma spectrometry system (MoGSS) was used. The MoGSS system comprised a 51 × 51 mm NaI: Tl detector (made by Saint Gobain) combined with an Ortec digiBase which records a 1024 channel spectral every second using Ortec's Maestro software (ORTEC 2005). The detector and digiBase are then combined with a GPS to record coordinates for each spectrum measurement using an SX Blue II differential GPS with a resolution of 0.6 m. When measuring the sites, the MoGSS system was kept at a consistent height of 0.5m from the ground to narrow the field of view. A walking speed of approximately 0.5 m s^{-1} and transect spacing of 0.5 to 1 m was maintained throughout the surveys, for further details on methods see Varley et al. (2017). MoGSS measured counts per second which can be turned into activity concentrations (Varley et al. 2017).

Above ground measurements: Electra for measuring beta dose rates

A Thermo Scientific Electra 1a portable survey meter combined with BP19RA probe was used to measure beta dose rates at all 21 sites. The Electra is used for environmental monitoring and takes measurements in counts per second and dose rates for both beta and gamma. At each site, ten measurements were taken across a randomised transect through the site (Scott & Dixon 2008). Counts were made for 60 seconds using a probe which has a monitoring area of 100 cm^2 coupled to a digital rate meter. Measurements were taken twice per measurement area at one centimetre above the ground. The first measurement was shielded with a casing of 12mm Perspex over the probe to shield from beta. The second measurement was conducted without the shield but covered with a protective file pocket to protect from physical contamination by particles but allowing contributions from both beta and gamma radiation to be recorded. The gamma measurement was then subtracted from the total beta plus gamma measurement. Nuvia calibrated the BP19RA probe against ^{90}Sr, ^{90}Y, ^{36}Cl and ^{14}C, with and without the protective file pocket and Perspex screen in place.

Handheld measurements for external gamma dose rate measurement

The external gamma dose rate at all 21 sites was measured with a handheld device representative of using the most commonly used equipment in radioecology for recording dose rates. Measurements were taken in a systematic sampling method across a transect through the site (Scott & Dixon 2008) using a handheld dosimeter MKS-01R and a Personal Radiation Detector PM1703GN/GNA/GNB at waist height to equalise distance from the ground.

Bumblebee dose assessment

A dose assessment was construcuted using the different measurements e.g. below-ground and above-ground, combined with exsisting information about bumblebee life history (e.g. nesting position and longevity per caste) and contributions from internal and external dose rates as calculated in Beresford et al. The dose rate measurements from the different techniques used in this chapter will enable the estimation of total dose to bumblebees which is important for chapter four. There is high variation in gamma and beta dose rates across the Chernobyl exclusion zone, which influences the internal and external dose rates wildlife receive. The internal and external dose rate combine to form the total dose rate which is important to for relating radiation effects.

The dose assessment was conducted by calculating dose rates to queens, workers and larvae, based on their ecology and life history. Bumblebees are annual eusocial insects; queens live for approximately a year, workers up to 41 days and larvae 28 days before becoming workers or males (Goulson 2009). If it is assumed that bumblebee workers forage for seven hours a day over the course of their lifespan, this is, therefore, equal to 30% of their time spent out of the nest. Queens leave the nest for initially foraging during colony development, mating and nest searching but remain in the nest the rest of the time, therefore equating to 98% of their time underground out of their total lifespan. Queens overwinter in the soil at a depth of 10cm and then colonies are formed, depending on the species, either in grass tussocks (carder species) or the ground (at a depth of between 10 -30cm) (Goulson 2009).

Underground dose calculations for external dose rate were conducted using the TLD measurements to account for shielding from the soil, and the highest value from the measured dose rates at 10 and 30cm were tested to determine if the depth of the bumblebee nest makes a substantial difference to the dose rate (Figure 3.5). The contribution from beta and gamma radiation to internal dose rates are approximately equal for bumblebees (Beresford et al. 2018). External dose rates were calculated for each life stage using a combination of the TLD information, Electra and MoGSS and weighted depending on occupancy (whether in soil or not). Above-ground beta dose rates were calculated using MoGSS for gamma and Electra.

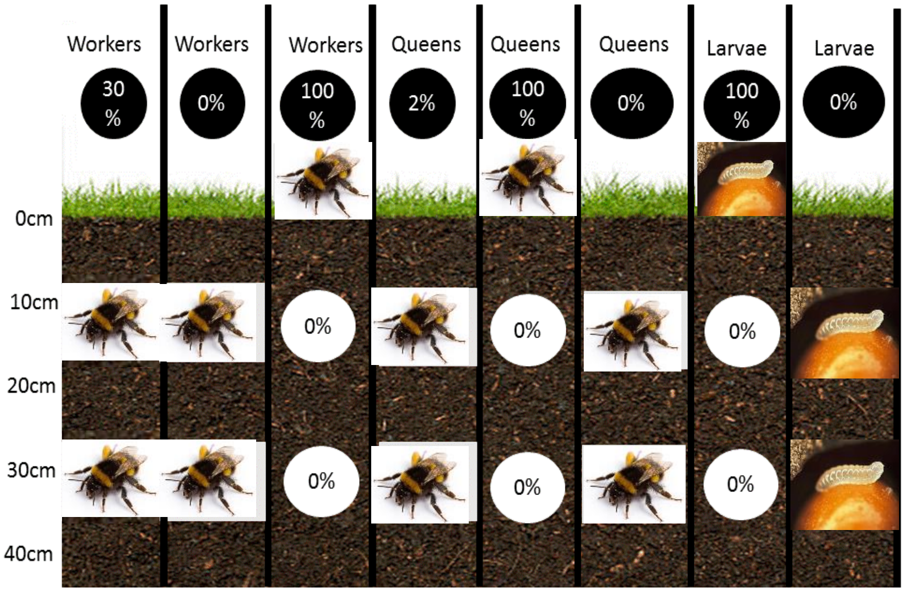

Figure 3.5 Schematic drawing of assumptions made during the dose assessment relating to bumblebee occupancy. Workers can be either part of their time foraging (therefore above ground), under-ground in the nest or on the surface of the soil in the nest (e.g. Carder species). Bumblebee queens and larvae are likely to be located either in the nest underground or on the soil surface (e.g. Carder species).

The ERICA tool

The ERICA tool is used to conduct dose assessments for wildlife, including bumblebees. This approch will test how different dose rates, e.g. how much difference the use of average versus maximum values measured across a site, and the how occupancy in the different areas of the ecosystem, i.e. nesting above ground and underground affects the dose rate to bumblebees.

ERICA requires the activity concentration of radionuclides in media, in this case soil, to calculate the internal and external dose rates to wildlife. This is determined though the use of a concentration ratio, which uses the activity concentration in the organism compared ot that in the environmental media.

The proposed approach for calculating total radiation dose rate to biota was defined as:

(I) Conservative using the highest dose rates for each site and the determining occupancy in the most highly contaminated area, either in the soil or on top of the soil.

(II) Realistic using the average values for each site and determining that occupancy is in the most highly contaminated area.

(III) Realistic and inform the dose assessment of occupancy using known ecological and life history information (e.g. time spent underground and lifespan).

The ERICA tool was used to test the generic concentration ratios in the ERICA Tool against the specified concentration ratio from a site in the CEZ (site 2) (Beresford et al. 2018). MoGSS mobile gamma spectrometry only measured ^{137}Cs activity concentrations, as this is one of the the main contributors to both internal and external dose rate in the CEZ, it would give an indication of the typical dose rates which were received by bumblebees.

In the ERICA tool, the size and shape of organisms can be designed to match that of the study system. Using this tool, queen bumblebees (which are significantly larger than worker bees) can be designed to test how the size of the organism influences the total dose rate.

3.4 Results

Two different comparisons of the dose rates measured in the Chernobyl Exclusion Zone are reported here. Firstly, the external dose rates at the five sites which had TLD measurements were compared using TLDs, Electra, MoGSS and the handheld monitor. Secondly, all sites were grouped by geographic location and distance from the reactor to assess the similarity of external dose rates as measured by Electra, MoGSS and handheld monitor measurements. All measured dose rates presented in Table 6.1 in the appendix.

3.5 Results: Comparison of methods for the five sites with TLD, Electra, MoGSS and external gamma

TLD measurements for sites in the Chernobyl Exclusion Zone

Beta and gamma dose rates were measured at two locations in the soil column (10 and 30cm deep) to be able to estimate dose rates for different bumblebee nest depths. Five sites were selected across the radiation dose rate gradient for TLD measurements (sites number 2, 3, 5, 10 and 20). At each site beta and gamma dose rates were measured twice in two randomly selected locations.

Beta and gamma dose rates at 10cm were consistently higher than the dose rates at 30cm. This indicates the majority of the contamination is located closer to 10cm than 30cm in the soil column which is in agreement with previous predictions (Figure 3.6). Beta and gamma dose rates were variable across the different sites indicating high spatial heterogeneity (Figure 3.6).

Site 2, located on the edge of the Red Forest, had the highest external dose rate and the most considerable variability of all the sites. Beta contributed significantly to dose rates at site 2 and was significantly higher than the gamma dose rate. Site 2 also had high spatial heterogeneity as the measurements located in different areas of the site had different dose rates (Figure 3.6). Site 3 had little variability spatially and in the soil column. Beta and gamma dose rates were relatively low at site 3 as dose rates did not deviate from 1 μGy h^{-1}. The beta contribution to dose rate was significantly higher than gamma at site 5. The measurements at Site 11 were different across the site, indicating spatial heterogeneity. The total dose rate at 10cm and 30 cm was similar, however, at 10cm the beta dose rate was lower than at 30cm, which was different from other sites. The total dose rate remains similar as the gamma dose rate was higher at 10cm rather than 30cm (Figure 3.6). Site 20 had low radiation dose rates for both beta and gamma, and little variability across the site and between depths.

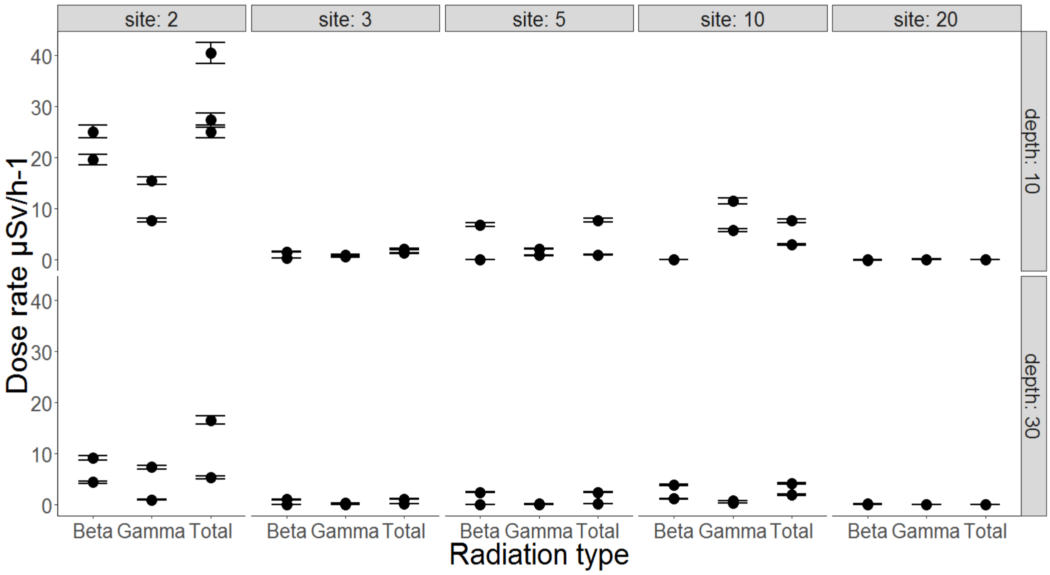

Figure 3.6. The measured external dose rate for beta and gamma using TLDs buried at depths of 10 cm and 30cm for 5 different sites across the Chernobyl Exclusion Zone. Each site has two measurement points, geographically located across the site. Each point contains shielded and unshielded TLDs allowing measurements of the contributions from beta and gamma

External gamma dose rate measured by the handheld device for sites measured with TLDs

The ambient gamma dose rates was measured ten times using a handheld device at the sites where TLDs were placed to enable comparisons with the TLD dose rates. Overall, the dose rate measurements recorded mirrored the measurements taken with the buried TLDs for gamma dose rate. Site 3 had a consistent low dose rate across the site with little variation. Site 2 had the highest dose rate and the highest amount of variation as the dose rates varied between 18 µGy h^{-1} and 40 µGy h^{-1}. Site 5 had little variability across the site, but the dose rates shown in Figure 3.7 were higher when compared to the TLD measurements in Figure 3.7. Site 11 showed little variability across the site, however, in Figure 3.7 as measured by the TLDs, one of the point measurements was significantly lower than the other which is not reflected in Figure 3.7. Site 20 had no variability, which was similar to the TLD measurements.

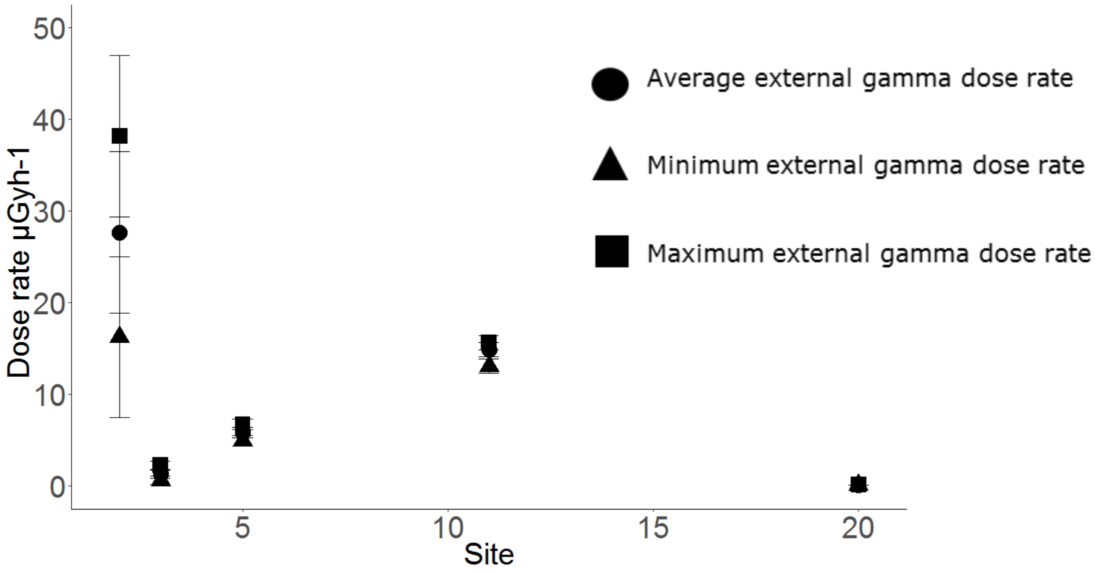

Figure 3.7 External gamma dose rate measurements taken from across the same sites in the Chernobyl Exclusion Zone as measured with the TLDs for comparison

External gamma dose rates measured by portable sodium iodine systems for sites with TLD measurements

The mobile gamma spectrometer system (MoGSS) measured the gamma radiation in counts per second, every second along with GPS coordinates to allow spatial mapping of the sites of interest in order to compare these dose rates to those measured in the ground with the TLDs. This investigation of the spatial heterogeneity of the sites in Chernobyl was conducted on a fine scale (Figure 3.8). Site 2 remained similar to the other figures, showing high variability in external gamma dose rate across the site. Although there were some measurements which were up to 30 µGy h^{-1}, the mean dose rate for the entire site remained low at 9 µGy h^{-1}. Other techniques have demonstrated that Site 3 has little spatial variability across the site. However, Figure 3.8 shows that although the average dose rate for the site remained low (approximately 1 µGy h^{-1}), some points within the site were up to 9 µGy h^{-1} but these were small areas and not detected by the ten point measurements made using the handheld dosimeter or the two selected sites for the TLD measurements. Site 5 was shown to have a slightly elevated dose rate but almost no variability across the site. Site 11, even though the mean dose rate was low, there were some measurements up to 10 µGy h^{-1} which followed the same pattern as Figure 3.6 and 3.7. Site 20, similarly to the other sites had little to no variability Maps of the MoGSS measurements are in Appendix.

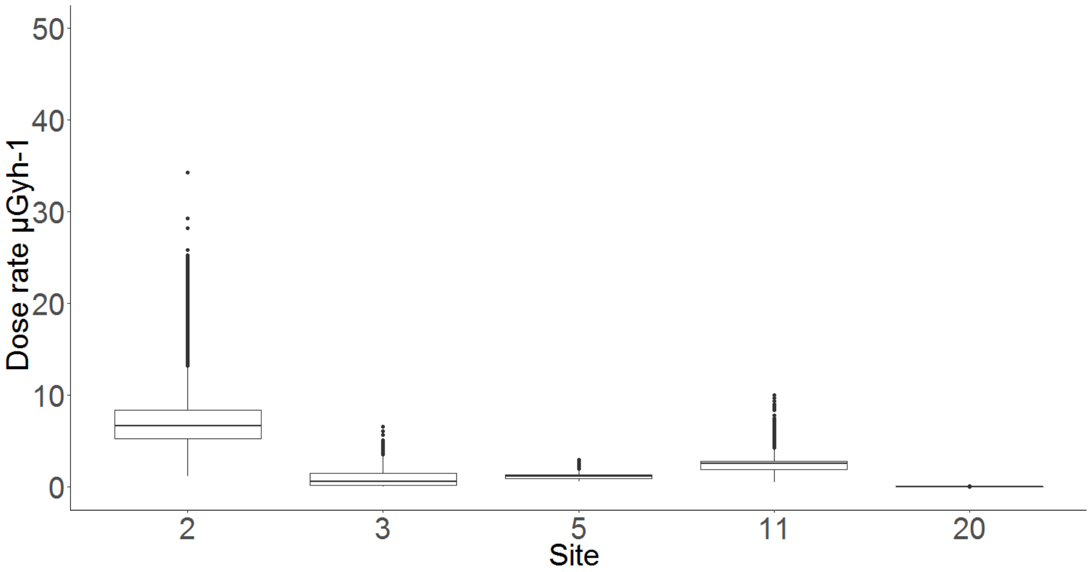

Figure 3.8 Portable sodium iodide measurements for external gamma dose rates at the ground surface for TLD sites taken from across the sites in the Chernobyl Exclusion Zone as measured for comparison with the TLDs

External beta dose rate measurements using Electra for sites also measured by TLDs

Figure 3.9 illustrates the range in beta dose rate measurements taken by the Electra system, one centimetre above the ground. The dose rates at Site 3 are similar to the beta dose rate measurements taken from the TLDs, and there was little variability across the site. Site 2, similar to the TLD measurements, has high variability across the site with beta dose rates up to 180 µGy h^{-1}, which is substantially higher than as measured by the TLDs. There are two reasons why the Electra measurement may be much higher than the TLD measurement for the beta dose rates. Firstly, these two methods are not directly comparable as the Electra measurements were made on the surface, compared to the TLDs which were measured at 10 and 30cm in the ground. The soil will shield some of the beta radiation when in the soil, therefore resulting in larger dose rates on top of the soil if the contamination in relatively shallow (i.e. 0 - 15cm). The difference in exposure scenario results in a difference in the measured values, depending on the location of the contamination. Secondly, there were two beta dose rate TLD measurements per site, whereas, Electra was used ten times across the site, therefore, increasing the likelihood of capturing any heterogeneity (Table 3.1).

Site 5 had an increased beta dose rate and little variability. Site 11 had substantial variation across the site and elevated beta dose rates, higher than those measured with TLDs in Figure 3.9. Site 20 had a comparatively low dose rate and little variation as observed in Figure 3.9.

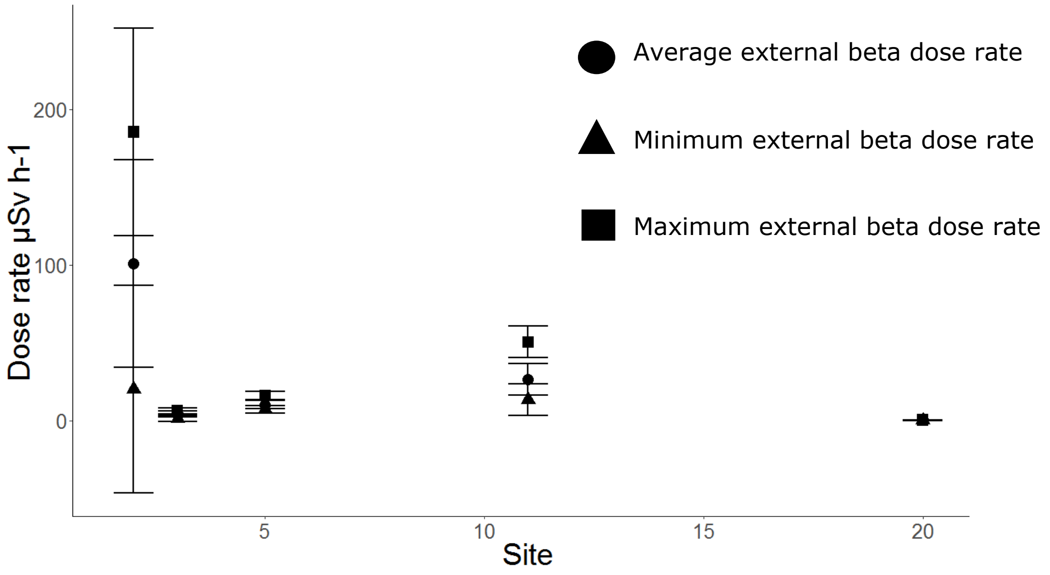

Figure 3.9 Electra measurements for external beta dose rates at the ground surface for TLD sites taken from across the same sites in the Chernobyl Exclusion Zone as measured with the TLDs for comparison

3.6 Results: Comparing all sites across the Chernobyl Exclusion zone grouped by geographic location

All the sites across the Chernobyl Exclusion Zone were grouped dependant on their geographic location, distance and direction from the Chernobyl Nuclear Power Plant (NPP). Group A consisted of sites located within 8 km of the Chernobyl NPP on the Western Trace, group B were located on the North Trace, group C were the furthest sites from the Chernobyl NPP on the Western trace (more than 8km away from the NPP) and group D were located Southwest of the Chernobyl NPP. Maps created using MoGSS data for each site are in Appendix.

External gamma radiation dose rates measured with a handheld monitor at all sites in Chernobyl

Figure 3.10 shows the range of measurements taken across all the sites using a handheld monitor for measuring external gamma radiation dose rates. Sites within group A, which were located close to the reactor generally had higher dose rates than the other groups. In particular sites 1 and 2 which were located close to the Red Forest had the highest dose rates and the highest variability in dose rate across each site. Site 3, located in the town of Pripyat approximately 7.5 km away from the Chernobyl Nuclear Power Plant had a much lower dose rate than the other sites in this group.

Sites within the group B, located on the Northern trace had similar dose rate ranges to site V, which is located in group A. Sites 6 and 7 had average dose rates of approximately 20 μGy h^{-1}, while site 6 had little variability across the site, 7 had a greater range of doses. Dose rates and variability were reduced for other sites in group B: K, T, U, X. Sites in group C which were located further down the Western Trace varied with distance away from the Chernobyl NPP. Sites 11 and 13 in group C were located directly on the Western trace. Site 11 had a higher overall dose rate with less variability than 13, which located further away from the Chernobyl NPP. Sites 14 and 15 were slightly off of the West trace and further away again from the Chernobyl NPP. Comparing sites 14 and 15 to 11 and 13 demonstrated that the external gamma dose rates as measured by the handheld monitor were significantly lower with little heterogeneity. Sites in group D which were located south of the reactor had the lowest dose rates out of the all the sites in the Chernobyl Exclusion Zone. Site 16 at Kopachi had the highest external gamma dose rates and was located closest to the Chernobyl NPP. Sites, 17, 18, 19, 20 and 21, had radiation dose close to UK background levels (Figure 3.10).

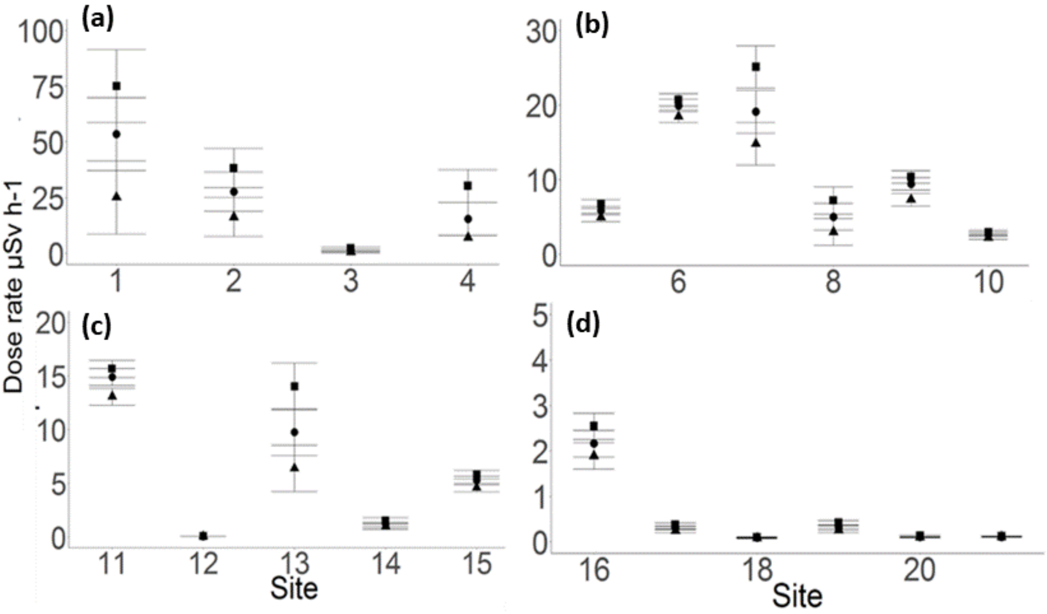

Figure 3.10 Comparing the average, maximum and minimum dose rates using the external handheld device making ten random measurement points across the site. Error bars are standard errors (a) within 8 km of the Chernobyl NPP, (b) North Trace (c) Furthest sites from the Chernobyl NPP on the Western trace (d) Southwest of the Chernobyl NPP. Circles are the average external gamma dose rate; the triangle is the minimum measured external gamma dose rate, and squares are the maximum measured external gamma dose rate

Mobile gamma spectrometry system (MoGSS) for all sites in the Chernobyl Exclusion Zone

A portable sodium iodide detector (MoGSS) was used to measure ^{137}Cs activity in the soil at a high spatial resolution by recording spectra every second and with each spectra linked to GPS coordinates (figure 3.11 a - d). The dose rates measured using MOGSS were consistently higher than measured using other techniques. Group A, which was closest to the reactor had the highest dose rates. Although some of the dose rates measured are high (1 and 2), the majority and, therefore, the average dose rate was much lower overall – similar to site 4. Group B, located on the north trace had substantially lower doses than group A, with a maximum dose rate of 5 μGy h^{-1}. Group C on the Western Trace also had lower dose rates and site 11 which was the closest to Chernobyl NPP had the highest and most variable dose rate out of this group. Group D, located in the south, have the lowest dose rates apart from site 19. Site 19 had not been identified as having a higher radiation dose in any of the previous surveys – up to 11 μGy h^{-1}. However, the average radiation dose rate at site 19 is lower than the other sites, at around 3 μGy h^{-1}.

