Investigating the impacts of Chernobyl-level radiation exposure on insect life history traits

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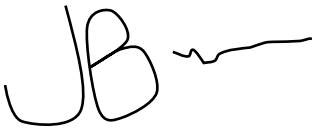
Statement of Originality

I hereby confirm that this thesis is an original piece of work conducted independently by Jessica Emily Burrows. I declare that all work contained is as a result of my own research and has not been submitted for any other degree. Where appropriate research material has been acknowledged and cited.

Signature of Candidate:



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Summary Abstract

The majority of our understanding of the effects of radiation are extrapolated from acute high-dose exposures or originate from human based studies. For example, chronic human exposures are well studied from external sources as well as internal e.g. radon in drinking water. We know comparatively less about the effects of radiation on non-human biota, especially those exposed to lower dose rates. This thesis consequently focused on the effects of dose rates found in some areas of the Chernobyl Exclusion Zone (CEZ), a heterogeneously radiologically contaminated landscape where dose rates range from 0.1 to 250 µGy h^{-1}.

This thesis primarily used the bumblebee *Bombus terrestris* as a model study system, to investigate whether low dose rates affect key life history traits in a vital pollinator. The dose rates used in these laboratory-based studies were between 40 and 200 µGy h^{-1}. I focused on key life history traits in order to provide a generalisable measure that could be used to investigate the effects of these dose rates in a consistent way across different species.

I found low dose radiation exposure causes a substantial change in bumblebee energy budgets through an upregulation in resource acquisition and metabolic rate. This change is further evidenced by a dramatic increase in haemolymph sugar concentrations at dose rates as low as 40 µGy h^{-1}. The gut microbiome was however largely unchanged by this increase in nectar, but did show an increase in microbial species richness in response to radiation. A response to chronic low doses of radiation was not unique to bumblebees as they also led to a significant increase in the number of eggs produced by *Drosophila melanogaster* within just 18 hours of radiation exposure. This was followed by a dramatic decrease in fecundity.

This thesis provides clear evidence that invertebrates experience substantial physiological effects as a result of low dose rates of radiation. I argue this result has ecological relevance because the dose rates at which these impacts were recorded can be currently found in the CEZ. This work could have implications for international policy as the dose rates that are currently considered safe for insects are set at 417 µGy h^{-1} by the International Commission on Radiological Protection. Whilst this thesis did not identify an exact mechanism driving physiological change in these species, I propose a possible explanation of an energetically costly recovery mechanism being activated by these lower doses.

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My first thank you must go to the Iapetus (named after the ancient ocean) doctoral training partnership for providing the funding that allowed the completion of this PhD project. I would also like to thank Professor John Wainwright who ensured I received all of the funding that I needed.

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This thesis is dedicated to my father David Edward Burrows. Throughout my many years as a student you came with me to every single open day, drove hours to collect me for weekend breaks when I needed them and provided endless support. This PhD is as much my achievement as it is yours. You taught me the one lesson that I will carry with me always, that happiness is more important than anything else. This one is for you Dad. I hope that it makes you as proud as I am to be your daughter.

In memory of my beloved grandparents, Adrienne May Burrows and Ronald Edward Burrows.

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List of Abbreviations

CEZ	Chernobyl Exclusion Zone
RAP	Reference Animal and Plant
NHB	Non-Human Biota
LET	Linear Energy Transfer
DCRL	Dose Rate Consideration Level
LET	Linear Energy Transfer
LNT	Linear No Threshold
ROS	Reactive Oxygen Species

Chapter 1: General Introduction

Ionising radiation is a natural part of life on earth, with it originating from both anthropogenic and natural sources. Ionising radiation includes alpha, beta, x-rays and gamma rays. When Ionising radiation interacts with biological cells damage can occur as a consequence, which can ultimately lead to whole organism effects. The most important target for the effects of ionising radiation is DNA (UNSCEAR, 2001). As a result, the effects of ionising radiation on human health are well characterised. Whilst we have a good understanding of the effects of radiation at high acute doses of radiation (Real et al, 2004), we know relatively less about chronic radiation exposure especially at lower environmentally relevant dose rates (Beresford et al, 2020 ; Basu, 2018 ; Geraskin, 2016; Tapio & Jacob, 2007). We additionally understand little about biological mechanisms which may be driving any effects found at lower dose rates (Lowe et al, 2022). This thesis sets out to explain why some organisms experience negative effects of radiation at chronic low dose rates found in our environment at radiologically contaminated sites (definitions provided in Table 1.1).

For many years the approach to environmental protection from ionising radiation has been based on an anthropogenic approach, that if humans are protected, then the environment as a result will be protected also (ICRP, 1991). This is now being deemed unacceptable and it is acknowledged that the environment must be protected in its own right (Valentin, 2003). As a result many countries are now establishing national requirements for the protection of the environment (Beresford et al, 2008) and creating robust international guidance and frameworks (ICRP, 2009 ; ICRP; 2017 ; IAEA, 2006). However, even with increasing international focus and research being conducted on non-human biota (NHB), there is still scientific debate surrounding dose rates of radiation that can cause adverse effects and the mechanisms driving them (Beresford et al, 2020).

These low doses can be found in several contaminated environments in which dose rates become elevated after nuclear disasters, these include Fukushima, Three Mile Island and Mayak (Russian Urals). However, the most well-known area where these low dose rates are found is in the exclusion zone which surrounds the Chernobyl nuclear power plant in northern

Ukraine. The Chernobyl nuclear accident occurred in 1986, releasing large volumes of anthropogenic radionuclides in to the environment and creating a large 4800 Km^2 exclusion zone (Beresford et al, 2021). The immediate consequences of the accident for both humans and the environment have been extensively investigated (IAEA, 1986 ; UNSCEAR, 1996 ; Beresford et al, 2020 ; Hinton et al, 2007 ; Horemans et al, 2019, Steinhauser et al, 2014).However, in the present-day dose rates have now dropped by orders of magnitude, ranging between <0.1 – 250 μGy hr^{-1} (Beresford et al, 2020). Many large mammal species have now recolonised this area after much biota was killed or fled after the initial accident (Geraskin, 2008; Ibster et al, 2016, Zelena et al, 2005). This recolonisation is largely attributed to the removal of human interreference within the zone (Webster et al, 2016), it also allows us to investigate the potential consequences of low dose exposure on these species physiology and use that knowledge to set new standards for radiological protection.

I will now explore some of the research conducted on the effects of radiation. I shall then explore the field of radioecology, to highlight key knowledge gaps about the effects of radiation on wildlife. In subsequent data chapters, I use bumblebees as a study system due to the growing body of evidence that suggests bumblebees are sensitive to radiation exposure. Therefore, in this introductory chapter I will explore bumblebees as a study system.

1.1 Mechanisms of radiation damage

Radiation primarily affects living organisms by inducing cellular damage. It is classified in to two forms: ionising and non-ionising. This thesis will focus on ionising radiation as this can have profound effects on organisms due to its interactions with biomolecules and its ability to induce atom ionisation (Reisz, 2014). A glossary of terms has been included with regards to discussions within this thesis on radiation (Table 1.1). Damage to cells can be caused by the action of radiation on DNA molecules either directly or indirectly. Direct effects involve radiation directly hitting the DNA molecule which disrupts its molecular structure and may lead to cell damage or death (Saha, 2013). A very small proportion of damaged cells that survive this process may then induce carcinogenesis, which is more common with large doses of radiation. During indirect effects, radiation hits water molecules within the cell, as well as

organic molecules (Koturbash, 2008). As a result, free radicals are produced, which have an unpaired electron structure. This structure is very reactive and therefore reacts with DNA to cause molecular structure damage. Indirect effects on DNA can as a result lead to impairment of function, cell death and carcinogenesis (Reisz, 2014). However, this depends on the number of free radicals that are produced, which in turn depends on the total dose an organism receives. This also depends on the quality of radiation and the conservative force acting on an ionised particle. This conservative force is known as Linear Energy Transfer (LET), with high LET particles having a high charge and low LET a low charge. The indirect mechanism is the most common cause of radiation damage as organisms are more likely to receive relatively low doses in comparison to higher doses experienced after nuclear accidents, and water composes nearly 70% of cells (Saha, 2013). Indirect effects can also involve reactive nitrogen species that occur as a result of ionisation of atoms on key molecules such as DNA.

Table 1.1: A glossary of radiological terms used within this thesis (CDC, 2022)

Term:	Definition:
Dose rate	The dose of radiation received per unit of time, usually reported as per hour.
Acute dose	Exposure to radiation in a short time period (usually minutes or hours).
Accumulated dose	The total dose received over the whole period of time an organism is exposed.
Chronic exposure	Exposure to radioactivity over a substantial period of time.
High dose radiation	In this thesis, this refers to dose rates used to cause cell death.
Low dose radiation	In this thesis, refers to dose rates below those known to cause cell death or immediate physiological/genetic effects.
Environmentally relevant dose rate	In this thesis, this refers to dose rates currently found in the Chernobyl Exclusion Zone: <0.1 – 250 µGy hr^{-1} (Beresford et al, 2020).
Gy (Gray)	A unit of measurement, which refers to the absorbed dose for any type of radiation: 1 µGy = 1000 mGy.
Sv (Sieverts)	A unit of measurement which refers to the absorbed dose, typically within human tissue:1 µSv = 0.001 mSv.

It is well known that high doses of radiation lead to cell death, with the effects of radiation on humans having been well studied to inform safety legislation (IAEA, 2022). The understanding of consequences of exposure to doses lower than these, often relates to the long-term risk of the development of biological and physiological alterations that can

manifest decades later as increased cancer risk (Derousky et al, 2015; Koturbash, 2008). Acute doses of radiation in invertebrates also have been well studied in terms of insect sterilisation techniques (Bakri et al, 2005 ; Copplestone et al, 2008). However, these studies present challenges when trying to determine effects of radiation at doses found in contaminated environments, which are significantly lower than in the experiments described (Larsson, 2012). Therefore, there needs to be more studies conducted at lower doses found in our environment. This is especially important for invertebrates, as most are much shorter lived than vertebrates and are mostly post-mitotic therefore they do not generally experience cancer. As a result, effects recorded in humans are not largely applicable to them. Invertebrates will still experience effects of radiation and it is important to understand the changes that this may cause to their biology and the mechanisms driving them.

1.2 Linear no-threshold model

The linear no-threshold (LNT) model is an underlying assumption of radiation protection that has been present for over 40 years, but remains controversial due to the difficulty in obtaining data at low dose rates. This assumption that there is no threshold for the effects of radiation was first proposed in a Nobel lecture in 1941, in which it was stated that there was 'no escape from the conclusion that there is no threshold' (Muller, 1941). The LNT model assumes that there is a linear relationship between total dose of radiation an organism receives and the risk of developing cancer (Tubiana et al, 2009). Therefore, even the lowest doses of radiation could potentially reduce an organism's lifespan or have other detrimental physiological effects. The LNT model was developed using a significant dose-response relationship between the doses of radiation that were received by survivors of atomic bombs and cancer risk (Webber & Zanzonico, 2017). The dose response is assumed to be linear to zero dose, despite data used to develop this model only existing for cancer risk of those exposed to high acute doses of more than 0.2 Gy (ICRP, 1977). As a result, it must be questioned whether we can extrapolate results from acute high dose studies to chronic low dose exposures as there is a large lack of data with regards to chronic low doses (Brechnigac & Doi, 2009). Whilst the LNT model was developed for the protection of humans, it is now a principle that has been extrapolated to studying effects of radiation on wildlife in the environment. However, it is unclear whether this is justifiable. Despite this the International Commission on Radiological

Protection (ICRP) states that available biological low dose data supports the LNT model (ICRP, 2008), however data from chronic low dose studies is not quite as clear (Webber & Zanzonico, 2017; Cuttler, 2010). Even though there is a lack of data, the LNT is largely accepted as it is a simple and pragmatic approach to radiological protection (NCRP, 2001). However, it must be remembered that the LNT applies to stochastic effects such as cancer induction. For wildlife, cancer is not generally a concern as most non-human biota do not live long enough to develop this disease, with the exception of larger mammals. Additionally, most invertebrates can't get cancer in its true sense because most of their cells are post mitotic and most effects on invertebrates focus on effects on fecundity. It is therefore more important that we establish where there are thresholds for stochastic effects. This would also help to direct research on mechanisms of internal radiation exposure identified by the Committee Examining Radiation Risks from Internal Emitters (CERRIE), which include genomic instability, by-stander effects, mutations in the germ line and epigenetic changes (CERRIE, 2004).

1.3 Radiation and our environment

Our environment and all organisms are exposed to ionising radiation from a variety of sources including cosmic rays from space and terrestrial radionuclides found within the earth's crust (Ojovan & Lee, 2014). Background radiation levels are not consistent and vary worldwide from ~ 2.3×10^{-4} to 0.3 µSv hr^{-1} (Figure 1.1). Underlying geology is a large factor in this variation, with some areas of Brazil, India and China having the highest background radiation levels due to high concentrations of radioactive minerals within their geology (Hendry et al, 2009). An example of this can be found in Brazil, India and Iran where monazite sand on beaches can lead to doses of ~ 50 µSv hr^{-1} (Ojovan & Lee, 2014).

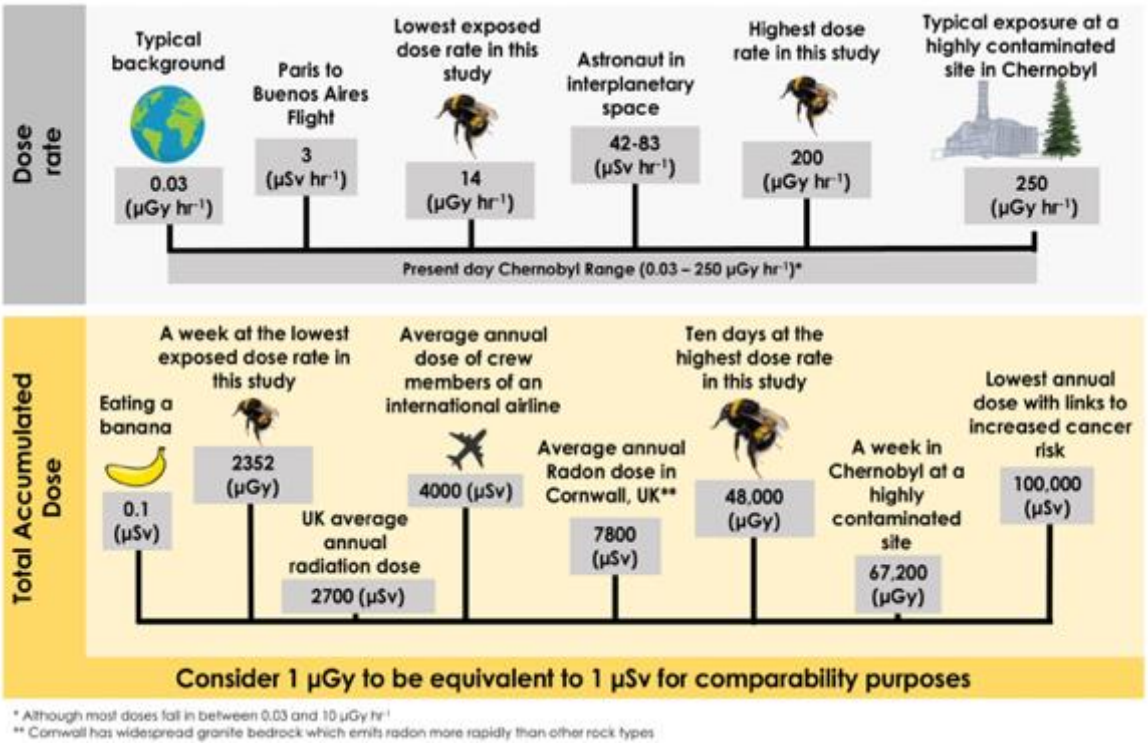

Figure 1.1: A diagram of various accumulated doses and dose rates experienced daily in our natural environment in comparison with dose rates used in the first data chapter of this thesis (Chapter 2). The annual Radon dose the UK has a cut off limit of 200 Bq m^{-3} (ANSTO., 2022 ; Chancellor *et al.*, 2018)

I will now explore some of the established literature on the effects of radiation that have been recorded in humans. Many of the dose rates explored here are common in radiologically contaminated sites in which many varied species can be found.

1.3.1 Air crew

Exposure to ionising radiation is an occupational risk factor for both commercial air crew and passenger's onboard aircraft. The atmospheric layer of the earth shields the effects of galactic cosmic rays with doses of only 0.06 µSv hr^{-1} reaching sea level (Lim, 2002). However, at cruising altitude for most Boeing 747 airliners (35,000ft) doses are much higher, at approximately 6 µSv with aircrew receiving approximately 0.3 µSv (Wollschlanger et al, 2018), which varies depending on length of journeys and routes taken (Table 1.2).

Table 1.2: Approximate dose rates received for flights of varying duration (Wohlschlaeger et al, 2018).

Destination, from London	Approximate flight time*, hr	Approximate dose for a return journey*, µSv
Paris	2	4
Glasgow	2.5	6
Malaga	5	15
Athens	7	25
Moscow	8	40
New York	15	100
Los Angeles	22	160
Johannesburg	23	75
Hong Kong	26	140
Sydney	40	160

Notes:
*Total time in the air for a direct return flight from London to the indicated destination.
The values in this table are approximate values for illustration purposes. Actual flight times may vary and the doses will depend on factors including flight profile and the specific route taken.

The effects on human health of low dose cosmic radiation experienced in flight include increased incidence of breast cancer (Lynge, 1996), prostate cancer and acute leukaemia (Band, 1996), and skin cancer in pilots (Haldorsen et al, 2000). However, most of these epidemiological studies examined only small cohorts and fail to account for confounding variables such as lifestyle and environmental factors (Lim, 2002).

1.3.2 Space exploration

Radiation is an important barrier to human space exploration due to the biological effects of high energy heavy ions (Cucinotta & Durante, 2006 ; Tao Lu, 2004, Zhou et al, 2013). Future deep space missions are anticipated to have detrimental effects on astronaut's health (Narici et al, 2015). For example, future missions to Mars could result in increased risk of cancer and risk to central nervous system function. During a three-year mission, astronauts will be exposed to whole body doses of 40.7 mSv or more (Tao Lu, 2004; Wilson et al, 1995). As planetary surfaces often lack an atmosphere, astronauts will experience higher doses of radiation than on earth. The dose rates on planetary surfaces are approximately 40 - 80 µSv hr^{-1}, which is less than dose rates found in deep space (Sagnatic et al, 2004). It should however be noted that there are several different types of ionising radiation within space, with galactic cosmic radiation originating from highly energetic protons, alpha particles, high charge and energy (HZE) nuclei, as well as secondary radiation from space craft (Chancellor et al, 2014).

1.3.3 Nuclear power plant worker

Due to strict guidelines from the International Commission on Radiological Protection (ICRP), occupational exposure in nuclear power plants and other radiological careers are limited to radiation doses of 100,000 μSv over five years, with a worker not to exceed 20,000 μSv in a given year (ICRP, 2007). Radiation workers within the United Kingdom receive an average annual occupational exposure of 0.18 mSv (UK Gov, 2010), with a statutory annual effective dose limit of 20 mSv per year (JSP 392, 2020). A study of over 400,000 radiation workers that are in contact with contaminated areas within nuclear power stations, found that there was a significantly increased risk of all cancers to workers, however the study could not adjust for confounding variables such as smoking, diet or environment (Cardis et al, 2005). Additionally, this study was conducted over 15 years ago with present day annual occupational exposures now significantly lower.

1.4.4 CT scans

Computed tomography (CT) scans are used in the medical profession for diagnostic evaluation. Ionising radiation exposure from a CT scan is substantially higher than a conventional x-ray. The dose a patient receives varies greatly between types of scan and area of the body being examined. The overall median effective dose of a scan is approximately 2,100 μSv for the head and 31,000 μSv for the abdomen and pelvis (Smith-Bindman et al, 2009). Low dose exposure to ionising radiation used in medical imaging has been correlated with tissue damage and carcinogenesis (Reisz, 2014). An estimated 1 in 270 women (1 in 600 men) who receive 20,000 μSv during a coronary CT will develop cancer from that CT (Smith-Bindman et al, 2009; Pauwels & Bourguignon, 2011), this risk increases in a linear fashion with doses above 50,000 μSv (Reisz, 2014). However, calculations on risk are based on extrapolations from data from individuals exposed to acute high doses of radiation. These also often are focussed on stochastic effects rather than non-stochastic effects which are more relevant for wildlife.