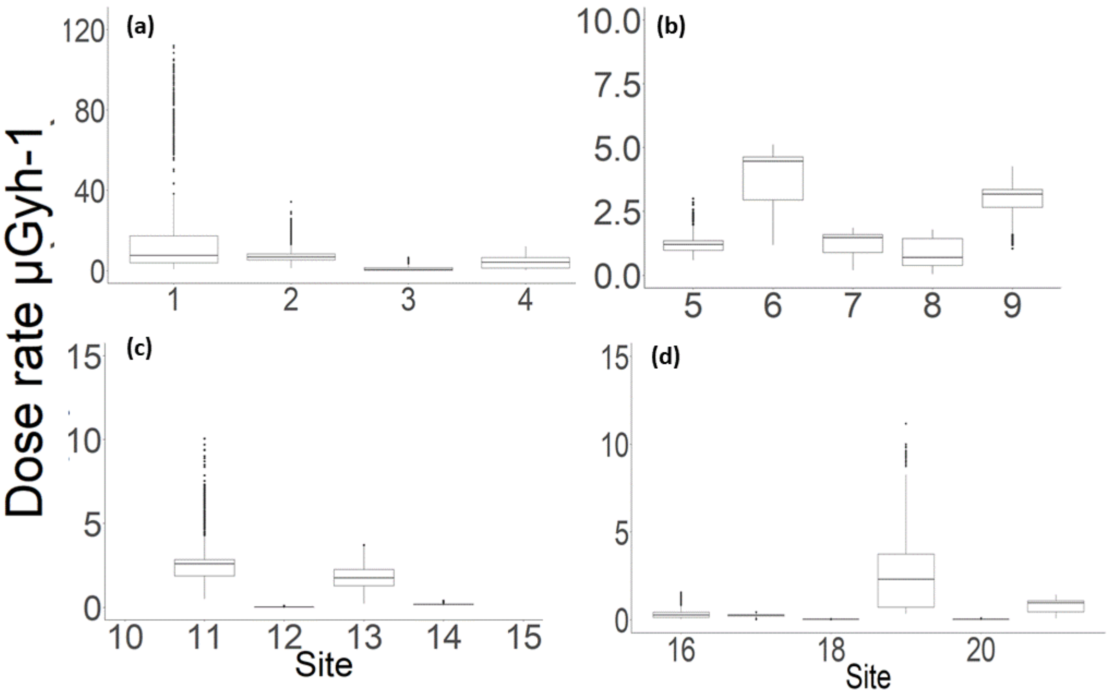

Figure 3.11 Distribution of dose rates across each site in the Chernobyl Exclusion Zone using the portable sodium iodine detector (MoGSS). (a) within 8 km of the Chernobyl NPP, (b) North Trace (c) Furthest sites from the Chernobyl NPP on the Western trace (d) Southwest of the Chernobyl NPP

Figure 3.12 offers greater insight into the distribution of dose rates across the four highest sites (1, 2 and 4) as all the dose rates measured across the site are plotted to identify the average dose rate and visually determine how frequently the highest dose rates are measured. Although all four sites contained higher dose rates than all the other sites in the Chernobyl Exclusion Zone. The majority of dose rates recorded using MoGGS across the site were lower. Therefore, the average was lower than the maximum dose rates. The majority of points at Site 2 were around 10 μGyh^{-1} with a second, smaller group of dose rates at 20 μGyh^{-1}. Site 1 had the highest dose rates out of all the sites measured for this chapter, but again, the majority of the dose rates are between 5 and 30 μGyh^{-1} with a small proportion of points between 30 μGyh^{-1} and 120 μGyh$^{-1.}$ Site 11 had a similar structure to site 2 but at lower dose rates, whereas, site 4 had two main groupings of dose rates, one at close to zero and the other was slightly higher at 10 μGyh^{-1}.

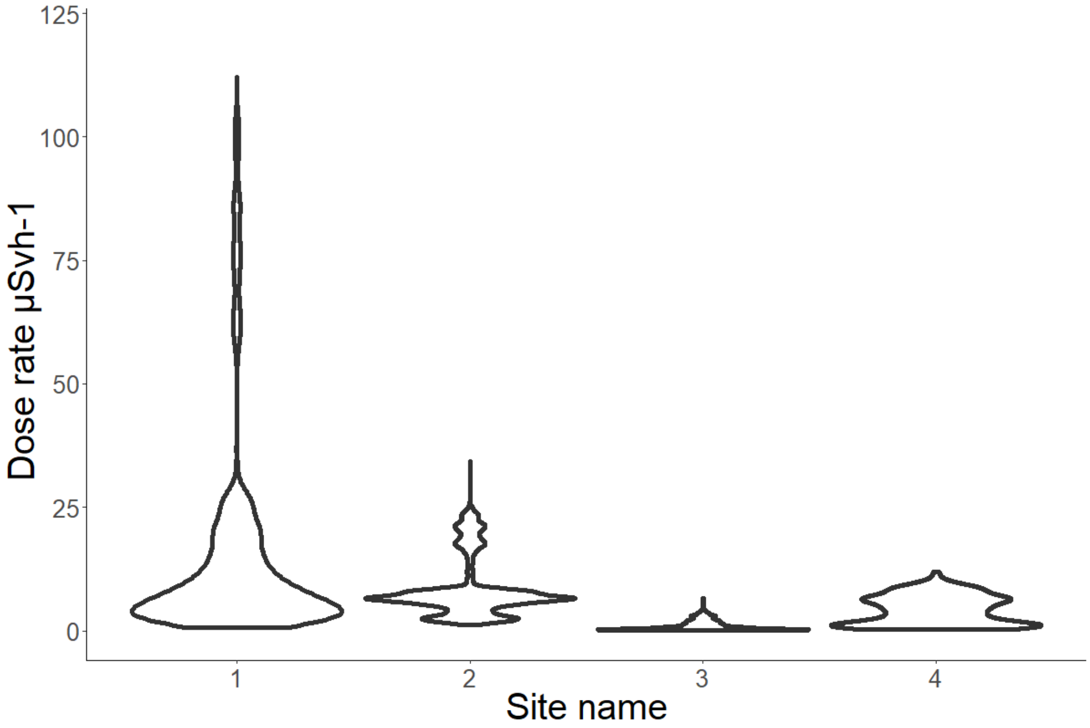

Figure 3.12. The density of external gamma dose rates across the most highly contaminated sites using sodium iodide detector (MOGSS)

External beta radiation dose rates measured with Electra at all sites in Chernobyl

The Electra was used to measure beta dose rates at 1 cm above the ground at all the CEZ field sites. For sites closest to the reactor on the Western Trace, the beta measurements were the highest (figure 3.13), apart from at site 3. Beta contamination was highly variable generally, more so than gamma contamination. The site with the highest beta dose rate measurements was site 4. Site 1 which had substantially higher dose rates than site 2 which had similar beta dose rates. Group B which is on the north trace had much lower beta dose rates than group A, apart from site 7 where the dose rates were higher, and the variability was increased. Sites 5, 6, 8, 9 and 10 all had contributions from beta ranging from zero to 50 µGy h^{-1}.

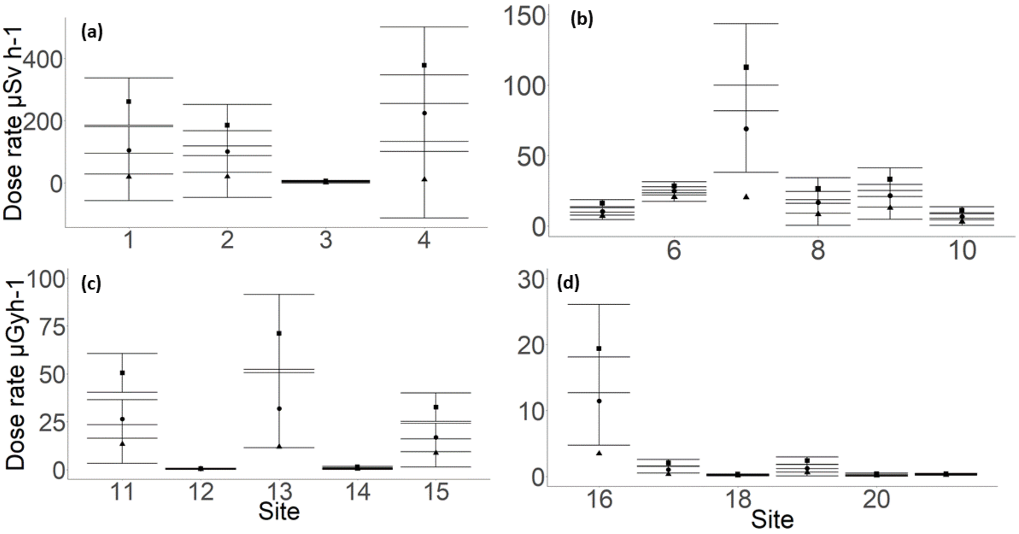

Figure 3.13 Comparing the average, maximum and minimum beta dose rates using the Electra making ten random measurement points across the site. Error bars are standard errors (a) within 8 km of the Chernobyl NPP, (b) North Trace (c) Furthest sites from the Chernobyl NPP on the Western trace (d) South west of the Chernobyl NPP. Circles are the average external beta dose rate, triangle is the minimum measured external beta dose rate and square the maximum measured external beta dose rate

Comparing average gamma external dose rates across techniques

Comparing the three different measuring techniques for the external gamma radiation dose rate demonstrates the high variability in dose rates across a spatially heterogeneously contaminated area such as the Chernobyl Exclusion Zone. Using the average values from these techniques is one way of estimating the likely dose rate to an organism living in this area. However, the variation between the minimum and maximum as measured by the different techniques increases when the dose rate is higher (Figure 3.14). Across the measurement techniques, the estimation for the average value was however generally within one order of magnitude. At one of the most highly contaminated sites (2), the average TLD and the average MoGSS techniques results were similar. However, the handheld external monitor recorded a dose rate approximately twice that of the other methods.

Similarly, at site 1 which was the most highly contaminated site, the average MoGSS value was much lower than the measurements obtained using the external hand monitor. This pattern continued across all sites of higher radiation contamination; generally speaking the TLD and MoGSS measured similar dose rates while the external handheld monitor consistently measured a higher dose rate (Appendix Table 6.1). For some sites (3, 7, 12, 14, 16, 17, 18, 20 and 21) there was little variation between measuring techniques, but this is likely to be due to the lower dose rate being seen (Appendix Table 6.1).

Comparing average beta dose rates across techniques

The two different measurement techniques used at the five sites with TLDs demonstrate that the averages of the values obtained using the two techniques were generally similar. However, these techniques were not directly comparable as Electra measurements were taken on the surface whereas, TLDs are placed in the ground. Therefore, depending on the location of the contamination, the TLDs may be shielded by the soil, and the beta dose rate may be reduced compared to surface measurements, or vice versa. The shielding is likely to be the reason why the TLD dose rates in Figure 3.15 are consistently lower than the Electra measurements. The differences between the two measurements are greater at sites with higher contamination; the higher the dose rates, the higher the discrepancy between methods. Sites 2 and 11 are the most contaminated sites, and the Electra method records a higher dose rate than the TLD (Figure 3.15), further data in Appendix 6.2 .

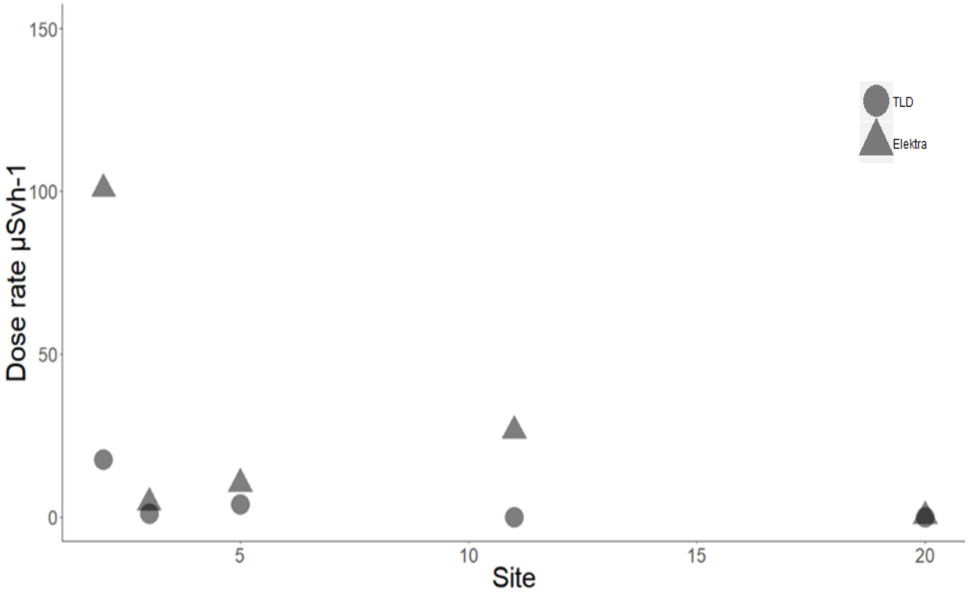

Figure 3.15 The average of two different measurement techniques for beta radiation across sites. TLD buried at 10cm in the ground and Electra probe system taken at 1cm above the ground

Estimating dose rates to bumblebees living in the Chernobyl Exclusion Zone using the different measurement techniques used in this study

Dose rates were calculated using a range of different techniques in this field study, and these dose rates were subsequently used in a dose calculation for bumblebees. Bumblebee ecology (e.g. occupancy and lifespan) was used to estimate the dose rate and the accumulated dose rate the different bumblebee castes would receive in their lifetime.

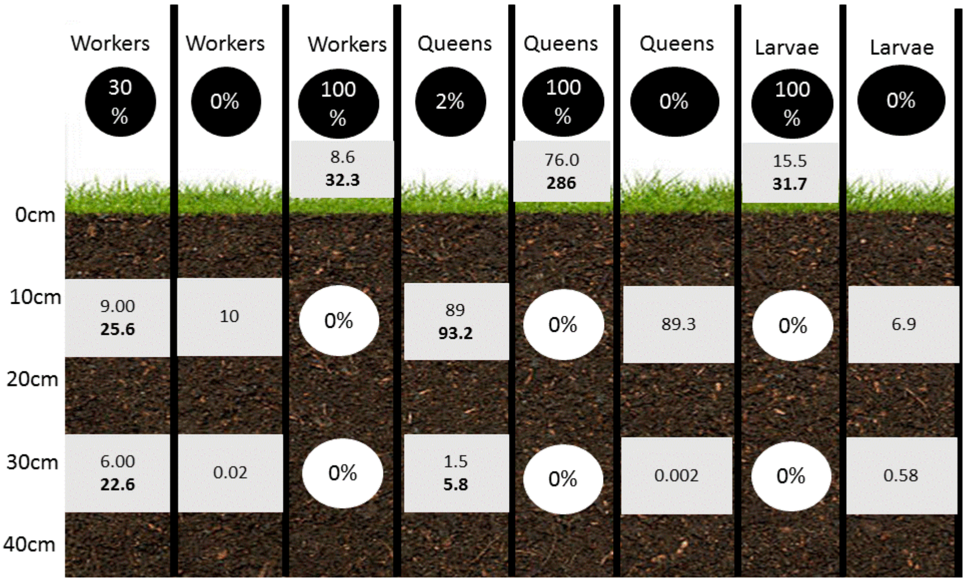

Figure 3.16 Site 2 dose calculations for external dose from beta and gamma for different occupancies of the varying castes of bumblebees assuming the nest site is either at 10 or 30 cm in the soil. Accumulated lifetime dose in mGy. Bold numbers calculated using the maximum dose rates across the site, non-bold numbers are calculated using the average values from across the site.

Different occupancies (i.e. above ground and underground) were tested for bumblebees (Figure 3.16) to determine which occupancies would result in the most substantial dose rates and lifetime accumulation. The data using the measured external dose rates from MOGSS, TLD and Electra demonstrate that the more time spent above the soil results in a more significant dose rate. These data suggest that the difference in occupancy could be particularly important for bumblebees that nest above the ground such as carder bees *B. pascorum* and *B. muscorum,* as well as tree nesting bumblebees *B. hypnorum.* Figure 3.16 also demonstrates the variability in the lifetime dose accumulated by the different castes. Queens, as they live substantially longer than workers and therefore had a higher accumulated lifetime dose of approximately 285 mGy at the most contaminated regions of site 2. The differences that using the maximum and average dose values from the measurements taken across the site have implications to the dose rate and accumulated total dose, as well as using informed occupancy data (excluding ground-nesting species) (Table 3.1).

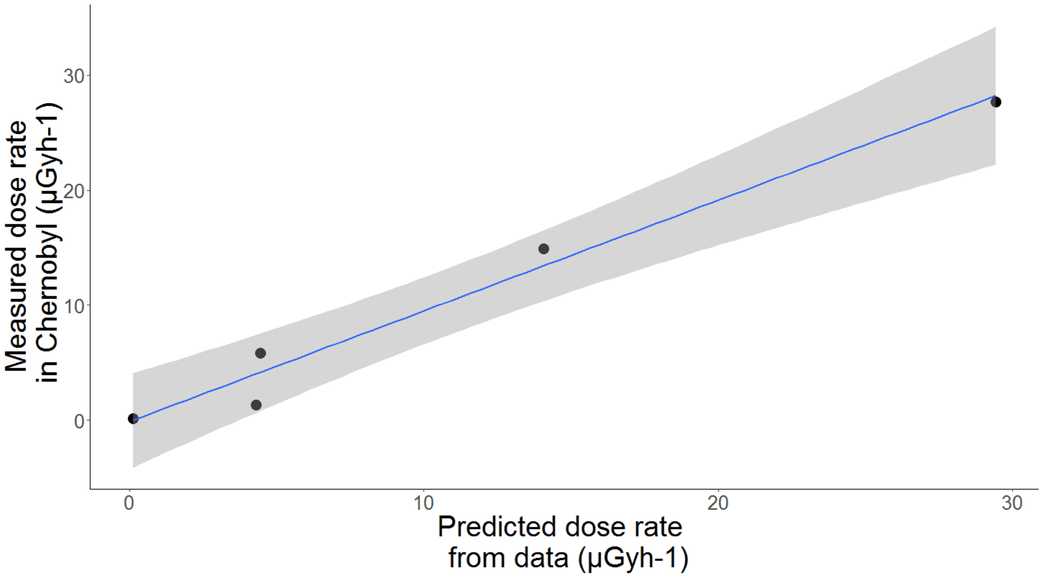

Figure 3.17 Predicted external dose rates from the dose assessment using dose rates collected by all techniques used in this study, compared to the dose rates measured in Chernobyl using an external handheld monitoring device (y-axis).

The predicted total dose rates to the different castes of bumblebees depended on their occupancy either in the soil or on top of the soil were not significantly different from the external measurements made with the handheld device in Chernobyl (Figure 3.17). The predicted dose rates were the calculations of the total external dose rate to bumblebees including contributions from beta and gamma took into account the niche occupancy of each caste.

Table 3.1 Calculated dose rates for bumblebees occupying different sites in the Chernobyl Exclusion Zone using mobile gamma spectrometry system (MoGSS), thermoluminescent dosimeters (TLD) and Electra system. Occupancy is assumed for queens 98% below ground and 2% on top of the soil, workers are assumed 30% on top of the soil, and 70% living in the soil and larvae are assumed to be 100% on top of the soil. All dose rates are given in (µGyh-1)

Site	**Caste**	**Internal**	**External average**	**External maximum**	**Total dose average**	**Total dose maximum**	***Lifetime total dose mGy (average)***	***Lifetime total dose***
F	Queen	1.23	1.52	1.64	2.76	3.16	***13.19***	***14.12***
F	Worker	1.20	1.36	2.93	2.56	4.29	***1.32***	***2.79***
F	Larvae	1.23	1.54	1.54	2.77	3.08	***1.02***	***1.02***
G	Queen	13.67	9.97	10.48	23.64	24.15	***89.06***	***93.26***
G	Worker	12.43	9.59	17.00	22.03	29.43	***9.60***	***16.45***
G	Larvae	13.76	10	10	23.76	23.76	***6.87***	***6.87***

Site	**Caste**	**Internal**	**External average**	**External maximum**	**Total dose average**	**Total dose maximum**	***Lifetime total dose mGy (average)***	***Lifetime total dose***
J	Queen	0.045	0.077	0.079	0.123	0.124	***0.662***	***0.673***
J	Worker	0.040	0.059	0.080	0.100	0.120	***0.057***	***0.07***
J	Larvae	0.046	0.079	0.079	0.125	0.125	***0.051***	***0.051***
K	Queen	2.68	1.52	1.55	4.21	4.24	***13.96***	***14.20***
K	Worker	2.51	1.53	1.94	4.05	4.45	***1.57***	***1.94***
K	Larvae	2.69	1.529	1.529	4.22	4.22	***1.07***	***1.07***
L	Queen	5.52	8.50	8.65	14.03	14.18	***72.76***	***73.99***
L	Worker	5.09	6.81	8.96	11.91	14.06	***6.60***	***8.59***
L	Larvae	5.55	8.62	8.62	14.18	14.18	***5.67***	***5.67***

The ERICA tool

Table 3.4 shows the results of the ERICA assessment undertaken for site 2, which was chosen for the ERICA assessment as it has previously been used in (Beresford et al. 2018a). Figure 3.17 demonstrated that the worst-case occupancy was above the ground - where bumblebees received the most substantial external dose rates. The ERICA assessment, however, predicted that bumblebees received larger doses when in the soil. On the soil, queens received 13.20 µGy h^{-1} and for queens spending 98% of their time in the soil and only 2% on the surface, received 26.00 µGy h^{-1}, which is almost double. This disparity could be due to the assumption ERICA makes that the activity is evenly distributed throughout the soil column. A volume source with a depth of 10 cm was assumed to be a good representation for aged radioactive deposits (Brown et al. 2008; Ulanovsky et al. 2008).

ERICA allows the manual creation of organisms, in this case, manually created organisms queen and bee, with their altered occupancy factors demonstrates the importance of using ecological information to inform dose assessments. When the occupancy was the same and assumed to be 100% on the soil, there was little variation in dose rates. However, the manually created organisms had significantly higher dose rates than the default due to the differences in occupancy. For example, in scenario 5, queen bumblebees are predicted to receive 7.19 µGy h^{-1}, worker bees 8.72 µGy h^{-1} and the default flying insect 3.37 µGy h^{-1}. The updated concentration ratios from Beresford et al. 2018, consistently increased the dose rate to bumblebees, but not by a significant amount (less than 1%). For example, the total dose rate to queens in scenario 1 was 13 µGy $h^{-1,}$ and with the addition of new concentration ratios, this increased to 13.2 µGy h^{-1} (Table 3.4).

The use of the maximum dose rates measured in site 2 by the MoGSS system predicted a significantly greater dose rate than the average dose rates measured across the site. For example, scenario 6 (Table 3.4) using the average activity concentration across the site and predicted that queens received an external dose rate of 7.24 µGy h^{-1} and workers received 8.75 µGy h^{-1}. This is substantially lower than using the maximum activity concentrations, where queens received 26 µGy h^{-1} and workers received 31.50 µGy h^{-1}.

The internal dose rates were small compared to the contribution from external dose rates. For example, scenario 1 (Table 3.4), the external dose rate to a queen bumblebee was 11.40 µGy h^{-1}, whereas the internal contribution from ^{137}Cs was 1.65 µGy h^{-1}. Therefore, the ERICA tool predicts that the contribution of ^{137}Cs to the internal dose is approximately 12%, which is significantly larger than the measured value used in Beresford et al. (2018), potentially due to the use of sampled soil cores compared to the methods used in this chapter of the activity concentrations from the whole site using MoGSS.

Table 3.2 Summary of internal, external and total dose rates as predicted from the ERICA integrated assessment tool using 137Cs activity concentrations measured by MoGSS in site 2. Scenarios tested how revised concentration ratios for 137Cs in Apidae species caught in the Chernobyl Exclusion Zone altered the dose rate when compared to the default concentration ratios for flying insects in the Chernobyl Exclusion Zone. Different geometries were tested of individuals to account for the differences between castes (worker, queen and default ERICA reference organisms flying insects). The use of either the maximum or the average dose rates across site 2 affected the dose rates calculated from ERICA.

		Scenario 1: worst predicted occupancy (on soil =100%) and maximum dose rate with ERICA default concentration ratio				
	Occupancy	Concentration Ratio ^{137}Cs	Activity ^{137}Cs (Bq kg^{-1})	External dose rate μGy h^{-1}	Internal dose μGy h^{-1}	**Total dose μGy h^{-1}**
Queen bee	100% = on soil	0.11	99549	11	1.7	**13**
Bee	100% = on soil	0.11	99549	12	1.3	**12.8**
Flying insect	100% = on soil	0.11	99549	12	1.4	**13.4**
		Scenario 2: worst predicted occupancy (on soil =100%) and maximum dose rate with Beresford et al. 2018 concentration ratio				
	Occupancy	Concentration Ratio ^{137}Cs	Activity ^{137}Cs (Bq kg^{-1})	External dose rate μGy h^{-1}	Internal dose μGy h^{-1}	**Total dose μGy h^{-1}**
Queen bee	100% = on soil	0.12	99549	11	1.8	**13.2**
Bee	100% = on soil	0.12	99549	12	1.4	**13.0**
Flying insect	100% = on soil	0.11	99549	12	1.4	**13.4**

		Scenario 3: worst predicted occupancy (on soil =100%) and average dose rate with ERICA default concentration ratio				
	Occupancy	Concentration Ratio ^{137}Cs	Activity ^{137}Cs (Bq kg^{-1})	External dose rate µGy h^{-1}	Internal dose µGy h^{-1}	**Total dose µGy h^{-1}**
Queen bee	100% = on soil	0.11	27671	3.1	0.5	**3.6**
Bee	100% = on soil	0.11	27671	3.2	0.4	**3.6**
Flying insect	100% = on soil	0.11	27671	3.3	0.4	**3.7**
		Scenario 4: worst predicted occupancy (on soil =100%) and average dose rate, with Beresford et al. 2018 concentration ratio				
	Occupancy	Concentration Ratio ^{137}Cs	Activity ^{137}Cs (Bq kg^{-1})	External dose rate µGy h^{-1}	Internal dose µGy h^{-1}	**Total dose µGy h^{-1}**
Queen bee	100% = on soil	0.12	27671	3.2	0.5	**3.7**
Bee	100% = on soil	0.12	27671	3.2	0.4	**3.6**
Flying insect	100% = on soil		27671	3.3	0.4	**3.7**
		Scenario 5: informed occupancy and average dose rate with ERICA default concentration ratio				
	Occupancy	Concentration Ratio ^{137}Cs	Activity ^{137}Cs (Bq kg^{-1})	External dose rate µGy h^{-1}	Internal dose µGy h^{-1}	**Total dose µGy h^{-1}**
Queen bee	98% in soil, 2% on soil	0.11	27671	6.7	0.5	**7.2**
Bee	70% in soil, 30% on soil	0.11	27671	8.4	0.4	**8.7**
Flying insect	100% = on soil	0.11	27671	3.3	0.4	**3.4**

		Scenario 6: informed occupancy and average dose rate with Beresford et al. 2018 concentration ratio				
	Occupancy	Concentration Ratio ^{137}Cs	Activity ^{137}Cs (Bq kg^{-1})	External dose rate µGy h^{-1}	Internal dose µGy h^{-1}	**Total dose µGy h^{-1}**
Queen bee	98% in soil, 2% on soil	0.12	27671	6.7	0.5	**7.2**
Bee	70% in soil, 30% on soil	0.12	27671	8.4	0.4	**8.8**
Flying insect	100% = on soil	0.11	27671	3.3	0.4	**3.4**

		Scenario 7: informed occupancy and maximum dose rate and Beresford et al. 2018 concentration ratio				
	Occupancy	Concentration Ratio ^{137}Cs	Activity ^{137}Cs (Bq kg^{-1})	External dose rate µGy h^{-1}	Internal dose µGy h^{-1}	**Total dose µGy h^{-1}**
Queen bee	98% in soil, 2% on soil	0.12	99549	24.2	1.8	**26**
Bee	70% in soil, 30% on soil	0.12	99549	30	1.5	**31.5**
Flying insect	100% = on soil	0.11	99549	11.9	1.5	**13.4**

		Scenario 8: informed occupancy and maximum dose rate with ERICA default concentration ratio				
	Occupancy	Concentration Ratio ^{137}Cs	Activity ^{137}Cs (Bq kg^{-1})	External dose rate µGy h^{-1}	Internal dose µGy h^{-1}	**Total dose µGy h^{-1}**
Queen bee	98% in soil, 2% on soil	0.11	99549	24.2	1.7	**25.9**
Bee	70% in soil, 30% on soil	0.11	99549	30	1.3	**31.4**
Flying insect	100% = on soil	0.11	99549	11.9	1.5	**13.4**

3.7 Discussion

The results from this chapter demonstrate that the contamination in the Chernobyl Exclusion Zone, even across a small spatial scale has high heterogeneity which results in difficulty establishing a reasonable estimate of dose rate to relate to radiation effects. The data demonstrate that the common-practice use of a hand held device to measure ambient external dose rates is not sufficient to estimate the total dose rate to bumblebees, however, due to bumblebees having a relatively small internal dose contribution, this method was within one order and can therefore be used as an indication. Therefore, although the hand held device gave a resonable estimate, it is not recommended on it’s own, as this study highlights the importance of the need to take into account the life history traits and the ecology of the study species. Life history traits can make a big difference to the total dose rate an individual receives as demonstrated by the dose assessments, for example, whether an organism lives on top of the soil or at a 10cm depth. It may also be better to give a range of doses/dose rates that non-human biota may receive when living in different areas of contaminated sites in radioecological studies.

The ICRP (International Commission on Radiological Protection) approach for radiological protection of the environment uses a series of reference animals and plants to represent a range of species; for which the bee is one. The dose assessments conducted within this study demonstrate that over the lifespan of a worker bumblebee, a large proportion of the total dose accumulated is acquired during the larval stage which is more radiosensitive. The accumulated lifetime dose for larvae varied depending on the site and where the organism lives, i.e. whether the species is ground nesting or on the soil surface.

To contextualise the accumulated dose from chronic exposure to previous studies which have used acute dose rate. When assumed that the larvae spend 100% of their life underground the accumulated dose ranged from 1 mGy to 7 mGy. For site 2, the highest site with TLDs, the accumulated dose was 31.73 mGy. However, comparing the accumulated dose rates to the accumulated doses administered acutely at which there have been recorded effects, these values are substantially lower than Ld_{50} values of 1-2Gy (delivered acutely) for sub adult wasps. The LD_{50} for sub-adult stages is much lower than the LD_{50} values for adult life stages which range from 20 – 3000 Gy (delivered acutely) for a range of insects (ICRP 2008).

The focus in dose assessments should not solely be on workers for eusocial bee colonies, as workers do not reproduce and are not as important compared to queens due to eusocial nature of bees and their ability to buffer against stress (honey bees and wasps) (Straub et al. 2015). There needs to be a greater focus in dose assessments to the larval stage and the queens. The larval stage is the most radiosensitive life stage in term of mortality (ICRP 2008) as this is when cells are dividing (Nauman & Whitten 1976). There is no current information for insect larvae in the ERICA Tool, but there is the option to create a manual organism to predict dose rates to the larvae stage. However, this requires significant base data which is not available at the moment (Stark et al. 2017).

The nesting ecology of bumblebees is poorly understood (Goulson 2010). Bumblebees usually nest in abandoned dwellings created by mice, voles, rabbits or birds (O'connor et al. 2017; Lye et al. 2012). The nests tend to be subterranean or under thick vegetation such as grass tussocks depending on the species (O'connor et al. 2017). Nests are therefore likely to be located within the top 30 centimetres of the soil, whereas over-wintering queens are likely to be within the top 10cm of the soil (Goulson 2009). Data have shown that the depth and location of the nest will make a big difference to the dose rate the colony receives.

Queen life history should be considered in dose assessments as it is very different from worker life history. Queens produce reproductives; new queens and males and therefore as the ovaries are one of the most radiosensitive organs they are more likely to suffer adverse effects than workers (Morley 2012). Queens are also the most ecologically important caste as fewer queens mean fewer colonies founded the following year and therefore a lower abundance of bumblebees in total (Baron et al. 2017). The risk of queen death throughout over-wintering is high, at least one queen from the original colony has to found her own nest successfully to ensure population continuation (Baron et al. 2017). Males were excluded from this analysis as although they reproduce sperm, their lifespan and traits are similar to workers and so the dose estimates can be considered to be the same for males and workers. To be able to take into account the different life stages of bumblebees assumptions have to be made using known ecological and life history information.

This study demonstrates that spending time above and below ground makes a difference to the average dose rate received while also demonstrating that the accumulated dose over a lifetime varies whether taking into account the queen or the workers. This study highlights the importance of these parameters in the dose assessment where lifespan (and accumulated dose) and life stage are usually not considered and/or reported.

Comparison of different techniques for measuring the external dose/dose rate

Generally, across all the five sites measured with the TLDs, the contribution from beta radiation was higher than from gamma. Low energy beta radiation is less important than gamma radiation for external dose as skin or chitin can stop it for invertebrates but beta is important for calculating internal dose. For beta dose rate, the internal dose rate exceeded the external dose rate. However, only 6% of the total dose rate for bumblebees is from internal dose rate (Beresford et al. 2018a). The sites with the highest contribution from beta radiation (1, 2, 4, 5, 11 and 13) are located relatively close to the reactor and on the north and west trace which concurs with predictions made stating that fuel particles containing strontium-90 landed close to the reactor (IAEA 2006). The mean dose rate from (Beresford et al. 2018a) was 19 μGyh^{-1} and the 95th percentile was 37 μGyh^{-1}. Comparing the results from this study to the same site (2), the average external dose rates measured ranged from 15.53μGyh^{-1} to 53.47 μGyh^{-1}.

In this chapter, there was not much difference between the dose rates measured with the handheld device and the total dose calculations incorporating life history for bumblebees used in the dose assessments (Table 5). This was further demonstrated by the ERICA tool, which used activity concentrations of ^{137}Cs as measured by MoGSS. The values predicted from the ERICA tool when using the average activity concentration were lower than predicted from my dose assessment incorporating life history traits when using the average dose rate. The dose rates predicted from ERICA for site 2 for the average dose rate received by worker bumblebees using the average values (8.75 μGy h^{-1}, Table 6) were significantly lower than the average dose rate measured for site 2 using the hand-held monitor (27.67 μGy h^{-1}). However, the ERICA predictions were similar to the average dose rate using MoGSS (8.65 μGy h^{-1}) and the 10cm TLDs (10 μGy h^{-1}), therefore, the handheld monitor gives a reasonable estimate of dose rates to bumblebees.

Site characteristics influence dose rate

Thermoluminescent dosimeters (TLDs) buried in the ground enabled dose rate estimates for both gamma and beta radiation to be made while taking into account shielding from the soil. This was subsequently compared to other techniques and incorporated into the dose calculation to measure dose rate across Chernobyl (6.6). The majority of the ^{137}Cs in sandy and soddy-podzolic soils has been shown to be located between 0-15cm (Varley et al. 2017). The location of the contamination is the reason why beta and gamma dose rates were shown to be higher at 10cm rather than 30cm. Furthermore, the shielding effect of soil on dose is well known (Stark et al. 2017). Therefore, it is unsurprising that the dose rates for both beta and gamma are higher as measured by the TLD at 10cm rather than 30cm. This study reaffirms that the majority of the gamma emitting radionuclides are in the upper portion of the soil column.

The vertical distribution of radioactivity impacts the external dose to organisms due to the effects of self- attenuation. In most models, the soil is defined as a semi-infinite layer of given thickness for which composition and contamination are assumed to be heterogeneous (Stark et al. 2017). The use of TLDs in this study demonstrates the heterogeneity within the soil column for both beta and gamma radionuclide distribution. Knowledge of the depth distribution can contribute towards remediation strategies and decisions as well as subsequent site use. Depth distribution can influence the environmental behaviour with regards to surface remobilisation which can be modelled using the E.D.E.N. tool (Varley et al. 2017; Beaugelin-Seiller et al. 2005).

Site 3 was the only site that was measured in an urban area and even though it was close to the reactor the dose rates here are substantially lower than measured at sites 1, 2 and 4. Dose rates are known to be significantly lower around urban areas due to photon absorption in building structures (IAEA 2006). However, the site that we measured, although surrounded by buildings, was still grassland. It might be the case that decontamination activities were carried out in this area after the Chernobyl accident which would mean that the dose rates are now lower.

The Chernobyl Exclusion Zone is in a dynamic state as radioactive isotopes undergo radiation decay and the contamination is subject to redistribution by physical, geochemical, and biological processes over time. Therefore, this constant change can cause further difficulties when conducting dose assessments from measurements of dose rates taken over the last 30 years since the accident, as radioisotopes undergo half-lives. This study supports the ERICA and ICRP guidance (Brown et al. 2008) that dose measurements and assessments must be undertaken for each radioecological study taking into account external and internal dose and the ecological and life history parameters to fully assess the dose rate and the accumulated dose to study organisms to establish an accurate dose-response relationship.

Dose assessments must be regularly undertaken as trees, grasses, other plants and fungi can trap/incorporate radionuclides during their life cycle. Although none of the sites had trees as they were all open meadow/grassland area, the soils under trees are thought to be strongly affected by processes such as canopy interception and translocation by precipitation, therefore, leading to areas of low and high radiocaesium inventory values in soil (Takada et al. 2016). This demonstrates that biotic processes such as the type of vegetation have to be taken into account when conducting dose assessments and add to further heterogeneity as they can influence the cycling and movement of radionuclides in the environment and this process is continuing. Processes such as forest fires also play a role in the remobilisation of radionuclides in the Chernobyl Exclusion Zone. As all organic materials in the contaminated areas contain radioactive material, any fire may further disperse radionuclides (Evangeliou et al. 2014).

Next steps

One problem with this study is that it is not known whether colonies reside in the sites in the CEZ which were measured, or whether the bees caught/observed were individuals foraging. The CEZ has patchy radiation and habitat suitable for bumblebees, i.e. wildflowers for foraging and tussock grass for nesting, and this is particularly patchy at sites of higher contamination as most the contamination resides within pine forests which are generally poor environments for bees to survive in. Therefore it is unlikely bumblebees are living in areas where the dose rates are higher than measured in this study. Therefore, bumblebees were assumed to come from nests relatively close to the area where they were caught and therefore were assumed to live in areas of similar contamination. Furthermore, given the patchiness in habitat quality, bees at the higher contamination sites may have to fly further to find resources. Spatially explicit wildlife exposure models using GIS to map likely bumblebee habitat across the Chernobyl Exclusion zone and simulate the movement of animals through the landscape to conduct habitat and exposure modelling would, therefore, be helpful in further improving dose estimates (Wickwire et al. 2011; Chow et al. 2005).

Conclusion

In conclusion, the data suggest that the common use of handheld monitors are a reasonable estimate for measuring dose rates to bumblebees but nevertheless, this study advocates undertaking comprehensive dose assessments as this may not be the case with all wildlife. Furthermore, this chapter advocates further research on understanding dose rates and the effects to sub-adult life stages. The dose rates for both beta and gamma across the CEZ showed high spatial heterogeneity and the data presented in this chapter data reinforce some of the issues associated with estimating dose rates to wildlife living in contaminated areas. Within the small spatial scales of the sampling sites, the variation in dose rate and the different contributors to external dose, either beta or gamma was significant. It is likely that a poor approach to dosimetry is not the reason why severe effects are being reported in the CEZ at substantially lower dose rates than expected for bumblebees.

Chapter 4

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

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

4.1 Abstract

There is disparity in the scientific literature as to the effects of chronic low dose radiation to wildlife. Laboratory studies predict effects to insects only occur at dose rates higher than are found in the 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 that laboratory investigations generally use acute exposures and radiation is studied as a single stressor. 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. The first aim of the work in this chapter is to test whether exposure to elevated radiation dose rates shortens longevity. The second aim is to investigate if virulence of the gut parasite *Crithidia bombi* is higher in areas of the CEZ with elevated radiation dose rates. The final aim is to test how exposure to parasitism (*C. bombi)* and increased radiation dose rates affects bumblebee fitness under laboratory conditions.

The field data indicated that the parasite burden (*C. bombi)* is increased and mean bumblebee age is reduced in areas of the CEZ with high radiation dose rates when compared to areas with low dose rates. To explore the relationships between longevity and parasitaemia, the field study was replicated under robust laboratory settings using environmentally relevant chronic radiation dose rates and infecting bumblebees with a *C. bombi* inoculum. The laboratory study reinforced the field observations, indicating that radiation-induced parasitaemia shortened lifespan. This reduction in longevity was mediated by shortened parasite incubation times and elevated *C. bombi* levels in the bumblebee gut. Moreover, radiation-induced parasitaemia led to greater weight loss in infected bumblebees. This chapter presents the first complementary laboratory and field study investigating the synergistic effects of radiation exposure and parasite infection on insects. The data suggest that when combined with other stressors radiation may have adverse fitness consequences at dose rates lower than previously thought (up to 300 μGyh^{-1}). These findings offer some resolution of the disparity in the results of field studies investigating radiation effects in the CEZ and those studying these effects in the laboratory.

4.2 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 (Copplestone et al. 2017, submitted). 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 (Garnier-Laplace et al. 2013). 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 (Andersson et al. 2009). These dose rates are sometimes more than an order of magnitude above those present in the present-day CEZ (ICRP 2008). 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 (Garnier-Laplace et al. 2013). 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 (Brechignac et al. 2016). Some of these limitations of laboratory studies were addressed in chapter two by conducting a laboratory experiment that used a range of lower dose rates, including those found in the CEZ, and exposing bumblebees for a significant proportion of their lifespan. The work presented in this chapter will address a greater number of the limitations of previous laboratory studies by both using environmentally relevant dose rates and including a co-stressor, the parasite *Crithidia bombi*.

In recent years, there have been studies on a variety of taxa which have found a range of adverse effects at dose rates below those expected from laboratory studies. These include studies on birds (Møller et al. 2012; Møller et al. 2011; Galván et al. 2011), small mammals (Lehmann et al. 2016) and large mammals (Moller & Mousseau 2013). There are fewer studies on terrestrial invertebrates, over 70% of all studies investigating the effects of chronic low dose radiation have been conducted on mammals, birds and terrestrial plants (Copplestone et al. 2008), therefore, to address the lack of available data for insects, bumblebees will be used in this study.

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 (Bezrukov et al. 2015; Møller & Mousseau 2009) and soil invertebrate abundance (Møller & Mousseau 2018; Mousseau et al. 2014) 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 μGy 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 (Møller et al. 2012; Møller & Mousseau 2009). 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 (Fuller et al. 2018a), abundance of invertebrates (Bonzom et al. 2016; Jackson et al. 2005), abundance of large mammals (Deryabina et al. 2015; Gashchak et al. 2016) and genotoxic endpoints in small mammals (Wickliffe et al. 2002; Rodgers & Baker 2000).

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 (Steel et al. 2013). The Chernobyl Exclusion Zone is of limited size (2,600km^2) 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.

As well the complications which generally face ecological studies and the taxonomical bias in existing studies, there are further issues associated with the dosimetry in heterogeneously contaminated areas such as the CEZ. To improve upon the issues associated with dosimetry, in chapter three, the dose rates that bumblebees were likely to receive were calculated, taking into account the internal and external dose rates, as well as life history parameters to give better estimates of dose rates. The total dose rates provided in chapter three were not dissimilar from the external dose rates measured with a handheld monitor, as frequently used in radioecological studies. The total dose rates which were determined from chapter three are used to provide dose rates for the field sites in this study.

The importance of habitat quality for field studies in Chernobyl

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 (Baker et al. 2001). Previous studies have used basic counts of trees (Møller & Mousseau 2007) 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 (Deryabina et al. 2015; Bezrukov et al. 2015; Møller & Mousseau 2015). 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. In this study, food resource assessments at the sites in the CEZ were conducted where and when the bumblebees were sampled. It is important to establish the suitability of habitat and quantify the amount and the quality of the floral resource available to bumblebees at the time of sampling.

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 (Venjakob et al. 2016; Grass et al. 2016; Fowler et al. 2016). Community composition, although varying over the course of a year, plays a vital role in driving pollinator abundance (Grass et al. 2016). 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 (Ricotta 2003). Species richness can be used to inform floral diversity and evenness (Jost 2010; Alatalo 1981). 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 (Kvålseth 2015; Ricotta 2003). There are a number of ways of calculating evenness and diversity (Alatalo 1981; Hill 1973) and each has their advantages and disadvantages. In this study, Pielou's measurement of evenness and (Pielou 1966) and the Shannon diversity index (Pielou 1966) will be used as complementary species richness estimates in this field study (Ricotta & Avena 2003).

Parasites as a co-stressor in the natural environment

Wildlife interact with a multitude of stressors which are omnipresent in the natural environment, and there is increasing evidence that the impacts of stressors can interact synergistically (Goulson et al. 2015; Vanhoudt et al. 2012; Holmstrup et al. 2010). Parasites are ubiquitous components of the natural environment and have been well studied for bumblebees (Conroy et al. 2016; Anthony et al. 2015). There are many studies which have used bees to investigate how multiple stressors interact and whether this interaction amplifies the individual negative fitness consequences of each stressor (Dance et al. 2017; Rolke et al. 2016; Barron 2015; Straub et al. 2015; Bryden et al. 2013). Wild pollinators such as bumblebees are an ideal model system to study the synergistic impacts of parasites and environmental stressors due to their ecological importance, the extensive work that has been conducted on life history traits and well-studied host-parasite systems. Most of this research investigating the consequences of various multiple stressors on bumblebees has been conducted using pesticides, as well as stressors such as temperature or food restriction (Dickel et al. 2018; Dance et al. 2017; David et al. 2016; Crall et al. 2017; Goulson et al. 2015; Henry et al. 2014; Pettis et al. 2012). In recent years, research has emerged testing how a range of environmental stressors can exacerbate the negative effects of parasitism (e.g. heavy metals (Szentgyörgyi et al. 2011), pesticides (Baron et al. 2017; David et al. 2016; Goulson, Nicholls, Botías & Rotheray 2015; Baron et al. 2014; Fauser-Misslin et al. 2014) and food restriction (Dance et al. 2017; Conroy et al. 2016; Riddell & Mallon 2006).

Previous research investigating interactions between radiation exposure and parasite infection

The interactions between infectious diseases and chemical pollution have been well studied (Abat et al. 2016; Morley 2010). However, the ways in which parasite dynamics in wildlife are affected by exposure to elevated radiation dose rates has not been extensively researched (Movila et al. 2012; Morley 2012). Within a polluted ecosystem such as the CEZ, relationships between the host and parasite will be dynamic and dependant on many different biotic and abiotic factors (Cable et al. 2017; Gergs et al. 2013; Lenihan et al. 1999).

Laboratory studies have demonstrated that exposing small mammals and birds to acute radiation can lead to increased susceptibility to disease; this increased susceptibility has been attributed to damage to the host's immune system by radiation (Morley 2012; Klemparskaya et al. 1961). Adult insects generally have higher resistance to radiation when compared to birds and mammals because, notwithstanding the gut and gonads, they are composed of differentiated cells which do not undergo division (Morley 2012; Bakri et al. 2005). Studies investigating the interactions between adult insects and pathogens have shown that radiation can influence parasite virulence both positively and negatively. For example, exposing the malarial parasite *Plasmodium gallinaceum* in mosquitoes to 50 Gy caused a decline in parasite numbers due to damage to the gut epithelium, preventing the parasite from encysting on the gut wall (Terzian 1953). Whereas, the susceptibility of mosquitoes to nematode infections was increased at 40 Gy, also due to radiation damaging the gut wall, because this enabled the nematode to pass quickly into the haemocoel and therefore increased virulence (Morley 2012; Pelgunov 1996).

Most of the field studies investigating the interaction between parasitism and radiation have been conducted on mammals, with no published studies on invertebrates. In 1992, bank voles in Chernobyl were found to have a higher helminth prevalence in highly contaminated areas compared to areas with low contamination: but this prevalence was reduced by 1995 (Morley 2012). Rabies prevalence in wolves in Chernobyl was found to be reduced in areas of high ^{137}Cs contamination: the relationship between infection prevalence and radiation dose rate has been attributed to either a direct reduction in viral viability or it may be driven indirectly if lower wolf density reduces transmission (Adamovich 1998).

Crithidia bombi

Crithidia bombi is a trypanosome parasite which resides in the gut of bumblebees and provides an ideal system for investigating the interactions between parasite virulence and radiation exposure. Bumblebees are annual eusocial insects and colonies can become infected with *C. bombi* when works forage outside the nest and pick up the infection from flowers which have been contaminated by previous visitations from infected workers (Koch et al. 2017). The parasite is then transmitted to others through contact networks which include the feeding interaction between workers and larvae, therefore resulting in larvae acting as a transmission hub (Folly et al. 2017). *C. bombi* infects its adult host and then develops in the gut before transmission stages are passed out in the host faeces (patency), from a minimum of two days after infection (Logan et al. 2005). Virulence in its broad sense is a measure of pathogen impact on host fitness. Although *C. bombi* is highly prevalent, it is generally benign. *C. bombi* exhibits condition dependent virulence (Brown et al. 2000a). If the host can compensate for the impacts through increased resource use, virulence is low. Under conditions of environmental stress, *C. bombi* has significant impacts on the host’s longevity and reproduction (Logan et al. 2005; Manley et al. 2017). Parasitaemia is defined as the parasite burden or abundance (Rynkiewicz et al. 2015). The work in this chapter tests whether the consequences of radiation exposure are exacerbated by exposure to the gut parasite *C. bombi* using a combination of field-based studies in the CEZ and a laboratory-based experiment.

Aims

This study aimed to help resolve the disparity in radioecology by using field studies based in the CEZ and complementary laboratory studies. The field studies aimed to test how bumblebee longevity and mean *C. bombi* parasitaemia were affected by living in the highly contaminated regions of the CEZ. Parasitaemia was measured as the *C. bombi* burden in bumblebee faeces, and bumblebee age was measured in wild bumblebees from different contaminated areas of the CEZ. The laboratory experiment tested if the radiation dose rate and infection by *C. bombi* synergistically interacted to exacerbate the fitness consequences of each stressor. In the laboratory, callow bumblebees which are newly eclosed from the nest, were infected with an inoculum of *C. bombi,* and each worker was assigned a radiation dose rate equivalent to the CEZ. Fitness traits such as longevity and weight loss were measured in the laboratory to test whether radiation and parasitism interact synergistically. The combined field and laboratory studies presented in this chapter address some of the key challenges facing radioecology. The addition of a co-stressor such as parasites may help to resolve the on-going controversy.

Hypotheses

1) Bumblebees in Chernobyl will not live as long in areas of high radiation contamination compared to areas of low contamination
2) Bumblebees in Chernobyl will have higher *Crithidia bombi* burdens in areas of high radiation contamination compared to areas of low contamination
3) Under laboratory settings, bumblebees will not live as long when exposed to *C. bombi* compared to those which are uninfected
4) Under laboratory settings, bumblebees will not live as long when exposed to high radiation dose rates compared to those which are unexposed
5) In an experimental test, bumblebees will not live as long when exposed to both high radiation dose rates and *C. bombi* as the two stressors will synergystically interact
6) Bumblebees exposed to high radiation dose rates and *C. bombi* will lose weight compared to those used as controls

4.3 Methods: Field studies

Bumblebee collection

This study was carried out in the Chernobyl Exclusion Zone (CEZ) (51°23’23.47”N, 30°5’38.57”E) in Northern Ukraine. Twenty-one sites were selected over a radiation dose rate gradient in the CEZ from 0.1 μGyh^{-1} (the same as background levels) to 30 μGyh^{-1}. The dosimetry work for these sites is explained in chapter 3. All sites ranged from 0.01 – 0.04 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 (Figure 4.1). 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.

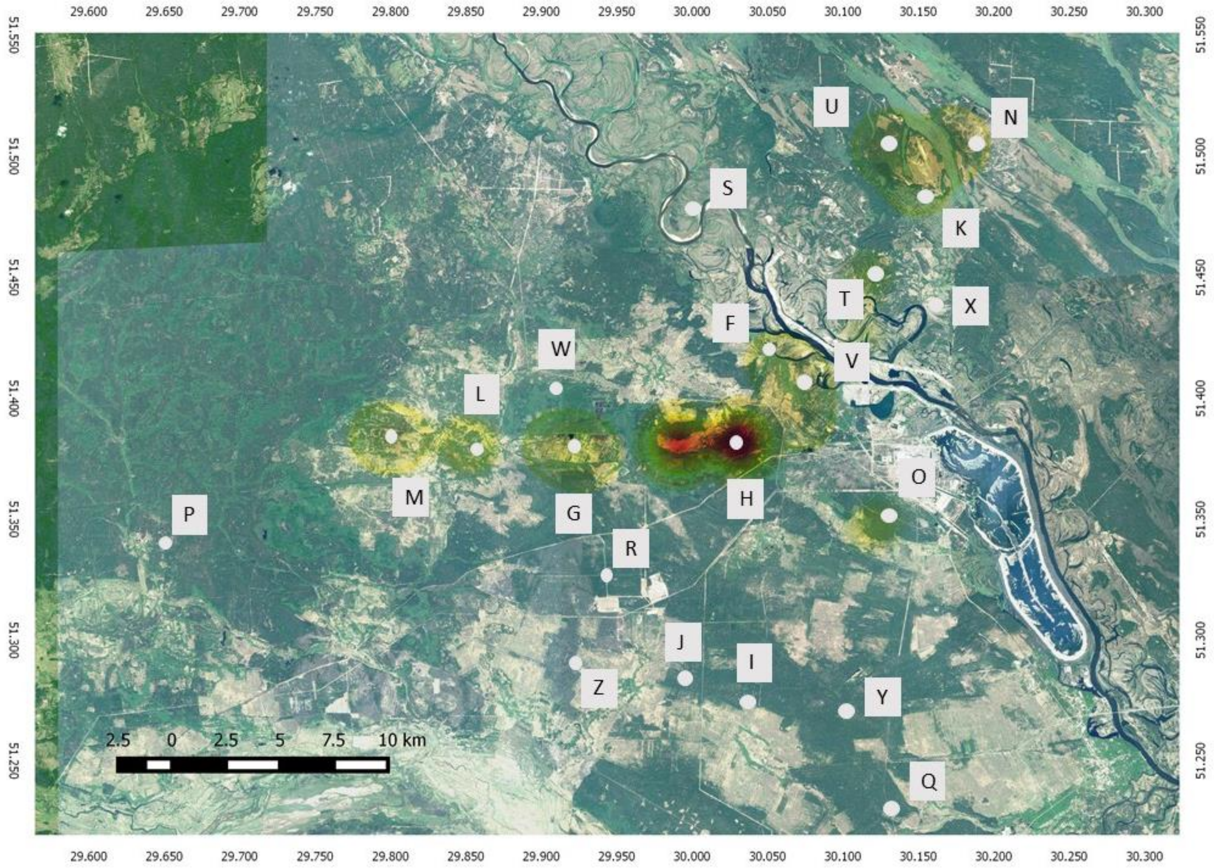

Figure 4.1 A map of sites where bumblebees were sampled across Chernobyl with a gradient of radiation taken from sampling using MoGGs data from Chapter 3

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 (Wray & Elle 2014; Kimoto et al. 2012).

Flowering plant species were identified, and the number of plants and their inflorescences were counted following methods outlined in Baldock et al. (2015). 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 (Oksanen 2015b). 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 (Oksanen 2015a)) 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 μGy h^{-1}), low (0.5 - 6 μGy h^{-1}) and high (6.1 – 30 μGy 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 (Cao et al. 2001). Vectors were fitted onto the NDMS ordination to visualise the direction and strength of the environmental effects.

Bumblebee species identification

Bumblebee species identification was conducted using morphological characteristics. Caste verification and further species confirmation were conducted by using genitalia identification for males (Konovalova 2010; Williams 2000).

Determining the age of wild bumblebees from Chernobyl

Age was estimated in wild bumblebees in the CEZ by estimating the amount of amount of wing wear on each bee wing. Wing wear has been shown to be a good proxy for establishing age in insects (Rhainds 2015; Foster & Cartar 2011; Higginson & Barnard 2004; Cartar 1992). 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 (Dukas & Dukas 2011). 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™ in a consistent left-right arrangement and analysed with a hand lens.