1.4 Radioecology

The previous sections of this introductory chapter have explored just some of the effects of radiation on humans, it is acknowledged that humans can experience increased doses from other situations including uranium mine workers and exposed populations from accidents (Howard et al, 2017 ; Vandenhove, 2002). However, the field of radioecology highlights that it is just as important to understand the impacts of radiation on non-human biota. The literature surrounding the effects of radiation on wildlife is comparatively sparse in relation to generating benchmarks for chronic exposure and as a result, there is significant debate within scientific literature surrounding dose rate thresholds of radiation necessary to cause different forms of biological damage, cause fitness loss in the wild and damage ecosystems (Copplestone et al, 2007). There is also a great disconnect in radioecology literature, as there are often discrepancies between laboratory experiments and field studies on wildlife (Garnier- Laplace et al, 2013). Often conclusions drawn from laboratory studies are contradictory to findings from the field, as laboratory studies typically only study high acute dose rates and they are often time limited (Garnier-Laplace et al, 2013). This makes drawing conclusions difficult as in the field wildlife is often exposed to chronic low dose rates of radiation, while dose rates studied in the laboratory are typically far higher than rates an organism would typically receive in a contaminated landscape (Geraskin, 2016). There is also disagreement between field studies in radiologically contaminated areas, such as the Chernobyl Exclusion Zone (CEZ). This is due to some field studies finding substantial deleterious effects on wildlife at dose rates equivalent to UK background (e.g. Moller & Mousseau, 2007; Moller & Mousseau, 2009), whilst others have detected minimal or no effects at all at dose rates of up to 150 µGy hr^{-1} (Bonzom et al, 2016; Chesser & Baker, 2006 ; Horemans et al, 2018 ; Deryabina et al, 2015; Murphy et al, 2011). Therefore, a key knowledge gap in radioecology is identifying mechanisms which drive any effects recorded at lower doses.

1.5 Nuclear Disasters

Whilst the nuclear industry maintains exceptionally high safety standards, in the past there have been several nuclear power plant accidents which have resulted in the contamination of the surrounding environment. The most notable of these accidents include Chernobyl (1986), Fukushima (2011), Windscale (1957) and Mayak - Russian Urals (1957). These

accidents notably increased environmental radiation levels in localised surrounding areas (Copplestone *et al.*, 2016; Rojavin et al, 2011). The Fukushima accident in Japan resulted in radioactive caesium contaminating a 1150 Km^2 area and Tritium also leaking from underground tanks in to water supplies (Kawasaki, 2021 ; UNSCEAR, 2013), with external doses of 10-15 mSv recorded in the first year after the accident (Ishikawa, 2021). In Russia, the Mayak accident led to the release of large quantities of various radionuclides including ^{137}Cs and ^{90}Sr, with a radioactive plume travelling for several kilometres exposing the environment to elevated dose rates (74 PBq) (UNSCEAR, 1996 ; Fesenko et al, 2019 ;Akleyev et al, 2017).

The most notable nuclear accident however occurred at the Chernobyl Nuclear Power Plant in 1986, when radioactivity was released by a damaged reactor over a time period of 10 days (IAEA, 1986). Immediately after the accident exposure rates were extremely high and were recorded at approximately 20 Gy per day (IAEA, 2006). Present day dose rates are now much lower typically between <0.1 and 250 µGy hr^{-1} (Beresford et al, 2020), with contamination dominated by Cs-137 and Sr-90 (Kashparov et al, 2018). The chronic low dose rates experienced in the CEZ, can be put into the context of the dose rates that are received by humans on a daily basis. For example, on a return flight from New York to Sydney a passenger will receive a total accumulated dose of 160 µSv (Wollschlanger et al, 2018), this is similar to the levels experienced after one hour in the Chernobyl Exclusion Zone in the most contaminated area. Also, radiation workers are typically exposed on a daily basis to a total dose rate of 2.2 µSv (Cardis et al, 2005). This highlights that knowledge gained on the effects of these chronic low dose rates in the CEZ landscape, could be extrapolated to other scenarios.

1.6 Research in the Chernobyl Exclusion Zone (CEZ)

To understand the magnitude of the effects of radiation on wildlife, research has mainly focused on the Chernobyl Exclusion Zone (CEZ), however there are increasing amounts of research occurring at other contaminated sites such as Fukushima (UNSCEAR, 2020 ; Strand et al, 2014) and Mayak accident (Fesenko, 2019 ; Orekhova and Modorov, 2017).The CEZ (c. 5000 kmsq) was established to restrict human access to the most radiologically contaminated

areas after the Chernobyl nuclear accident in 1986. High dose rates that are known to cause radiation damage are still found in the zone (UNSCEAR, 2008), however this is only within 1-5% of its surface area (Beresford, 2016). Therefore, there is little knowledge of the long-term effects of chronic radiation on many important species within the CEZ. This highlights a need for longer term data sets, which could provide information on biological impacts over time.

A focus of studies in the CEZ has been small mammals and birds, as prior to the accident they were thought to be the most radio-sensitive taxonomic groups (Beresford, 2016; Moller et al, 2016). However, when rodents were investigated in the 1990s a few years after the accident, in highly contaminated field sites (up to 830 μGy h^{-1}) several studies found no evidence of genetic change in the form of chromosomal aberrations in comparison to control sites. These investigations primarily examined changes in mitochondrial function (Baker et al, 2001; Wickliffe et al, 2002). Whilst no effects have been found in the CEZ, generally effects of radiation on vertebrates first appear at the molecular level. It should also be noted that often molecular data cannot be used to estimate effects on populations, ecosystems or even individuals, as there are usually more complex mechanisms involved in their response to stresses and translating molecular damage into fitness loss is extremely difficult (Clements and Rohr, 2009). Recent work has used adverse outcome pathways to attempt to link molecular to physiological effects (Tollefsen et al, 2022). These pathways use a conceptual framework to organise and report linkages between stressors, their biological targets and the adverse outcome (Villineuve et al, 2014 ; Ankley et al, 2010). These frameworks however still need to be critically assessed. Therefore, further laboratory and field-based studies are still needed to understand if physiological effects translate to populations and affect their abundance. This could then have implications for the ecosystem as a whole.

A study conducted on larger mammals in the zone found significant declines in abundance as radiation levels increased (Moller and Mousseau, 2013). This study however covered only 16.1km of transects, which were examined only once. A further study which considered long term-census data found no relationship between radiation dose rate and mammal abundances, with data collected over transects which were 20 times larger and repeated over 3 years (Deryabina et al, 2015). Recent work has also found that Eurasian lynx are found in the CEZ in numbers of 2.2-2.7 individuals per 100 km^2, numbers that are similar to other areas

in Europe where conditions are ideal for lynx. This suggests the CEZ is providing an ideal habitat for this species to thrive (Gashchak et al, 2022). Similarly, remote camera surveys found no impact of radiation on mammal abundance within the zone by monitoring mammal tracks in the snow (Webster et al, 2016). Whilst these studies suggest that there is no impact of radiation on mammal abundance, it is difficult to confidently show there are no effects of radiation on mammals as there is no consistency in methodologies or dosimetry. It should also be highlighted that these studies investigate some mammals with large home range sizes. It could also be argued that radiation causes poorer resource quality within certain areas, which could influence the food chain. Additionally, radiation could affect mammal fitness which when coupled with poor resource could lead to sink populations. However, this requires further investigation. More recent work has shown that large range animals are present in the zone which shows resources are present for them (Gaschak et al, 2022). It should also be noted that there are large differences in the ecology of the species used in these studies, and therefore the niches they exploit in the environment. This can lead to substantial differences in the doses of ionising radiation that is absorbed by that species even if the individual is present in the same place at the same time (Geraskin, 2016). Therefore, further research is required on mammals to understand chronic impacts on key species abundance and biology.

Other work in the Chernobyl Exclusion zone on non-human biota includes work on floral resources and plants within the dynamic environment. The accident can be clearly identified in tree growth rings highlighting the years of extreme drought that followed (Holiaka et al, 2020). In meadow plant communities found within this habitat there has been a shift in plant communities to more radioresistant tree species, whilst with increasing dose rates there has also been found to be a decrease in the number of plants and number of different species within meadow communities(Geraskin, 2016). However, it should be noted that there is a general shift from agricultural land in the CEZ to scrub due to land no longer being managed and natural succession taking place. There have additionally been studies conducted at the cellular level with evidence of single and double strand breaks recorded in plant roots (Georgieva et al, 2017). Further work has been conducted on specific plant species in order to understand how they respond to low doses of radiation, including a study on *Hypericum perforatum* (St John's Wort) that found a decrease in asexual reproduction (Geraskin, 2016).

Whilst the majority of research in the CEZ focuses on the terrestrial environment, there are ponds, lakes and other freshwater present across the landscape. Therefore, some work has also considered effects on the aquatic environment. A study on the genetic diversity of zebra mussel found no significant effect of radiation (Fetisov et al, 1992). However, this study provided no dose rates or dose assessments (Turlure et al, 2014). More recent work, which again found no effect of radiation on population genetic diversity in the crustacean *Asellus aquaticus* (Fuller et al, 2019). There was additionally no effect of radiation recorded on *A. aquaticus* development and reproduction (Fuller et al, 2018; Fuller et al, 2017). However, some work has identified effects on aquatic species, with *Daphnia* exhibiting higher genetic diversity in water bodies with higher radiation dose rates (Goodman et al, 2022). There has also been effects of radiation recorded on aquatic based vertebrates at dose rates found in Chernobyl, with an increase in infertile eggs in brown frogs recorded shortly after the accident and an increase in the number of micronuclei in reproductive tissues (Eliseeva et al, 1994; Voitovich and Afonin, 2000). It is important to identify effects of radiation on aquatic invertebrates as these are relevant not only to the CEZ but also because aquatic environments can retain contaminants for decades (Dallas et al, 2012).

1.7 Invertebrates in the Chernobyl Exclusion Zone (CEZ)

There have been very few studies conducted on the effects of chronic radiation exposure in the CEZ on invertebrates. Immediately after the disaster there were some short-term studies conducted on soil dwelling invertebrates due to most radionuclides from the accident moving to leaf litter and remaining in the top soil layer (Geraskin, 2016; Krivolutsky, 1996 ; Krivoltuzkii & Pokarzhevskii, 1992). There are additionally several properties of soil which influence the radionuclide mobility and bioavailability, which include the type of soil, pH, water content and the vegetation present (Geraskin, 2016). Laboratory studies have also shown negative effects on soil invertebrate reproductive fitness of adults, increased egg mortality and mortality of early life stages at a dose rate of 30Gy (Hinton et al, 2007). The CEZ is host to many radionuclides which can be found in soil: ^{137}Cs, ^{90}Sr and ^{240}Pu. Of these ^{137}Cs in particular is highly soluble so penetrates soil material easily. The soil-based invertebrates found in the CEZ have been suggested to have sensitive juvenile stages, however current dose rates have been found to have no impact on soil invertebrate feeding activity (Beresford et al, 2022). The

recovery of soil invertebrates is supported by data collected 4 years after the accident, which found that as dose rates declined over time, the population density recovered (Kriolutzkii & Pokarzhevskii 1992). The recovery of population density after the accident was also further confirmed in 2011 by the finding that decomposition of uncontaminated leaf litter increased along a spatial gradient of dose rates across sites within the zone. This highlights an increase in the number of soil decomposers with total dose rate. This indicated that after two decades there was no detrimental impacts on organic matter decay and therefore invertebrates within the soil (Bonzom et al, 2016). However, other studies have reported a negative relationship between litter mass loss and level of ambient radiation (Moussaeu et al, 2014). In contrast to the first study however the range of dose rates in the study by Mousseau was much higher and two of the most contaminated sites appear to drive the negative relationship between loss of litter mass and increasing radiation level (Geraskin, 2016). More recently a study found lower abundance of soil invertebrates under contaminated wood at dose rates of 1 μGy h^{-1} (Moller and Mousseau, 2018). Therefore, there is still large uncertainty in literature on the effects of radiation on these soil dwelling species.

Primarily studies have investigated the impacts of radiation on terrestrial invertebrates and their population abundance and diversity. However, studies investigating the biological effects on individuals within these populations are in comparison exceptionally sparse. There have been very few field studies undertaken in the CEZ focusing specifically on pollinators such as bumblebees. The abundance of bumblebees has been reported to decline across the landscape of the CEZ with increasing radiation dose rates, with effects such as low population abundance controversially reported at levels as low as background with insufficient data to support findings (Moller et al, 2012). This decline in abundance with increasing dose rate seemingly supports other work on the abundance of pollinators such as butterflies. The decline in pollinators was found to be negatively correlated with fruit production of trees in the CEZ, suggesting impacts on ecosystem services (Moller & Mousseau, 2009). It should be noted however that both of these studies failed to account for important confounding variables such as habitat suitability and quality. Effects were described at dose rates that could only be recorded in the 'red forest' area of the CEZ, this forest is to the west of the CEZ reactor and had the highest deposition of radionuclides (Arkhipov et al, 1994), which will have strongly affected results. Therefore, it is important for future to work to consider confounding

variables that could influence effects, not just for bumblebees, but for all non-human biota in radiologically contaminated areas.

The studies that have been conducted on invertebrates in the CEZ highlight a need for robust experimental designs when investigating environmentally relevant dose rates. For example, a criticism of the studies conducted on wildlife in the CEZ is that for many the dose estimation of exposure is considered to be exceptionally poor (Beresford et al, 2019). Many studies use handheld monitors to record dose rates without considering calculating internal exposure as well as considering confounding factors such as the mobility of species (Beresford et al, 2020). As a result, there is a need to develop robust dosimetry models, similar to those that already exist for humans. Additionally, studies in the CEZ rarely consider whether the effects we are seeing now are due to existing dose rates or are residual effects following the accident (Horemans et al, 2019). This could be addressed through reconstructing past exposures or through the combination of field and laboratory studies. The combination of field and laboratory studies would also address an issue that many studies fail to recognise that the CEZ is a multi-stressor environment (Gagnaire et al, 2017; Holmstrup et al, 2010). Organisms will not just be exposed to radiation but also separately to environmental factors such as changing seasons or food scarcity due to environmental succession (Beresford et al, 2020). By studying the effects of radiation in the field and in the laboratory it allows for the control of confounding factors and reduction of noise in field data sets.

1.8 Laboratory studies conducted on terrestrial invertebrates

Whilst studies in the Chernobyl Exclusion zone have found effects of radiation on wildlife from dose rates of 100 μGy h^{-1}, laboratory studies have primarily reported effects from dose rates almost eight times higher (Garnier-Laplace et al, 2013). There have been very few studies conducted on terrestrial invertebrates in the field, but there has been a range conducted in the laboratory. These studies however have considered primarily high dose rates of more than 950 μGy hr^{-1}, higher than dose rates recorded in areas such as the CEZ (Copplestone et al, 2008). Studies primarily consider acute dose rates, which are used to determine the radiation dose that is necessary to kill 50% of a sample of organisms. The main focus is often to understand effects on sterility for practical application to sterilise insect pests (Dyck et al,

2005; Gad, 2014). For example, a total dose 5 Gy has been found to reduce fertility and fecundity of the pest species southern green shield bug (*Nezara viridula*) (Zunic et al, 2002).

There have however been some studies conducted in the laboratory on the Pale-blue grass butterfly (*Zizeeria maha*), which has been used in the contaminated area at Fukushima as an indicator species of ionising radiation impacts (Hiyema et al, 2012). In field-based studies it was suggested that maximum dose rates of 8 µGy hr^{-1} during juvenile development led to mild morphological abnormalities in butterflies collected one month after the accident (Hiymea et al, 2013), however confounding environmental variables were not considered (UNSCEAR, 2020). Laboratory based studies were also conducted on butterflies to investigate effects of feeding adults Cs-137 contaminated leaves taken from Fukushima, it was found that leaves with 43-450 kBq/kg led to increased morphological abnormalities in butterflies (Gurung et al, 2019). Further studies however identified that contaminated leaves showed an increase in sodium levels with radiation exposure caused by radiation stress and nutritional imbalances, which could have resulted in the observed abnormalities (Sakuchi et al, 2021). This highlights that even laboratory-based studies need to consider confounding variables alongside radiation exposure.

Recent unpublished work suggests that laboratory studies often under predict the effects of radiation on wildlife, in particular for bumblebees. At a dose rate administration as low as 100 µGy hr^{-1}, a reduction in queen production has been detected (Raines, thesis). This study also addressed current disparity between laboratory and field-based studies by analysing additional stressors in optimum conditions. It was found that when exposed to dose rates below 300 µGy hr^{-}, bumblebees had increased parasite loads of the common gut parasite *Crithidia bombi* (Raines, thesis). It is still unknown as to whether observed effects are due to the effect of radiation on the hosts immune response or a change in parasite characteristics. These dose rates were previously considered to not cause harm in laboratory studies, therefore this study is an important indicator of further work that needs to be conducted in order to conduct ecologically relevant benchmarks (ICRP, 2008). Similar effects to those of radiation on bumblebees has been recorded due to the stressor of neonicotinoids, in which they cause reductions in colony size and queen production (Whitehorn et al, 2012). Therefore,

studies on the effects of the unique stressor of radiation could be applicable to other stressors.

Despite the importance of bees for sustaining ecosystems through pollination services (Kremen et al, 2007 ; Goulson, 2010), there have been very few published laboratory studies conducted on the effects of radiation on bumblebees (Raines et al, 2020). There has however been work conducted on the effects of radiation on honeybees (*Apis mellifera*), but often information is limited to data on bioaccumulation of radionuclides (Haarman, 1997). Often dose rates used are much higher than those found in the environment, with effects such as egg hatching failure reported with a total dose of 75 Gy and an increase of non-hatching eggs from doses of 4 Gy (Lee, 1957). A recent study compared the effect of radiation on honeybee biomarkers for a range of dose rates from 0.18 µGy hr^{-1} to 24,500 µGy hr^{-1}. This study found that antioxidant and immune system biomarkers decreased as dose rate increased, with some evidence of variations in metabolism biomarkers (Gaganire et al, 2019). These studies used acute doses of radiation, which creates problems when trying to extrapolate to invertebrates exposed to much lower doses of radiation in contaminated environments. Therefore, it is vital that laboratory studies investigate effects at chronic lower dose rates to increase environmental relevance.

1.9 Radiological protection

Radiological protection is a science-based discipline in which research is conducted to protect both humans and the environment from harmful effects of ionising radiation. The International Commission on Radiological Protection (ICRP) has developed a system for the protection of the environment which is similar in concept to the reference person approach used for humans (Larsson, 2012). The reference animal and plant (RAP) approach came in to action in 2008 to account for a diversity of species when classifying the effects of radiation on the environment (ICRP,2007). There are several RAPs which are hypothetical organisms that are representative of typical environments, e.g. earthworm represents a soil environment (Table 1.3). Each of these reference animals or plants are organised in to 'bands' called Derived Consideration Reference Levels (DCRL), these bands represent the dose rate range at

which negative effects are expected to start occurring to individual organisms (ICRP, 2007). These bands are now often used in assessments of protection of the environment (Gwyn et al, 2023).

Table 1.3: Dose Consideration Reference Levels for Reference Animals and Plants (ICRP, 2007).

			DCRL µGy hr^{-1}		
Wildlife Group	Ecosystem	RAP	4 - 42	42 - 417	417 - 4167
Large terrestrial mammal	Terrestrial	Deer			
Small terrestrial mammal	Terrestrial	Rat			
Aquatic birds	Freshwater/ Marine	Duck			
Large terrestrial plant	Terrestrial	Pine Tree			
Amphibians	Freshwater/ Terrestrial	Frog			
Pelagic fish	Freshwater/ Marine	Trout			
Benthic fish	Freshwater/Marine	Flatfish			
Small terrestrial plant	Terrestrial	Grass			
Seaweeds	Marine	Brown Seaweed			
Terrestrial insects	Terrestrial	Bee			
Crustacean	Freshwater/Marine	Crab			
Terrestrial annelids	Terrestrial	Earthworm			

A reference bee is assumed to not be affected by radiation below 417 µGy hr^{-1} and therefore whole populations are assumed to be protected below this rate (ICRP, 2014 ; Zinger et al, 2008). However, some studies from radiologically contaminated areas such as the Chernobyl exclusion zone have reported effects on taxa at dose rates starting from 0.1 µGy hr^{-1} (Moller et al, 2012 ; Moller and Mousseau, 2009 ; Moller and Mousseau, 2018). These dose rates are highly controversial as they were recorded near to highly contaminated areas within the exclusion zone and as dose rates at which effects were recorded are comparable to natural background (Beresford et al, 2020), calling dosimetry in to question as these dose rates had never before been recorded in the CEZ. However, this study highlights a need to examine low dose radiation effects on environmentally important species, such as bumblebees, in order to understand at what dose rates, I may begin to see changes in key life history traits.