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 (Bates et al. 2015)) 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.

Measuring Crithidia bombi burden in bumblebees caught across different regions of the Chernobyl Exclusion Zone

Bumblebees (n =204) collected in seasons July 2016 (n= 37 at 10 sites), and June 2017 (n = 167 at 15 sites) were analysed for the presence of *C. bombi:* equating to a mean of 10 bumblebees per site per season (range: 3 – 18). The bumblebees collected in 2016 were a subset of those used for the wing wear analysis, whereas, bumblebees collected in June 2017 were not included in any other analysis. Individual bumblebees were placed in clean tubes until they defecated; post-defecation the species identity was recorded, and individuals were either released or euthanised. Faeces were collected from the tube using a 60μL haematocrit tube which was sealed and kept chilled until return to the laboratory. The faeces were subsequently examined within two hours of capture for the presence of *C. bombi,* and parasite burden was counted. Faeces were placed on a haemocytometer, and the presence or absence of *C. bombi* was noted. The number of *C. bombi* cells in five 0.1 μl central squares in the grid were counted using a microscope with 400x magnification (Whitehorn et al. 2013). These *C. bombi* counts were multiplied by 50 to express them as parasite cell burden per 1 μ l.

Testing the relationship between radiation dose rate in Chernobyl and Crithidia bombi burden

To test how the *C. bombi* burden varied in bumblebees caught across different contaminated areas of the CEZ, a generalised linear mixed effects model with a Poisson distribution (lme4 (Bates et al. 2015; Bolker et al. 2012) was constructed. Bumblebee species, radiation dose rate and sample period were fixed factors and site was used as a random effect. Prevalence of *C. bombi* in the sampled population was tested for an association with radiation dose rate and sampling period. Each bumblebee was used as a replicate in the model, and allocated a binary, either an infected or non-infected response. The factors affecting prevalence were tested by constructing a generalised linear model with a binomial distribution and radiation dose rate and sample period were predictor variables.

4.4 Methods: Laboratory-based experiment

Experiment to determine if radiation dose rate and parasite intensity interact to the detriment of bumblebee fitness

In order to test whether parasite infection and radiation dose rate affected bumblebee fitness under a controlled laboratory setting, ten *Bombus terrestris audax* colonies were obtained from the supplier Biobest in May 2017. To standardise colony conditions and ease the extraction of newly eclosed workers, upon arrival the colony was anaesthetised with CO_2 to enable removal of the majority of workers already present in the box; this left the queen, the brood and a cohort of up to ten workers. Each colony was then moved to a controlled radiation facility and stored in the non-irradiated control section with a temperature of 25°C and a light cycle of 16 hours from 7 am – 11 pm to mimic summer conditions. Radiation dose rates were calculated using Electronic Personal Dosimeter Model 23-1 located on top of each individual bee house at the time of the experiment.

To ensure colonies were uninfected before that start of the experiment a random subset (mean n = 5, range 3 - 6) of workers from each colony were tested for the presence of *C. bombi* by inspecting faeces. Colonies were then retested for infection after one week to verify this result before the start of the experiment. The random subset of the workers from the original colonies were housed in groups of threes to create six microcolonies and fed 15 µl of *C. bombi* inoculum each to provide a consistent source of *C. bombi* throughout the subsequent experiment. The six microcolonies were placed in the control area of the radiation facility and were fed, with housing changed every three days.

To source *C. bombi* for the inoculum, ten wild *Bombus terrestris* queens were caught from the grounds of the University of Stirling, Scotland (56°08′45″N 3°55′10″W) in May 2017 and held in individual tubes. When these bumblebee queens had defecated, the faeces were collected, and 5 µl of faces containing *C. bombi* were diluted with 10 µl of sterilised sugar water (Brown et al. 2000) and fed to the workers in the microcolony. The workers in the microcolony were subsequently tested daily for the presence of *C. bombi* until the infection was evident in the faeces as examined under the microscope. All further inoculum for the experiment was comprised from the infected faeces of three randomly selected stock worker bees from across the microcolonies.

In order to ensure all newly eclosed bumblebee workers were the same age at the time of infection and irradiation workers were removed from the brood daily. Newly eclosed workers were either white or pale yellow indicating their age. Once removed from the nest, the newly eclosed workers were weighed and then housed in separate containers (Figure 4.2), fed *ad libitum* with pollen and nectar and randomly assigned a radiation treatment ranging from 20 µGy h^{-1} to 400 µGy h^{-1} or positioned in the control area where they were not exposed to radiation but subject to the same environmental conditions (Figure 4.3). On day three of the irradiation, the newly eclosed workers were re-weighed, placed into clean housing, starved for two hours and fed 15 µl inoculum in sucrose water. Individuals in the non–infected control group were also re-weighed, placed in the clean housing and starved for two hours and then fed 15 µl sucrose water (but not the inoculum). After being fed the inoculum, workers remained at the radiation treatment until death, and every three days individual workers were weighed, and housing was changed. In order to quantify the time to patency (parasite appearance in faeces) and parasite burden, the faeces of bees exposed to *C. bombi* were tested for parasite presence daily until it was detected and thenceforth ever 1-2 days. The date of death was recorded for each bee to record longevity.

Figure 4.2 Bumblebee housing was changed every three days. Housing comprised of a plastic pot with air holes in the top, filter paper to ease housing change and cotton wool for bedding. 40% concentration sucrose water was available in the falcon tube, and 1g of pollen bought from Biobest™.

Figure 4.3 Radiation facility at Stirling University. Individual bumblebees in their housing were randomly assigned a radiation treatment. Different radiation dose rates generated by calculating distance from the ^{137}Cs source verified using an electronic personal dosimeter.

Statistical approach to test how Crithidia bombi and increased radiation dose rates are associated with parasitaemia and life history traits in infected bumblebees

Initially, models were constructed to test how fitness traits were affected by radiation dose rate and *C. bombi* infection. The time between infection and when *C. bombi* was recorded in the faeces (time to patency) was tested using a linear mixed effects model (lme4 (Bates et al. 2014)). The model included the parameters dose rate, bumblebee start weight, and an interaction term to test if radiation dose influenced the relationship between start weight and time until patency. The colony of origin was used as a random effect to account for colony variation in response to *C. bombi*.

The factors influencing the rate of increase in *C. bombi* burden after the point of patency were tested using a linear mixed effects model (lme4 (Bates et al. 2014)). The fixed factors in this model were dose rate, starting weight, time until patency, and finally, a second order polynomial term for days of the experiment since patency, to allow for a non-linear relationship. Interaction terms were specified to test whether the dose rate individuals were exposed to altered the relationship between parasite burden and the days of the experiment. Similarly, an interaction term was fitted to test whether the relationship between time until patency and parasite burden was affected by radiation dose rate. Lastly, to test whether the relationship between start weight and *C. bombi* burden was influenced by the radiation dose rate, the interaction between dose rate and days until patency was tested. Colony identify was fitted as a random effect to account for infection susceptibility differences between colonies. Furthermore, an additional random effect was fitted for each individual bumblebee, accounting for the repeated measures on each bee during the experiment.

In order to test factors affecting weight change over the course of the experiment, the parameters radiation dose rate, *C. bombi* burden at day 10 of the experiment and day of the experiment were fitted as fixed factors in a linear mixed effects model (lme4 (Bates et al. 2014)). Interactions were specified to test whether the relationship between the day of the experiment and body weight was mediated by dose rate, whether the effect of parasite burden at different days of the experiment was mediated by the radiation exposure and whether the parasite burden mediated the relationship between parasite burden at different days and weight change. Furthermore, a three-way interaction was fitted to test whether radiation exposure further influenced the impact of parasitism (burden) on the rate of change (experimental day) in body weight. The original colony of the worker and individual worker was specified as a random effect in this model.

Structural equation modelling (SEM) for Crithidia bombi counts in laboratory bumblebees

Structural equation modelling (SEM) is useful for testing *a priori* defined models and quantifying the relative importance of explanatory variables (Lefcheck 2016). SEM can also test whether a given effect, for example, radiation dose rate has a direct or indirect relationship with life history parameters. Piecewise SEMs (confirmatory path analysis) (Lefcheck 2016) were used to test relationships between radiation dose rate, infection parameters and bumblebee fitness responses. Infection parameters used in this experiment were *C. bombi* burden at day ten after patency and the number of days until patency (the number of days from exposure to the inoculum to when *C. bombi* was first noted in the faeces). The fitness responses measured in this study were longevity and the proportional weight change over the course of the experiment.

A conceptual model was defined (meta-model, Figure 4.4) as an *a priori* defined model, detailing potential cause-effect relationships based on work conducted in the field in this chapter and biological relevance in the literature. From this evidence, two hypotheses were determined to test using an SEM:

(1) Bumblebees which have been exposed to higher radiation dose rates whilst being infected with *C. bombi* are likely to have higher *C. bombi* burdens.

(2) Bumblebees exposed to higher radiation dose rates and *C. bombi* should lose weight, i.e. a proxy for condition, as *C. bombi* exhibits condition dependent virulence and therefore will exacerbate fitness loss. A loss of condition could either be attributed to higher infection intensity and impaired resistance or higher virulence per parasite which would result in lower tolerance to infection.

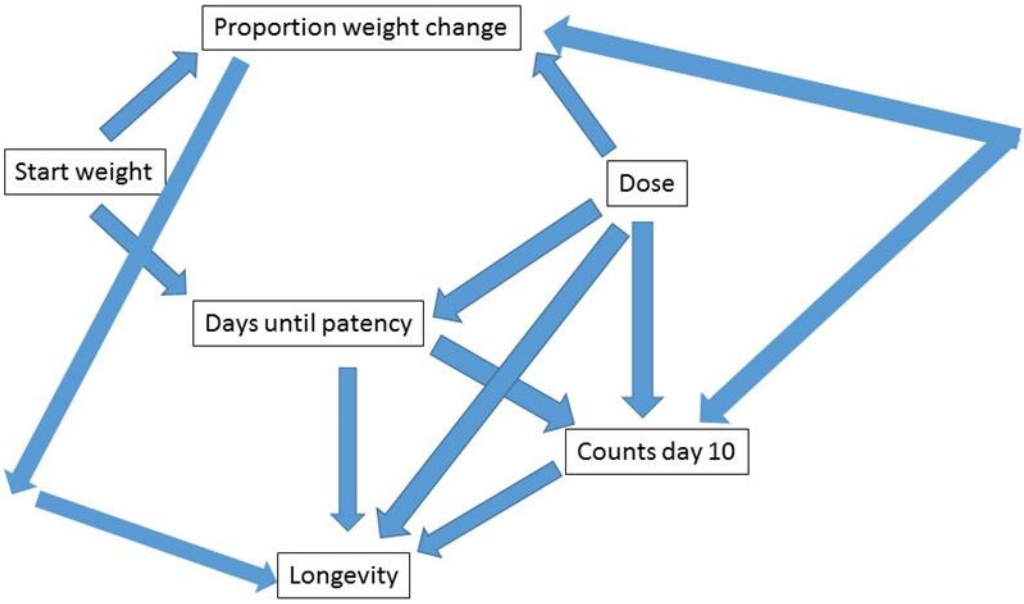

Figure 4.4 A conceptual meta-model demonstrating allowed pathways for structural equation modelling the relationships between radiation dose rate and infection status using parasite C. bombi. Days until patency (time it took for infection to be identifiable in faeces) to be influenced by dose rate and starting weight. The C. bombi burden at day ten to be influenced by the dose rate, days until patency and the proportional weight loss over the course of the experiment. Longevity is predicted to be influenced by the proportonal weight change over the course of the

Before SEM analyses, all explanatory variables were examined for normality and transformed when necessary. Logarithmic transformation of days from infection until *C. bombi* was noted in the faeces was conducted to improve the linearity of its relations with other variables. To construct the SEM, linear models with a Gaussian error structure were used. Models were initially constructed using linear mixed effects models, but the fit was not significantly improved by using a random effect of the original colony to account for differences in genetic susceptibility to *C. bombi* infection (Wilfert & Jiggins 2010; Wilfert et al. 2007). All predictors were standardised to one standard deviation before statistical analyses to compare effect sizes of each predictor. Multicollinearity was checked among predictors using corrplot, ensuring all correlations were less than 0.5 (Wei & Simko 2016).

During model validation, missing paths were evaluated. The evaluation process suggested possible relevant relationships between variables that were not specified in the initial model. These pathways were then either added to the model if they were considered to be causal or otherwise allowed to covary freely. Upon model validation, a significant missing pathway was identified, the relationship between individual starting weight of the bumblebee and individual longevity. Fisher's C [Shipley's test of directed separation] was used to evaluate SEM fit (where higher P values (>0.05) indicate that the data supports the model), i.e., H0= no difference between the data and the hypothesised paths. However, it should be noted that alternative models including parameters which were not measured could also support the data.

Statistical approach to test how Crithidia bombi and increased radiation dose rates are associated with life history traits comparing infected and non-infected bumblebees

Models were constructed to test whether infection with *C. bombi* and elevated dose rates were associated with reduced longevity and increased weight loss compared to non-infected bumblebees. All predictor variables were scaled to one standard deviation and colony of origin for each worker was included as a random effect. A linear mixed effects (lme4 (Bates et al. 2014) maximal model was constructed to test how longevity was affected by increased radiation dose rate and infection by *C. bombi.* Fixed factors included radiation dose rate, infection status, bumblebee start weight of each individual and weight change between the start and the end of the experiment. Interaction terms were allowed to test whether the association between radiation dose rate and longevity was dependant on the start weight of the individual bumblebee. Interaction terms were also specified to test whether the relationship between proportional weight change and longevity was dependant on the starting weight of the individual. Finally, an interaction was specified to test whether start weight influenced the association between longevity and radiation dose.

To test how parasite virulence, as measured by host weight loss was influenced by radiation exposure over the course of the experiment, a maximal model was created with radiation dose rate, individual start weight and infection status as fixed factors. Interactions were included to determine whether the relationship between radiation dose rate and weight change was dependent on infection status and whether individual start weight influenced the association between weight change and infection status. Finally, an interaction was included to test whether start weight affected the association between weight loss and radiation dose.

Model selection and diagnostics

Model selection was undertaken using Akaike Information Criteria (AIC) values, which were compared for candidate models. When AIC values for two models differed by more than two points, the model with the lowest AIC was selected, and when the models did not differ by more than two points, the most parsimonious model was chosen. Likelihood ratio tests following term removal from the final models were used to establish p values and chi-square values for model terms. Model diagnostics were used to verify fit and ensure all assumptions had been met. The interpretation of the importance of factors in the model was based on the size of the estimate. All analyses were performed in R (version 3.5.1) (R Core Team 2018).

4.5 Results: A field study assessing habitat quality, bumblebee population age structure and *C. bombi* parasitaemia in the CEZ

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

This study 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 ± 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 ± 0.037. The total number of inflorescences across each site, indicating the amount of food resource available, varied from 26 to 33986 inflorescences per m^2 with a mean of 1137 inflorescences per m^2 (± 2361 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 ± 0.04, $\chi^2 = 1.65$, p = 0.20; figure 4.5).

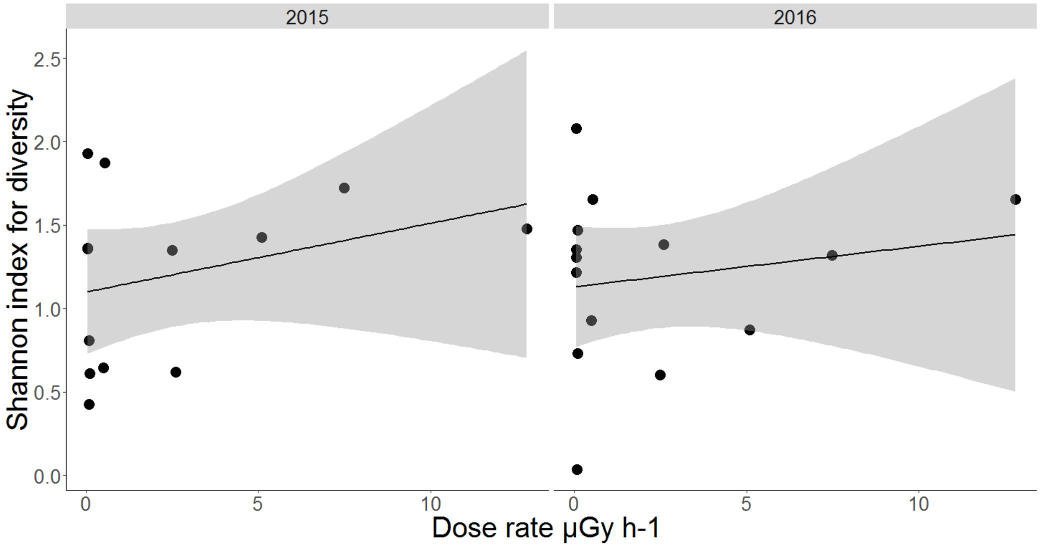

Figure 4.5 No effect of radiation dose on floral plant species diversity in the Chernobyl Exclusion Zone for two different sample periods (July 2015 and June 2016). Diversity determined by Shannon index for plant species at sites across a gradient of radiation. The points are the raw data and the trend line is from the model fit accounting for site and sample period, with 95% confidence intervals.

There was a non-significant positive relationship between Pielou's measurement of floral species evenness and increasing radiation dose rate (slope = 0.01 ± 0.009, χ^2 = 2.04, p = 0.15; figure 4.6). There was also a non–significant negative relationship between the total number of inflorescences and radiation dose rate (slope = 951.2 ± 2210.5, χ^2 = 0.18, p = 0.66; Figure 4.7). For a table of flowering plant species identified see appendix 1.

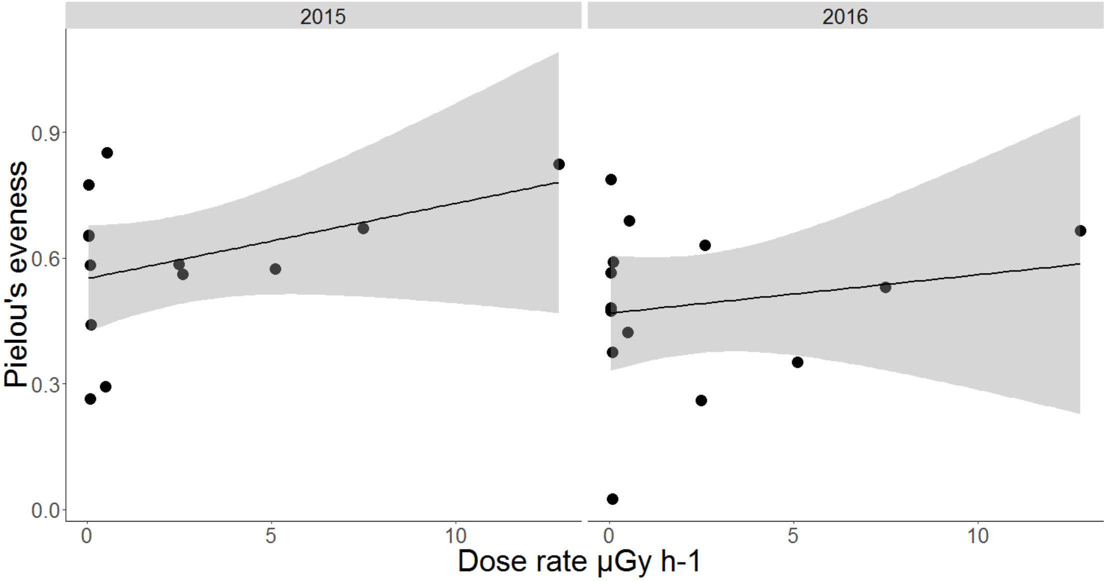

Figure 4.6 No effect of radiation dose on floral plant species evenness in the Chernobyl Exclusion Zone for two different sample periods (July 2015 and June 2016). Evenness was determined by Pielou's measurement of evenness of different plant species at sites across a gradient of radiation. The points are the raw data and the trend line is from the model fit accounting for site and sample period, with 95% confidence intervals.

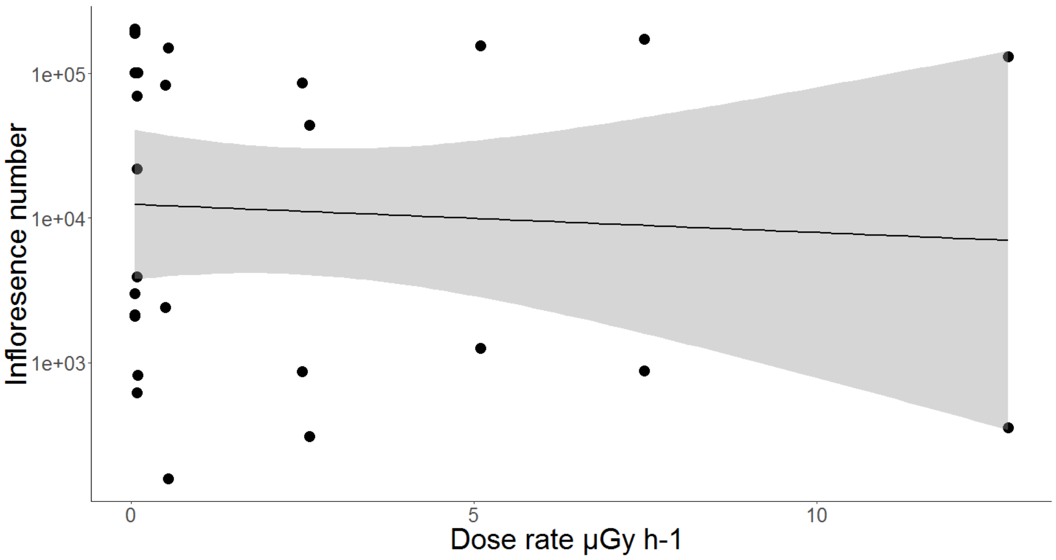

Figure 4.7 No relationship between the total numbers of inflorescences on all flowering plants across each transect in the sites in the Chernobyl Exclusion Zone from both sampling periods (2015 and 2016). The points are the raw data and the trend line is from the model fit accounting for site and sample period, with 95% confidence intervals.

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%, Table 4.1. The most common species present were *Myositis sp. (Myosotis stricta* and *Myosotis ramosissima)*, *Viccia 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 (Figure 4.8, Table 4.2). When the data were subset only to include these common species as there was not enough statistical power to perform a PCA analysis otherwise.

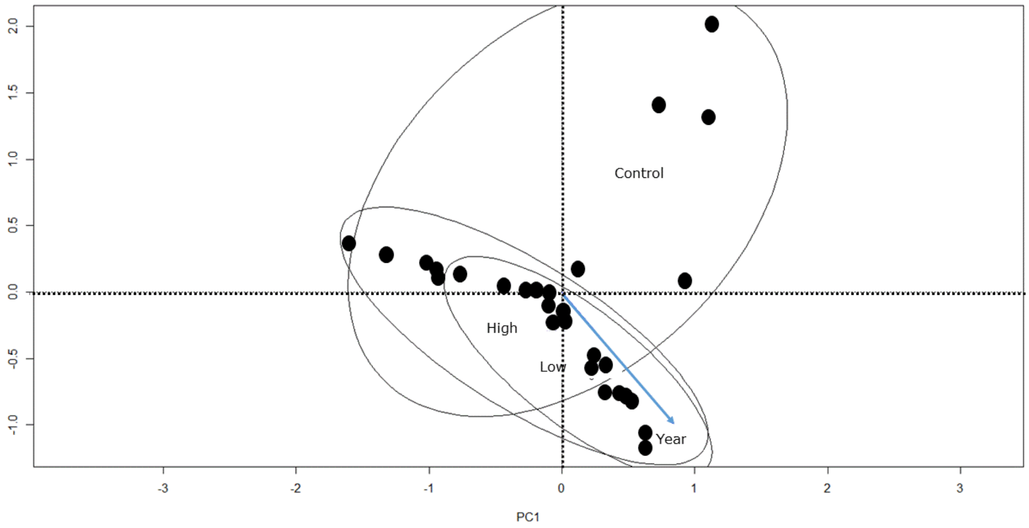

Figure 4.8 No relationship between radiation dose rate and floral species composition. Principal components analysis for ordination using the first two principal components (PCs) based on floral species composition. The data points represent each site, and the ovals are grouped by the radiation dose rate as the environmental variable. Control (background (up to 0.2 µGy h-1), low (0.5 - 6 µGy h-1) and high (6.1 – 30 µGy h-1). The blue arrow represents the year of sampling.

Table 4.1 Results from the principal components analysis (PCA) testing the influence of floral species composition on ordination. Total inertia for PCA = 84, sum of all eigenvalues = 26, results are scaled. Only plant species and not environmental factors were included in the principal components analysis.

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
Eigenvalue	7.93	6.88	5.31	5.25	4.95	4.81	4.49	4.20
% of total variance explained	9.45	8.10	6.32	6.25	5.89	5.72	5.34	5.00

Table 4.2 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 (Oksanen 2015a)

Predictors	PC1	PC2	R^2	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 μGyh^{-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 ± 0.01, $\chi^2_{(1)} = 7.00$, p = 0.008, Figure 4.9). The model predicted that a 5 μGyh^{-1} increase in radiation dose rates led to a 4% reduction in the amount of wing wear accumulated.

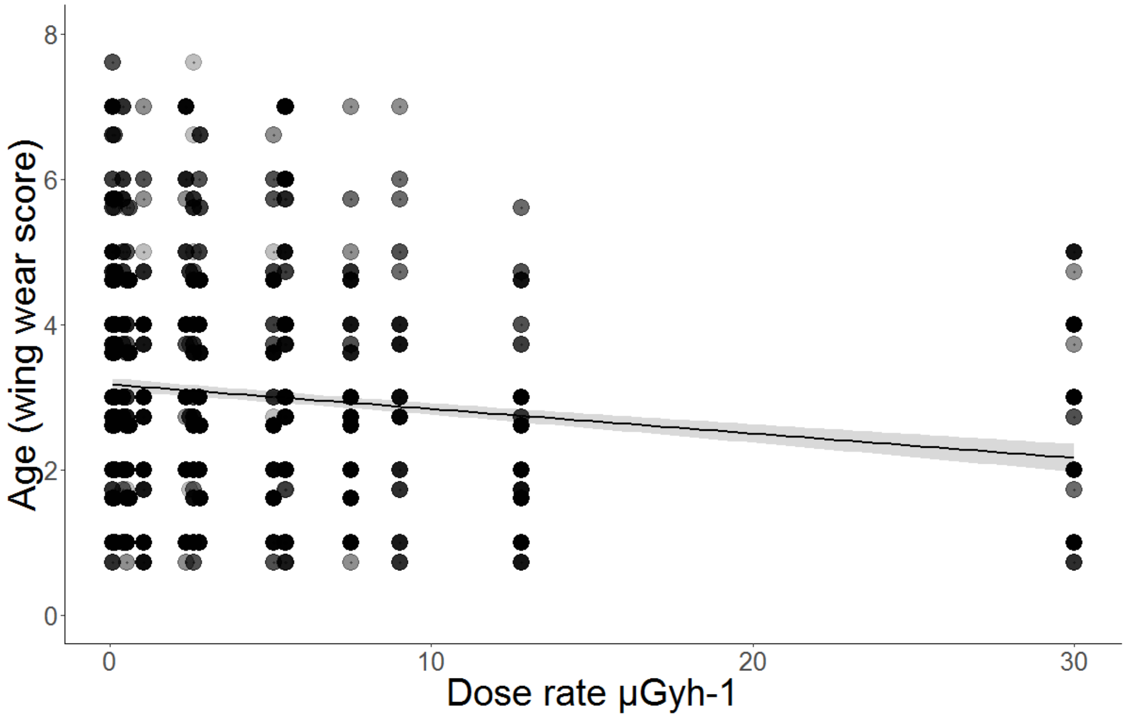

Figure 4.9 The negative association between radiation dose rate and age. Wing wear as a proxy for age measured in worker bumblebees caught in different sites across a gradient of radiation in the Chernobyl Exclusion Zone. The points are the raw data, and the trend line is from the model fit accounting 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 ± 0.23), July 2016 (2.65 ±0.19) and September 2016 (intercept = 4.61 ± 0.24), with significantly higher wing wear in June 2015 than July 2016 ($\chi^2_{(2)}$ = 32, p = <0.001, Figure 4.10). 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 4.10).