A study conducted on bumblebees at dose rates relevant to contaminated environments found substantial life history effects below 400 µGy hr^{-1} (Raines et al, 2020). This work has highlighted that it must be questioned whether these dose bands are adequate for the protection of the environment. Additionally, there were no data available for research undertaken on bees at the time of this ICRP publication when the DCRL was set. The data

provided was extrapolated from experiments conducted on wasps, weevil and fruit flies (ICRP, 2007). The ICRP system has been designed to evolve with new information so new data will be incorporated in to the system. Consequently, the ICRP has also acknowledged that work needs to be done to help establish DCRLs further and there is now ongoing revision to RAP data (ICRP, 2021). It should also be noted that the RAP bee is a representative of all insects and not just the bee, therefore more investigation is required to ensure that any effects recorded are found in multiple species. The bee is also reported to be a soil dwelling organism for the purpose of dose conversion factors, as bumblebees and other solitary bees nest underground and because this maximises the external dose from a radiological protection perspective (ICRP,2008). Surface deposited radionuclides however tend to lead to an exponential decrease through the soil of radioactivity, so that the bulk of the contamination is on or near the surface of the soil. Consequently, a burrowing animal may move below the contaminated layer and therefore due to soil density receive a reduced dose in comparison to the surface. Therefore, soil could also decrease the effect of radiation on bees especially during development phases of larvae within the nest (IAEA, 2006). This lack of data and identification of varied life stages highlights a need to conduct research to ensure DCRLS are at the correct range to protect species.

1.10 Study systems

This introductory chapter has explored ionising radiation and its effects on humans, its effect after the occurrence of nuclear accidents and how the Chernobyl nuclear accident has led to research on effects in wild species and ecosystems. Whilst there are other contaminated landscapes this introduction was restricted to wildlife research in Chernobyl due to the dose rates investigated in the data chapters. The contamination in Chernobyl is primarily from radioactive caesium, which was the radiation source used in this thesis. Other contaminated landscapes have more of a mixture of radiation and so were of less relevance. This introduction will now begin to focus on different study systems and theories that should be explored in future research to understand the effects of low dose radiation exposure.

1.11 Life History theory

Life history theory explains how organisms, such as ecologically important bumblebees, allocate energy resources to survival and reproduction in order maximise their own fitness (Kavanagh & Kahl, 2018). These resources are essential for both somatic and germline cells. Throughout an organism's lifecycle they take on nutrients which are metabolised and then allocated to a key life history function which includes, reproduction, maintenance, development, and resource storage (Boggs, 2009). Examples of organism maintenance include functions vital for survival such as defence, flight, and basic metabolism. Organisms must then live on these restricted energy budgets and prioritise the allocation of their energy whilst also managing trade-offs with other functions. Additionally, the quality and quantity of food can have large impacts on life history of insects as it heavily influences key aspects of reproduction (Fischer et al, 2004). Therefore, it is vital that we understand the impacts of stressors on the allocation of resources to better understand impacts on life history. Especially, as environments in which organisms are found are often not constant and therefore access to resource can fluctuate which influences the response of organisms (Boyce et al, 2006). In ladybirds for example it has been found that restricted access to nutrients as larvae can lead to compensatory feeding behaviours as adults, as well as increased mortality in response to food stress as an adult (Dmitrew & Rowe, 2007). We have however only just begun to understand the extent to which compensatory feeding in non-human biota is affected by variations in environment. However, similar compensatory feeding effects have been recorded in humans highlighting any effects on this could be useful outside of non-human biota (Stubbs et al, 2004).

1.12 Bumblebees

Bumblebees are key species which are vital for entire ecosystems due to the way they support plant diversity (Ollerton, 2017). They are a social group that can be found in temperate and alpine regions, where they play key roles as pollinators. Pollination is an ecosystem service and is essential not only for agriculture but also for the persistence of critical wild habitats (Klein et al, 2007). Bumblebees are some of the most effective pollinators as they are capable of buzz pollination, a specialised behaviour that releases pollen from poricidal anthers (De Luca & Vallejo-Marin, 2013). Bumblebees are in decline globally due to a variety of factors such as climate change, habitat degradation, increased spread of parasites and the usage of

neonicotinoids (Simmons & Angelini, 2017). One of the primary drivers of bumblebee decline is also nutritional stress which occurs as a result of the loss of foraging habitat (Woodard, 2019). It is therefore important I understand the impact of stressors driving their decline on their biology in order to preserve species.

Bumblebees are social hymenopterans and so live within a colony, which consists of a queen and several workers (Wilson, 1971). Whilst similar to honeybees in their eusocial nature, they differ in that honeybees have far larger hives and have a queen that lives in the hive with daughters for up to four years (Wilson, 1971). Bumblebee Queens specialise in colony reproduction, whereas workers specialise in rearing offspring and sourcing nutritional resources for the colony. Bumblebee workers vary greatly in size which then determines their role, larger worker bumblebees engage more in foraging activities whilst smaller workers often remain in the nest to carry out care taking activities for larvae (Goulson, 2003). Males are also present within the colony, but they have much shorter lifespans and live primarily to inseminate females (Stubblefield & Seger, 1994). Most bumblebee colonies establish a nest on the ground surface within a cavity e.g., a tree trunk, or more commonly below-ground, these nesting sites are chosen based on suitability and size rather than distance to areas with the most floral resource (Pugesek & Crone, 2021). Whilst bumblebees do not seek out nesting sites based on resource, the size of their nest and the eventual size of workers produced by a colony is largely dependent on the availability of high-quality floral resources (Pereboom et al, 2003). When resource is abundant, colonies tend to be larger which means more workers available for food provisioning, brood care, and the defence of the colony (Owen et al, 1980).

1.12.1 Bumblebees: Nutrition

Bumblebees forage from floral resources throughout their entire life cycle in order to support their survival, but also for nesting resources (Pope & Jha, 2018; Goulson et al, 2011). The survival of colonies is dependent on workers being able to successfully gather adequate nutrition for themselves and their offspring. Additionally, workers need to raise the temperature of their flight muscles to 30^{o}C for foraging (Heinrich, 1979), which requires a large expenditure of energy and the amount relies on the temperature of the surrounding environment (Woods, 2005). The diet of an individual bumblebee consists of a mixture of

pollen and nectar, which is obtained during foraging flights. Bumblebees obtain essential proteins from pollen, with floral resources providing a concentration of between 2.5 to 61% (Roulston & Cane, 2000). The pollen also provides bumblebees with a range of nutrients such as lipids, vitamins, and minerals. When individuals feed more on protein rich diets, colonies are generally more reproductively successful (Kitaoka & Nieh, 2008). Nectar obtained from floral resources provides the main source of carbohydrates to bumblebees, which is their primary energy source to fuel metabolically costly activities such as flight and nesting activities (Goulson, 2010). There are three sugars present within nectar which include glucose, fructose, and sucrose (Bernardello et al, 2004). The percent concentration of each of these sugars varies with different plant species (Percival 1961). During foraging flight bumblebees will use olfactory cues to detect floral resources with the highest concentration of sucrose (Wolff et al, 2006) and the highest protein content (Cook et al, 2003; Arenas & Farina, 2012).

Once nectar is consumed it is stored within the honey stomach, from which workers will regurgitate nectar directly in to nectar stores termed 'honey pots' within the colony. However, some nectar is used to sustain the individual, which travels down through the crop in to the mid gut where sucrose is broken down in to glucose and fructose (Figure 1.2). These sugars are then absorbed in to the haemolymph where they are either metabolised in to energy or stored within the fat body. These sugars are vital in times of stress, for example when a bumblebee experiences starvation conditions glycogen is converted to trehalose and released in to the blood stream to maintain metabolic rate (Bede et al, 2007). When starved trehalose is used first as the primary source of energy for flight muscles and maintain homeostasis, when this is used up there is a rapid decline in both glucose and trehalose in the haemolymph (Park et al, 2013).

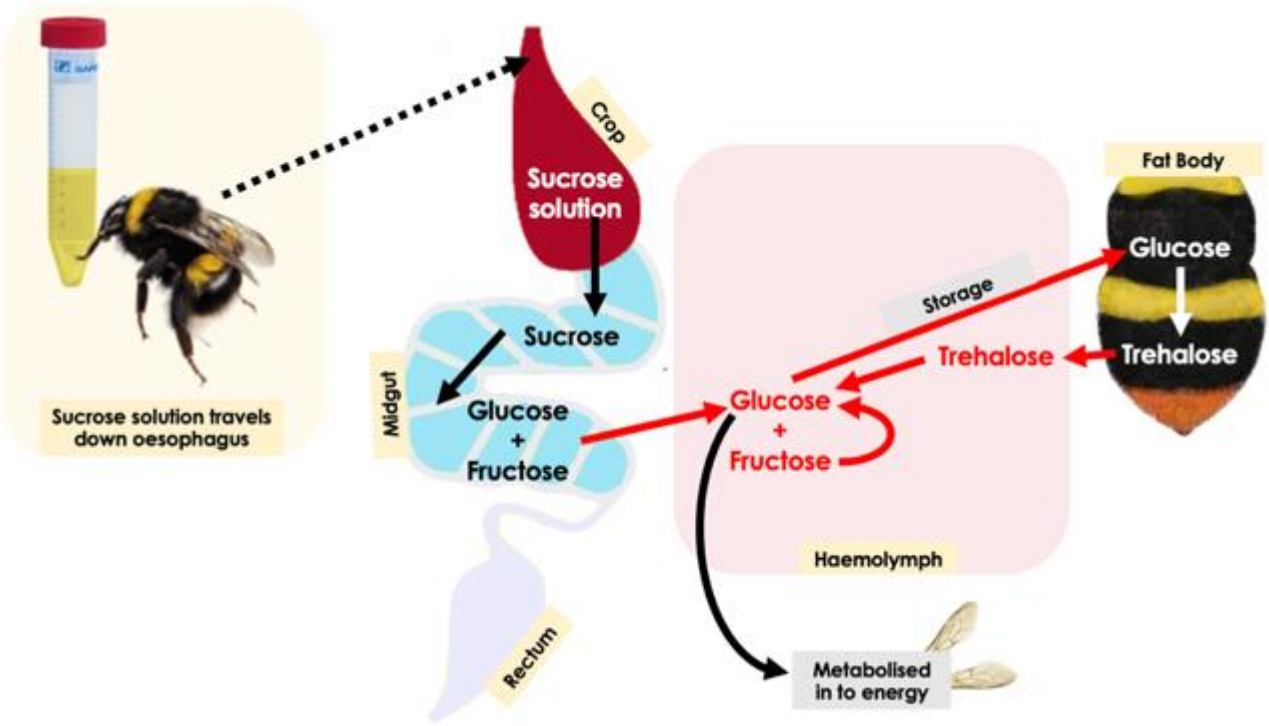

Figure 1.2: The breakdown of nectar (sucrose solution) as it passes through the bumblebee digestive system and enters the blood stream. The diagram shows that sucrose is broken down within the midgut in to glucose and fructose where it then passes through the gut barrier in to the haemolymph. In the haemolymph glucose and fructose are either directly used for energy or stored in the form of glucose in the fat body. When carbohydrate levels drop within the bumblebee, glucose is converted in to trehalose in the fat body before being transported to the haemolymph where it is converted back to glucose and fructose (Yu et al, 2008).

The bumblebee gut also benefits bumblebee nutrition through the presence of a specialised microbiome which assists with digestion of complex sugars (Hammer, 2021), bumblebees can effectively digest and absorb most of the content from pollen and nectar (Hammer et al, 2021). The gut microbiome has many useful functions which include digestion and detoxification of pathogens that are often present in nectar (Rothman et al, 2020), as well as bacterium such as *Gilliamella* which digests complex polysaccharides that otherwise would have been indigestible by the bumblebee (Kwong et al, 2014). Bumblebees obtain their microbiome from their parent colony, with the founding queen transferring her key bacterium (Kwong and Moran, 2016). As the colony develops newly emerged bumblebees obtain their core microbiome through the fecal-oral route and through contact with nest material, including the most common bacteria *Snodgrassella* and *Gilliamella* (Billiet et al, 2017). Whilst the bumblebee microbiota is quite robust, it can become destabilised during times of stress, such as with exposure to pathogens in the environment (Parmentier et al, 2016). This leads to a loss of core symbionts and their replacement by non-core microbes

which is often linked to disease in hosts (Levy et al, 2017). Therefore, it is vital for understanding mechanisms of radiation effects that we obtain a better understanding of whether stressors experienced by bumblebees affect this aspect of their biology.

1.12.2 Bumblebees: Responses to stress

Bumblebees are an ideal model system to study the impacts of differing stressors due to extensive research on their biology and their well-studied life history. As there are some species of bumblebees that are considered threatened, it is vital we understand how any stress effects their physiology and the mechanisms driving any changes. Bumblebees can be negatively affected by a multitude of stressors, for example in the agricultural landscape many different neonicotinoids have been found to change foraging behaviour and reduce foraging efficiency (Henry et al, 2012; Sandrock et al, 2014). Further work has shown that they also suffer impaired immune function following exposure (Simmons & Angelini, 2017). Similar effects have been recorded when bumblebees were exposed to immune stimulation, bumblebees responded to this stress by increasing energy consumption by 7.5% (Tyler et al, 2006). This suggests that individuals may sacrifice some physiological systems to ensure consistent supplies of energy. A common stressor within environments is that of food related stress, which will have key impacts on bumblebee life history. When a queen bumblebee has access to restricted nutritional resource during the early stages of her development it has been found that queens are 50% less likely to survive hibernation (Woodard et al, 2019). Increasing global temperatures will also affect bumblebee nutrition through higher temperatures leading to reduced foraging activities (Gerard et al, 2022). It is therefore important to understand how bumblebees re-allocate resources.

Bumblebees are also found in contaminated landscapes such as the Chernobyl Exclusion Zone for which there is a growing body of evidence that they exhibit responses to this novel stressor. Laboratory work has shown a significant reduction in the number of queens produced at dose rates found in some areas of the CEZ (Raines et al, 2020). Additionally, when the stressor of radiation was combined with that of the common bumblebee gut parasite, there was found to be a decline in lifespan as a result of shortened parasite incubation levels (Raines, unpublished). Therefore by investigating the effect of novel stressors such as

radiation, we can also better understand the mechanisms driving previously recorded physiological responses.

1.13 Drosophila as another key study organism

Whilst bumblebees are an important organism for which to investigate the effects of radiation due to their well-studied life history and importance to ecosystem services. We need to explore the effects of radiation on more than one species to understand if any impacts on life history are unique to bumblebees due to social aspects of their biology. I propose that the organism *Drosophila melanogaster* is an ideal organism to provide generality to studying the effects of radiation. Additionally, some of the first work examining the effects of x-rays found mutations within *D. melanogaster* which led to the Nobel prize being awarded (Muller, 1946). *D. melanogaster* is still to do this day one of the most used model organisms due to its rapid generation times and extensive knowledge about its genetics (Tolwinski, 2017). The genome of *D. melanogaster* was sequenced in the year 2000 and contains over 14000 genes (Vaiserman et al, 2021). This will allow the study of the effects of radiation on various aspects of its physiology through generations to better understand long term impacts. The generation time of *D. melanogaster* is approximately 10 days from fertilised egg to development in to an eclosed adult, the maximum lifespan of this fly is then between 60 to 80 days (Fernandez-Moreno et al, 2007). This short lifespan allows the investigation of the schedule of reproductive activity, for example high doses of radiation have been shown to lead to decreased body weight in F1 generations (Vaiserman et al, 2004; Vaiserman et al, 2021). High doses of radon have also been shown to reduce fecundity (Piementel et al, 2003). This key species has also been recorded in contaminated environments such as the Chernobyl Exclusion Zone, which means that it could be an ideal organism to try to understand the effects of radiation on life history traits (Mosse et al, 2006).

1.14 Aims and Objectives

The overall aim of this thesis is to identify whether radiation dose rates that are found within the Chernobyl Exclusion Zone have any fitness impacts on the ecologically important bumblebee. I also aim to identify at what dose rates I may begin to see any physiological changes within individuals of the species *Bombus terrestris*. I also set out to find any potential

mechanisms that may be driving effects, whilst also identifying core universal biomarkers of radiation. To do this I drew on life history theory, which assumes that investment in fitness related traits is heavily associated with resource availability and allocation. As a result I investigated the effect of radiation exposure on the consumption of energy and its allocation to metabolic rate. I then followed the journey of nutrition through the bumblebee, to understand how it was being used biochemically. Many of the sugars obtained by bumblebees through nectar are broken down within the gut, so I explored how the microbiome within it were also affected by nutrition and radiation exposure. I also aimed to understand if the effects recorded in bumblebees were unique to this species or whether they could be extrapolated to other insects. So for the final chapter I conducted a study on *Drosophila melanogaster* to investigate radiation life history effects on reproduction.

1.15 Research Hypothesis

The central hypothesis addressed in the second chapter of this thesis is:

- I hypothesise that radiation exposure negatively affect life history traits, through impacts on the bumblebee energy budget.

The second chapter titled "Ecologically relevant radiation exposure triggers elevated metabolic rate and nectar consumption in bumblebees" focuses on nutritional investment which is a key aspect of life history theory. This chapter addresses a key knowledge gap within radiation literature in which various exposure pathways and differing life history responses often makes general predictions about the effects of low dose radiation difficult. This chapter sets out to provide a more generalisable way of assessing radiation effects rather than simply choosing a single life history trait, such as reproduction.

For the third chapter of this thesis, the following hypothesis was investigated:

- Radiation drives an increase in nectar consumption that bumblebees are using biochemically.

In the third chapter titled “The biochemical and nutritional consequences of increased metabolic activity as a result of radiation exposure in bumblebees” I followed nectar consumed by bumblebees through the digestive system, to understand if nectar was being immediately used biochemically, stored or used to fuel a metabolically costly recovery process as a result of radiation exposure. This study included a resource limitation treatment in order to understand if a metabolically costly recovery process was being elicited as this would result in a faster run down of resources within bumblebees.

In the fourth chapter I explore the hypothesis:

- Ecologically relevant radiation exposure either indirectly affects the bumblebee microbiome through increased nectar consumption or is directly affecting bacteria within the gut through damage.

The fourth chapter follows the nectar solution as it passes down through the gut. It was hypothesized that this extra nectar passing along the gut would alter bacterial abundance. I therefore performed 16S Sequencing of the V4 region to investigate changes in gut microbial community composition in response to radiation exposure.

To investigate whether the responses recorded throughout the thesis are unique to bumblebees, due to unique aspects of their physiology and biology, I set out to understand if low dose radiation affects another key species. For the fifth and final data chapter of this thesis I addressed the hypothesis:

- The effects of radiation on reproduction can be found again in another species, particularly *Drosophila melanogaster*.
- Reproductive output changes with ageing under radiation exposure.

For the fifth chapter titled “Levels of radiation exposure similar to those which can be found in the Chernobyl Exclusion Zone cause reduced fecundity and developmental success in *Drosophila melanogaster”*, I selected this well-established model organism to allow a better assessment of the generality of radiation effects. I aimed to understand how radiation

impacts reproduction over a substantial proportion of the fly life span to provide a better understanding of the effects experienced by an individual living in a contaminated environment.

1.15.1 Structure

The first chapter of this thesis briefly introduces ionising radiation and its known effects on humans, provides a background on the effects of low dose radiation in contaminated environments, explores bumblebee biology/physiology and states the research aims of the data chapters in this thesis. The chapters 2 - 5 are data chapters written in the form of journal articles from experimental work carried out at the University of Stirling. The second chapter has been peer-reviewed and published in the Journal of Functional Ecology. The conclusions from these four data chapters are drawn together in the final thesis conclusion in Chapter 6.

Chapter 2: Ecologically relevant radiation exposure triggers elevated metabolic rate and nectar consumption in bumblebees

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Key Words: Ionising radiation, Life history, Insects, Resource allocation, Pollinator, Energy budget, Eco-toxicology, Radiological contamination.

2.1 Abstract

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

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

(3) I monitored metabolic rate, movement and nectar intake of bumblebees before, during and after 10 days of radiation exposure. Subsequently, I monitored feeding and body mass across a dose rate gradient to investigate the dose rate threshold for these effects. I studied dose rates up to 200 µGy hr^{-1}: a range found today in some areas of the Chernobyl Exclusion Zone.

(4) Chronic low dose radiation affected bumblebee energy budgets. At 200 µGy hr^{-1} nectar consumption elevated by 56% relative to controls, metabolic CO_2 production increased by 18%, and time spent active rose by 30%. Once radiation exposure stopped, feeding remained elevated but CO_2 production and activity returned to baseline. My analysis indicates that elevated metabolic rate was not driven by increased activity but was instead closely associated with feeding increases. My data suggest bumblebee nectar consumption was affected across the 50-200 µGy hr^{-1} range.

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

landscapes, including Chernobyl. I demonstrate bumblebees are more sensitive to radiation than assumed by existing international frameworks for environmental radiological protection.