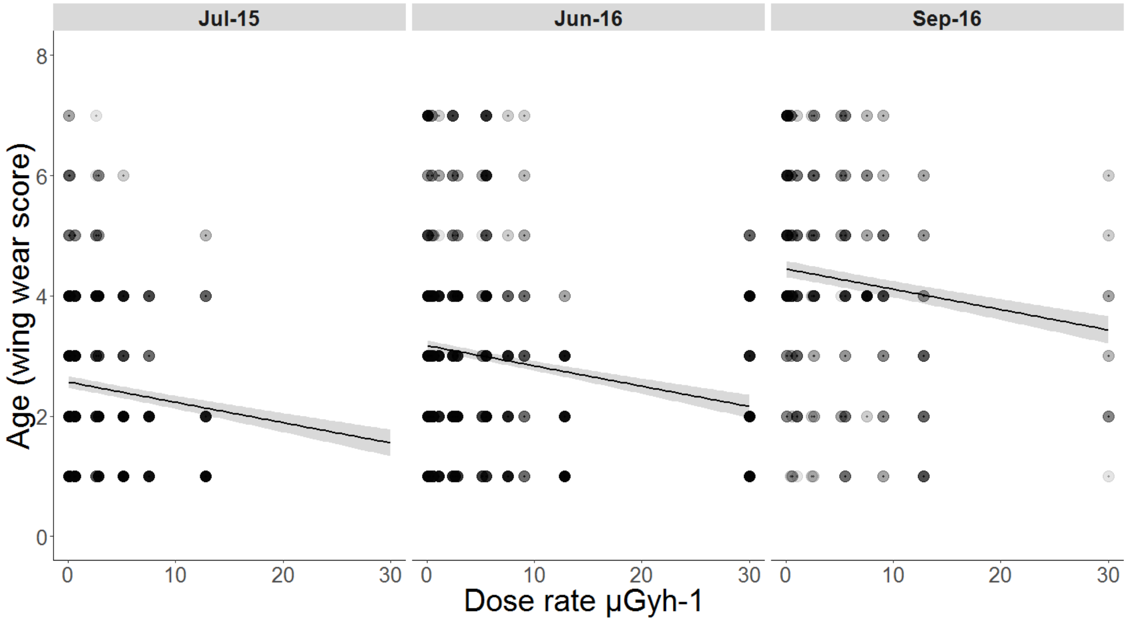

Figure 4.10 The association between average age for worker bumblebees and radiation dose rate broken down by sampling period. Sampling periods were July 2015, June 2016 and September 2016. The interaction between sample period and radiation dose was included in the model, but the interaction term was not significant. The negative effect of radiation dose rate occurred in all sampling periods, September was the latest sample in the calendar year and had the highest wing wear score, and therefore bumblebees are likely to be older. The points and the trend line are from the model fit with dose rate and sample time as fixed factors and site as a random effect. The shading of the points refers to how much data is present at each radiation dose and for each wing wear score, the darker the colour, the more data underlies that point.

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 (Table 4.3). 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.

Table 4.3 Results of the analysis to determine how the impact of radiation dose rate on mean bee age (wing wear) varies across the population age distribution. Wing wear was categorised into seven different groups depending on the wing area missing. Included in the model are radiation dose rate and sample year as predictor variables.

	Intercept	Slope	SE	X^2	P –value
All bumblebees	2.62	-0.041	0.014	7.00	0.0081
6 and below	2.53	-0.029	0.011	6.14	0.0132
5 and below	2.46	-0.026	0.010	6.19	0.0128
4 and below	2.39	-0.024	0.010	4.67	0.0305
3 and below	1.86	-0.009	0.005	3.17	0.0746
2 and below	1.54	-0.002	0.003	0.561	0.453

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 ± 0.07) was significantly higher than in males (4.91 ± 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 ± 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 ± 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, Table 10). 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. subterraneous* and *B. sylvarum* were found to have significantly different intercepts from *B. terrestris* (Table 4.4). 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).

Table 4.4 A comparison of worker abundance and mean wing wear (age) across the 13 species sampled in the CEZ. Slope estimate and standard error determined from a linear mixed effects model including dose rate, site and species as well year as a random effect. Species at the intercept is B. terrestris as this was the ubiquitous species.

Species	**% Occurrence across sampling seasons**	**Intercept**	**Standard error ±**
Intercept **(*B. terrestris*)**		2.86	0.176
B. distinguendus	0.46	-0.216	0.155
B. hortorum	0.53	-0.830	0.30*
B. humilis	0.11	-0.939	0.72
B. hypnorum	0.28	-0.200	0.423
B. lapidarius	8.45	-0.200	0.423**
B. lucorum	16.93	-0.337	0.066**
B. muscorum	1.29	0.200	0.188*
B. pascorum	10.49	-0.057	0.080
B. pratorum	0.05	1.115	1.00
B. ruderarius	2.83	-0.397	0.120*
B. soroeensis	4.07	0.569	0.272**
B. subterraneus	1.47	0.767	0.272*
B. sylvarum	3.57	0.503	0.128*

Higher C. bombi loads in bumblebees from in areas of higher radiation exposure

To test whether the *C. bombi* burden in the faeces of bumblebees was affected by increasing radiation dose rates, 206 bumblebees were sampled in June 2016 and July 2017. Parasitaemia ranged from 0 to 9,920 *C. bombi* cells per 1µl of faeces; the mean burden was 1,800 *C. bombi* cells per 1µl of faeces. Radiation dose rate was positively associated with faecal *C. bombi* burden (slope = 0.05 ± 0.01, $\chi^2_{(1)} = 11.51$, $p = 0.0006$; Figure 4.11). At 10µGy h^{-1}, the model predicted that bumblebees had 15% higher C. bombi burden than at uncontaminated sites and at the highest dose rate of 30 µGy h^{-1} bumblebees had 44% higher counts compared to the control sites.

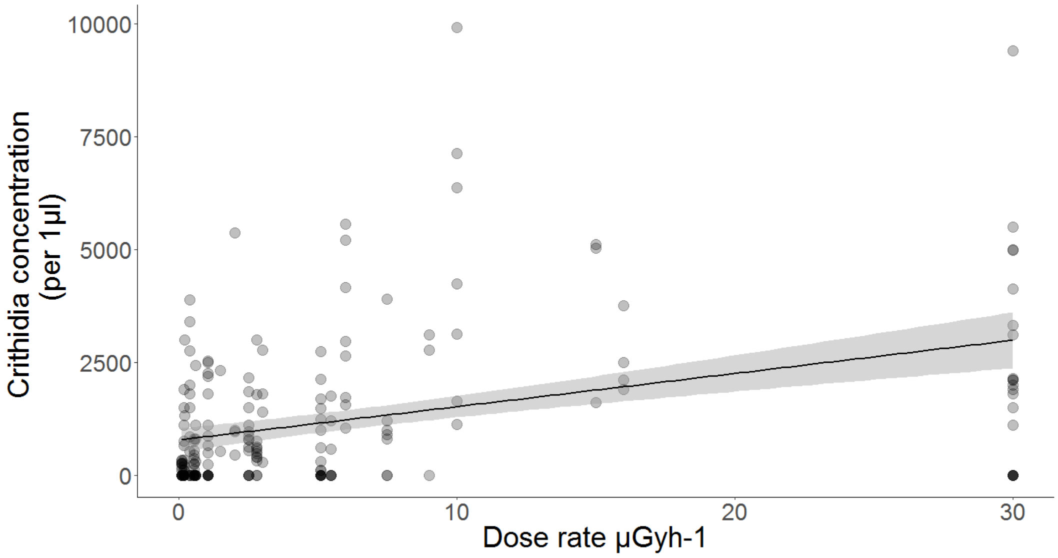

Figure 4.11 The positive relationship between increasing radiation dose rates and the burden of C. bombi in the faeces of bumblebees for June 2016 and July 2017. The points are the raw data, and the fitted line is the model fit with 95% confidence intervals accounting for the site as a random effect.

The interaction between radiation dose rate and sampling year was significant, demonstrating that the radiation dose rate effect was inconsistent across years ($\chi^2 = 64.90$, p = <0.001; Figure 4.12). Separate models were run for each sampling period and radiation was significantly associated with parasitaemia in both 2016 (slope = 0.121 ± 0.01, $\chi^2 = 9.46$, p = 0.002) and 2017 (slope = 0.057 ± 0.21 $\chi^2 = 6.09$, p = 0.013). Therefore, the positive effect of radiation on *C. bombi* burden remained in both years even though, mean infection burden and prevalence were considerably higher in 2015 than in 2016, possibly due to epidemiological differences across years.

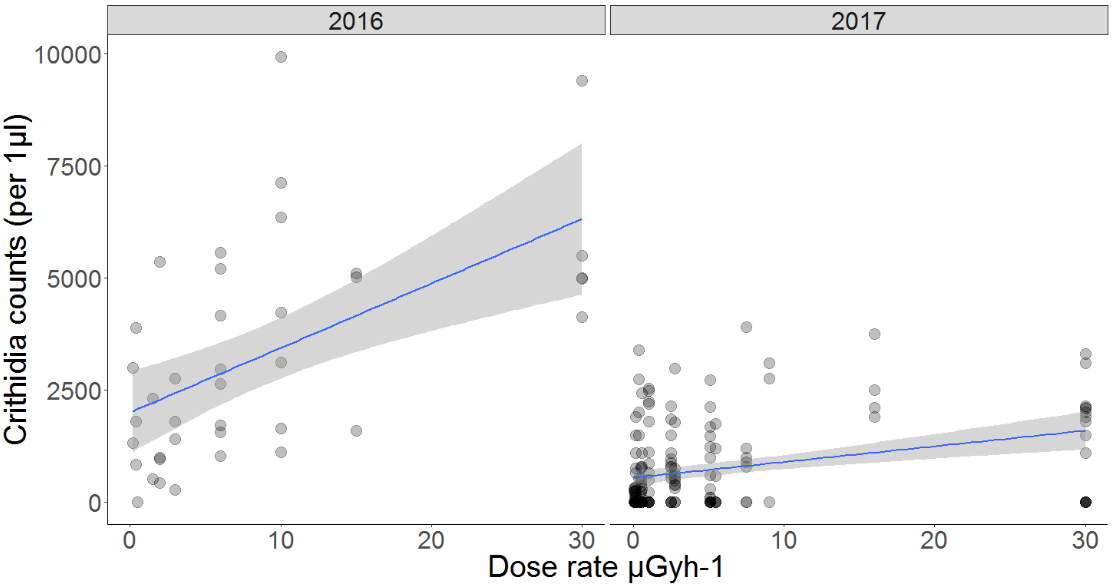

Figure 4.12 Higher C. bombi burdens in bumblebees caught in 2016 when compared to 2017. The counts of C. bombi in individual bumblebees collected across the CEZ separated by sampling year. The effect of radiation dose on C. bombi burden is significant for each sampling year. Points are the raw data, and the fitted line is the model fit with 95% confidence intervals.

C. bombi was present in the faeces of 96% of worker bumblebees in 2016 and 40% in 2017. There was no association between *C. bombi* prevalence and radiation dose rate (slope = 0.019 ± 0.019, $\chi^2_{(1)} = 1.06$, p = 0.30). *C. bombi* prevalence was significantly higher in sampling period 2016 rather than 2017 (intercept = -3.17 ± 1.02, $\chi^2_{(1)} = 24.22$, p = <0.001, figure 4.12). Therefore, the infection dynamics varied over different seasons; both prevalence and burden of *C. bombi* were higher in 2016 compared to 2017.

4.6 Results: An experimental test of the effects of radiation dose rate and *C. bombi* infection on bumblebees

To test how *C. bombi* and increased radiation exposure impacted bumblebee fitness under robust experimental conditions, 156 bumblebee workers were extracted from ten colonies. Bumblebees were exposed to *C. bombi* and either shielded from radiation or allocated to a radiation dose rate treatment on a radiation gradient.

The impact of radiation on time until patency

This study tested how the experimental parameters influenced the time between infection and when the parasite *C. bombi* was present in the faeces (time till patency). Radiation dose rate reduced the number of days until bumblebees were patent ($\chi^2{}_{(1)} = 3.92$, $p = 0.047$, Table 11). Time until patency was reduced by 10% in bumblebees exposed to 100 μGyh^{-1}. Start weight had a positive impact: heavier bees took longer to develop patent infections ($\chi^2{}_{(1)} = 6.27$, $p = 0.012$, Table 11).

The impact of radiation on the rate of C. bombi burden change over time

To test how *C. bombi* burden changed over the course of the experiment, the relationship was tested between radiation dose rate and *C. bombi* burden. Bumblebee start weight and days across the experiment from patency were fixed factors, and the latter was fitted as a polynomial term to allow curvature of the relationship. Radiation dose rate had a positive association with *C. bombi* burden across the full course of the experiment ($\chi^2{}_{(1)} = 5.75$, $p = 0.016$; Table 4.5, figure 4.13). Parasite burden was negatively associated with start weight: lighter bees had higher burdens ($\chi^2{}_{(1)} = 5.75$, $p = 0.016$; Table 4.5). The polynomial term of days from patency time was significantly associated with *C. bombi* burden*.* The two parameters in this curve differ in their meaning: the significant linear term demonstrated that burden initially increased (Table 4.5), whereas the significant quadratic term demonstrated that there was a decline in the rate of increase and burden plateaued at the end of the experiment (Table 4.5, Figure 4.13).

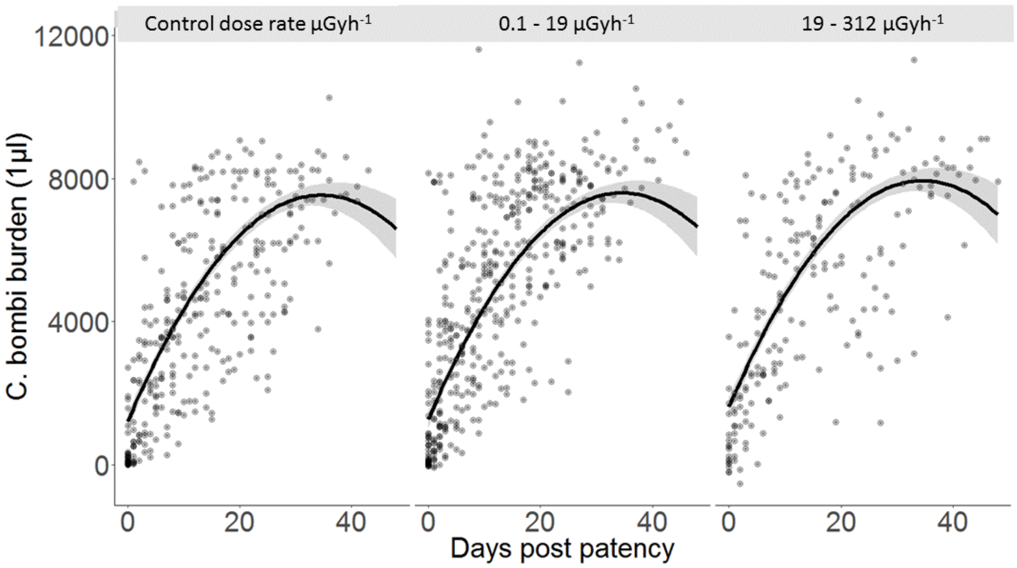

Figure 4.13 Bumblebees exposed to higher radiation dose rates start with a high C. bombi *burden after infection and the burden remains higher throughout the lifespan when compared to unexposed bumblebees. Points and fitted line predicted from a model including start weight.*

Weight

To investigate whether radiation exposure influenced how bumblebee weight changed over the course of the experiment, a model was constructed including the day of the experiment, the radiation dose rate, the *C. bombi* burden at day 10 of the experiment and an interaction was included to test if radiation dose rate influenced the relationship between weight and *C. bombi* burden. Radiation dose rate had a negative effect on weight ($\chi^2_{(1)} = 4.55$, $p = 0.032$; Table 4.5): exposure to higher dose rates led to weight loss. There was no significant direct relationship between *C. bombi* burden at day 10 and body weight ($\chi^2_{(1)} = 0.17$, $p = 0.675$; Table 4.5); however, body weight was influenced by a significant interaction between *C. bombi* burden and dose rate ($\chi^2_{(2)} = 6.11$, $p = 0.013$; Table 4.5, Figure 4.14). The interaction demonstrated that at low and medium radiation doses (up to 19 μGyh^{-1}), bumblebees suffered weight loss as *C. bombi* burden increased; however, at higher dose rates (19-312 μGyh^{-1}), this relationship was reversed so that increasing parasitemia was associated with greater body weight.

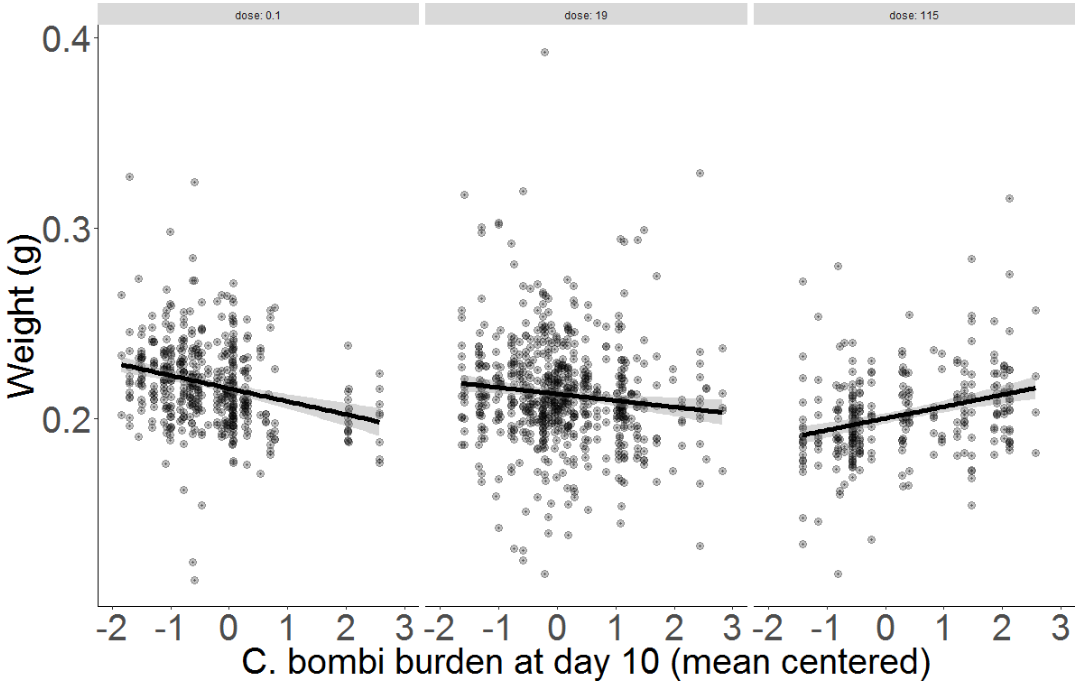

Figure 4.14 The interaction between radiation dose rate and the amount of weight lost by individual bumblebees. Bumblebees lost weight as they were exposed to increasing radiation dose rates when they had low C. bombi burdens, however they gained weight as radiation dose rate increased when they had high parasitaemia levels. Points and fitted line predicted from a model.

Table 4.5 The model parameters from all models testing the factors influencing time until patency, C. bombi *burden and weight change over the course of the experiment. Shaded areas represent terms remaining the final model.*

	Intercept	**Slope**	**SE**	**X^2**	**P value**
Time to patency	4.12				
Dose rate		-0.0049	0.001	3.92	0.047
Start weight (scaled)		0.267	0.135	6.27	0.012
Dose rate by start weight (scaled)		0.0004	0.001	0.045	0.831
***C. bombi* burden**	4757.36				
Dose rate (scaled)		219.69	92.54	5.75	0.016
Start weight (scaled)		-221.15	100	4.64	0.031
Days of the experiment (poly,1)		68811	1929	327.03	<0.001
Days of the experiment (poly,2)		-20437	1832	93.17	<0.001
Days until patency		36.31	72.31	0.010	0.919
Dose rate interaction with days of the experiment (poly 1)		766.40	2096	2.81	0.42
Dose rate interaction with days of the experiment (poly 2)		3273	1796	2.79	0.24
Radiation dose by time until patency		37.71	74.22	0.252	0.615
Weight change	0.209				
Dose rate (scaled)		-0.0089	0.004	4.55	0.032
C. bombi burden at day 10		-0.0013	0.003	0.17	0.675
Dose rate by *C. bombi* day 10 burden interaction		0.0069	0.002	6.11	0.013
Dose rate (scaled) and days of experiment interaction		-0.0002	0.0001	3.10	0.082
Burden at 10 days and days of experiment		0.0001	0.0001	1.43	0.23
Dose rate by burden at 10 days		0.005	0.002	1.02	0.31
Dose rate by burden at ten days by day of experiment		0.0001	0.0001	1.36	0.24

Structural equation modelling

Having already demonstrated that *C. bombi* parasite load is higher and mean wing wear (a proxy for age) is lower in bumblebees caught in more highly contaminated regions of the CEZ. The experimental data analysis so far has demonstrated that radiation dose rate was associated with weight loss, but depended on the start weight of the individual and also reduced the number of days between parasite infection and patency. Furthermore, the data from both the laboratory and field study suggested that radiation dose rate is positively associated with *C. bombi* burden. To test these results and investigate direct and indirect relationships between the fitness parameters a structural equation model (SEM) was used. The SEM tested if the effect of exposure to ionising radiation and the infection with the parasite *C. bombi* was well supported by the data (Fisher's C = 6.53 p = 0.58).

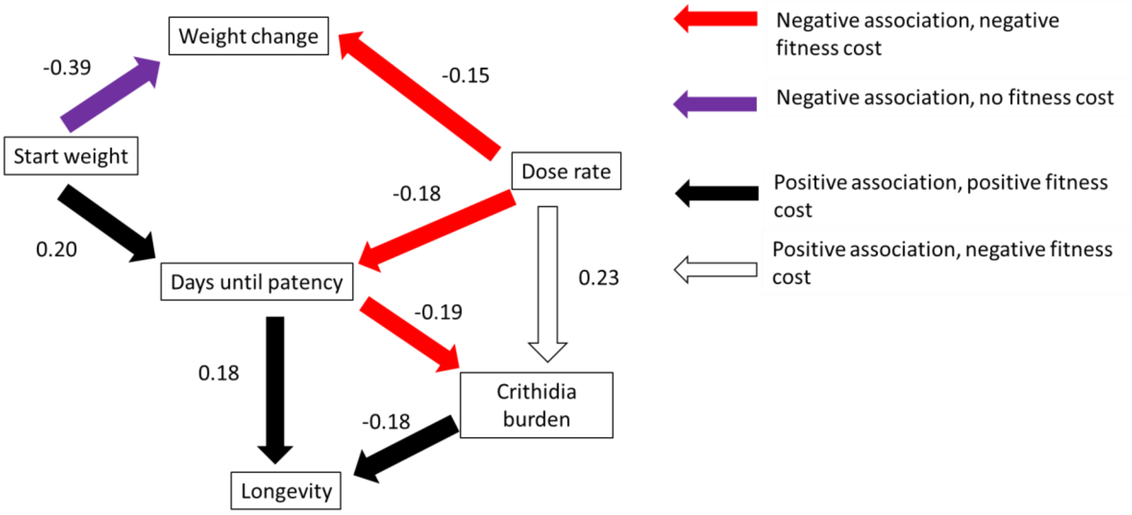

Figure 4.15 The output from the structural equation model (SEM) exploring the effects of radiation dose rate and infection on fitness (longevity and weight change) of individual bumblebees. Boxes represent measured variables and arrows represent unidirectional relationships among variables colour coded to help identify fitness consequences. The effect sizes are presented with errors in Table 12.

The data from the laboratory experiment in the SEM analysis supported the field data presented in this chapter: *C. bombi* burden was higher in bumblebees exposed to elevated levels of radiation and radiation dose rate was associated with reduced longevity of bumblebees. All statistics and parameter estimates are reported in Table 4.6, and a diagram of interactions and effects of all significant predictors and responses is demonstrated in Figure 4.15. The data demonstrate that increasing radiation dose rates reduced the number of days it took for *C. bombi* to be visible in the faeces (patency). Reduction in time to patency had a negative relationship with individual worker longevity. Therefore, bumblebees which are exposed to increased radiation dose rates become infected more quickly which subsequently reduced their longevity. Radiation dose rate was positively associated with *C. bombi* burden and, to further support the field data, *C. bombi* faecal burden had an adverse effect on longevity. Radiation dose rate increased the weight loss in bumblebees. Bumblebees with larger start weights took longer to become patent (time from infection until *C. bombi* was noted in the faeces). Finally, bumblebees with larger start weights lost a greater amount of weight than smaller bumblebees.

Table 4.6 Summary standardised coefficients for all parameters included in the Structural Equation Model (Figure 4.15). C. bombi *burden is the burden at day 10 post-patency.*

Response	**Predictor**	**β**	**Std error**	**P value**
***C. bombi* Burden**	Dose rate	0.228	0.076	0.0003 **
***C. bombi* Burden**	Patency	-0.191	0.076	0.0132*
Weight change	Start weight	-0.399	0.073	<0.0001 ***
Weight change	Dose rate	-0.15	0.073	0.0356 *
Patency	Start weight	0.209	0.077	0.0078 **
Patency	Dose rate	-0.183	0.077	0.0193 *
Longevity	Patency	0.180	0.080	0.0268 *
Longevity	*C. bombi* burden	-0.187	0.083	0.0262 *
Longevity	Weight change	0.150	0.078	0.050*
Longevity	Dose rate	0.117	0.083	0.161

Comparison of non-infected bumblebees and infected bumblebees across a radiation gradient

The previous analyses considered bumblebees that were infected with *C. bombi* and how radiation exposure influenced their fitness. However, the experiment also contained uninfected bees that were not used in the analyses described so far. This final analysis section compared the performance of uninfected and infected bumblebees (regardless of the infection burden) and investigated how these effects were influenced by exposure to does rates along a radiation exposure gradient. Table 4.7 demonstrates how bumblebees were distributed across treatment groups. Bumblebees were exposed to either *C. bombi* or remained uninfected and either shielded from radiation or allocated a radiation dose on a radiation gradient.

Table 4.7 Experimental setup and the number of individual bumblebees in each different treatment groups. Treatment groups were either infected or uninfected and shielded from radiation or radiation gradient.

Treatment	Control radiation	Radiation
Uninfected	18	48
Infected	35	121

Exposure to *Crithidia bombi* and increased radiation dose rate reduces the longevity of bumblebees

All bumblebees in the infection treatment group became infected after being fed the inoculum (n = 156). Bumblebees which were both uninfected and not exposed to radiation (n =18) were used as controls in this experiment (Table 4.7). First, the full dataset was tested in order to determine whether there was an effect of radiation exposure on bumblebee longevity, irrespective of infection status: there was no overall relationship between radiation dose rate and longevity (intercept = -0.01 ± 0.01, $\chi^2_{(1)} = 1.41$, p = 0.23). Furthermore, there was no evidence of a radiation dose rate by infection status interaction, which might have suggested that parasitism influenced the impact of radiation exposure (slope = -0.039 ± 0.03, $\chi^2_{(1)} = 2.74$, p = 0.25; Figure 4.16). Bumblebee longevity was substantially reduced by exposure to *C. bombi* irrespective of the radiation treatment (intercept= -9.93 ± 2.16, $\chi^2_{(1)} = 15.47$, p = <0.0001): being infected with *C. bombi* was associated with a 21% reduction in longevity.

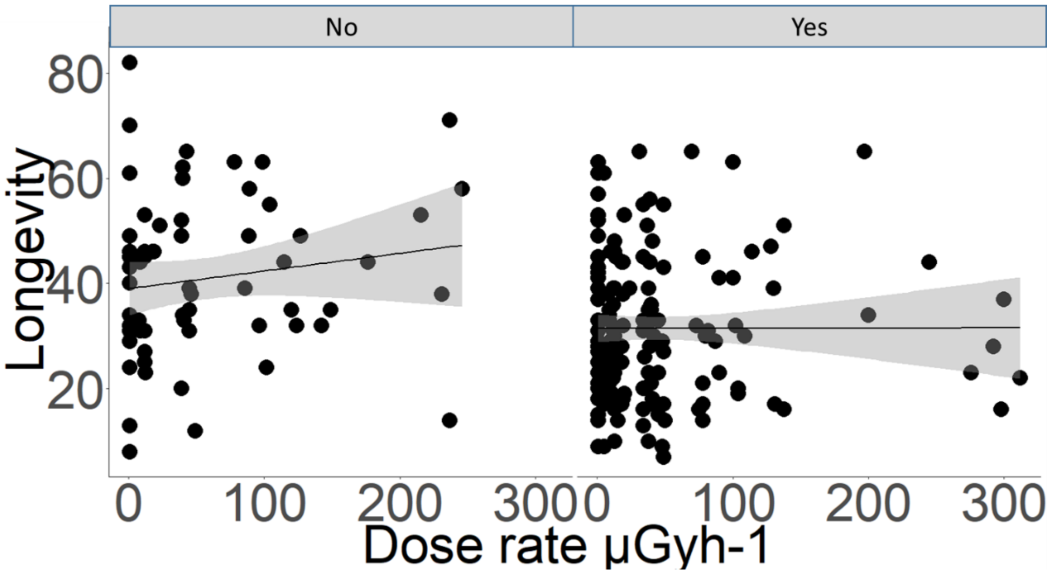

Figure 4.16 The interaction between radiation dose rate and infection status of bumblebees with Crithidia bombi. Points are actual data and the line is the model with with 95% confidence intervals.

Exposure to *Crithidia bombi* and increased radiation dose rate reduces the amount of weight gained over the course of the experiment

Bumblebees exposed to low dose rates or in the control area gained weight throughout the experiment, but bumblebees at higher dose rates lost weight. Bumblebees exposed to radiation dose rates of 100 μGy h^{-1} showed a 10% weight loss irrespective of whether they have been exposed to *C. bombi* or not (slope = -0.0005 ± 0.0002, $\chi^2_{(2)}$ = 4.36 , p = 0.036; Figure 4.16).

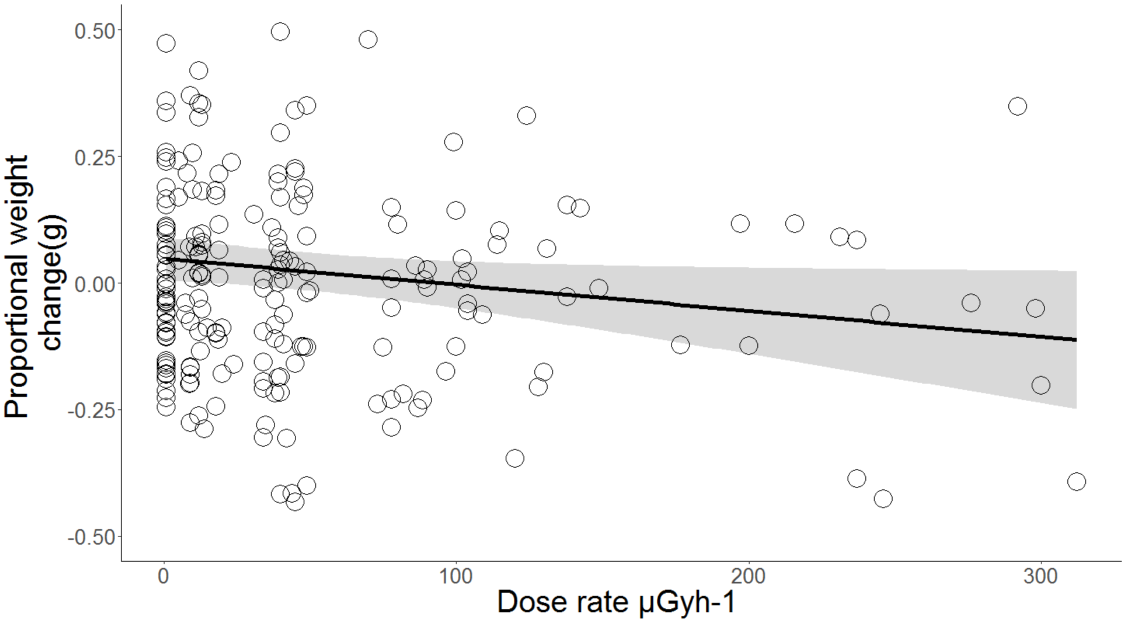

Figure 4.17 The negative association between radiation dose rate and weight change. Bumblebees exposed to low doses of radiation gained weight but bumblebees exposed to high radiation dose rates lost weight, regardless of infection status. Infected and uninfected bumblebees were included in our analysis and demonstrate that increased levels of radiation are associated with greater proportional weight loss. The points and the fitted line are from the model fit accounting for original colony, radiation dose and start weight. The shaded areas are the 95% confidence intervals.

4.7 Discussion

The work in this chapter demonstrated that radiation exposure and parasite infection could have synergistic negative impacts on fitness in bumblebees at lower dose rates than previously anticipated. Radiation exposure increased the number of *C. bombi* parasite cells in the gut in both wild bumblebees from the Chernobyl Exclusion Zone and in the laboratory under robust experiment conditions. The structural equation model suggests that this increase in *C. bombi* burden leads to a reduction in bumblebee longevity. Furthermore, the work in the CEZ suggested that this infection-mediated cost of radiation exposure to bumblebee lifespan also occurred in the field.

In the CEZ, mean bumblebee age was lower in areas with higher dose rates when compared to lower dose rates. When bumblebees of the highest age classes were systematically removed and the data reanalysed, the data showed that not only were bumblebees missing from the highest age class, but the overall mean age decreased indicating reduced longevity. Bumblebees with the middle scoring wing wear also become increasingly rarer as the radiation dose increases, therefore, indicating a higher risk of death and reduced longevity. Reduced longevity due to of increased radiation dose rate is supported by the experimental work conducted in this chapter. This study provides evidence that altered parasite virulence may drive the reduced lifespan effect of increased radiation dose rate as bumblebees exposed to high dose rates had shortened incubation times and a higher parasite burden. Furthermore, increased radiation dose rates also resulted in a weight loss when compared to bumblebees which were not exposed to radiation which may be another independent symptom of fitness loss, but there was no evidence in the analyses that this was mediated by parasitism.

What the studies in this chapter mean for the controversy in radioecology

Controversy exists in radioecology as laboratory studies predict that the effects of radiation exposure to invertebrates occur at dose rates which are much higher than occur in the environment (ICRP 2008). Whilst some studies, mostly based in the CEZ, support this hypothesis and find no effects to invertebrates at elevated radiation dose rates (e.g. Fuller et al. 2018; Fuller et al. 2017; Lecomte-Pradines et al. 2014), a different subset of studies from the CEZ have detected significant effects of radiation exposure at dose rates just above UK background, for example, (Møller & Mousseau 2018; Møller et al. 2012; Møller & Mousseau 2009). The combination of studies in this chapter helps to resolve this disparity and address many of the challenges facing radioecology. The work presented in this chapter demonstrates the need for complementary field and laboratory studies and the consideration of co-stressors in laboratory experiments.

Laboratory studies have often been criticised for having little environmental relevance, using inappropriate dose rates and not taking into account the synergistic consequences of co-stressors. This study exposed bumblebees to a gradient of radiation focussed at dose rates found in present-day Chernobyl (0 - 400 μGy h^{-1}). The laboratory experiment tested the consequences of parasite virulence as a co-stressor with radiation. The parasite, *C. bombi* is frequently encountered in the environment and prevalence can reach over 80% in the early summer (Popp et al. 2012). In this laboratory study, I detected negative effects of radiation at lower dose rates than previously predicted from laboratory studies using invertebrates. However, the majority of previous laboratory studies have been limited by using radiation as a single stressor (ICRP 2008). By incorporating an ecologically relevant co-stressor in this study (i.e. parasite infection), the data suggest that there are adverse effects of radiation at lower doses to bumblebees when bees are also managing the physiological challenges of infection. This is the first experiment to investigate the effects of radiation and a co-stressor which have been conducted on bumblebees at these dose levels.

The data from this study demonstrate that radiation and parasitism interact synergistically, rather than additively. The fitness consequences of bumblebees exposed to a single stressor (parasitism or elevated dose rates) were shown to be small and statistically non-significant, whereas, when bumblebees were exposed to both stressors, the effect size was larger, by over an order of magnitude. If this study can be generalised widely, the data may suggest that many previous laboratory studies have systematically underestimated the ecological impacts of low dose radiation exposure because they have typically studied organisms in optimal conditions in the absence of parasites.

Although the work in this chapter detected effects to bumblebees at lower dose rates than predicted from previous laboratory studies, similar to chapter 2, 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 μGyh^{-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 (Brechignac 2016; Brechignac et al. 2016). This argument can be countered by appropriately powered experiments with sufficient site replication, as demonstrated by the work conducted in this chapter. 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. Therefore, increasing the strength of inference that elevated radiation dose rates drive the reduction in lifespan and the increase in parasite infection intensity observed in this experiment.

Dosimetry has previously been shown to be a key challenge to radioecological field studies and is thought to be an explanation as to why effects are detected at very low dose rates in the field (Beresford & Copplestone 2011). Many field studies only measure the external dose rate and use this when comparing to any observed effects instead of considering the total dose rate. Often studies also do not take into account the life history and the ecology of the study species and therefore may overpredict the effects of radiation dose rate. To address this issue, dosimetry for the field study was improved as demonstrated in chapter 3, and thus this work provides confidence that the dose rates at which the effects were measured in this chapter are good estimates that this is what the bumblebees have received. While effects detected in the field were at lower dose rates than those observed in my laboratory studies, this could be for a number of reasons. The bumblebees in the laboratory were kept individually. Therefore, it is unknown how the effects of parasitism would affect colony fitness. Furthermore, there may be other stressors present in the CEZ which may also synergistically interact therefore causing further detriment to bumblebee fitness.

A reduction resistance most likely explains why bumblebees have high *C. bombi* burdens and less time between infection and patency

It is unlikely that the increased parasitaemia of *C. bombi* in bumblebees is a result of the effects of radiation on the parasite rather than the host. Trypanosomes are known to be highly resistant to DNA damage caused by ionising radiation (Grynberg et al. 2012; Moloo 1982). Trypanosome parasites have an efficient double-strand DNA repair apparatus, at 500 Gy of radiation, trypanosomes have demonstrated the ability to reconstruct their DNA (Grynberg et al. 2012; Morley 2012). There are also some examples where exposure to chronic low dose radiation exposure has resulted in a decline in infections, and furthermore, a small number of studies have been found to increase the virulence of certain parasites through hormesis (Morley 2012). If *C. bombi* exhibits a hormetic response to the radiation, this could increase burden by enhanced virulence and replication. However, it is likely that the radiation is affecting the host, rather than *C. bombi* due to known radioresistance of protozoa and the reasons outlined below.

Hosts can use two different strategies to defend themselves against parasites: tolerance and resistance (Miller & Cotter 2018; Råberg et al. 2007). Tolerance is the host’s ability to limit the disease severity from the pathogen, while resistance is the ability to limit parasite burden within the host. Resistance has an adverse effect on parasites, whereas tolerance does not (Miller & Cotter 2018; Kutzer & Armitage 2016; Råberg et al. 2009).

In this study, the increased parasite burden with increased radiation dose rates suggests a resistance failure (Kutzer & Armitage 2016). Resistance, for example, is mediated through the release of antimicrobial peptides as part of the humoral response (Schmid-Hempel 2003). There is no evidence for a failure of tolerance in this study; tolerance is measured as an increase in fitness (for example, weight or longevity) against increasing parasite infection (Kutzer & Armitage 2016). In this study, I tested whether there was a parasite burden by radiation interaction on bumblebee weight, although the test was significant, the results were not consistent as the slope reversed at high radiation dose rates. Tolerance strategies, for example, involve reduced host movement, conservation of energy and changes in behaviour to forage for specific nutrients (Miller & Cotter 2018).

A possible explanation as to the decrease in resistance of bumblebees to *C. bombi* is the reduction in time to patency. A reduction in time to patency in bumblebees exposed to elevated radiation dose rates resulted in increased *C. bombi* burden in the gut of the bumblebees. One proposed mechanism is that exposure to elevated radiation dose rates compromises the structure and permeability of the gut. The gastrointestinal tract in invertebrates is comprised of barrier epithelium which helps prevent the invasion of pathogens (Davis & Engström 2012). The protective role of the barrier epithelia is twofold: providing an impenetrable physical barrier and a chemical barrier, in the form of expressed antimicrobial peptides and reactive oxygen (AMP) (Davis & Engström 2012; Bosch et al. 2009). After oral intake, *C. bombi* resides in the hindgut of the host and is transmitted via the faeces (Cisarovsky & Schmid-Hempel 2014). Infection by *C. bombi* is extracellular, developing within a few days and resulting in large numbers of parasite cells lining the walls of the midgut and rectum (Riddell et al. 2011).

The effects of radiation could be causing an alteration in the epithelial integrity. The midgut is important in water regulation and retention, and when compromised, the insect quickly loses turgidity and dies (Miller & Cotter 2018). Adult insects have a relatively high resistance to radiation, because, except the gut and gonads they are composed of differentiated cells that do not undergo replacement (Morley 2012). The increased gut radiosensitivity to radiation damage could provide a mechanism as to why *C. bombi* may exhibit greater virulence on bumblebees exposed to higher levels of radiation. Radiation exposure is associated with damage to tissue which includes increased permeability of biological barriers such as the gut epithelium (Morley 2012). It has been shown that the gut epithelium is often sufficient to stop further progress should the pathogen defeat the morphological defences of insects (Evans et al. 2006). A compromised epithelium can increase the susceptibility of the host to infection, reduce infection time, the magnitude of the radiation exposure and the sensitivity of the host to the particular infectious agent (Morley 2012).

Previous work on other insect species has shown that the immune system is compromised shortly after exposure to radiation. Exposing *Hemerocampa leucostigma* larvae to *B. thuringiensis* and a radiation dose rate of 300 Gy over four days resulted in a depression in haemocyte counts (another common immune function response), but this was not apparent until ten days post exposure (Rossmoore & Hoffman 1971). However, the doses used by Rossmoore & Hoffman (1971) were delivered acutely and at much higher dose rates than used in this experiment. Although Rossmoore & Hoffman (1971) used acute and higher dose rates (300 Gy) than were used in this experiment, this reduction in immunocompetence could be a pivotal factor affecting both the reduced time between infection and patency in faecal samples and reduction in longevity (Rossmoore & Hoffman 1971; Morley 2012).

Other studies using acute radiation dose investigating nematode infections in adult mosquitoes demonstrate that there is increased susceptibility to infection following radiation exposure as the parasite can easily pass through the damaged gut wall (Morley 2012; Terzian 1953). Similarly, in *Glossina* spp., acting as a vector for *Trypanosoma* spp., the trypanosome survived and multiplied in the intestines and haemolymph of irradiated adults which consequentially resulted in rapid migration in the haemocoel and fitness consequences to the host (Morley 2012; Moloo 1982). Alteration to the ultrastructure of the host's gut accompanied by an increase in virus-like particles are known to facilitate the establishment of trypanosomes in insects (Morley 2012); therefore a change in gut structure could explain the reduced time until patency demonstrated in this experiment and that bumblebees exposed to higher radiation dose rates have overall higher counts of *C. bombi.*

Bacterial gut symbionts are imperative to help resistance to infection (Cariveau et al. 2014). Recently, there has been evidence that the diversity of the gut microbiome in bank voles was elevated by increased radiation dose rates in the CEZ (Lavrinienko et al. 2018). Although the fitness consequences of this alteration to the gut microbiome are unknown, it has been demonstrated that gut microbiota in bumblebees can determine resistance against *C. bombi* (Mockler et al. 2018; Cariveau et al. 2014)*.* If the gut microbiota were affected by increased radiation dose rate, this might decrease resistance to *C. bombi* and could explain the effects such as reduced time to patency and increased *C. bombi* burden.

Infected bumblebees have reduced longevity

The analysis in this study found that bumblebees which were infected with *C. bombi* had a shorter lifespan than uninfected bumblebees, independent of radiation exposure. The findings in this chapter align with Brown et al. (2000) that *C. bombi* exhibits condition dependent virulence. Although the bumblebees were fed *ad libitum*, they were kept in individual housing, the lack of natural eusocial behaviour and collective foraging may have produced inherent stress on the bumblebee, therefore, allowing *C. bombi* to exhibit condition-dependant virulence. The way in which *C. bombi* reduces host survival is unclear; it may reduce gut resource content for the host. Secondly, it may destroy intestinal cells, therefore, inhibiting utilisation of gut contents and thirdly, it may induce the host immune system and the energy required for could reduce lifespan (Brown et al. 2000b). The latter mechanism, the activation of the immune system can reduce fitness has been previously demonstrated in bumblebees and is well supported in the literature (Brown et al. 2000b).

Transmission rates

Transmission rates of *C. bombi* vary throughout the year; parasite prevalence is highest during the middle of summer and declines towards the end of the summer into autumn (Popp et al. 2012). If in areas of elevated radiation dose rates *C. bombi* burden is larger and the time to patency is reduced, this could result in a higher chance of transmission in areas of higher dose rates when compared to areas of low contamination. Transmission is mediated through a combination of contact with flowers (Koch et al. 2017; Cisarovsky & Schmid-Hempel 2014) and/or transmission through nestmates (Otterstatter & Thomson 2007). Previous studies that a shorter time until patency increases the chances of infection in further hosts (i.e. bumblebees in a colony) (Schmid-hempel & Schmid-hempel 1993). Increased burdens and increased transmission of parasites in the wild is likely to temporally advance the size of the epidemic (Ribeiro & Valenzuela 2011). The field data suggest that increased burdens could be either a consequence of impaired resistance or an increased chance of transmission. However, the laboratory data confirm that the increased burden is likely due to decreased host resistance, but this reduced resistance would likely increase transmission in the wild.

In the wild, bumblebees usually contract *C. bombi* within the first two weeks of the start of foraging activity (Brown et al. 2000). The dynamics of infection during the colony's initial stages could determine the ultimate success of the hive because colonies start out with small numbers of workers (Otterstatter & Thomson 2007). Adverse effects of pathogens may lead to increased mortality and food shortages ultimately exacerbating the consequences of infection (Otterstatter & Thomson 2007). In turn, these adverse effects of parasitism could lead to consequences on an ecosystem level by reducing pollination services. There are two fundamental ways increased *C. bombi* burdens could reduce pollination services. Firstly, reduced longevity and higher mortality in workers reduces the number of pollinators overall (Koch & Brown et al. 2017). Secondly, *C. bombi* infection has been shown to shift the floral preferences of bumblebees, for example, iridoid glycoside concentration in nectar which can help self-medicate against infection (Koch & Brown et al. 2017; Richardson et al. 2016; Schmid-Hempel & Stauffer 1998). Further work is necessary to understand the epidemiological implications of alteration in *C. bombi* burden and reduced time to patency for transmission at a population level.

Wing wear in males associated with the stage of colony life cycle at which they were produced

Males bumblebees caught in the Chernobyl Exclusion Zone were excluded from the wing wear analysis. All captured males had a low wing wear score; due to males (and new queens) were produced at the end of the colony life cycle and they do not forage as much as workers (Goulson 2010). The majority of males were caught in September 2016. Therefore, they are younger than workers and all of a similar age. This reaffirms the use of wing wear as a proxy for age. Worker lifespan ranges between 13 and 41 days in the wild (Goulson 2010), and workers are produced for the majority of the colony lifespan until colonies reach a peak level and then switch to producing reproductives in the latter part of the annual colony cycle towards the end of the summer (Goulson 2010). The data support a negative effect of elevated radiation dose rates on lifespan in worker bumblebees but not males. On the one hand, it could be that radiation harms workers, but not males. However, it is more likely that this reflects the fact that males were not as old at the time of sampling.

No reduction of floral resource, community composition and habitat quality with increased radiation

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 μGyh^{-1} but did not detect an effect on plant populations (Evseeva et al. 2009). 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 (Boratyski et al. 2016).

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 (Møller et al. 2016; Geras'kin et al. 2012). 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 such as described as Ballantyne et al. (2017) to understand if there is an ecosystem-level consequence to living in contaminated areas in the CEZ is recommended.

4.8 Conclusion

This study demonstrated that the burden of the gut parasite *C. bombi* was increased, and longevity was reduced in bumblebees by exposure to elevated radiation dose rates demonstrating a relationship between radiation and parasitism. This relationship was supported by data collected in wild bumblebees in the CEZ and under a complementary robust experimental scenario. In the field, reduced longevity was demonstrated by a reduction in the mean age in bumblebees caught in areas with higher dose rates in the CEZ. Furthermore, reduced longevity was supported indirectly by the structural equation model which demonstrated that increased parasitaemia and a shorter time until patency reduces lifespan.

Moreover, exposure to increased radiation dose rates in the presence of *C bombi* parasitaemia leads to more significant weight loss in individual bumblebees. The relationship between radiation and parasitism resulted in adverse fitness consequences which occurred at dose rates lower than previously thought. These findings offer some resolution as to the disparity between the studies investigating radiation effects undertaken in the CEZ and the differences between laboratory and field studies.

Chapter 5

General Discussion

5 General Discussion

The work from this PhD thesis aimed to provide clarity to the controversy surrounding the consequences to wildlife of living in radiologically contaminated areas such as the Chernobyl Exclusion Zone (CEZ). The research in this thesis presents the first contribution to the knowledge of the effects of radiation to bumblebees both as a single stressor and when combined with a common bumblebee co-stressor, the parasite C. bombi. The combined use of complementary laboratory and field studies emphasises the importance of testing field studies under controlled laboratory conditions and vice versa. In the laboratory, exposure of bumblebees to radiation dose rates encompassing those found in the CEZ resulted in the loss of reproductive fitness. The field data demonstrated that when bumblebees were caught in areas with elevated dose rates, mean age, and therefore indicating a reduction in longevity, was reduced. Bumblebees in highly contaminated areas had higher *C. bombi* loads than bumblebees caught in areas where the dose rates were close to UK background. The field results were supported by experimental work in the laboratory which demonstrated that parasite burden was higher and the time between infection and when the parasite was patent in the faeces was reduced in bumblebees exposed to radiation, factors which subsequently resulted in reduced longevity.

5.1 Disconnect in radioecology

In recent years, there has been contention in radioecology regarding the differences between laboratory and a subset of field studies. Laboratory studies predict the effects of radiation exposure to bumblebees only occur at much higher dose rates than occur in the present-day CEZ (ICRP 2008). However, a subset of field studies have detected effects at low dose rates, whereas others have not detected effects. For example, studies investigating the relationship between radiation dose in the CEZ and bumblebee abundance have found a severe adverse effect at dose rates close to UK background (Møller et al. 2012; Møller & Mousseau 2009). This thesis aimed to provide insight into this conflict to understand if these findings are correct and to explore mechanisms that may explain these data. Using the findings in this thesis, I propose three different reasons as to why there is this disagreement in radioecology.

(I) Some laboratory studies may erroneously under-predict the effects of radiation on wildlife exposure conditions which do not represent the environment

(II) Some field studies may overpredict the effects of radiation to wildlife due poor dosimetry and lack of consideration for confounding factors

(III) Some field studies, due design limitations, are poorly placed to detect effects on wildlife.

Laboratory studies under-predict the effects of radiation on wildlife

The work conducted on bees in this thesis suggests that laboratory studies may often under-predict the effects of radiation to wildlife in the natural environment due to the differences in dose exposure, the measured endpoints and use of optimum experimental conditions. Laboratory studies investigating the effects of radiation to invertebrates have typically used acute dose rates and data from pest insect sterilisation programmes (ICRP 2008; Bakri et al. 2005). Endpoints such as sterility or mortality have typically been measured, and these results have been extrapolated to predict what may occur at a lower dose rate such as those found in areas like the CEZ. Extrapolation of effects from high dose rates to low doses may be problematic due to the paucity of the data; resulting in a high degree of uncertainty (Brèchignac & Doi 2009). The problems with extrapolation have been demonstrated in the thesis which is the first study to provide information as to the effects of radiation on bumblebee fitness. In this thesis, radiation exposure resulted in the production of zero queens at the highest dose rate of 2800 µGy h^{-1}. At the lower dose rates, bumblebee colonies still produced queens when exposed to radiation, but there was a measured decline as low as 100 µGy h^{-1}. It is unlikely that extrapolation from previous reproductive fitness data from laboratory studies focussing on sterility and using acute dose rates would have predicted a loss in reproductive fitness mediated by the reduction in the number of queens produced at the end of colony life.

Further uncertainty results from the extrapolation of laboratory studies to the natural environment as laboratory studies frequently maintain the study organism in optimum conditions and use radiation as a single stressor (Brèchignac & Doi 2009). Maintaining organisms in an optimum environment, for example, consistent temperature and *ad libitum* feeding could potentially mask the effects of radiation (Dyck et al. 2005; Folt & Chen 1999). Optimum conditions and single stressors is not a unique problem to studies investigating the effects of radiation, the importance of multiple stressors has been acknowledged in other ecotoxicological fields (Holmstrup et al. 2010). Bumblebees are naturally exposed to a number of stressors, ranging from sub-optimal temperature, foraging pressures, parasites and predation (Klein et al. 2017). The effects of radiation to wildlife has most commonly been considered in a single stressor context even though; there have recommendations to investigate radiation in a multiple pollution context (Vanhoudt et al. 2012; Brèchignac & Doi 2009).

In Chapter 4 of this thesis, the introduction of *C. bombi* as a co-stressor resulted in fitness consequences at dose rates (up to 300 μGy h^{-1}) previously predicted from laboratory studies not to cause harm (ICRP 2008). Longevity was measured in both chapter 2 at a colony, worker and queen level while considering radiation as a single stressor and chapter 4 at a worker level when radiation dose rate was combined with a parasite. Although there are limitations to comparing these two results, a negative association with radiation exposure was detected in chapter 4 but not chapter 2, therefore circumstantially supporting the hypothesis that the fitness consequences to radiation in a multiple stressor context are more damaging compared to considering radiation as a single stressor. The results from chapter 4 further demonstrate the importance of considering co-stressors in a laboratory context to be able to extrapolate to the natural environment. The data from the field suggest that the effects of radiation may occur to bumblebees at lower doses (up to 30 μGy h^{-1}) than in the laboratory. This difference between laboratory and field studies indicates that there are likely additional co-stressors in the natural environment of the CEZ which may further interact with bumblebees causing a greater fitness loss in the field.

Some field studies over predict the effects of radiation to wildlife

The work in this thesis did not find evidence for radiation effects to bumblebees as low as reported in previous field studies (Møller et al. 2012; Møller & Mousseau 2009). The work conducted to improve dosimetry approaches in chapter 3 found that using a handheld monitor gave a reasonable estimate of the external dose rate bumblebees are likely to receive. However, based on the work in this thesis, dose assessments are advocated when undertaking radioecological studies in the CEZ to estimate total dose to organisms. In conclusion, it is not likely that a poor approach to dosimetry is the reason studies detect effects at low radiation dose rates to bumblebees in the CEZ.