2.2. Introduction

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

We know relatively little about the effects of radiation on most animals compared to humans (Basu, 2018). Many laboratory radiation-effect studies, on invertebrates in particular, have been delivered at high acute dose rates (typically > 60 Gy d^{-1}) (ICRP, 2008; Andersson *et al.*, 2009). Furthermore, the wide diversity of species studied, with contrasting ecologies, varying radiation exposure pathways, and differing potential life history responses, makes general

predictions about the likely effects of environmental contamination difficult. This diversity may contribute to the conflicting results from contemporary Chernobyl Exclusion Zone studies of how radiation affects animal life history and population-level metrics: findings range from no effect (Baker *et al.*, 2001; Bonzom *et al.*, 2016; Fuller *et al.*, 2019) to significant negative consequences at comparatively low dose rates (Møller *et al.*, 2007; Kesäniemi *et al.*, 2019). Investigations include those at the population level (e.g. pollinator abundance (Møller, Barnier and Mousseau, 2012)), organismal physiology (e.g. sperm abnormalities in birds (Hermosell *et al.*, 2013)), and cytogenetical effects (e.g. chromosomal aberrations in bank voles (Rykabon and Goncharova, 2006)). The mechanisms by which the dose rates currently found at Chernobyl could negatively affect animal life history are currently unclear (Smith, Willey and Hancock, 2012). To better assess radiation effects on organismal ecology we require understanding of biological processes that bridge the gap between the molecular signatures of exposure that are difficult to interpret, and fitness-related traits that appear to be inconsistently affected.

In this study I use a novel experimental approach to assess the impact of ecologically-relevant radiation exposure. I draw on life history theory, which assumes an organism's investment in fitness-related traits is constrained by resource availability and by decisions on resource allocation between these traits (van Noordwijk and de Jong, 1986). Both resource acquisition and the manner in which resource trade-offs are resolved can change dramatically when organisms are exposed to stressors, potentially due to a re-allocation of resources towards traits promoting survival (Eeva, Hakkarainen and Laaksonen, 2006; Hladun *et al.*, 2012; Fritsch, Jankowiak and Wysocki, 2019). For example, the challenges associated with responding to pathogen infection and pesticide pollution alter feeding behaviour, changing resource acquisition and metabolic rate (Tyler, Adams and Mallon, 2006; Bashir-Tanoli and Tinsley, 2014; Baas and Kooijman, 2015). I hypothesised that studying whether resource use and acquisition are influenced by radiation would be a proximate way of assessing radiation effects that has the potential to be more generalisable across species than picking single life history traits such as lifespan, fecundity or immune defence. Radiation effects on resource use might be manifested as either a decrease in energy use if radiation triggers major metabolic impairment, or as an increase in energy expenditure if radiation triggers metabolically costly recovery processes or stress responses. Whilst effects of ionising

radiation on metabolic rate have not been studied in invertebrates, ultraviolet exposure has been observed to elevate CO_2 production in mosquitos, which suggests metabolically active processes can respond to radiation exposure (Villena *et al.*, 2018).

I studied the ecologically important bumblebee *Bombus terrestris*, a species found in the Chernobyl Exclusion Zone and in which Chernobyl-level radiation has been shown to reduce reproduction (Møller and Mousseau, 2009; Raines *et al.*, 2020). Bumblebees are eusocial pollinators for which resources are essential for colony growth, maintenance and nest cell provisioning (Konzmann and Lunau, 2014). Whilst their eusocial biology makes bumblebees rather unusual, many physiological responses of individual bumblebees may be generalisable to other species with solitary ecology. Floral nectar is a key energy source for bumblebees, which usually varies in sugar content between 15 and 64% (Seely, 1995); large quantities of nectar are needed to fuel a high mass specific metabolic rate and rapid colony growth trajectories (Duncan, Krasnov and McMaster, 2002; Goulson, 2010). Bumblebees, like many insects, exhibit discontinuous gas exchange, in which release of carbon dioxide and uptake of oxygen occurs cyclically (Miller, 1981).

The International Commission on Radiological Protection uses eusocial bees as a Reference Animal to generalise the likely ecological effects of radiation to all insects: this framework currently assumes insects are unaffected by radiation below 417 µGy hr^{-1} (ICRP, 2008). When this sensitivity threshold was set there were no data on radiation effects for bumblebees below 417 µGy hr^{-1}, instead data were taken from studies on insects of similar size to justify this threshold (ICRP, 2008). Additionally, data for this threshold was extrapolated from studies using short term exposures to higher dose rates which solely focused on responses of death and sterility. Yet some studies within the Chernobyl Exclusion Zone controversially suggest that there are significant reductions in bumblebee abundance at dose rates typical of natural background (0.01 - 1 µGy hr^{-1}) (Møller and Mousseau, 2009; Møller, Barnier and Mousseau, 2012). Recent laboratory work demonstrates impairment of bumblebee queen production down to 50 µGy hr^{-1} (Raines *et al.*, 2020).

I hypothesised that bumblebee metabolism responds to environmentally-relevant ionising radiation exposure. Therefore I investigated whether radiation exposure alters metabolic rate

and nectar consumption, whilst also measuring bumblebee activity and body mass changes; furthermore, I tested whether radiation effects persisted once exposure stopped. I also hypothesised that altered metabolic requirements might change bumblebee nectar preferences, so I conducted experiments using nectar solutions of varying concentration.

2.3 Materials and Methods

I investigated radiation effects on bumblebee energy budgets via two complimentary experiments. The first investigated the effect of three environmentally-relevant dose rates on bumblebee metabolic rate, nectar consumption and activity. The second used an exposure gradient to test for a dose rate threshold in the effect of radiation on feeding on nectar solutions ranging from 20% – 50% (w/v).

2.3.1 Study system husbandry

I purchased *Bombus terrestris audax* colonies from Biobest® for each experiment (experiment 1, n = 10; experiment 2, n = 5). To identify newly eclosed bumblebees, on arrival every colony was anaesthetised with CO_2 and all bumblebees marked using commercial bumblebee paints. Each day following marking newly eclosed (unmarked) bumblebees were removed and weighed by placing the individual in to a pre-weighed tube and then subtracting the weight of that tube for final mass. Bees were then individually housed prior to experiments in clear plastic containers (55mm (l) x 55mm (w) x 60mm (h)) with access to *ad libitum* pollen, nectar solution and cotton wool as nesting material. Bumblebees remained in these containers throughout subsequent experiments; containers were cleaned every 5-7 days. The nectar solution was 40% w/v sucrose in distilled water, provided in a 12 ml falcon tube with a hole punctured in the side for feeding. I verified bumblebees were uninfected by the common gut parasite *Crithidia bombi* by microscopically inspecting faeces from a random sample of workers per colony (minimum n = 18); all tested negative. This research was conducted with the approval of the University of Stirling Animal welfare and Ethical Review Body (AWERB, Project Number: 122 (19 20).

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

To test effects of ecologically-relevant radiation dose rates on bumblebee energy budgets I measured feeding rate (nectar volume consumed) and metabolic rate (CO_2 production). Bumblebees were placed in the University of Stirling environmentally-controlled radiation facility (12 hr light: dark cycle (07h – 19h)) on shelves at different distances from a ^{137}Cs source to deliver controlled doses of gamma radiation (three distances within the radiation field and one control group outside the radiation field, Figure 2). Dose rates were verified prior to the experiment by placing dosimeters at each bumblebee position on each shelf. Bumblebees were kept in containers in two adjacent rows on each shelf (one row closer to the source and one further away) and moved between rows every two days to ensure no systematic dose rate variation occurred. Due to the 110mm position difference between the front of one row and the back of the other, the maximum a dose rate could vary within this space was +/- 9 µGy h^{-1} at 200 µGy h^{-1}, +/- 3 µGy h^{-1} at 100 µGy h^{-1} , then dropping to +/1 at 40 µGy h^{-1}. The maximum dose was chosen as it represents one of the highest doses recorded in the CEZ. Part of the same environment-controlled room with the same conditions, but not exposed to radiation, was used to house the control treatment (Figure 2). Background radiation levels at the University of Stirling are 0.11 ± 0.01 µGy h^{-1} (Raines *et al.*, 2020). Nine data loggers around the facility recorded temperature and humidity every 2 minutes; the mean of these environmental variables was calculated for each bumblebee from the nearest data logger for the 2 days before each feeding rate measurement (mean = 25.6°C, range ± 0.3) and humidity (mean = 32.1%, range ± 13.1). The mortality rate of bumblebees throughout the experiment was 7.8%, with only 5.3% mortality between days 1 and 20 (n = 288 bumblebees). There was an average of 30 bumblebees taken from each original colony and an average of 2 bumblebees died from each of the colonies.

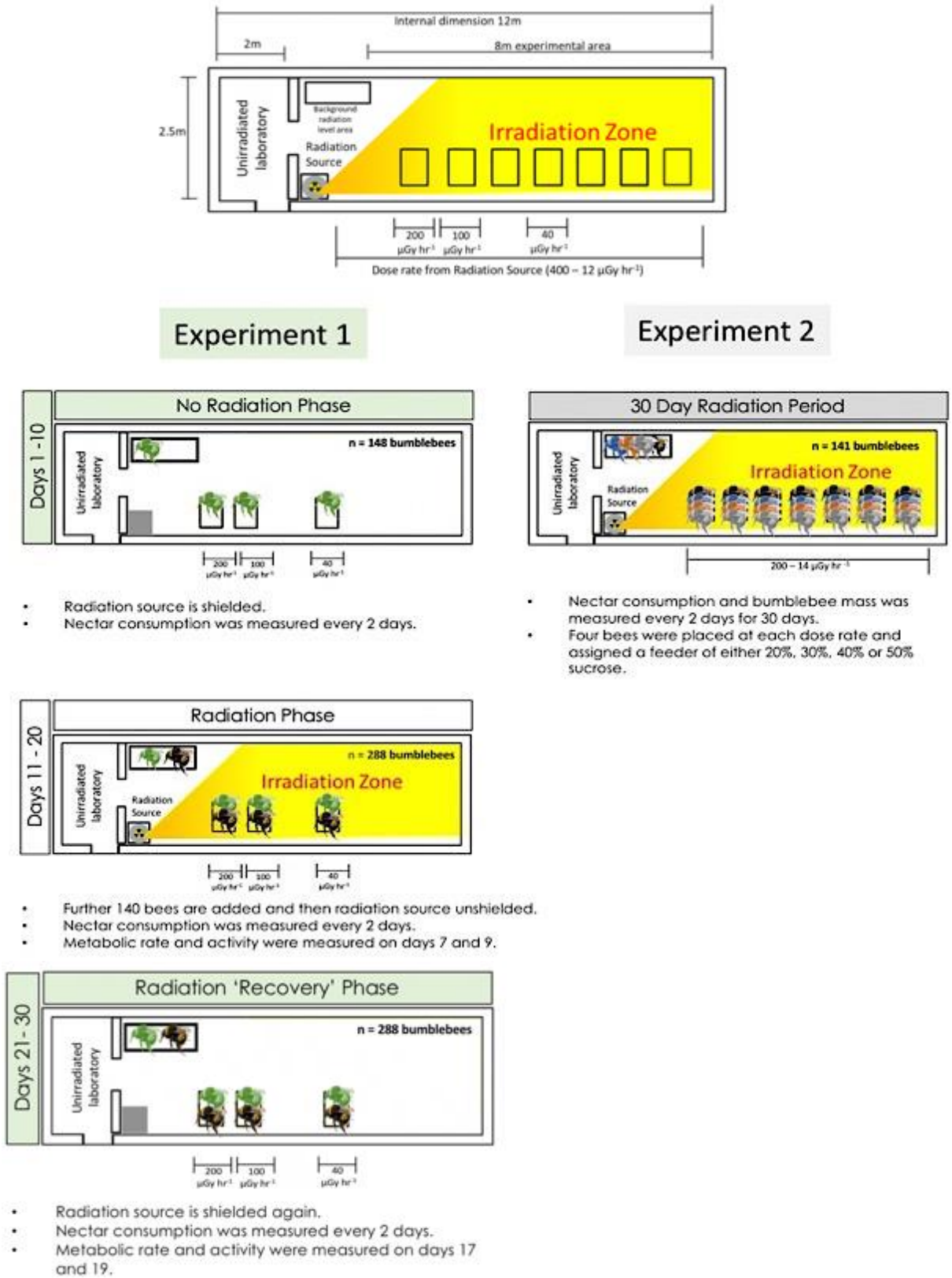

Figure 2: A diagram of the radiation facility at the University of Stirling**.** The top image represents the radiation facility with dimensions. The subsequent diagrams represent the two experiments and their design. For

experiment 1: black boxes represent shelving units on which bumblebees were placed. Green bumblebees represent those that entered the experiment on day 1 and black bumblebees represent those that entered in the radiation phase. For experiment 2: the different coloured bees represent each of the four sucrose feeding treatments of 20%, 30%, 40% and 50%.

The experiment involved three 10-day phases. First, to verify no confounding environmental effects influenced metrics, a 'no radiation' phase in which bumblebees were placed at assigned 'dose rate' positions but the radiation source remained shielded and no radiation was delivered (n = 148 bumblebees). Bumblebees were assigned positions in a stratified random way so neither age (days since eclosion) nor body mass (at eclosion) differed between dose rate groups ($F_{(3, 145)}$ = 2.84, p = 0.10 and $F_{(3, 145)}$ = 0.87, p = 0.36 respectively). Then followed a 'radiation' phase with four dose rate treatments (200, 100, 40 and 0.11 µGy h^{-1}) for 10 days; at this time 140 more bumblebees were added (n = 288 in total; n = 72 per dose rate). The maximum dose rate was chosen as one of the highest dose rates recorded in the CEZ, whilst the lowest was chosen as the lowest dose that has recorded effects in bumblebees (Raines, thesis). Again, no differences existed between dose rates for age ($F_{(3, 285)}$ = 0.10, p = 0.75) or mass ($F_{(3, 285)}$ = 0.16, p = 0.69). The final experimental phase tested if effects on bumblebees were transient: bumblebee 'recovery' from radiation was monitored for 10 days whilst the source was again shielded. The dose rate of the radiation source is 402 µGy h^{-1} and radiation was delivered chronically. During the 10-day irradiation phase the total accumulated doses were 48000 µGy (200 µGy h^{-1}), 24000 µGy (100 µGy h^{-1}), 9600 µGy (40 µGy h^{-1}). During this phase room temperature was maintained (mean = 25.6°C, range ± 0.3).

To measure bumblebee feeding I weighed nectar tubes every two days; feeders were re-filled when empty and changed every four days (tube shown in Figure 1.2). From when the radiation phase started, for half of the individuals (n=144), I tested if radiation influenced bumblebee nectar preference between a high and low concentration sucrose solution (5% vs 40% w/v) by providing two 14 ml feeders. A nectar concentration of 5% is very low, but values below 10% can be found in some plant species (Nicholson & Thornburg, 2007).

To assess bumblebee metabolic rate and activity levels I measured bumblebee CO_2 production whilst simultaneously filming movement for a 60-bumblebee subset (30 from 200 µGy h^{-1} and 30 controls) on days 7 and 9 of both 'radiation' and 'recovery' phases (days chosen

to detect an effect after several days of radiation and due to lack of time not repeated on more days). Bumblebees selected were a mixture of those that entered the experiment at the start of the 'radiation' phase and those present for all three phases. Twelve bumblebees died in total between the first and fourth measurement and were replaced by another randomly chosen bumblebee from that treatment to maintain sample size. CO_2 output was measured using an infrared gas analyser (IRGA: EGM-4; PP Systems, Amesbury, MA, USA). Bumblebees were taken from the radiation facility to an adjacent room and housed in transparent plastic cylinders (34.36 cm^3) individually connected to the IRGA with tubing in an open flow system. This room was not temperature controlled, therefore air was drawn through tubing from the adjacent climate-controlled facility, through the chambers containing bumblebees, and then to the IRGA using an air pump (flow rate = 0.6 l min^{-1}). Air flow temperature (mean = 25.2^oC, range ± 1.3) and humidity (mean = 32.2%, range ± 9.1) was recorded and averaged for the 5-minute duration of all measurements. Bumblebees were left to acclimatise for five minutes prior to recordings. CO_2 levels were measured from a single chamber at a time using batches of four chambers; a manifold was used to switch recording between chambers. CO_2 was measured every 1.6 seconds for 5 minutes; to calculate bumblebee CO_2 output I subtracted ambient CO_2 measurements recorded from air flowing through an empty reference chamber for 30 seconds immediately after each recording. I converted each bumblebee's mean CO_2 output to µmol min^{-1} using flow rate and the ideal gas law (PV=nRT) which accounts for system pressure and volume. To monitor bumblebee activity during the 5-minute metabolic rate recording, a video camera (FHD camcorder, 1080p, 30MP) filmed movements. Subsequently the video was reviewed: total time bumblebees spent inactive (standing still or only moving legs, antennae or wings) or active (walking in the tube or buzzing) was recorded. Additionally, total distance each bumblebee walked was recorded from the video based on tube length. To minimise time out of radiation exposure bumblebees were only removed from the radiation facility for a maximum of 30 minutes for each measurement period. Within these 30 minutes a group of four bumblebees first had a 5-minute acclimatisation period followed by sequential 5-minute metabolic rate measurements on each of the bees, with 30 second background measurements taken in between.

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

2.3.3. Experiment 2: Dose-rate threshold of the radiation effect on bumblebee nectar consumption

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

2.3.4 Statistical analysis

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

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

Radiation effects on nectar consumption and metabolic rate were analysed using linear mixed effects models in lmer from package lme4 (Bates *et al.*, 2015). The pre-exposure phase was used as a baseline for comparison of radiation and recovery phases. Models investigated radiation and recovery phases separately. Predictors included dose rate (continuous variable), time within a phase and their interaction. I included covariates for bumblebee mass and age at the start of the experiment, and assessed their interactions with dose rate to test for condition-dependent effects. Access to a second feeder was included as a factor. Environmental variables temperature, humidity (at the nearest data logger) and their interactions with each other were also included. Two-way interactions between dose rate and the environmental variables temperature and humidity were included in models analysing nectar consumed during the radiation phase from the 40% and 5% feeders to verify they did not influence dose rate effects. I tested if radiation effects varied between radiation and recovery phases by combining data for both phases, then adding a phase term to the model alongside its interactions with dose rate and day. To investigate whether radiation dose rate influenced bumblebee activity and distance covered in the chamber during metabolic rate measurements, I constructed zero-inflated gaussian generalized linear mixed models in glmmTMB (Brooks *et al.*, 2017); the response variable was number of seconds active (in 5

min), predictors were as above. A gaussian zero inflated model was selected as 38.3% of observations were zero movement.

I tested associations between variation in feeding, metabolic rate and activity using Bayesian multi-response mixed effects models in MCMCglmm (Hadfield, 2010). A multi-response model was used to examine covariance between all response variables. Response variables were: total sucrose consumption in the 2 days before metabolic rate measurement; mean CO_2 output; and a two-vector response encompassing number of seconds active and inactive during metabolic rate measurements (error distributions were: gaussian, gaussian and binomial respectively). Fixed effects enabled independent intercepts for each response variable, and for each to be independently affected by radiation treatment (control vs 200 μGy h^{-1}) and bumblebee starting mass. This model just compared control vs 200 μGy h^{-1} as these are the only dose rates for which I have metabolic rate data. Models had three random effects: colony, bumblebee and residual error; for each I specified a trait interaction to estimate variances and covariances between response variables in an unstructured covariance matrix. I used parameter expanded priors (prior specification for variances) for colony and bumblebee random effects. My analysis focussed on correlations between traits in the residual error term, reflecting how between-replicate variation in the three response variables was associated. Markov chains ran for 60,000 iterations, discarding the first 10,000 interactions and sampling every 50 iterations. Parameter modes and p values were calculated from the posterior. Standard diagnostics verified low correlation between posterior samples (<0.1), chain convergence and insensitivity to prior specification.

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

To test for a lower dose rate threshold for radiation effects on nectar consumption general linear mixed effects models (using lmer) included covariates for nectar concentration, dose rate and days-within-experiment, alongside their interactions up to three-way. Additional covariates were bumblebee mass and age at the experiment start. I also tested if consumption was influenced by the interaction between dose rate and weight at the start of the experiment. Further models investigated if bumblebee mass, dry weight and thoracic temperature were affected by radiation exposure, with the same predictor structure (except

for models with mass response variables where start age was excluded due to variable collinearity). To test if environmental variables influenced dose rate effects found, the two-way interactions with dose rate for humidity and temperature were included for models analysing nectar consumption and mass change.

2.3 Results

2.3.1. Experiment 1: The effect of radiation on bumblebee nectar consumption

Before radiation exposure commenced I verified that positional effects within the radiation facility did not influence nectar consumption. 148 bumblebees were assigned positions where they would subsequently receive radiation and were then monitored for 10 days whilst the radiation source was shielded. Nectar consumption was not associated with future dose rate, demonstrating no confounding unmeasured environmental differences around the facility (Table S2.1).

During the radiation exposure phase, dose rate was significantly associated with elevated consumption of 40% nectar solution (Figure 2.1; Table S2.2; $\chi^2_{(1)} = 39.74$, $P = 2.90 \times 10^{-10}$). It did however take 5 days for effects on nectar consumption to significantly establish (Day 5 data: $\chi^2_{(1)} = 16.67$, $P = 7.5 \times 10^{-5}$). This effect of radiation on feeding became stronger as time exposed increased (Figure 2.1; Table S2.2; dose rate by day interaction, $\chi^2_{(1)} = 38.25$, $P = 6.22 \times 10^{-10}$). After 10 days exposure to 200 µGy hr^{-1} bumblebee consumption increased by 56% compared to controls. Furthermore, for the initial 148 bumblebees, pairwise differences between nectar consumption one day before radiation started and one day after were not significant for any dose rate treatment (Table S2.5).

Bumblebees with higher body mass consumed more nectar (Table S2.2; $\chi^2_{(1)} = 16.94$, $P = 3.85 \times 10^{-5}$), but mass did not affect response to radiation (Table S2.2; dose rate by bumblebee mass, $\chi^2_{(1)} = 0.14$, $P = 0.70$). Slight temperature and humidity variation in the controlled environment facility affected feeding; both variables were positively associated with nectar volume consumed, however their effects were independent of radiation dose rate (Table S2.2). I tested whether radiation affected bumblebee preference for nectar sucrose concentration: half the bumblebees had a second feeder containing 5% nectar in addition to the 40% nectar feeder (which the analyses above focussed on). When comparing how much a bumblebee consumed from both the 40% and 5% feeders, across all feeding records (n=2275) bumblebees consumed 52.1% (± 2.1 SE) from the 40% feeder. There was no significant effect of radiation on the volume of nectar consumed from the 5% feeder (Table S2.6; $\chi^2_{(1)} = 0.37$, $P = 0.54$).

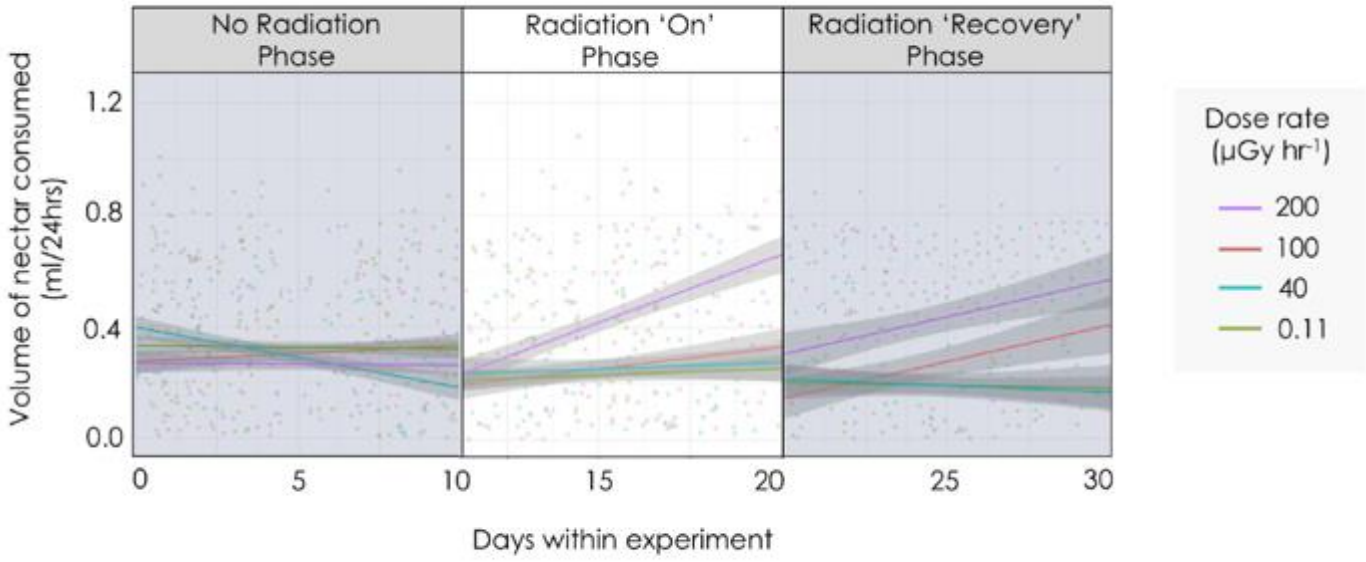

Figure 2.1. Exposure to radiation dose rates elevated bumblebee nectar consumption (40% sucrose), both during a 10-day irradiation phase and throughout the subsequent 10-day recovery. Data are presented for consumption during the no radiation phase (graph on the left), radiation 'On' phase (graph in centre) and 'Recovery' phase (graph on the right). The trend lines and shaded 95% confidence intervals were calculated from a mixed effects model with the same terms as shown in Table S2.2. The figure was generated from an analysis on each phase to provide an independent estimate of the dose rate effect. Plotted coloured points represent raw data values and were jittered. Grid lines are for ease of axis interpretation. For pre-radiation phase: n= 444 observations on n= 148 bumblebees. For radiation 'On' and 'Recovery' phases: n= 864 observations on n = 288 bumblebees.

I assessed 40% nectar consumption during a 10-day recovery phase after radiation exposure stopped: the effect of the previously-delivered dose rate persisted (Table S2.7; $\chi^2_{(1)} = 21.35$,

$P = 3.84 \times 10^{-6}$). Similar to the radiation phase, effects of prior dose-rate on appetite continued to increase with time for the higher dose rates, despite bumblebees no longer being exposed (Figure 2.1; dose rate by day interaction, $\chi^2_{(1)} = 12.48$, $P = 4.11 \times 10^{-4}$). As further evidence that elevated nectar consumption persisted once radiation exposure stopped, I pooled radiation and recovery phase data and found, after accounting for temporal changes, no difference in the effect of radiation between phases (Table S2.8).

2.3.2. Experiment 1: The effect of radiation on bumblebee metabolic rate and activity

I assessed metabolic rate by measuring CO_2 production in a subset of 60 bumblebees split equally between 0.11 and 200 µGy hr^{-1}. During the exposure phase, CO_2 production was 18% higher in bumblebees receiving 200 µGy hr^{-1} than in controls (Table S2.9; $\chi^2_{(1)} = 4.80$, $P = 0.03$). The strength of this effect was consistent on both days 7 and 10 of exposure (Table S2.9; radiation exposure by day interaction, $\chi^2_{(1)} = 0.11$, $P = 0.75$). Across both treatments CO_2 production fell significantly between days 7 and 10; it was also affected by small variations in air temperature. However when examining the relationship between the temperature effect and radiation exposure, this change in temperature was independent of dose rate (Table S2.9). During the recovery phase (when radiation ceased) the difference in CO_2 production between the control and 200 µGy hr^{-1} treatments was no longer significant (Table S2.10; $\chi^2_{(1)} = 1.66$, $P = 0.20$). Indeed, there was a significant change in the effect of radiation between the exposure and recovery phases (Table S2.11; radiation exposure by phase interaction, $\chi^2_{(1)} = 5.54$, $P = 0.02$).

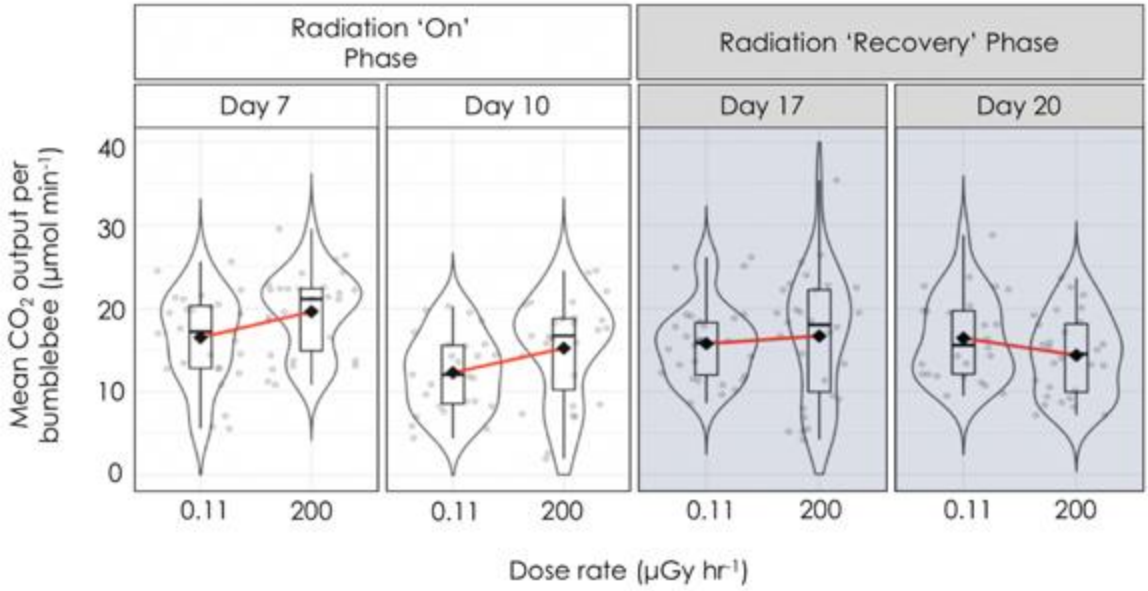

Figure 2.2. Bumblebee mean metabolic rate elevated with radiation exposure, a difference that disappeared when exposure stopped during the 'recovery' phase. Graphs show differences in mean carbon dioxide output for bumblebees on days 7 and 10 of the radiation phase (left) and recovery phase (right). Points on each graph show mean carbon dioxide output per bumblebee. The model analysing these data is shown in Table S2.12, which combines both the radiation and recovery phase data; the fit is represented by the red line and black diamonds, highlighting differences between dose rates in mean CO_2 output. n= 240 observations, n = 60 bumblebees.

Bumblebees exposed to radiation moved more during the metabolic rate assays. Across all activity observations bumblebees were inactive for 28% of the time. My zero-inflated analysis demonstrated no effect of radiation on the probability of bees moving (Table S2.12). However, for those bees that did move, time active was 30% higher following exposure to 200 µGy hr^{-1} compared to controls (Table S2.12; $\chi^2_{(1)}$ = 2.10, P = 0.04). This difference disappeared by 7 days into the 'recovery' phase (Figure 2.3; Table S2.13). I found quantitatively the same results when considering the distance a bumblebee travelled as a metric of movement (Tables S2.14 & S2.15).

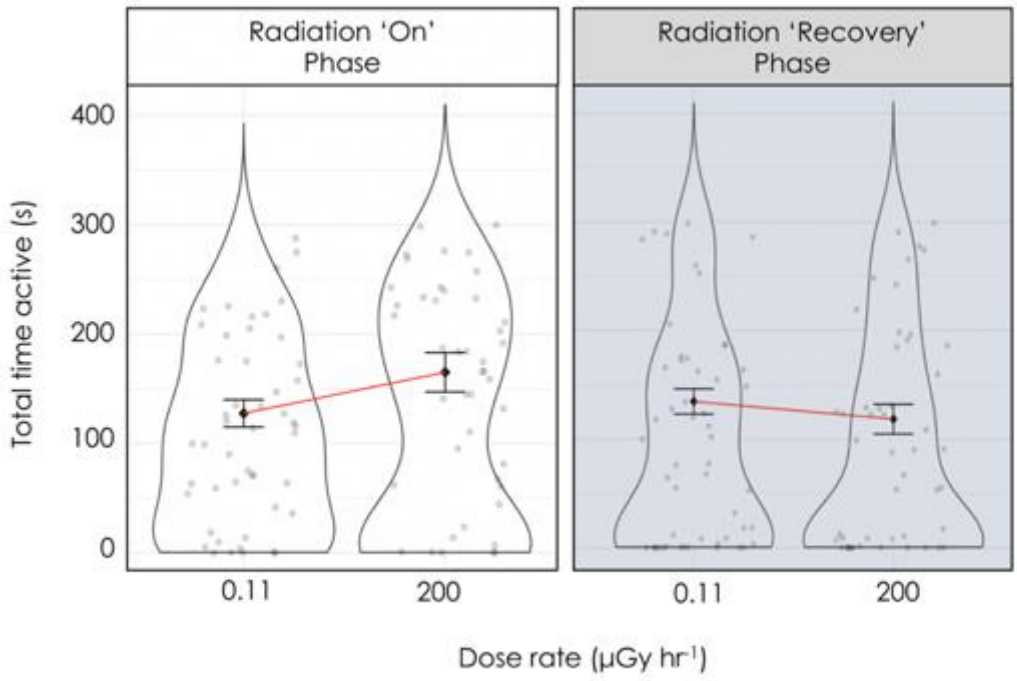

Figure 2.3. The time bumblebees spent active increased under radiation exposure. The left panel shows the 'on' phase when bumblebees were exposed to radiation and the right panel shows the 'recovery' phase when radiation exposure stopped. The single black point and whiskers represents mean time a bumblebee spent moving and the standard error calculated from the gaussian part of the zero-inflated model. The red line denotes differences in mean time a bee was active between control bumblebees and irradiated bumblebees. Grey points represent raw data. The model from which this was calculated is the minimal model presented in Table S2.16, which combines both the radiation recovery data. n= 240 observations, n = 60 bumblebees.

To investigate links between radiation-induced changes in bumblebee metabolic rate, nectar consumption and activity I assessed the extent that variation in these measures was correlated during the radiation phase. Across all bumblebees, nectar consumption in the 2 days prior to metabolic rate measurements was significantly positively associated with CO_2 output (correlation +0.31, 95% HPD $1.36x10^{-3}$ – 0.47; P_{MCMC} = 0.03); however, metabolic rate was not correlated with bumblebee activity levels during those measurements (correlation +0.01, 95% HPD -0.21 – 0.23; P_{MCMC} = 0.49). There was a weak but non-significant positive association between nectar consumption leading up to measurement and bumblebee activity levels (correlation +0.22, 95% HPD -0.12 – 0.40; P_{MCMC} = 0.12). Recovery phase results were qualitatively similar, though associations were weaker.

2.3.3 Experiment 2: Dose-rate threshold of the radiation effect on bumblebee nectar consumption

I then undertook a completely new experiment in which I investigated if I could determine if a detectable dose rate threshold existed for the effect of radiation on nectar consumption that I found above. I conducted an independent experiment on 141 bumblebees to investigate appetite effects along a dose rate gradient (192 – 0.11 μGy hr^{-1}). I also tested if radiation effects changed in response to increased sugar availability, by giving bumblebees one of four different nectar concentrations. The methodology for this was the same as above in terms of weighing of feeders and bumblebees to monitor consumption.

Increasing dose rate was again associated with increased nectar consumption during 30 days exposure (Figure 2.4a; Table S2.16; $\chi^2_{(1)} = 4.89$, $P = 0.03$). Whilst there was an overall trend that higher dose rates were associated with greater feeding, in this experiment the magnitude of this response varied with time and for different nectar concentrations (Table S2.16; Figure S2.3; concentration of nectar by dose rate by days within experiment, $\chi^2_{(1)} = 6.03$, $P = 0.01$). After 10 days exposure, the dose rate effect was only evident for bumblebees consuming 50% nectar, whereas after 20 days it was the 40% nectar group that showed a compelling trend. (Figure S2.3).

Clearly, the major driver of the increase in Figure 2.4a is because there was a substantial effect of radiation on feeding at higher dose rates. However, I investigated this dataset to determine whether the lower and intermediate dose rates also generated a statistically significant uplift in feeding. To do this I systematically removed data points from the analysis in increments of 10 μGy hr^{-1}, starting with the highest dose rates, thereby restricting my analysis to progressively lower dose rates. This process inevitably reduced our sample size and statistical power. Whilst Figure 2.4b appears to be opposite to Figure 2.4a, it actually shows the magnitude of the effect per 1 μGy, this suggests that per μGy the impact of low dose rates is actually greater than higher dose rates but certainty at low dose rates is limited by lack of statistical power. For three of the truncated data sets between 192 and 100 μGy hr^{-1} the radiation effect remained significant (Table S2.17). To compare the effect-size at different dose rates I calculated the increase in nectar consumption per unit of exposure (μGy hr^{-1}) for each truncated data set; this parameter remained approximately consistent down to dose rates of 50 μGy hr^{-1} (Figure 2.4b). This does not show that the total radiation effect on

appetite at 50 μGy hr^{-1} was the same as at 192 μGy hr^{-1} but instead that the effect of increasing dose rate was broadly linear between 50 and 192 μGy hr^{-1}. At the lowest exposed dose rates I studied (14 - 30 μGy hr^{-1}), my estimates of feeding elevation per μGy hr^{-1} became substantially larger, though sample sizes for these analyses were small and confidence intervals much broader (Figure 2.4b).

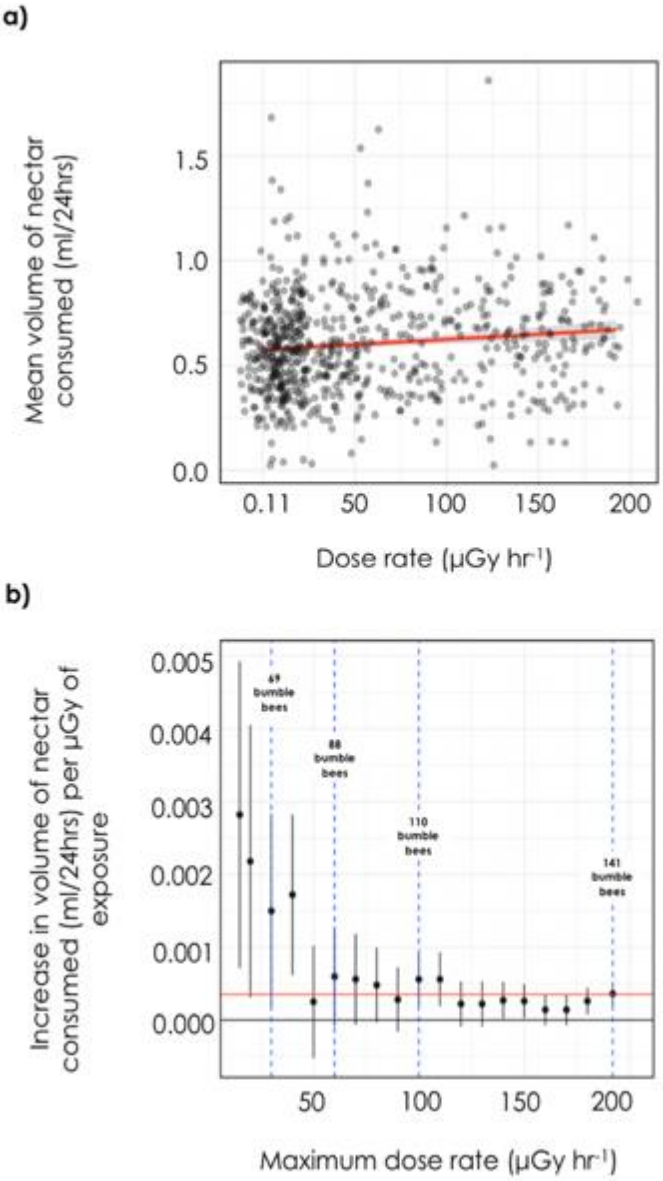

Figure 2.4a) The mean volume of nectar consumed by bumblebees increased in a dependent manner with dose rate. Data are pooled across the 30-day experimental period. Plotted points represent raw data values and were jittered. The red line represents model fit from Table S2.17. **b)** The increase in nectar consumed per µGy of exposure. Parameter estimates were calculated by progressively omitting the highest doses of radiation from the model presented in Table S2.16. The red line denotes mean increase in volume consumed, calculated from all data up to 192 µGy for reference. Blue dashed lines denote the number of bumblebees remaining in the analysis when the doses above were removed. Error bars denote standard error. n= 847 observations (8 repeated measures per bumblebee, n = 141 bumblebees.

In general bumblebees lost mass during the experiment (Table S2.18; $\chi^2_{(1)}$ = 18.09, *P* = 2.17x10^{-5}), but dose rate did not influence this mass loss (dose rate by days interaction, $\chi^2_{(1)}$ = 0.53, *P* = 0.47). In contrast, when I assessed the effect of radiation on bumblebee dry weight at the end of the experiment there was a significant effect of dose rate mediated by the effect

of bumblebee starting mass (wet weight). Higher dose rates were associated with greater dry mass for bumblebees that started the experiment at mid and heavy mass, but there was little effect of dose rate for light bumblebees (Figure 2.5; Table S2.19; dose rate by starting mass interaction; $\chi^2_{(1)} = 18.71$, $P = 1.76 \times 10^{-5}$). Bumblebees regulate body temperature partly by endothermic heat generation; whilst there was a marginal trend for bumblebees at higher dose rates to be warmer, this was not significant (Table S2.20; $\chi^2_{(1)} = 2.54$, $P = 0.11$).