The data presented in this thesis disagrees with two specific papers which have studied bumblebee abundance in the CEZ and detected effects at low dose rates (Møller et al. 2012; Møller & Mousseau 2009). I propose that thie disparity between the work conducted in this thesis and these field studies is due to a lack of considerations for confounding factors such as habitat suitability. The work in this thesis selected comparable sites which were suitable for bumblebee nesting and foraging. During the fieldwork in the CEZ for chapter 4, I did not find comparable, or suitable sites where bumblebees would be likely to forage and nest at the radiation dose rates reported in these studies (Møller et al. 2012; Møller & Mousseau 2009). Habitat type was categorised into agricultural grasslands, deciduous forest or coniferous forest (Møller et al. 2012; Møller & Mousseau 2009). These different habitats will inherently influence bumblebee abundance and biodiversity (Kaluza et al. 2018). The abandoned agricultural land in the CEZ is now a rich habitat for bumblebees for both nesting and foraging because succession has resulted in an abundance of wild flowering plants as shown in chapter 4. Nearly all the areas with the highest radiation dose rates are located in forested areas dominated by Scots pine (*Pinus sylvestris)* (Laćan et al. 2015; Evangeliou et al. 2014). At lower dose rates there are some patches in the agricultural fields with young pines and birch (Evangeliou et al. 2014), and urban forests in the abandoned town of Pripyat consist of fast-growing Lombardy poplars (*Populus nigra*) and horse chestnut *(Aesculus hippocastaneum)* (Laćan et al. 2015).

In Møller et al. (2012) and Møller et al. (2009), point counts were undertaken across the CEZ, but I contend that the type of habitat surrounding the area where the bumblebee abundance counts were made will strongly influence bumblebee abundance and diversity. Previous research has demonstrated that bumblebee abundance and species richness significantly declines with increasing forest cover (Diaz-Forero et al. 2013; Winfree et al. 2007) and agricultural fields have the highest bumblebee abundance compared to extensive forest and suburban developments (Diaz-Forero et al. 2013; Winfree et al. 2007). Observing bumblebee abundance at sites which do not have comparable habitat is likely to confound the association with radiation dose rate. The general lack of consideration for habitat type and quality in radioecology studies, therefore, potentially confounding the associations with radiation exposure does not only apply to bumblebee studies. Research has shown that pine tree plantations with limited tree diversity have significantly lower species richness for a range of taxa and biodiversity (Bremer & Farley 2010). Studies measuring large mammal abundance across the CEZ will also be significantly influenced by habitat quality and similarly to (Møller et al. 2012; Møller & Mousseau 2009), have identified the general habitat in the study area (Webster et al. 2016; Deryabina et al. 2015; Moller & Mousseau 2013).

Some field studies may be poorly designed to detect effects of radiation to wildlife

The studies conducted by Møller et al. (2012) and Møller & Mousseau (2009) mentioned above, which detect adverse effects of radiation in the CEZ at very low dose rates conflict with studies, also based in the CEZ which do not detect effects of radiation, even at the highest dose rates. Therefore, I propose that it may also be the case that some field studies are designed so that they do not have enough power detect an effect of radiation.

The lack of power in ecological studies is a well-acknowledged problem (Lemoine et al. 2016; Martínez-Abraín 2008), and this is particularly relevant for radioecological studies in an area such as the CEZ where sites are frequently selected because of the limitations of areas with an increased radiation dose rate. This problem is exacerbated when studying aquatic animals as there are a limited number of lakes in the CEZ and fewer with a range of radiation dose rates. Low statistical power in ecological studies increases the chances of committing a type II error (Lemoine et al. 2016). There is a general confusion when sampling units of observation and units of inference are confused leading to pseudoreplication and lack of independence. The actual sample size of a study is the number of observations of that unit of inference (Steel et al. 2013). Multiple observations of samples within each unit of inference (e.g. site or pond) are not independent. Recent studies in the CEZ which do not detect an effect of radiation have relatively low sample sizes. For example, n = 6 lakes were sampled for freshwater invertebrates (Fuller et al. 2018), n= 7 (Baker et al. 2001) and n= 3 (Matson et al. 2000) sites were sampled for small mammals in the CEZ and outside the CEZ as controls. Fluctuating asymmetry requires a large sample size to be able to detect an effect (Fuller et al. 2017) sampled aquatic invertebrates across a dose gradient of 0.064 to 27 µGy h-1. A mean of 300 invertebrates were sampled from 6 ponds across the CEZ. However, the ponds are the units of independent samples, and therefore this is unlikely to have enough power to detect an effect, therefore could result in a type II error (Grabowski & Porto 2017; Steel et al. 2013; Nakagawa & Cuthill 2007).

Reduced power in radioecological studies with limited independent sampling units highlight the difficulty of conductin ecological studies in the CEZ. One solution is the use of complementary laboratory studies to test whether the effect or lack of effect is reproducible under experimental conditions. In the field study in chapter four, twenty sites were sampled for bumblebees and floral resource over three different sampling periods this was to test that the habitat was similar, to ensure these habitat quality metrics did not co-vary with radiation dose rate and also ensure there was enough statistical power in the experimental design. In chapter four field study was replicated in the laboratory under robust experimental conditions and recapitulated the field result therefore giving greater confidence in the field study.

5.2 What do these studies mean for radiological protection of the environment?

At an international level, the work in this thesis provides the first low dose information to test the allocation of the DCRL (Derived Consideration Reference Level) set by International Commission on Radiological Protection (ICRP) for the Reference bee. The current DCRL, which predicts where there is likely to be an adverse effect of radiation for bees is currently set between 400 – 4000 μGy h^{-1}. As stated in chapter 2, I advocate that the DCRL be reduced to 40 - 400 μGy h^{-1} which would put bumblebees in the same radiosensitivity band as fish, frogs and grass (ICRP 2008). The reallocation of the DCRLs is reaffirmed by the work conducted in chapter 4 of this thesis, providing further evidence that bumblebees suffer adverse effects of radiation at lower doses than previously anticipated. The DCRL for the reference bee was allocated on a precautionary basis by the ICRP, as there were no data available at the lower doses to inform this placement. The data in this thesis provide the first data to test this placement, detecting effects at substantially lower dose rates than previously measured. The majority of the laboratory studies which have contributed data towards the placement of the DCRLs have used acute dose rates and have not been conducted on bees (ICRP 2008). The work in this thesis included substantially lower dose rates ranging from 10 μGyh^{-1} to 3000 μGyh^{-1} to include dose rates found in the CEZ and the dose rates of the DCRL. The lowest dose rate in the laboratory that an adverse effect of radiation was detected was at 100 μGy$h^{-1,}$ which resulted in a 6% decline in reproduction.

The DCRLs are used for decision-making processes and apply to different exposure situations (Copplestone et al. 2015; Strand et al. 2009). The ICRP has three scenarios to describe exposure situations; planned, existing and emergency (ICRP 2007). Everyday situations which are regulated such as decommissioning, management and disposal of radioactive waste and remedying contaminated land are classed as planned exposure situations (ICRP 2007). An existing exposure situation exists when a decision on control has to be taken, which include residues from past practices which operated outside the ICRP’s recommendations, for example, the present-day CEZ (ICRP 2007). An emergency exposure situation occurs during the operation of practice and requires urgent action, for example, when the Chernobyl Nuclear Power Plant exploded (ICRP 2007).

There are different consequences of the findings of this thesis to each scenario. The dose rates at which effects were detected are likely to be higher than those that occur in planned exposure situations as these dose rates are only likely to be present in sites of existing and emergency exposure situations. However, the DCRLs do influence values used for dose assessments to non-human biota; these dose assessments are conducted by regulators prospectively when assessing regulated releases in planned exposure situations to ensure the protection of wildlife, particularly Natura 2000 sites under the Birds and Habitats Directive (EC 2010; EC 1992). The ERICA tool is frequently used to conduct dose assessments to assess the risk to wildlife and uses screening values to ensure protection of the environment of 10 μGyh^{-1} (Larsson 2008; Zinger et al. 2008; Brown et al. 2008). The studies in this thesis did not detect effects below 10 μGyh^{-1}, therefore, confirming that the conservative 10 μGyh^{-1} screening value and the current regulatory approach is fit for purpose.

The UK Government, Department for Business, Energy and Industrial Strategy (BEIS) have proposed new build nuclear power stations and the development of a Geological Disposal Facility (GDF) in the UK (BEIS 2018; DECC 2014). The GDF will provide a storage solution deep in the ground for higher activity radioactive wastes. (BEIS 2018; DECC 2014). There is a site selection process underway for the GDF which will require detailed dose assessments to ensure that people and the environment are protected. The work in this chapter supports previous assessments that there will no discernible impact on the environment from the GDF, as calculated from the ERICA Tool (Torudd & Saetre 2013).

5.3 What are the implications of this work for the population level?

It is essential to consider to what extent the effects in this thesis will cause damage to bumblebee populations living in the CEZ. In chapter 2, there was a 6% decrease of reproduction at 100 µGy h^{-1} and 84% decrease in reproduction at 2800 µGy h^{-1}. It is probable that exposure to 2800 µGy h^{-1} would cause significant population declines in bumblebees, however, this dose rate it highly unlikely to be found in the environment and was only present immediately after the Chernobyl accident. The 6% decrease in queen production, however, is more negligible. Research has demonstrated that a 26% reduction in the number of queens laying effects will likely lead to cause population extinction (Baron et al. 2017). For bumblebee populations to survive, a colony needs to produce at least one new queen who successfully founds a colony the next year, in the absence of density dependence (Baron et al. 2017). The authors of this study acknowledge that their estimates are likely conservative. The estimates from the laboratory study in chapter 2 were acknowledged to be conservative due to optimum colony conditions when compared to the natural environment. In conclusion, although a 6% decrease in reproduction is relatively small, the effect size may be much more significant in the CEZ when co-stressors are considered, and therefore at 100 µGy h^{-1} it is possible that this dose rate will impact bumblebee populations.

Bombus spp. bees contract *Crithidia bombi* either from their nest mates or by foraging on contaminated flowers (Yourth & Schmid-Hempel 2006). Although *C. bombi* is highly prevalent in the environment, laboratory experiments have shown that it is relatively benign (Yourth & Schmid-Hempel 2006). In the CEZ based field study, chapter 4 determined that *C. bombi* load was higher in bumblebees from areas with higher contamination. This was supported by the laboratory-based study which demonstrated that bumblebees exposed to high radiation levels had high *C. bombi* loads. Laboratory studies have shown that increased *C. bombi* will cause fitness loss when bumblebees are exposed to stressful conditions, *C. bombi* exhibits condition dependent virulence (Brown et al. 2000). However, it is unknown what impacts bumblebee parasites have on fitness in the wild but it is hypothesised that increased parasite prevalence is a contributing factor to be wild bee declines (Goulson et al. 2015). When the bumblebee is exposed to a stressor, the parasite reduces longevity and can affect reproduction. In chapter 4, I demonstrated that exposure to low dose chronic ionising radiation and exposure to the parasite *C. bombi* resulted in fitness loss in bumblebees. Individuals had reduced longevity and increased the weight when exposed to both stressors. Increased virulence under environmental stress will have implications at a population level. Other reported consequences of the increased virulence in *C. bombi* are increased weight loss in hibernating queens, reduced colony founding success, colony size, male production and reduction in overall fitness by up to 40% (Brown et al. 2003).

There is no reason to assume that bumblebees are more inherently radiosensitive than other insects. The eusociality life history has been shown to make bumblebees more resilient against environmental stressors (Straub et al. 2015), as colonies can tolerate the loss of workers as long as reproduction is maintained. Radiosensitivity variation between different insects has been recorded in the IDIDADS dataset: Acrididae and Blaberidae were shown to be significantly less radioresistant than other insects by an order of magnitude (Bakri et al. 2005). The use of low chronic dose rates, sensitive endpoints and co-stressors in both a laboratory and field studies is necessary to understand what is happening in the environment, and therefore as a result of the work in this thesis, I advocate similar studies using multiple stressors to be undertaken of other invertebrates species to test whether bumblebees are more radiosensitive than other invertebrates.

5.4 The benefits of taking a more holistic approach to measuring the effects of radiation on bumblebees

The work in this thesis undertook a holistic approach to understand the effects of low dose chronic ionising radiation to bumblebees and examined some of the endpoints recommended by the ICRP, such as reproduction, longevity as well as additional fitness measurements such as condition. Instead of using acute radiation exposure and binary endpoints such as mortality and sterility, experiments were designed to identify alterations to life history traits. For example, effects were investigated at both a colony level and individual level. Colonies that were exposed to increased radiation dose rates produced fewer queens than those not exposed; it was only at 3000 μGy h^{-1} that reproduction failed completely. Furthermore, when testing whether radiation dose rate and *C. bombi* infection synergistically interacted, the data demonstrated that longevity was reduced and mediated by a high parasite burden and the reduction in time from infection to patency.

In the field, bumblebees did not live as long when exposed to increased radiation dose rates. This reduction in longevity and therefore fitness, differs from previous laboratory studies which have measured mortality so as to determine the LD_{50} values. Reductions in fitness are more relevant when understanding how chronic low dose radiation can affect a population (Buechel & Schmid-hempel 2016). To provide a more holistic view of the CEZ, in the field, floral resource quality measurements were taken across the sites at which bumblebees were sampled to investigate whether floral habitat quality co-varied with radiation dose. There was no association between floral traits at each site and radiation dose.

The experiments conducted in this thesis provide a stepping stone towards the ecosystem approach to the radiological protection of the environment which has been recently advocated (Bradshaw et al. 2014; Brechignac 2016; Brèchignac & Doi 2009). Individual-level endpoints as used in the RAP approach are not sufficient to gain a full understanding of what may be occurring in the natural environment of the CEZ. The next step which could improve the understanding of these effects at a population or ecosystem level in the CEZ would use the application of Bayesian modelling techniques to calculate the probability of population extinction, as presented in Baron et al. (2017). Investigating whether exposure to increased radiation dose rate alters pollination services in the CEZ by a combination of field studies and testing under experimental conditions would be a useful natural progression of this work to gain understanding at an ecosystem level.

5.5 Conclusion

This thesis provides the first detailed investigation of the effects of low dose chronic ionising radiation on bumblebees. The combination of laboratory and field studies reveals that reductions in fitness rather than the binary endpoints of mortality or sterility occur at lower doses than previously anticipated. From this, I advocate a reduction in the International Commission on Radiological Protection reference level (DCRL) for the bee RAP and therefore reduce the dose band at which negtive effects are predicted from 4,000- 400 μGy h^{-1} to 400- 40 μGy h^{-1} which would place the Reference bee dose band at the same level as grass, frogs and fish. The work in this thesis also supports the screening value of 10 μGy h^{-1} used in regulatory assessments to ensure the environment is protected.

To test whether controversy in radioecology is likely a result of a poor approach to dosimetry, I tested different ways of measuring radiation dose to bumblebees living in the heterogeneously contaminated CEZ. The data demonstrated that commonly-used handheld devices give a reasonable estimate to bumblebees, and therefore are not likely to be the reason some studies detect effects at low dose rates.

With regards to the controversy in radioecology, the addition of multiple stressors in experiments and the use environmentally relevant dose rates in laboratory studies demonstrate that bumblebees suffer a reduction in fitness at lower dose rates than previously anticipated. However, the effects presented in this thesis were not measured at dose rates as low as those predicted in other studies and questions are raised about confounding factors in their experimental design.

In conclusion, bumblebees in the CEZ suffer a loss of fitness as both a direct effect of radiation and as a result of synergistic interactions between natural stressors therefore exacerbating the consequence to both the individual, the colony and potentially the population.

Chapter 6

Appendix

6. Appendix

Contamination maps of the Chernobyl Exclusion Zone

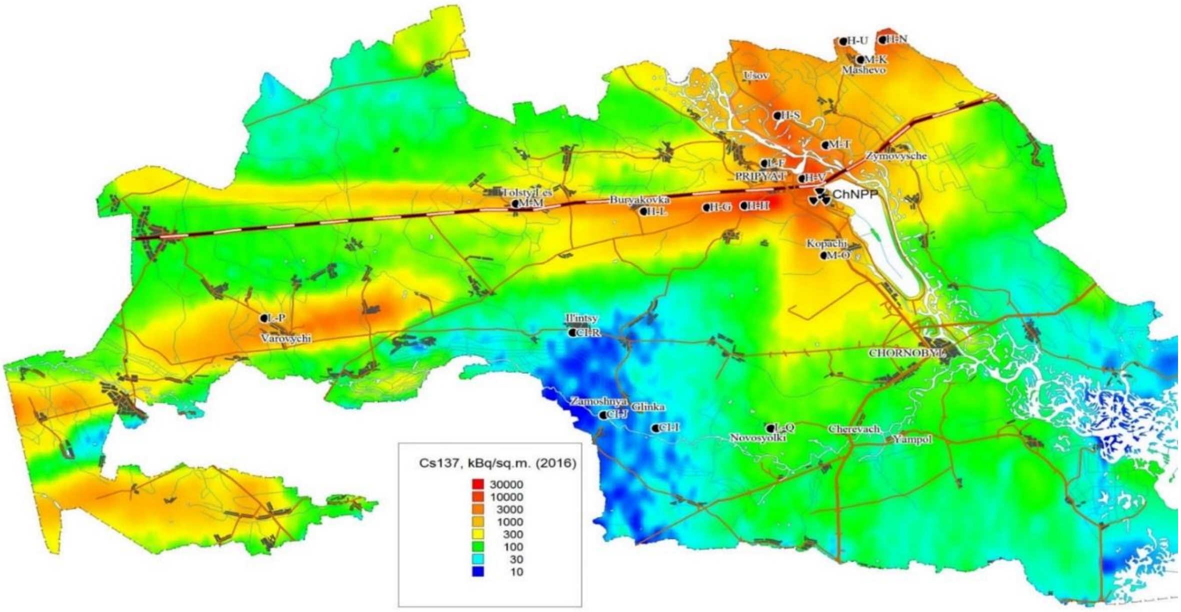

Figure 6.1 Map of ^{137}Caesium contamination of the Chernobyl Exclusion Zone with sites located (Sergey Gaschak)

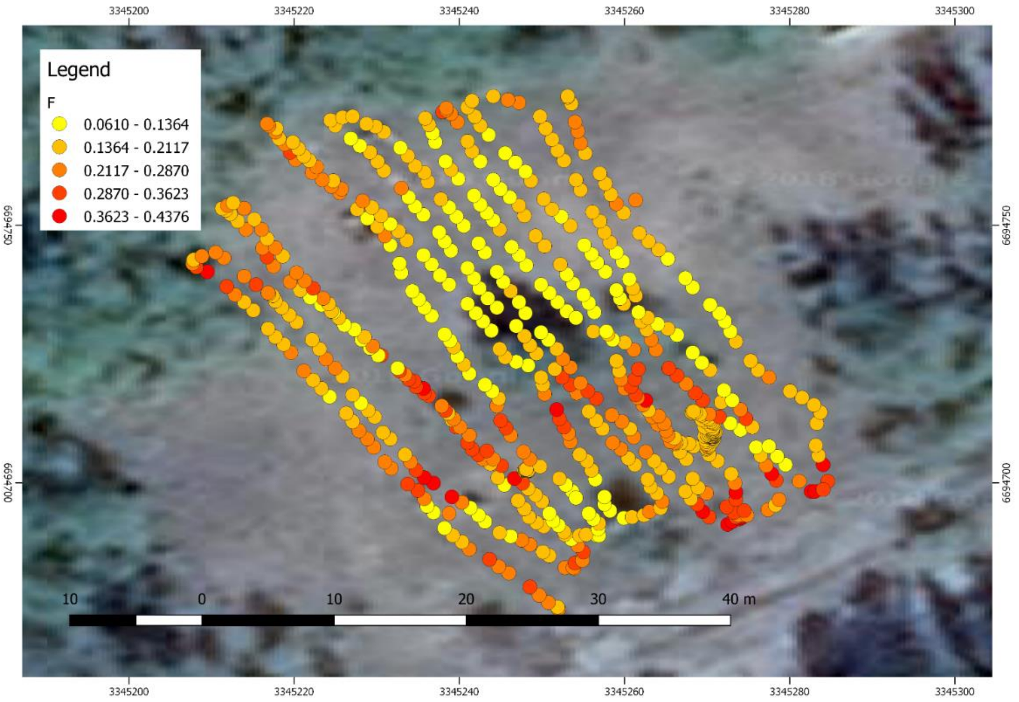

Figure 6.2 Map of site F created using data from Mobile gamma spectrometry system (MoGSS) for ^{137}Cs dose rate (μGyh^{-1})

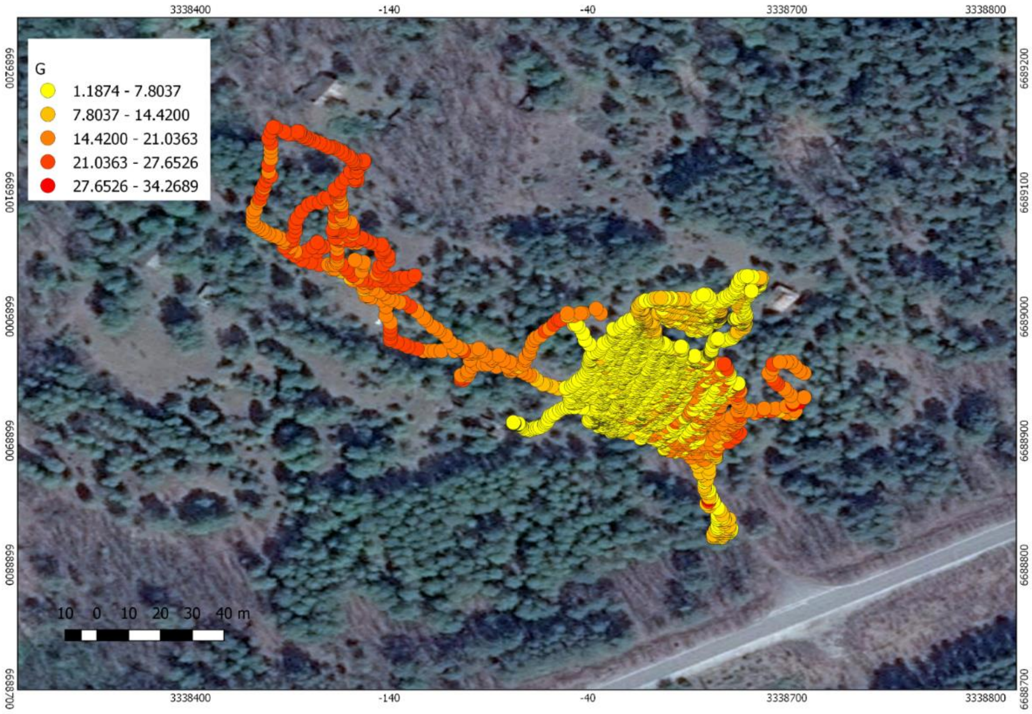

Figure 6.3 Map of site G created using data from Mobile gamma spectrometry system (MoGSS) for ^{137}Cs dose rate (μGyh^{-1})

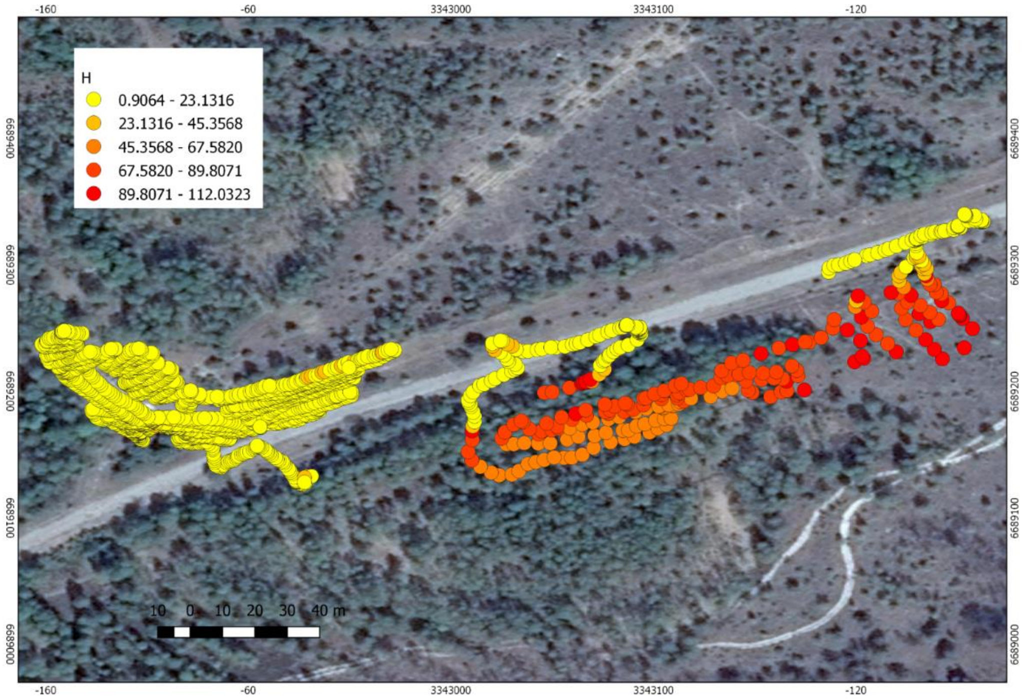

Figure 6.4 Map of site H created using data from Mobile gamma spectrometry system (MoGSS) for ^{137}Cs dose rate (µGyh^{-1})

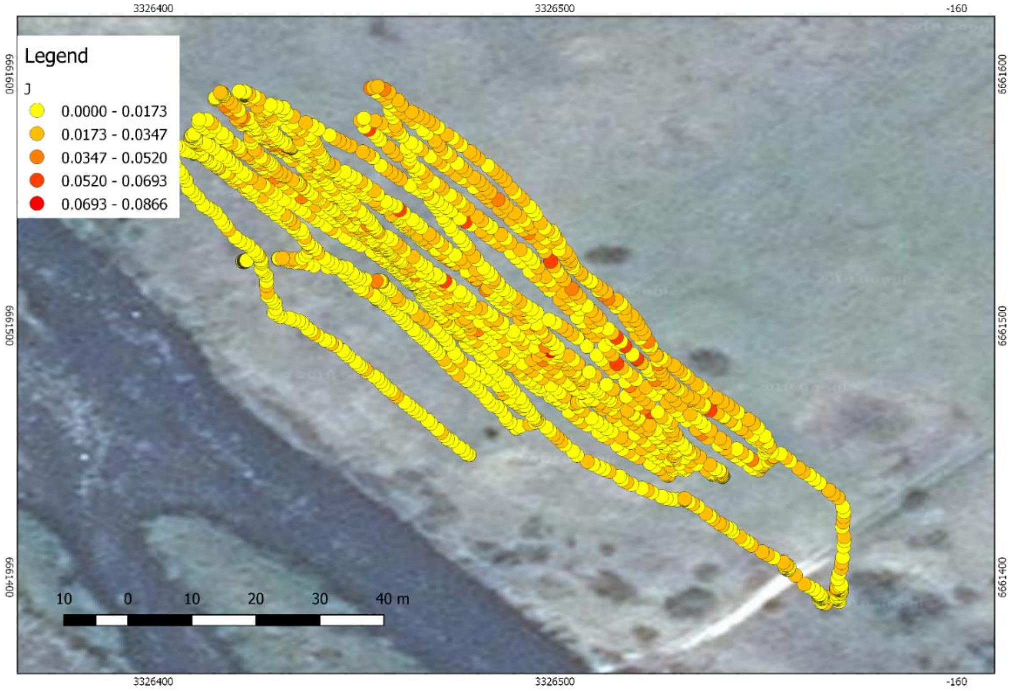

Figure 6.5 Map of site J created using data from Mobile gamma spectrometry system (MoGSS) for ^{137}Cs dose rate (µGyh^{-1})