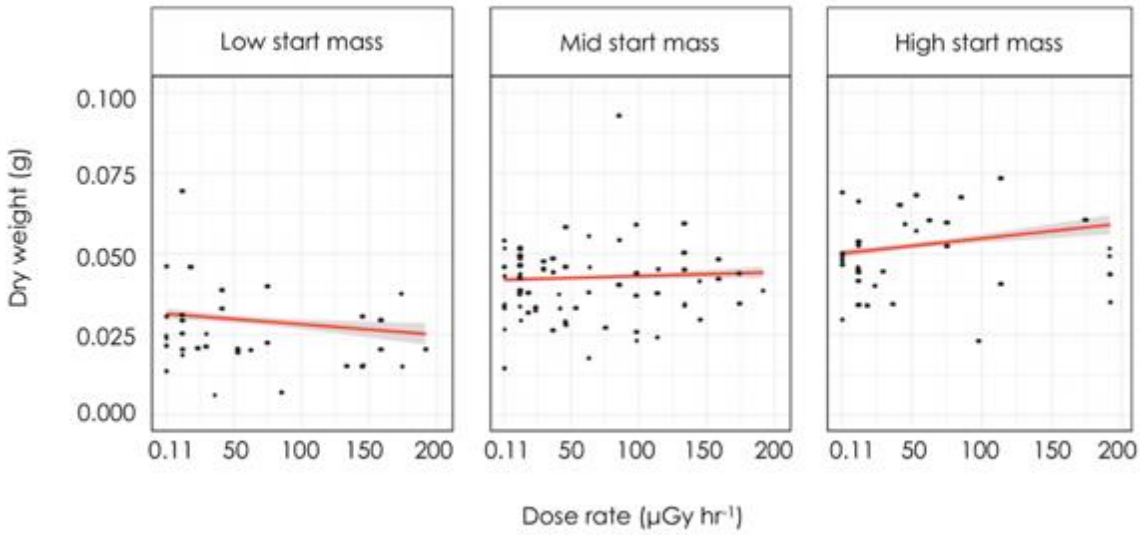

Figure 2.5. Exposure to radiation elevated dry weight of bumblebees depending on their mass when they entered the experiment. Data are presented for the mass of bees as they entered the experiment and categorised into low (0-0.12g), mid (0.12 – 0.2g) and high (>0.2g) start weights. The trend lines and shaded 95% confidence intervals were calculated from a mixed effects model with the same terms as shown in Table S2.19. The figure was generated by fitting a categorical factor for weight of a bumblebee when it entered the experiment, alongside a start weight by dose rate interaction, to provide an independent estimate of the dose rate effect for each weight category, n= 121 bumblebees.

2.4 Discussion

I present evidence that ionising radiation significantly affects insect metabolism and energy budgets, demonstrating that field-realistic radiation exposure influences fundamental metabolic processes in an ecologically important species. Substantial increases in bumblebee nectar consumption occurred during irradiation and remained even after exposure. Radiation-induced increases in food intake and metabolism might potentially influence many life history traits through changes in resource budgets. I observed significant energy budget

changes at 100-200 μGy hr^{-1}; dose rates found today in more highly contaminated areas of the Chernobyl Exclusion Zone (Beresford, Scott and Copplestone, 2020). These effects occurred at dose rates below those currently considered safe for bumblebees by the International Commission on Radiological Protection (ICRP, 2008).

Bumblebee nectar consumption increased by 52% at 200 μGy hr^{-1} (accumulated dose of 48000 μGy), compared to controls following 10 days of irradiation. Bumblebees must have stored, metabolised or excreted this additional nectar. Whilst my ability to detect resource storage was limited, radiation dose rate did drive an increase in dry weight (at least for larger bees) during 30 days exposure. However, I detected no radiation effect on wet mass (weight of alive individual) of live bumblebees during the experiment. This change in dry weight (weight of deceased bee once dried to remove moisture) and not the wet mass of a live bumblebee suggests some material is accumulating within the bumblebees during radiation exposure; one potential explanation is that a stress response is occurring that has led to some excess nectar being stored as fat. Indeed, fat storage occurs in bumblebees under other stresses such as parasite infection (Vesterlund and Sorvari, 2014); however further work is required to test whether this is the case for radiation exposure. Whilst the effect on dry mass should be expected to be seen also in wet mass, bumblebee tissues have a high-water content. Therefore, the removal of water allows the better assessment of fat material. We additionally did not assess excretion; therefore, I cannot rule out that some of the additional nectar passed straight through the gut. I found no evidence that bumblebees used additional nectar for endothermic heat generation because body temperature was not significantly influenced by radiation. However, metabolic rate increased by 18% at 200 μGy hr^{-1} (accumulated dose of 48000 μGy); because nectar consumption increased by 52%, this suggests that only about one third of the additional sucrose eaten contributed to metabolic rate elevation. Whilst bumblebee activity increased by 30% at 200 μGy hr^{-1}, residual variance in activity was not associated with between-individual metabolic rate variation, indicating that increased movement was not the main driver of elevated metabolism. Instead, between-bumblebee variation in metabolic rate was significantly correlated with nectar consumption, suggesting radiation-induced feeding elevation may be a response to fuel unmeasured energetically costly radiation responses. Bumblebees do indeed suffer adverse fitness effects from radiation at these dose rates, such as impaired reproduction (Raines *et al.*, 2020). I

speculate that the increased bumblebee movement I observed happened either because increased appetite triggered food searching, or as a direct effect of radiation on behaviour.

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

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

The post-exposure period during which radiation effects on feeding persisted is a substantial proportion of a worker bumblebee's 22-69 day lifespan (Smeets and Duchateau, 2003). Bumblebees in radiologically contaminated landscapes such as Chernobyl may spend their entire life exposed to the dose rates I studied; therefore radiation-induced feeding increases

might escalate over their lifecycle. Increased nectar consumption would require more and longer foraging flights. Increased foraging might invoke other costs for bumblebees, such as elevating parasite transmission, which generally occurs on flowers during feeding (Shykoff and Schmid-Hempel, 1991). Worker foraging efficiency would be reduced by higher metabolic rates, which might also increase resource requirements of larvae in developing broods. Radiation-induced metabolic rate elevation could directly impact bumblebee life expectancy, as workers with higher resting metabolic rates die sooner (Kelemen *et al.*, 2019). Radiation effects like these may well impair bumblebee colony reproduction, as has been shown in the laboratory (Raines *et al.*, 2020). Nevertheless, my current study did not address colony-level fitness, therefore it is not possible to extrapolate directly from the physiological and behavioural effects of radiation I observed on workers to the potential consequences for bumblebee populations in the field. It remains possible that the cumulative impacts of the effects I detected could have wider detrimental impacts on pollination ecosystem services in radiologically contaminated environments.

I tested for a lower dose rate threshold at which increased nectar consumption disappeared. Feeding increases were significant between 100 - 200 μGy hr^{-1}. Below 100 μGy hr^{-1}, with smaller sample sizes, feeding elevation was not statistically significant, but the effect per unit of radiation exposure stayed relatively constant down to 50 μGy hr^{-1}(Figure 2.4b), indicating radiation effects may persist into this dose rate range or effects may be delayed at this dose rate. Whilst this graph appears to show that the effects below 50 μGy hr^{-1} there is a loss of statistical power due to smaller sample sizes, therefore future studies should use higher sample sizes to find at what dose rate this effect begins. My findings have policy implications for the International Commission on Radiological Protection's environmental protection framework, which classifies dose rates below 417 μGy hr^{-1} as safe for bumblebees and other insects. My data corroborate recommendations of Raines *et al* (2020) that this threshold should be lowered.

The Chernobyl Exclusion Zone landscape is heterogeneously contaminated with ambient external dose rates ranging from typical background levels up to 250 μGy hr^{-1} (Beresford, Scott and Copplestone, 2020). In contaminated environments radiation exposure occurs both externally from gamma radiation, but also from other routes including internal accumulation

of radionuclides. However, 95% of dose exposure to bumblebees at Chernobyl is from external gamma, indicating that my experimental design mimics natural exposure effectively (Beresford *et al.*, 2020). It is possible that there is some food chain transfer within bumblebees but this has yet to be investigated. Many studies from Chernobyl report negative radiation effects on organisms (Møller *et al.*, 2007; Møller and Mousseau, 2009; Møller, Barnier and Mousseau, 2012; Hermosell *et al.*, 2013; Kesäniemi *et al.*, 2019). Yet the mechanisms driving these effects generally remain unclear. My study identifies a process that may underpin some of these radiation impacts in contaminated environments. Resource availability is a dominant constraint on life history trait investment (van Noordwijk and de Jong, 1986). I have discovered that radiation increases bumblebee resource requirements, elevating metabolism and feeding. The extent to which these effects can indeed be generalised to other insects and animals more widely, will depend on whether they are specific to bumblebees (with their eusocial biology). Whilst eusociality does make bumblebees atypical, the fact that I studied individual workers (rather than colony-level traits) means that my results may well be relevant to other organisms with solitary ecology.

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

Chapter 3: The biochemical and nutritional consequences of increased metabolic activity as a result of radiation exposure in bumblebees

Key Words: Ionising radiation, Nutrition, Haemolymph Sugar, *Bombus terrestris*, Biochemistry, Adaptive response, Eco-toxicology, Radiological contamination.

3.1 Abstract

(1) Ecologically relevant radiation exposure increases metabolic rate and nectar consumption in bumblebees. However, our understanding of how radiation impacts the utilisation and storage of nutrients is limited. It is important to understand these impacts in bumblebees as sugar obtained from nectar is fundamental in driving elevated metabolic rates in response to radiation exposure. Additionally, the fat body of insects acts as their metabolic centre and therefore investigating lipid storage under stress is vital to understand metabolic needs.

(2) Here I studied the presence, storage and transport of important nutrients in bumblebees under ecologically-relevant radiation exposure treatments. I included a resource limitation treatment in order to understand if bumblebee responses to radiation are metabolically costly.

(3) I conducted biochemical tests on bumblebee body tissue (excluding organs) and haemolymph after 14 days of radiation exposure. I investigated effects of radiation exposure on the levels of energy stored in tissues. I additionally explored whether radiation influenced the energetic response to nutrient limitation. To do this I manipulated resource acquisition for half of the bumblebees in the final four days of the experiment in order to create a 'resource limitation' treatment. For this study I used dose rates that are ecologically relevant to contaminated landscapes such as at Chernobyl.

(4) I verified my previous findings that radiation exposure elevates both bumblebee metabolic rate and nectar consumption in bumblebees. my data indicated there was no effect of radiation on the storage of energy within tissue: carbohydrate, glycogen, lipid and proteins. In contrast, radiation exposure significantly increased haemolymph sugar concentrations. My data suggest that these effects start at doses as low as 40 µGy h^{-1}. The four-day resource limitation treatment reduced bumblebee weight but did not alter the magnitude of the radiation effects on nutrient levels.

(5) My data enhance understanding of the impact of radiation exposure on bumblebee metabolic processes. I demonstrate that the additional sugar consumed during radiation exposure enters the bumblebee haemolymph but does not lead to additional storage within the tissues. Accompanied by my observation of radiation-induced metabolic rate increase,

this suggests that additional sucrose consumed is rapidly metabolised in an as yet unrecognised physiological process. I hypothesise that the gut microbiome may contribute to the changes I observed in sugar metabolism. This study therefore contributes to identifying the mechanism that is driving changes in bumblebee life history in response to radiation exposure.

3.2. Introduction

Food is the critical source of nutrients for insect survival and any reduction in its provision can negatively affect growth, reproduction and lifespan (Chang, 2015). The reduction in availability of food occurs commonly in the environment, such as with changes in seasons or local ecological damage. Any reduction in food availability can cause organisms extreme stress. However, when an insect is exposed to an ecological stressor such as limited resource, it can often adapt physiologically, for example glycogen which is associated with potassium is stored in fat cells, liver and muscles. Therefore it can be mobilised during low temperature stress to prevent cellular damage (Denlinger, 1991). In response to these common stressors it has been found that insect responses broadly fall into two categories: behavioural changes or physiological counter-measures (Zhang et al, 2019). These physiological measures can include the regulation of metabolism and biochemical substances within the body in order to assist with the endurance of the stressor (Yang et al, 2016). By studying the effects of important ecological stressors on biochemical changes, we can better understand adaptive responses to physiological stress.

In this study I investigated the combination of a novel stressor and resource limitation, to understand if bumblebees alter their nutrient utilisation to fuel metabolically costly recovery mechanisms. Bumblebees are floral generalists that forage solitarily to visit a wide range of plant species to collect pollen for protein and nectar for sugars. When foraging, bumblebee workers should seek out the highest concentration of sugar, however this can be affected by various factors including age and physiological state of the individual and colony (Simpson and Raubenheimer, 2012). This foraging strategy provides them with the required proteins, lipids and micronutrients, with floral nectar providing the main source of carbohydrates (Vaudo et al, 2016). The most common carbohydrate in nectar, which bees can only obtain from plant sources, is sucrose. When bumblebees consume nectar, the disaccharide sucrose is broken down into the monosaccharide's glucose and fructose within the midgut (Figure 3).

These sugars then diffuse down concentration gradients across the midgut epithelium into the haemolymph where they are used as a primary energy source, or are stored in the fat body until carbohydrate concentration levels drop (Steward et al, 2014). Carbohydrates are a key source of energy in bumblebee physiology and are primarily found as glycogen, trehalose and glucose. In particular, trehalose is used for the storage of carbohydrates, as well as in the transport of carbohydrate in the haemolymph (Figure 3; Yu et al, 2008). Additionally, when the intensity of stresses such as starvation increases, glycogen is often converted to trehalose to maintain energy metabolism (Bede et al, 2007). This conversion often occurs to meet small short-term deficits in nutrition, however for longer term and more severe deficits fat is often metabolised. The storage of fat is also key for normal life processes in insects (Park et al, 2013), with even moderate nutrient deprivation leading to changes in bumblebee energy storage strategy for which there is an increased requirement to store reserves as fat (Lorenz, 2001).

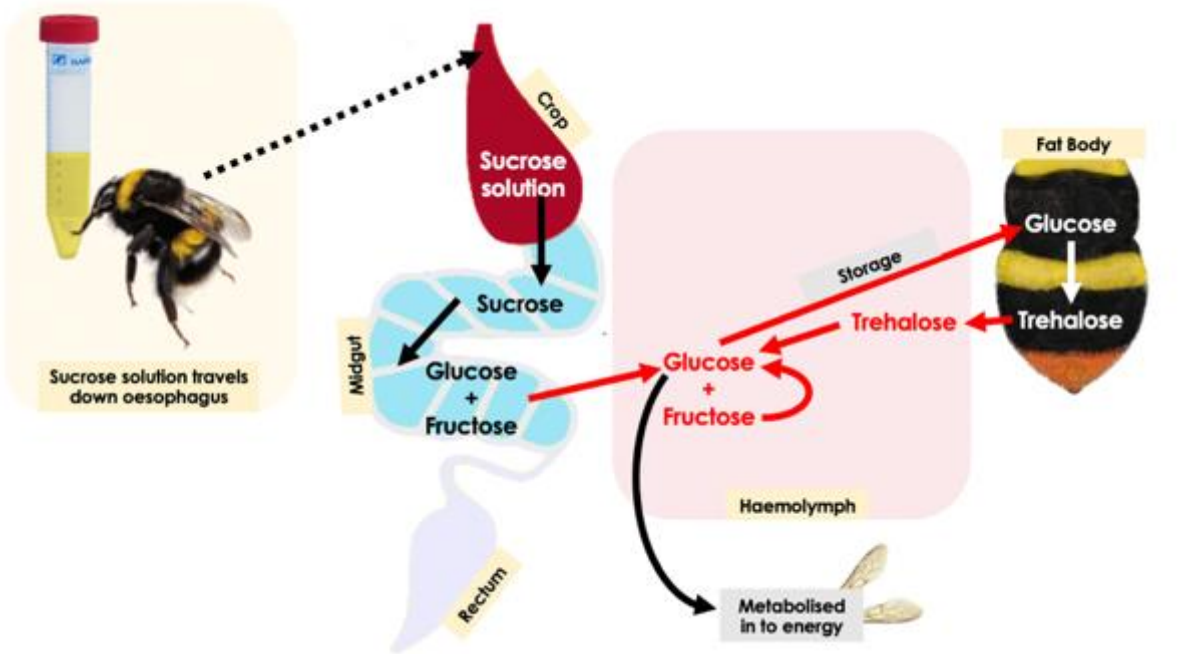

Figure 3. The mobilisation of energy within the bumblebee digestive system, haemolymph and fat body. When a bumblebee feeds on nectar, it travels down the oesophagus and is stored in the crop. Within the crop there is some pre-digestion of sucrose into monosaccharides via salivary enzymes. When carbohydrate concentrations in the haemolymph drop, the passage of nutrients from the crop to the midgut is facilitated through the contraction of the gut muscles. In the midgut, sucrose is converted into glucose and fructose. After this, both

diffuse into the haemolymph. Glucose is one of the main sources of energy within the haemolymph and so any excess that is not used to fuel cellular metabolism is converted into trehalose within the fat body and stored for future use. This figure can also be seen in the introduction (Figure 1.2)

Some studies have investigated the effect of stress on sugar levels found in the haemolymph for a variety of bee species. In honeybees, individuals respond to starvation stress by using and depleting haemolymph glucose levels (Wang et al, 2016), yet when under normal conditions honeybees keep haemolymph glucose and trehalose concentrations constant (Blatt and Roces, 2001). Most bee species can forage across large areas of landscape to obtain the resources they require. It is therefore important to understand their ability to regulate their nutrient usage and storage under unfavourable conditions. When exposed to neonicotinoid pesticides, honeybees have shown evidence of altered nutritional and metabolic physiology, including a reduction in lipid storage in a dose dependant manner (Cook, 2019). Previous studies have shown how stressors such as chemical pesticides may lead to colony collapse in honeybees by causing nutritional imbalances (Branchicella et al, 2019). However, there is a lack of literature surrounding these effects on bumblebees. Nutrition plays an important role in life history of insects as reproduction is influenced by the quality and quantity of food (Fischer et al, 2004), therefore it is vital that I understand the effect of stressors on nutrient storage and use, especially in ecologically important species such as bumblebees.

A unique stressor for which there is growing evidence of its effect on bumblebee physiology and life history, is low dose ionising radiation. Organisms are constantly exposed to low levels of radiation from a variety of natural sources, however large-scale nuclear accidents such as that which occurred at Chernobyl in 1986 have dramatically increased environmental exposure in specific areas. This novel stressor therefore should be investigated to better understand its impacts on species living in radiologically contaminated environments. In the CEZ, it has already been reported that radiation has led to a reduction in pollinator abundance at dose rates as low as UK background (Møller, Barnier and Mousseau, 2012). Further laboratory work has also shown effects on bumblebees at doses of 100 $\mu Gy\ h^{-1}$, which include a 6% decline in bumblebee colony queen production and significantly delayed colony growth (Raines et al, 2020). When bumblebees were exposed to both the effect of radiation and the stressor of parasitism in a laboratory setting, there was also found to be a significant decline

in lifespan, potentially driven by shortened parasite incubation times and elevated parasite levels within the gut (Raines, 2020). Recently, studies have also shown that dose rates as low as 40 µGy h^{-1} elevate bumblebee metabolic rate and the volume of nectar bumblebees consume (Chapter 2 ; Burrows et al, 2022). These impacts of radiation at lower dose rates highlight a real need to further understand the mechanisms driving these physiological changes.

My previous study described a metabolic syndrome that occurs as a result of radiation exposure in bumblebees, which includes an increase in nutrient acquisition that remains after exposure and metabolic rate which recovers after exposure (Burrows et al, 2022). Here, I set out to investigate this radiation-induced upregulation of energy use. There are a number of possibilities for what happens to this additional consumed sugar and therefore different explanations as to why this feeding change occurs, these include: (a) sugar was just simply excreted; (b) sugar is being stored within tissues; (c) sugar is being immediately used to fuel an energetic response; or (d) a potentially metabolically costly recovery process is occurring as a result of radiation exposure.

I hypothesise that if radiation is activating a fundamentally important energy intensive recovery process, then it will be prioritised under nutrient limitation conditions, leading to the rapid depletion of energy storage. I used biochemical measures on tissue and haemolymph samples to estimate energy reserves, alongside the mobilisation and transport of carbohydrate resources. I therefore designed the experiment to investigate the answers to these questions by combining radiation-exposure treatment with two resource-level treatment groups: bumblebees which had access to abundant resource and bumblebees with limited nutritional resource. Both of these resource treatment groups were kept in the same conditions and exposed to environmentally relevant radiation exposure. I predict that the upregulation in energy consumption is being used to fuel a metabolically costly response to radiation exposure, and as a result there will be less sugar being stored within tissues.

3.3. Materials and Methods

I conducted multiple biochemical tests on bumblebee tissue and haemolymph samples in order to understand how radiation exposure influences the storage and processing of energetic resources within bumblebees. I additionally investigated physiological measurements relevant to energy storage in bumblebees such as metabolic rate, the volume of sucrose consumed and scaled mass index. These measures were used to verify previous findings (Burrows et al, 2022) and also to investigate the presence of a metabolic syndrome in response to radiation exposure.

3.3.1 Study system husbandry

For this study a total of 6 *Bombus terrestris audax* colonies were purchased from Biobest® and these produced 362 bumblebees for this experiment. Immediately upon arrival a total of 20 bumblebees from each colony were tested for the common gut parasite *Crithidia bombi* by microscopically inspecting faeces; all samples tested negative. Subsequently each colony was anesthetised with CO_2 and all bumblebees present were marked using commercial bee paints. Each day following this all newly eclosed bumblebees were identified and removed from the colony, they were then weighed and their thorax width measured. Each bumblebee was kept in an individual container (55mm (l) x 55mm (w) x 60mm (h)) throughout the experiment with access to *ad libitum* pollen, nectar solution and cotton wool as nesting material. These containers were cleaned every 5 days. Nectar solution was provided (40% w/v of sucrose in distilled water) in a 12 ml falcon tube with a hole punctured in the side for feeding.

3.3.2 Irradiation Treatment

In order to measure the effects of radiation on bumblebee energy storage I placed a total of 362 bumblebees in an environmentally controlled radiation facility at the University of Stirling. Environmental variables were constantly monitored by data loggers and varied minimally: temperature (mean = 25.7^{o}C, range ± 0.8) and humidity (mean = 39.1%, range ± 14.2). Within this controlled facility (12 hr light: dark cycle (07h – 19h)) bumblebees were kept at three different distances (n = 90 bumblebees at each dose rate) from a ^{137}Cs source in order for them to receive gamma radiation at three dose rates (200 µGy h^{-1}, 100 µGy h^{-1} and 40 µGy

h^{-1}); a different section of the radiation facility, which is not exposed to radiation, was used to house a control treatment. The dose rate of the source is 402 µGy h^{-1}. Bumblebees were exposed for 14 days and received accumulated doses of 67200 µGy (200 µGy h^{-1}), 33600 µGy (100 µGy h^{-1}), and 13400 µGy (40 µGy h^{-1}). This control section experiences the exact same environmental conditions but was only exposed to the background radiation rate at the University of Stirling, which is 0.11 ± 0.01 µGy h^{-1} (Raines *et al.,* 2020). All dose rate locations were verified using a dosimeter. All containers in the radiation treatments were kept in two parallel rows on shelving units that were 110mm wide in the direction of the radiation field. Containers were swapped between front and back rows daily to eliminate consistent differences in dose rate. The maximum dose rate variability caused by this position effect was +/- 9 µGy h^{-1} at 200 µGy h^{-1}, dropping to +/1 at 40 µGy h^{-1}.

All bumblebees were assigned to a dose rate in a stratified and random way so that neither age, nor mass at the start of the experiment varied between dose rate groups ($F_{(3, 356)} = 0.14$, $p = 0.82$). To assess the extent of environmental variation in the radiation facility during the experiment 9 data loggers were used to record temperature (mean = 25.78°C, range ± 2) and humidity (mean = 34.8% RH, range ± 2.89). The spatial and temporal variation in these environmental metrics was minimal. Three bumblebees (one at 100 µGy h^{-1} and two at 0.11 µGy h^{-1}) died during the experimental period and were not replaced.

Bumblebees were exposed to radiation treatments for 14 days (see Figure 3.1). For the first 10 days all bumblebees were fed on 40% w/v sucrose solution. However, on day 10 I manipulated resource acquisition. Whilst half of the bumblebees at each radiation dose rate remained on 40% w/v sucrose solution (n = 179 bees in total), for the other half I used a 'resource limitation' treatment where the 40% w/v feeder was replaced with a 5% w/v feeder for the final four days of irradiation (n = 179 bees in total). The 5% sucrose concentration was chosen as it was the lowest sucrose concentration to sustain life. The four-day duration was chosen as several experiments using 20 bumblebees each time recorded a 25% death rate after four days. I measured bumblebee feeding throughout the experiment by weighing and replacing nectar tubes on day 1, day 7 and day 10. All bumblebees received new (pre-weighed) feeders on day 10; feeders were reweighed on day 14 before experiment

termination. Throughout the experiment, all feeders were checked daily and changed every three days.

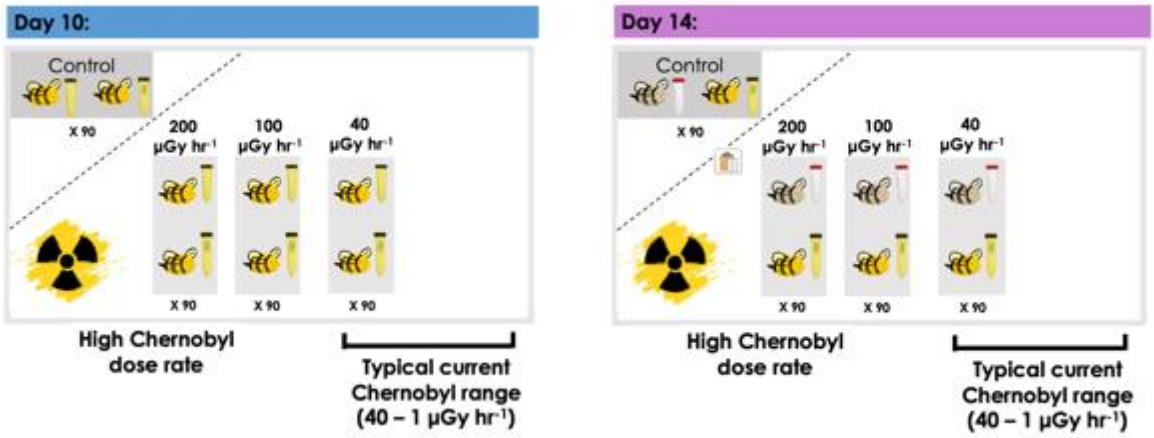

Figure 3.1 A visual representation of the experimental design. The left panel represents the experiment from day 0 to day 10, in which 90 bumblebees are places at 3 dose rates (200, 100 and 40 µGy h^{-1}). A further 90 bumblebees were placed in a control area where they were unaffected by the radiation source (0.11 µGy h^{-1}). Each bumblebee had access to a 40% (w/v) feeder. The right panel represents the experimental design from day 10 to day 14. All bumblebees remained at the position but half were given low concentration feeders 5% (w/v) represented by the empty feeding tube.

In order to quantify any changes in body mass, all bumblebees were weighed on a balance measuring to 0.1 mg on days 7, 10 and 14. The analytical balance was used for all weight measurements to 0.1mg (Denver Instrument, model PI-225DA). The measure was taken by placing an individual in a pre-weighed tube and then subtracting the weight of that tube to give final mass. I then calculated scaled mass index for each bumblebee using these mass measurements and thorax width which was recorded at eclosion (Peig & Green, 2009). Scaled mass index was used as it is considered to give a good index of condition by relating the mass of a bee to its size.

To assess bumblebee metabolic rate, I measured CO_2 production of all bumblebees prior to experiment termination on day 14 using the methodology of Burrows et al, (2022). CO_2 output was measured using an infrared gas analyser (IRGA: EGM-4; PP Systems, Amesbury, MA, USA). All bumblebees were placed into experimental chambers for five minutes before measurements were taken. Air flow temperature (mean = 25.42°C, range ± 7.2) was recorded and averaged for the 5-minute duration of all measurements. I took the mean CO_2 output for each bumblebee and converted it to µmol min^{-1}. This value was then converted using the flow

rate of the infrared gas analyser and ideal gas law (PV=nRT) to account for any changes in pressure and volume of air within the system.

3.3.3 Haemolyph extraction

After recording metabolic rate on day 14 I collected haemolymph samples for subsequent haemolymph sugar quantification. I removed each bumblebee from the radiation field and anesthetised it with CO_2. I then made a small incision in the thorax using a sterile scalpel blade. A total of 5µl of haemolymph was collected from this incision using a graduated glass micropipette. After the extraction of haemolymph, the bumblebee was immediately euthanised and preserved by placing it in a 1.5ml Eppendorf tube and dropping this into liquid nitrogen before being stored at -80 ^{o}C for later analysis. The haemolymph sample was transferred from the graduated micropipette to an Eppendorf tube, it was then also dropped into liquid nitrogen and stored at -80 ^{o}C until testing took place.

3.3.4 Bumblebee tissue and gut preparation

After the completion of the experiment I undertook analysis of the tissues and haemolymph in order to estimate energy reserves within each bumblebee. I began by preparing tissue for the investigation of energy storage through biochemical analysis.

Prior to this analysis each bumblebee was slowly defrosted on ice to prevent tissue damage. I prepared the tissue by removing the bumblebee gut (whole gut - mouth to anus) in order to prevent tissue contamination with gut material. This also allowed us to take measurements on the bumblebee gut in order to assess potential impacts of the increased flow of nectar solution through it. The gut of each bumblebee was removed under a microscope by creating a long incision along the bumblebee abdomen with a scalpel blade. The midgut was then isolated and placed on a pre-weighed weigh boat before mass was determined to 4 decimal places (µg) . In order to measure the weight of gut contents, a piece of filter paper was selected and pre-weighed. The gut was then gently squeezed on to this filter paper using tweezers. This was then weighed on the analytical balance to measure gut content. I then re-weighed the gut in order to measure its weight when empty. Additionally, due to observed colour variation when midguts were removed, each gut was photographed and a scale used

to quantify this variation. The guts were graded from 1 (lightest colour gut – almost visually clear) to 5 (darkest gut – black in colour) (see Figure 3.2).

Figure 3.2. The colour of guts removed from bumblebees exposed to radiation. The guts were graded between 1 and 5 based on their colour, with gut colour 1 the lightest (far left) and gut colour 5 the darkest (far right). For the purposes of these images the honey stomach of bumblebees is attached to provide a clearer view of the colour of the digestive system. All guts are imaged within a weighing boat prior to gut contents and stomach being removed.

3.3.5 Energy Storage in Tissue Samples

I used the methodology of Houslay et al, (2017) to measure energy storage in bumblebees by quantifying carbohydrate, glycogen, lipid and protein content of bumblebee tissues. After the gut dissection (above) bees were refrozen in an Eppendorf tube at -80^{o}C. To begin this analysis of energy storage within the bumblebee, the tissue (with the digestive system already removed) was taken from the freezer and dropped again into liquid nitrogen. The tube containing the bumblebee was then removed from the nitrogen and tissue crushed within the tube to a powder form using a micropestle. In order to break open cells within the sample I then added 1 ml of Lysis buffer (100mM KH_2PO_4, 1mM of DTT (dithiothreitol) and 1mM of EDTA (ethylenedianimetetra-acetic acid)), this buffer was set to a pH of 7.4. To separate out insoluble matter I then centrifuged these samples at 1107 RCF at 4^{o}C. The soluble fraction was used for analysis of carbohydrates through the anthrone assay. The pellet from this reaction was then used to assess glycogen content of bumblebee tissues.

The total carbohydrate of bumblebee tissue was measured using the anthrone assay. To complete this assay I first took a total of 180 µl of the lysis buffer homogenate and combined

it with 20 µl of 20% (w/v) sodium sulphate solution (mixed with distilled water) and then mixed with 1500 µl of 1:2 (v/v) chloroform: methanol solution to solubilise carbohydrates. An Anthrone assay was then performed by taking 150 µl of the chloroform:methanol supernatant to a 96-well plate. In order to evaporate liquid and leave sugars behind, this plate was then incubated at room temperature for 30 minutes. For the next step I added 240 µl of anthrone reagent (Sigma-Aldrich), these plates were then incubated for 15 minutes at room temperature. The microplate was then further heated at 70^{o}C for 15 minutes before absorbance was read at 625nm; D-glucose used to create a standard curve to calibrate the result.

In order to measure glycogen content of the bumblebee tissue, I collected the pellet fraction created by the carbohydrate test and transferred it to a new Eppendorf tube. This pellet was then washed with 80% methanol three times, vortexed and centrifuged at 31,483 RCF at 4^{o}C. I carefully removed the supernatant ensuring the pellet was not disturbed and then added 1ml of Anthrone Reagent (Sigma-Aldrich). The pellet was then incubated for 20 minutes at 70^{o}C. The samples were subsequently cooled on ice and collected via a plastic syringe. This liquid was forced through a low-protein binding membrane syringe filter by gently plunging the syringe handle. The filtrate was then transferred to a 96 well plate. The final absorbance was read at 625nm. D-glucose was used to create a standard curve for calibration purposes.

For the determination of lipid content in bumblebee tissues I conducted a Vanillin assay. For this assay the preparation of a reagent is required through the combination of 1.2g/L of vanillin powder and 68% ortho-phosphoric acid. This reagent is created and stored within a foil covered tube in order to protect it from light. In order to measure lipid content of tissues I subsequently took 100µl of homogenate from the chloroform:methanol extract to a 96 well microplate and heated it at 70^{o}C for 30 minutes. Then 10 µl of 98% sulfuric acid was added to each well and incubated at 70^{o}C for 5 minutes before being left on ice. I added 190 µl of Vanillin reagent, incubated for a further 15 minutes and measured absorbance at 525 nm. To convert absorbance to lipid concentration I created a standard curve using a dilution series of Triolein.

In order to determine the protein content of each bumblebee sample I performed a Bradford Assay. For this analysis I collected 2.5 µl of the original lysis buffer homogenate and combined

it with 200 µl of pre-mixed Bradford reagent (Sigma-Aldrich). This was then added to a 96-well plate. The optical density of each well was immediately measured at 595nm. A dilution series of known concentrations of Bovine Serum Albumin (BSA) was used for the creation of a standard curve to determine the relationship between absorbance values and protein concentration.

All absorbance readings were taken with a FLUOstar Omega microplate reader (BMG Labtech, Germany). The final optical density of each sample was recorded as the mean of four replicate measurements.

3.3.6 Haemolymph sugar measurements within haemolymph

In order to measure the levels of haemolymph sugars I focussed on the four main sugars in insect haemolymph: glucose, fructose, sucrose and trehalose. I used a modified version of the methodology of Phillips et al, (2018) to analyse haemolymph samples collected from each bumblebee. All measurements were carried out at room temperature (20^{o}C), unless stated otherwise. The four haemolymph techniques were verified prior to experimentation through samples taken from 20 bumblebees not used within the experiment.

In order to determine final concentrations of glucose, fructose and trehalose reagents were mixed based on the final concentrations of reagents (Table 1) presented in Phillips et al, (2018). When these enzymes are mixed with the sugars being measured they create a coloured product which can be assessed through optical density measures. For this analysis I refer to the mixtures of reagents as stains due to this colour change produced.

To ensure all enzymes kept their activity all solutions were kept on ice prior to use throughout the analysis. As the reagents ATP and NAD can become unstable in water solution, these reagents were mixed in small concentrations and kept cold on ice until being used on the same day. When creating the core reagent solution used for this analysis (Table 1; yellow box), all non-enzyme reagents were made up to 1 litre for ease of weighing, then diluted down to create 1ml for final analysis. A stock solution was created of reagents at 100x concentration before being diluted 100x for usage. This stock solution was mixed in TAE buffer (Sigma-

Aldrich) rather than water, however for optimal ATP stability the buffer was adjusted to a pH of 7.6.

All reagents required to convert Trehalose and Fructose (Trehalase and glucose-6-phosphate isomerase) were stored within the freezer until they were slowly thawed over 5 hours for use. For liquid enzymes no dilutions were conducted before addition to stain mixes. The GPI powder was reconstituted in 100x concentration TAE buffer. For each of the three stains, 24 µl was added to 100 µl to the sugar reagent solution (Table 3.1, yellow highlight) before being added to 876 µl of TAE buffer to make 1ml of solution ready for the addition of haemolymph.

Table 3.1. The reagents used to produce formazan dye from Glucose, Trehalose and Fructose and create a coloured stain. Reagent list taken from (Phillips et al, 2018). The highlighted yellow cells represent reagents required for all three tests with white cells showing reagents needed to convert to Trehalose and Fructose.

Haemolymph sugar reagents				
Reagent	**Final Concentration**	**Glucose**	**Trehalose**	**Fructose**
thiazolyl blue tetrazolium bromide (MTT)	1.2mM	+	+	+
1-methoxy-5-methyl phenazinium methyl sulphate (mPMS)	0.06 mM	+	+	+
magnesium chloride (MgCl2)	4mM	+	+	+
nicotinamide adenine dinucleotide (NAD)	0.8mM	+	+	+
adenosine triphosphate (ATP)	4mM	+	+	+
hexokinase (HK)	8U	+	+	+
glucose-6-phosphate dehydrogenase (G6PD)	4U	+	+	+
phosphogluconate dehydrogenase (6PGD)	8U	+	+	+
trehalase	21U		+	
glucose-6-phosphate isomerase (GPI)	4U			+
TAE buffer	0.2M pH 7.6	To 1ml	To 1ml	To 1ml

To measure the amount of sucrose within each haemolymph sample, the sucrose disaccharide must be cleaved into fructose and glucose before measuring the amount of each sugar. To convert sucrose to these two monosaccharides I used a solution of 20 U/ml of invertase and 0.02M of Na-acetate buffer, which was then set to a pH of 4.6. This created hydrolysed glucose which I then measured. To do this I created a glucose stain which consisted of: 2.4 mM MTT, 0.12 mM mPMS, 8mM $MGCl_2$, 1.6mM NAD, 8mM ATP, 16U Hexokinase, 8U G6PD, 16U 6PGD. This solution was then made up to 1ml with 0.04M TAE buffer set at a pH of 7.6.

A total of 1 µl of bumblebee haemolymph was then added to 20 µl of freshly prepared reagent stain for each sugar. Four 21 µl replicates were then added to individual wells of a 96-well (flat-bottomed) assay microplate which was then incubated in the dark at room temperature for four hours. To stop the reaction, 40 µl of 10% SDS in 0.001M HCL solution was mixed with the stain. The optical density of the stain was then measured at 570nm with a FLUOstar Omega microplate reader (BMG Labtech, Germany) within 30 minutes of stopping the reaction. For standard curves, water and reagent blanks were added as zeroes. To create the standard curve, pure forms of each sugar were taken for each dilution series and 1 µl added of each to TAE buffer. The dilution series started from 0 to100 µg / ml. The final optical density of each sample for each sugar was recorded as the mean of four replicate wells.

3.3.7 Statistical analysis

All analysis was conducted using R version 4.2.2 (R Development Core Team, 2020). All models were validated through the comparison of Q-Q plots and histograms. Where appropriate, model simplification was conducted by eliminating terms from the model and then using likelihood-ratio tests to compare the fit of each model with and without the term of interest in order to generate a P value.

In order to test whether bumblebee body-mass was influenced by the resource limitation treatment I investigated bumblebee weight change between day 10 and day 14, when the experiment was terminated. I tested if weight change was influenced by the resource treatment and if the magnitude of this effect varied between the dose rate treatments using linear models. Predictors included dose rate (as a factor), whether a bee was in a resource limited or abundant treatment group, and their interaction. To test for the impact of environmental conditions on weight change I also included temperature and humidity recorded twenty-four hours before each weight measurement.

My experiment tested the impact of radiation exposure treatment (and resource treatment) on multiple response variables; in order to investigate these effects, I used multivariate analysis of variance (MANOVA) to account for multiple testing. For this analysis all response variables were mean centred and scaled by the standard deviation to aid parameter

interpretation. The response variables included five physiological measures: standard mass index, metabolic rate, sucrose consumed during the experiment, weight of the bumblebee gut without its contents and the weight of gut contents. I also assessed eight biochemical response variables, measured on either bumblebee tissue (glycogen, carbohydrate, lipid and protein), or on bumblebee haemolymph (glucose, fructose, trehalose, sucrose). Predictors within each MANOVA included dose rate (as a factor), whether a bee was in a resource limited or abundant treatment group and the mass of each bumblebee recorded at eclosion. The interaction between dose rate and whether a bee was in a resource limited or abundant treatment group, as well as the main effect of temperature and humidity of the experimental facility were removed during model simplification. The removal of any terms was verified through the additional multivariate tests: Pillai, Wilks, Hotelling-Lawley and Roy.

To test for the effect of radiation on the colour of bumblebee guts, I classified each gut on a grading colour scale of 1-5. This grading was conducted blind to the treatment group of each bumblebee. I used the polr command from the MASS package (Venables & Ripley, 2002), in R to conduct ordered logistic regression. This model assessed how the explanatory variables influenced the proportional odds of observations falling into the different response (colour) categories. Predictors in this model included dose rate (as a factor), whether a bee was in a resource limited or abundant treatment group, and their interaction. I additionally accounted for bumblebee size by including the mass of each bumblebee recorded at eclosion. To test for any effects of environmental variables on results and as bumblebees were in different spatial positions around the facility, I included the average temperature and humidity recorded at the nearest data logger to each bumblebee (9 data loggers) as predictors.

3.4 Results

I firstly verified that limitation of resource did affect bumblebees . During this four-day resource limitation treatment there was a 25.9% decrease in mean bumblebee mass between day 10 and day 14 (Table S3.1; resource limitation treatment, $\chi^2_{(1)} = 5.356$, $P = 0.021$). The dose rate of radiation a bumblebee received had no effect on this mass loss (Table S3.1; dose, $\chi^2_{(1)} = 0.359$, $P = 0.783$). Furthermore, there was also no effect of radiation exposure on the magnitude of the effect of the resource limitation treatment (Table S3.1; dose rate by resource limitation interaction: $\chi^2_{(1)} = 0.232$, $P = 0.630$).