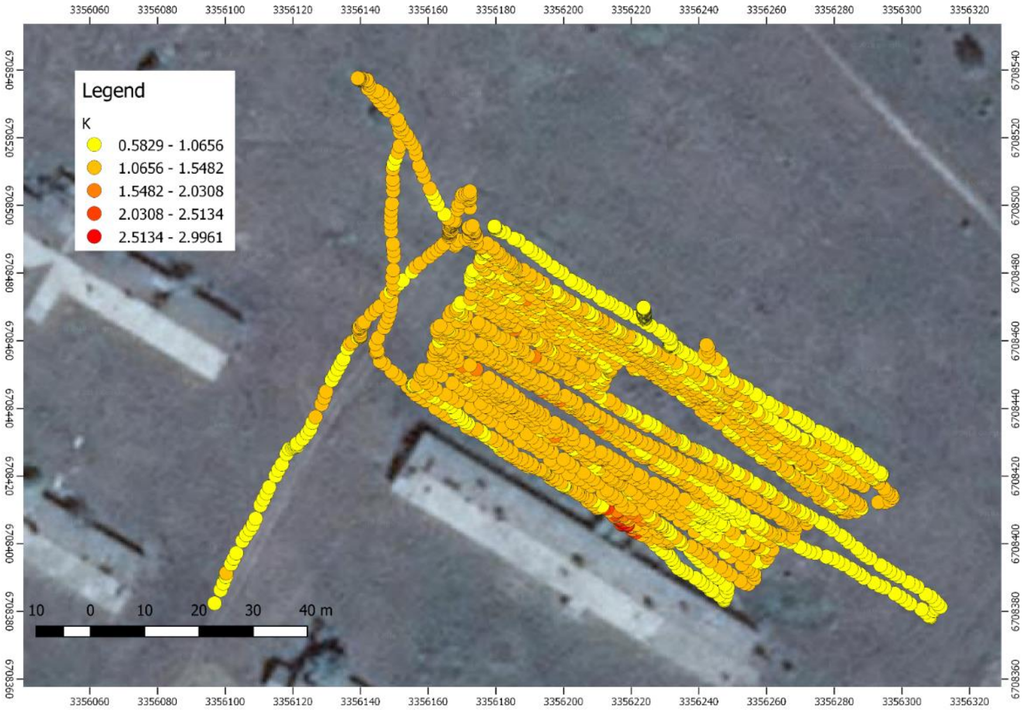

Figure 6.6 Map of site K created using data from Mobile gamma spectrometry system (MoGSS) for ^{137}Cs dose rate (µGyh^{-1})

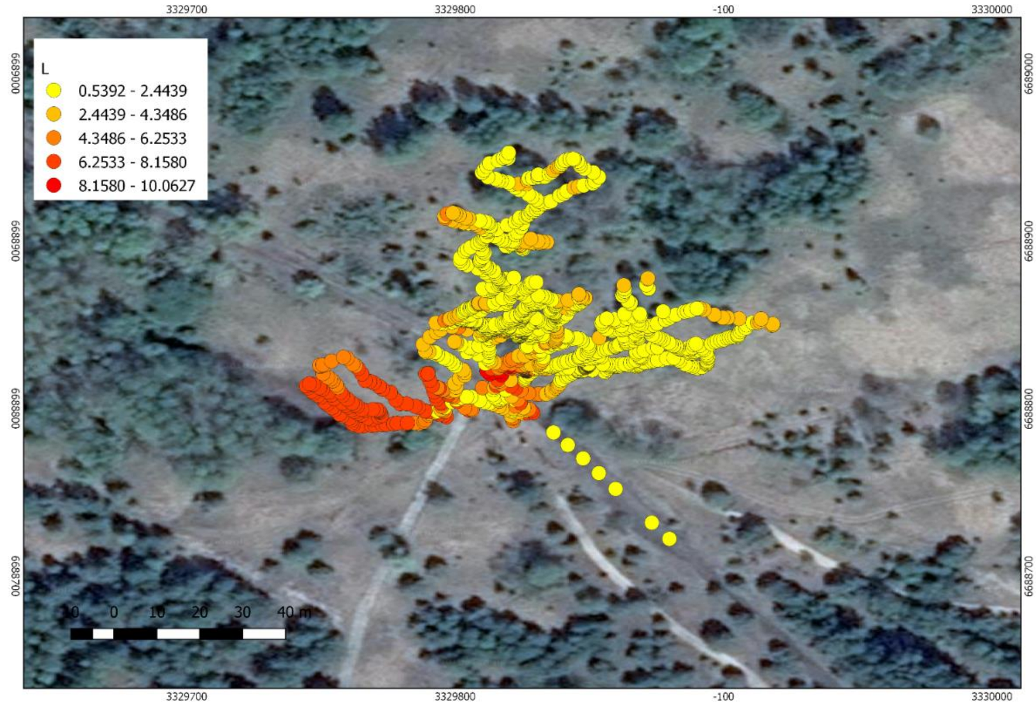

Figure 6.7 Map of site L created using data from Mobile gamma spectrometry system (MoGSS) for ^{137}Cs dose rate (µGyh^{-1})

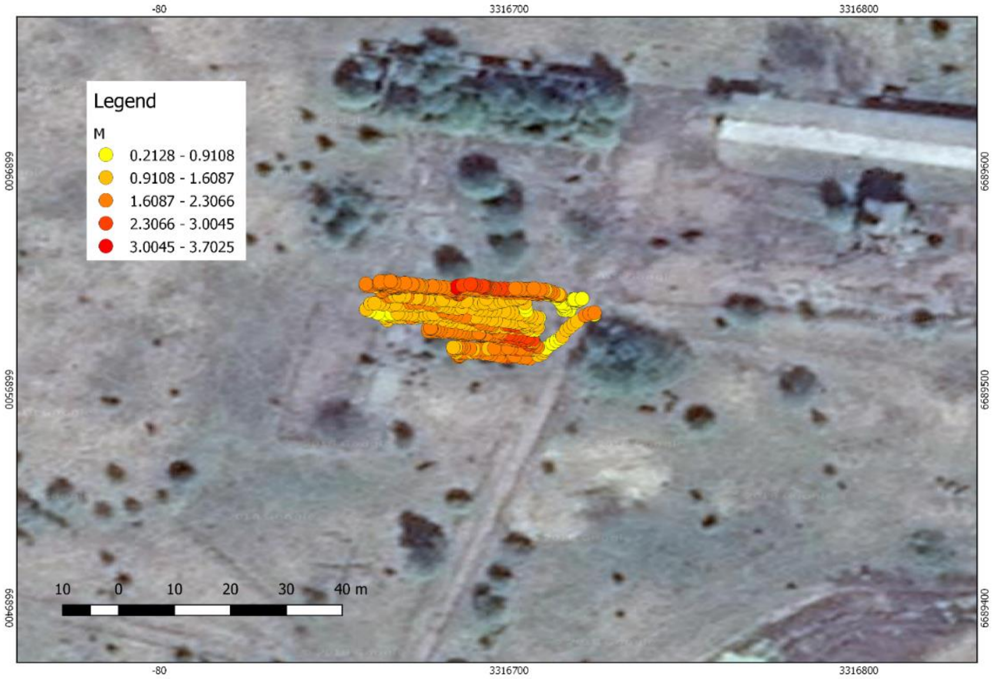

Figure 6.8 Map of site M created using data from Mobile gamma spectrometry system (MoGSS) for ^{137}Cs dose rate (µGyh^{-1})

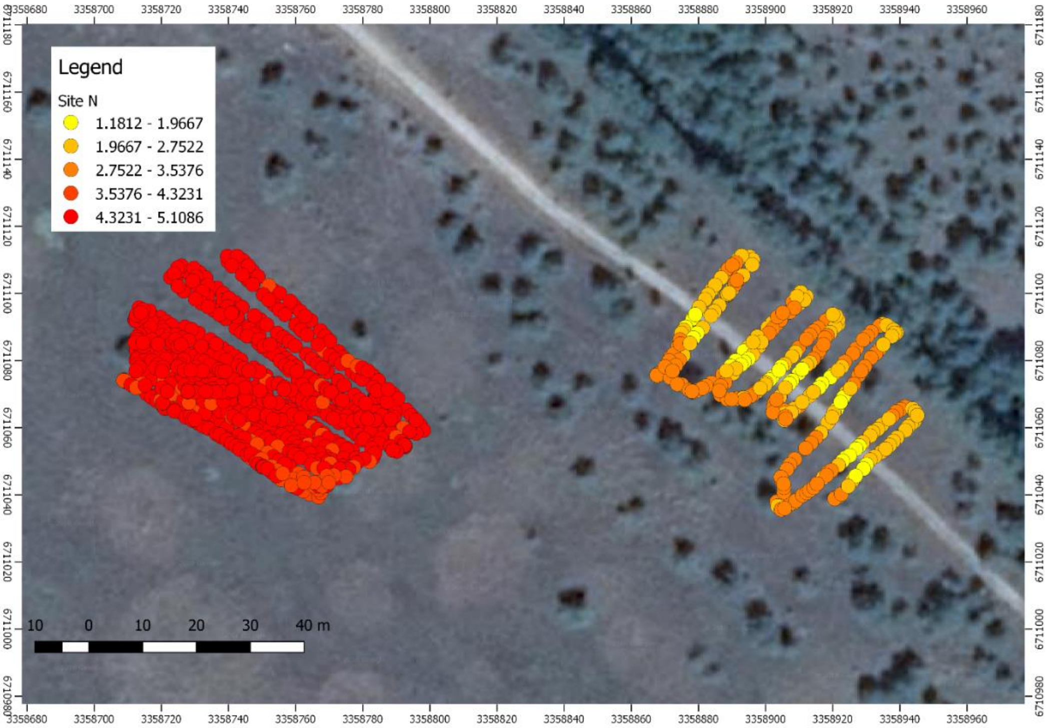

Figure 6.9 Map of site N created using data from Mobile gamma spectrometry system (MoGSS) for ^{137}Cs dose rate (µGyh^{-1})

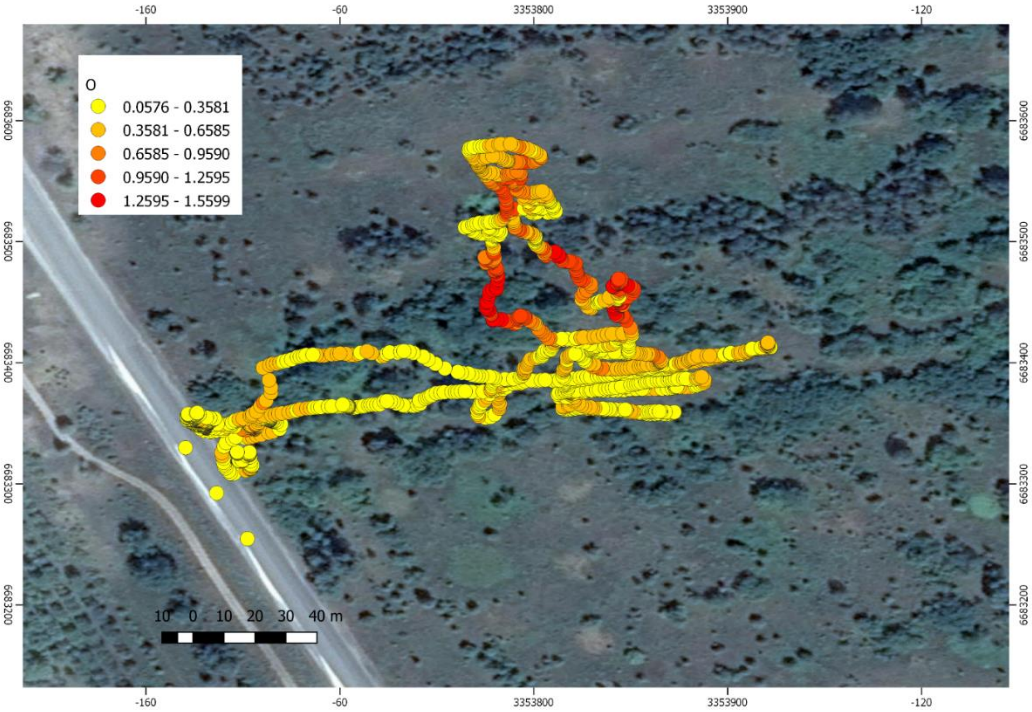

Figure 6.10 Map of site O created using data from Mobile gamma spectrometry system (MoGSS) for ^{137}Cs dose rate (µGyh^{-1})

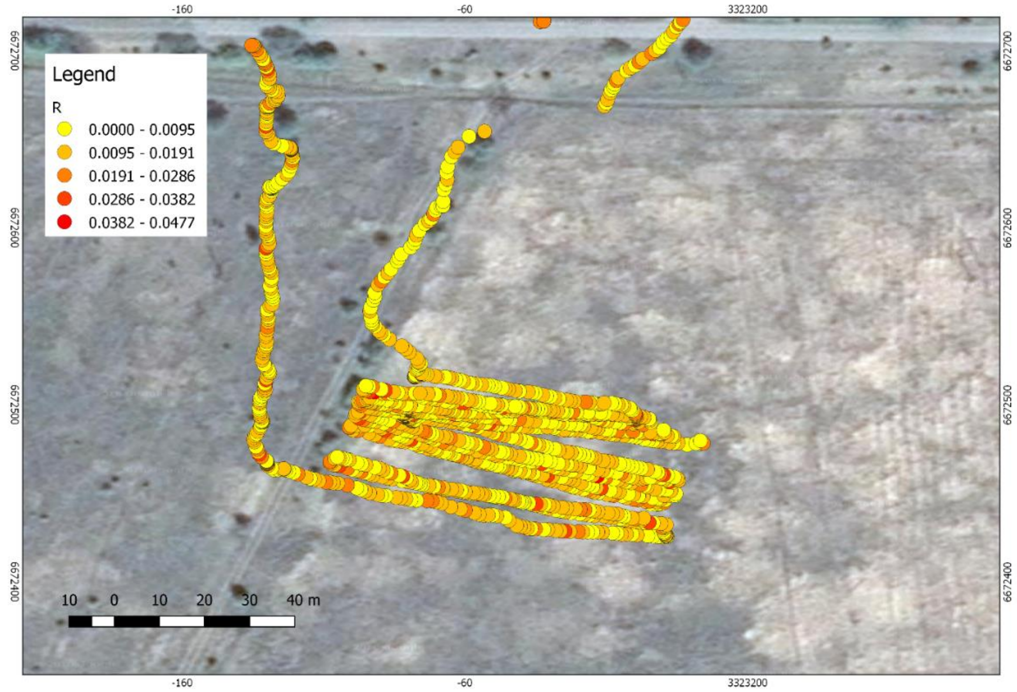

Figure 6.11 Map of site R created using data from Mobile gamma spectrometry system (MoGSS) for ^{137}Cs dose rate (µGyh^{-1})

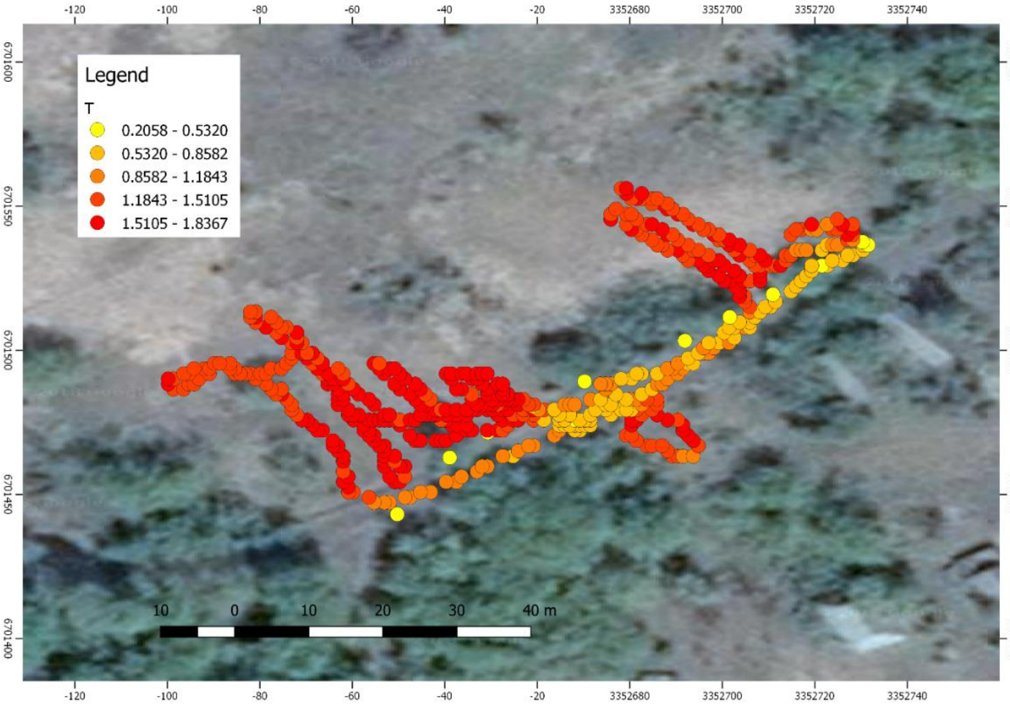

Figure 6.12 Map of site T created using data from Mobile gamma spectrometry system (MoGSS) for ^{137}Cs dose rate (μGyh^{-1})

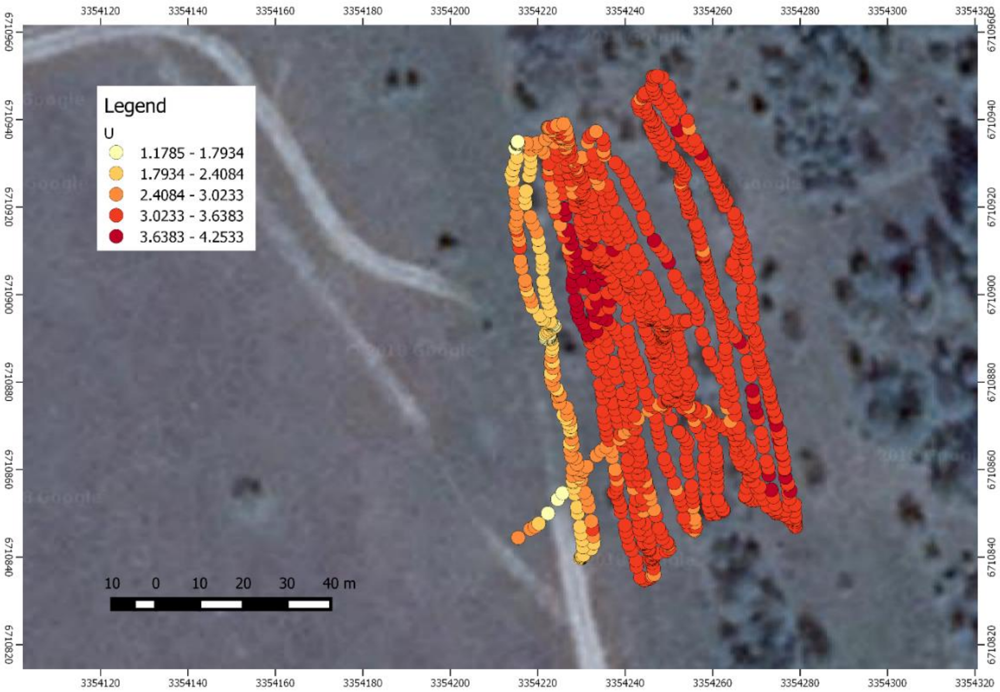

Figure 6.13 Map of site T created using data from Mobile gamma spectrometry system (MoGSS) for ^{137}Cs dose rate (μGyh^{-1})

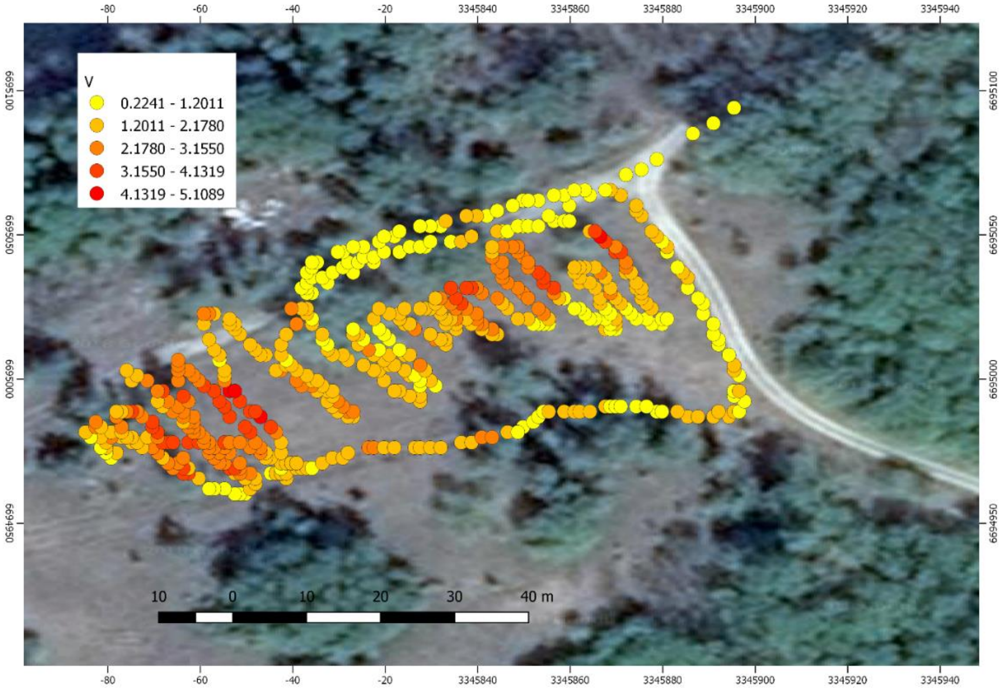

Figure 6.14 Map of site V created using data from Mobile gamma spectrometry system (MoGSS) for ^{137}Cs dose rate (μGyh^{-1})

Table 6.1 Results from the three different techniques measuring external gamma ambient dose rate; portable sodium iodine detector (MoGSS), shielded thermoluminescent dosimeters (TLD) in the ground and external hand-held monitor for all sites in the Chernobyl Exclusion Zone

	MoGSS					**TLD**		**External**
Site name	**Number of counts**	**Minimum dose rate**	**Average dose rate**	**95% percentile**	**Maximum dose rate**	**Average dose rate 10cm**	**Average dose rate 30cm**	**Average external dose rate**
F	2095	0.04	0.97	3.103	6.60	1.54	0.213	1.32
G	3238	1.18	8.65	21.59	34.26	10	4.148	27.67
H	2144	0.66	15.53	75.04	112			53.47
I	3155	0.000	0.015	0.030	0.081			0.08
J	2084	0.000	0.015	0.02	0.086	0.08	0.002	0.11
K	2626	0.582	1.183	1.562	2.996	1.53	0.106	5.86
L	3270	0.488	2.588	5.421	10.06	8.63	0.550	14.88
M	2095	0.212	1.754	3.027	3.702			9.75
N	1037	1.181	4.00		5.102			19.92

Site name	**Number of counts**	**Minimum dose rate**	**Average dose rate**	**95% percentile**	**Maximum dose rate**	**Average dose rate 10cm**	**Average dose rate 30cm**	**Average external dose rate**
O	3903	0.009	0.327	0.894	1.559			2.16
P	1783	0.096	0.179	0.245	0.388			1.17
Q	900	0.005	0.231	0.339	0.432			0.31
R	1442	0.000	0.011	0.023	0.477			0.09
S	745	0.193	1.276	1.697	1.834			19.12
T	792	0.041	0.857	1.627	1.777			5.03
U	1572	1.044	2.971	3.590	4.253			9.43
V	682	0.224	4.239	8.997	12.00			15.48
Y	1113	0.316	2.581	6.951	11.16			0.31
Z	2040	0.063	0.811	1.174	1.415			0.11

Table 6.2 Results from the two different techniques measuring external beta ambient dose rate; thermoluminescent dosimeters (TLD) and Electra probe system for all sites in the Chernobyl Exclusion Zone

Site name	Average TLD beta dose rate 10cm	Average TLD beta dose rate 30cm	Average Electra (beta) dose rate	Minimum Electra (beta) dose rate	Maximum Electra (beta) dose rate
F	0.934	0.213	4.325	1.3	6.3
G	17.52	5.869	100.7	20.25	185
H			104.7	19.5	261
I			0.4	0.247	0.553
J	0.013	0.08	0.220	0.1	0.40
K	3.869	1.257	10.16	7.125	16.12
L	0	2.491	26.47	13.37	50.5
M			31.93	12	71.12
N			24.87	20.5	28.37
O			11.43	3.46	19.37
P			0.81	0.4	1.39
Q			1.05	0.35	2.05
R			0.21	0.14	0.32
S			68.89	20.25	112.6

Site name	**Average TLD beta dose rate 10cm**	**Average TLD beta dose rate 30cm**	**Average Electra (beta) dose rate**	**Minimum Electra (beta) dose rate**	**Maximum Electra (beta) dose rate**
T			16.73	8.25	26.37
U			21.4	12.75	33.12
V			224.3	10.75	377.8
Y			1.24	0.63	2.4
Z			0.31	0.23	0.383

Flowering species of Chernobyl for Chapter 4

Table 6.1 Flowering plant species identification for all sites and the number of times across sites each flowering plant species was recorded

Flower species	2015	2016	Flower species	2015	2016
Achillea millefolium	16	0	*Lythrum salicaria*	1	0
Ajuga genevensis	0	1	*Melandrium album*	0	5
Ajuga reptans	0	2	*Moehringia trinervia*	0	12
Arabidopsis thaliana	0	2	*Myosotis arvensis*	3	1
Arabis glabra	0	7	*Myosotis ramosissima*	0	34
Berteroa incana	0	1	*Myosotis stricta*	0	41
Brassica oleracea	0	4	*no flowers*	0	20
Campanula patula	0	15	*Nonea pulla*	0	13
Campanula rotundifolia	1	0	*Oenothera biennis*	4	0
Cardueae	1	0	*Polygala vulgaris*	0	19
Carum carvi	1	0	*Potentilla argentea*	0	13
Centaurea	30	0	*Potentilla erecta*	0	2
Centaurea maculosa	2	0	*Pulicaria dysenterica*	2	0
Chamaenerion	6	0	*Ranunculus acris*	0	36
Coronaria floscuculi	0	12	*Rhinanthus minor*	1	9
Crepis	13	0	*Scabiosa caucasica*	4	0
Crepis biennis	0	5	*Sedum acre*	0	4
Crepis capillaris	1	0	*Sisyrinchium montanum*	0	11
Crepis tectorum	0	3	*Stellaria fragilis*	0	23
Dianthus carthusianorum	2	0	*Stellaria media*	0	9
Echinops sphaerocephalus	4	0	*Tanacetum vulgare*	2	0
Echium vulgare	1	0	*Taraxacum*	2	0
Epilobium parviflorum	1	0	*Thymus serpyllum*	1	0
Erodium cicutarium	0	3	*Tnagopogon major*	0	1
Erysimum cheiranthoides	0	1	*Trifolium dubium*	0	16
Euphorbia esula	0	1	*Trifolium montanum*	0	1
Filipendula denudata	0	1	*Trifolium pratense*	0	9
Galium palustre	0	4	*Trifolium repense*	0	1
Galium saxatile	7	0	*Valeriana officinalis*	0	1
Galium verum	11	0	*Verbascum nigrum*	5	0
Genista tinctoria	3	0	*Verbascum thapsus*	8	0
Glechoma hederacea	0	1	*Verbena hastata*	14	0
Gratiola officinalis	0	1	*Veronica chamaedrys*	0	4
Hireaceum pilosella	0	16	*Veronica prostrata*	0	1
Hypericum perforatum	13	0	*Vicia americana*	2	0
Hypochaeris radicata	0	7	*Vicia cracca*	3	16
Iris sibirica	0	1	*Vicia tetrasperma*	0	19
Jacobaea vulgaris	3	0	*Viola odorata*	1	0
Jasione montana	15	0	*Viola tricolor*	0	86
Lamium purpureum	3	0			
Leontodon hispidus	0	10			
Leucanthemum vulgare	5	0			
Linaria vulgaris	6	0			

Appendix wing wear scores

Figure 6.15 Bee wing with a score of 1

Figure 6.16 Bee wing with a score of 2

Figure 6.17 Bee wing with a score of 3

Figure 6.18 Bee wing with a score of 4

Figure 6.19 Bee wing with a score of 5

Figure 6.20 Bee wing with a score of 6

Figure 6.21 Bee wing with a score of 7

Chapter 7

Reference List

7. Reference list

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