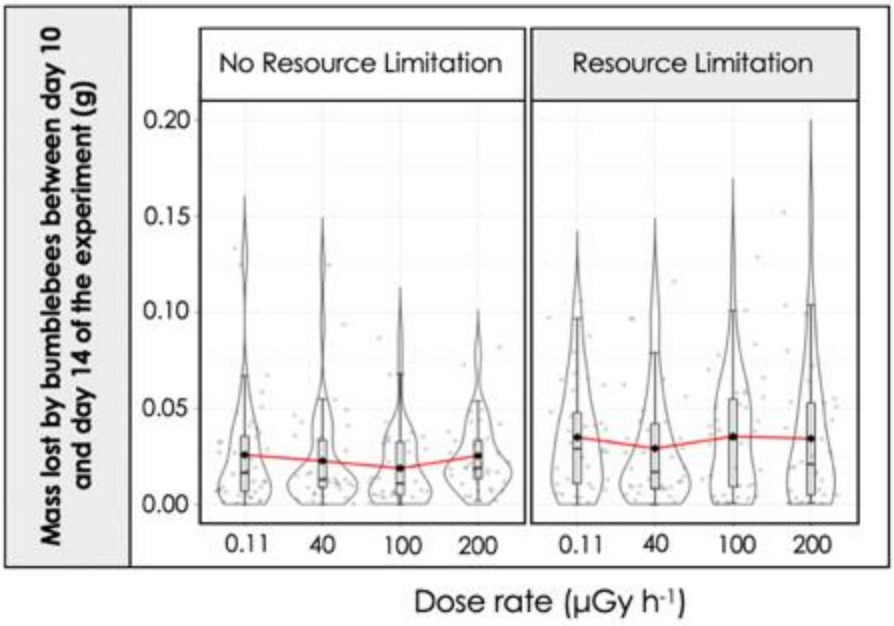

Figure 3.3. The impact of resource limitation on bumblebee mass. All bumblebees lost mass over the final four-day period of the experiment (during which half the bees received a resource limitation treatment). High positive scores on each graph indicate greater mass loss. Graphs represent mass loss between day 10 and 14 of the experiment. The left panel represents bumblebees fed 40% sucrose in a no resource limitation treatment and the right panel shows bumblebees fed a resource limited (5% sucrose) diet. Mass loss was higher for bees in the resource limitation treatment, than for those on 40% sucrose; this effect did not differ between the radiation treatments. Points on each graph show mean change in mass per bumblebee. Axis was rounded to two decimal places. The model analysing these data is shown in Table S3.1, the fit is represented by the red line and black diamonds, highlighting that there is no effect of radiation on bumblebee mass. n = 359 bumblebees.

Due to the number of different measurements taken I first assessed the correlation between all these response variables. I found significant positive pairwise correlation between all tissue measurements: glycogen, carbohydrate, lipid and protein content (Figure 3.4). The measures of glycogen and carbohydrates were also correlated with the weight of the

bumblebee gut and metabolic rate. Additionally, all measurements of the concentration of the four sugars within the haemolymph were also highly correlated with each other and with the scaled mass index of the bumblebee (Figure 3.4). The volume of sucrose consumed was also correlated with glycogen content within tissues, with all sugar measures taken from the haemolymph and with the metabolic rate (mean CO_2). The mass of the contents of the gut was correlated with gut weight when empty, as well as with the carbohydrate and glycogen content of tissues. These correlation diagrams were used to visualise relationships between measures within the bumblebee.

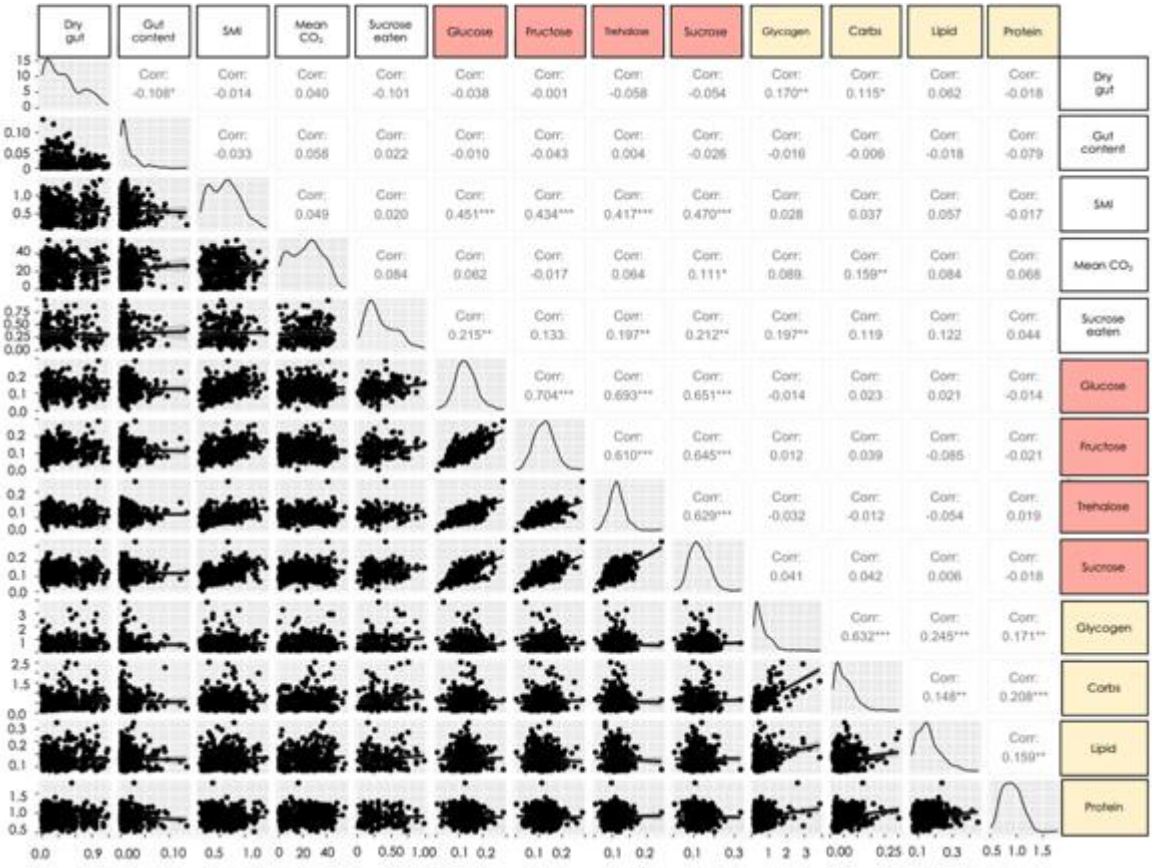

Figure 3.4. The correlation between all variables measured at the end of the 14-day experimental period in which bumblebees were exposed to radiation and experienced the resource manipulation. Graphs to the left of the plot are density plots of continuous variables. The values to the right of the graph represent correlation between each variable. This plot includes measurements taken on bumblebee physiology: Dry gut (the weight of the bumblebee gut without its contents), gut content weight, SMI (scaled mass index), Mean CO_2 (Metabolic rate), and sucrose eaten (volume consumed during the final two days of the experiment. This plot also includes measures taken on bumblebee tissue, which are represented by yellow squares: Glycogen, Carbs (Carbohydrate), Lipid and Protein. The red squares on this plot represent sugar concentration measures recorded from bumblebee haemolymph: Glucose, Fructose, Trehalose and Sucrose. The correlation between measures is denoted by ***. n = 359 bumblebees. All variables in this plot are mean centred and standardised.

In order to verify the effects of radiation exposure on bumblebee energy budget that I found previously (Burrows et al, 2022), I measured the metabolic rate and volume of sucrose consumed by each individual. Radiation exposure was again associated with a strong increase in sucrose consumption: there was a 26.6% increase in feeding at 200 μGy hr^{-1} compared to the controls (Figure 3.5c; Table S3.2a; $t_{(1)}$ = 10.381, *P* = 2 x 10^{-16}). Radiation also elevated metabolic rate, with an increase of 37.2% at 200 μGy hr^{-1} relative to controls (Figure 3.5a; Table S3.2b; $t_{(1)}$ = 1.693, *P* = 0.002). However, the increase in metabolic rate was not significant for lower dose rates of 100 and 40 μGy hr^{-1} (Table S3.2b). There was no effect of the resource limitation treatment on either volume of nectar consumed by bumblebees or their metabolic rate; nor was there any evidence that any effects of radiation are different between the two diet treatments (dose rate by diet treatment interactions: Table S3.2a; Table S3.2b). The scaled mass index (SMI) of bumblebees did not change in response to radiation exposure (Figure 3.5b; Table S3.2c; dose rate of 200 μGy hr^{-1}, $t_{(1)}$ = -0.202, *P* = 0.840) nor in response to the resource limitation treatment (Table S3.2c; , $t_{(1)}$ = 0.620, *P* = 0.536). This is in contrast to the result above (Figure 3.3) that shows that bumblebees lose bodyweight in response to the resource limitation treatment.

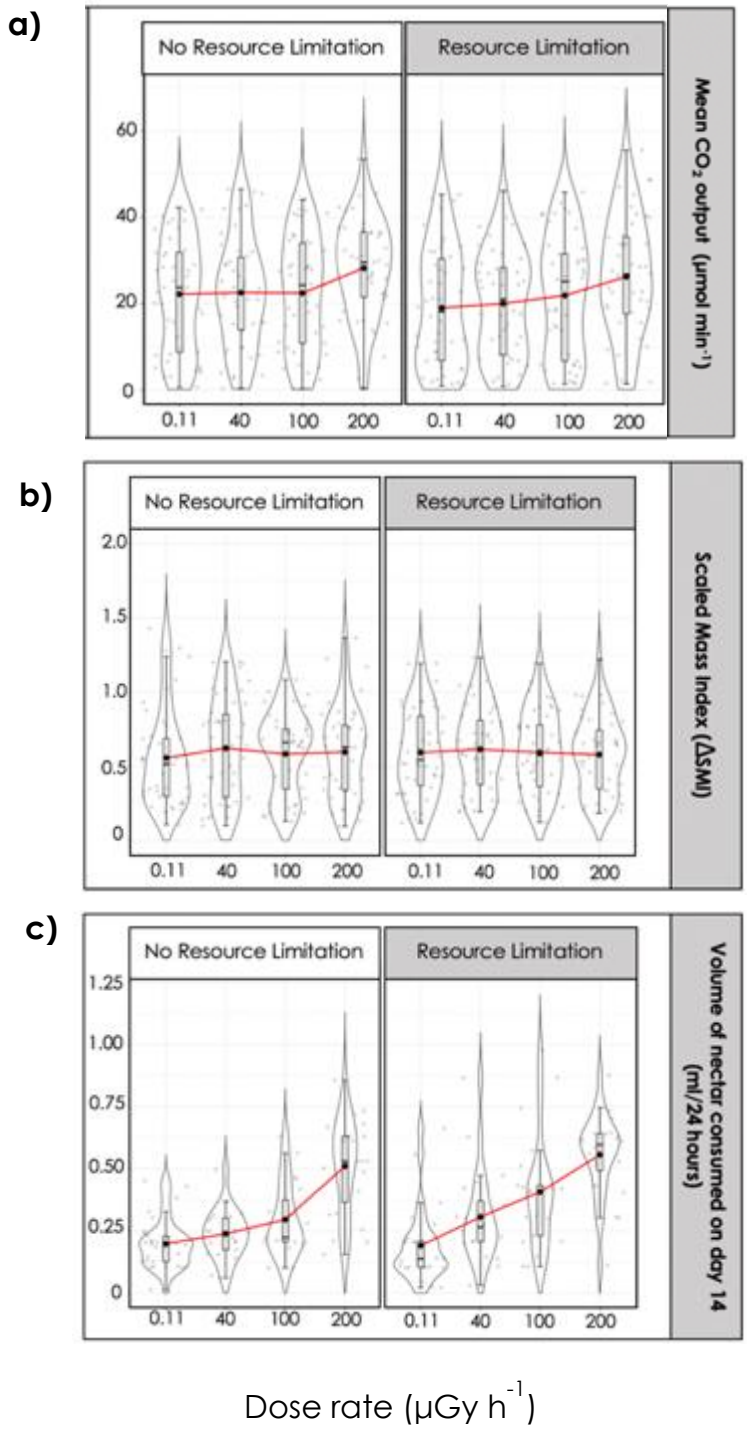

Figure 3.5a). Bumblebee metabolic rate (mean CO_2 output) increased with radiation exposure ($t_{(1)}$ = 1.693, *P* = 0.002) but was unaffected by the resource limitation treatment (right). Points on each graph show mean CO_2 output per bumblebee (Table S2.3b). The model analysing these data is shown in Table S3.2b. b) The scaled mass index (SMI) of bumblebees was unchanged by irradiation and resource limitation (right) ($t_{(1)}$ = -0.202, *P* = 0.840). Points on each graph show the SMI of each bumblebee. The model analysing these data is shown in Table S3.2a. c) The volume of nectar consumed by bumblebees between day 12 and 14 of the experiment increased with radiation exposure both for bumblebees with plentiful resource (left) and those with limited resource (right) ($t_{(1)}$ = 10.381, *P* = 2 x 10^{-16}). Points on each graph show mean volume of nectar consumed per bumblebee on day 14 of the experiment. The model analysing these data is shown in Table S3.2c. For each graph the red line and black diamonds show mean estimates for each dose rate in each of the treatments. n = 359 bumblebees.

To quantify energy storage within bumblebees I then undertook measurements on bumblebee tissue to quantify their protein, glycogen, carbohydrate and lipid content. I found no significant effects of dose rate treatments on any of these measures. Total lipid stored in bumblebees exposed to 200 µGy hr^{-1} was 16.6% higher than in control, however this difference was not significant (Figure 3.6a, Table S3.2d; $t_{(1)}$ = 1.842, *P* = 0.067). The resource limitation treatment did not generally affect these energy storage metrics, with the exception of carbohydrate storage, which was 16% lower in the resource limitation treatment compared to bumblebees with plentiful resource (Figure 3.6a, Table S3.2e; resource limitation treatment, $t_{(1)}$ = 2.071, *P* = 0.040). In my analysis of all four tissue measures I included the interaction between dose rate and the resource limitation treatment; this was to test if the hypothesised resource-costly response to radiation exposure would result in stronger depletion of these storage metrics under nutrient limitation. I found no significant interaction between dose rate and nutrient limitation for any of the tissue measures (Table S3.2d, Table S3.2e, Table S3.2f, Table S3.2g).

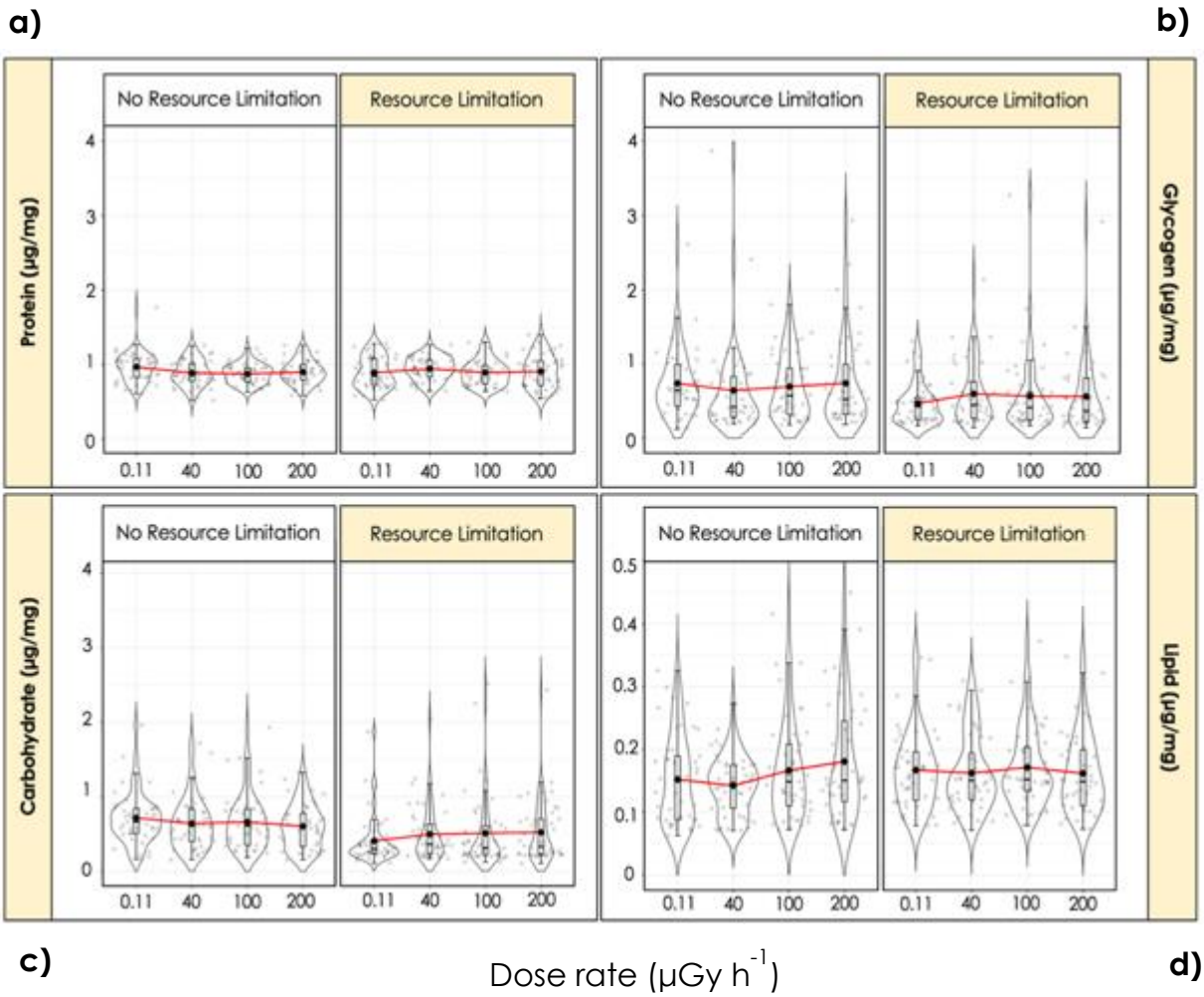

Figure 3.6a). Bumblebee storage of protein was unaffected by both radiation exposure and resource limitation (right panel). Points on each graph show mean protein content per mg of wet weight of bee. The model analysing these data is shown in Table S3.2g. b) The storage of glycogen did not alter with exposure to radiation or with the reduction of available resource (right panel). Points on each graph show the mean measure of glycogen per mg of wet weight of bee. The model analysing these data is shown in Table S3.2f. c) The storage of carbohydrates in bumblebee tissue decreased when resource was limited (right panel) but was not significantly affected by radiation exposure. Points on each graph show the mean measure of carbohydrate per mg of wet weight of bee. The model analysing these data is shown in Table S3.2e. d) Lipids stored within bumblebee tissue increased with exposure to the highest dose rate studied, however lipid storage was again not significantly affected by resource limitation. Points on each graph show the mean measure of lipid per mg of wet weight of bee. The model analysing these data is shown in Table S3.2d. For each panel the red line and black diamonds show mean estimates for each dose rate in each of the treatments. n = 359 bumblebees for each energy storage measure.

I then undertook a series of biochemical tests on haemolymph collected from each of these bumblebees to quantify the levels of sugars in the haemolymph (glucose, fructose, sucrose and trehalose). For all sugars there was no significant effect of resource limitation treatment on the concentration within the haemolymph; similar trends were seen in both resource treatment groups (Figure 3.7). Bumblebee mass (as recorded at eclosion before the experiment started) was positively correlated with the concentration of all sugars (Table S3.2h ; Table S3.2i, Table S3.2j, Table S3.2k).

In contrast to the measures of energetic reserve in whole tissue, for three of the haemolymph sugar measures there was a significant positive correlation with increasing radiation exposure. For glucose measures there was a significant increase of 28.4% at 200 µGy hr^{-1} in comparison to controls, but a 8.7% increase was also recorded at dose rates of 40 µGy hr^{-1} (Figure 3.7a, Table S3.2h; $t_{(1)}$ = 2.711, *P* = 0.007). The concentration of sucrose within the haemolymph increased slightly with radiation exposure but this effect was not significant (Table S3.2i; $t_{(1)}$ = 0.758, *P* = 0.105). The concentration of fructose and trehalose increased significantly under the irradiation treatments: by 8.8% for fructose (Table S3.2j, $t_{(1)}$ = 8.574, *P* = $2x10^{16}$) and 14.3% for trehalose (Table S3.2k, $t_{(1)}$ = 4.403, *P* = $1.84x10^{5}$) at 100 µGy hr^{-1} compared to the control bumblebees (0.11 µGy hr^{-1}). However, whilst there was a small increase in concentration of both these sugars in the haemolymph at 40 µGy hr^{-1}, this difference was not significant (Table S3.2j; Table S3.2k). For all haemolymph sugar measures the interaction between dose rate and the nutrient limitation treatment was not significant (Table S3.2h , Table S3.2i, Table S3.2j, Table S3.2k).

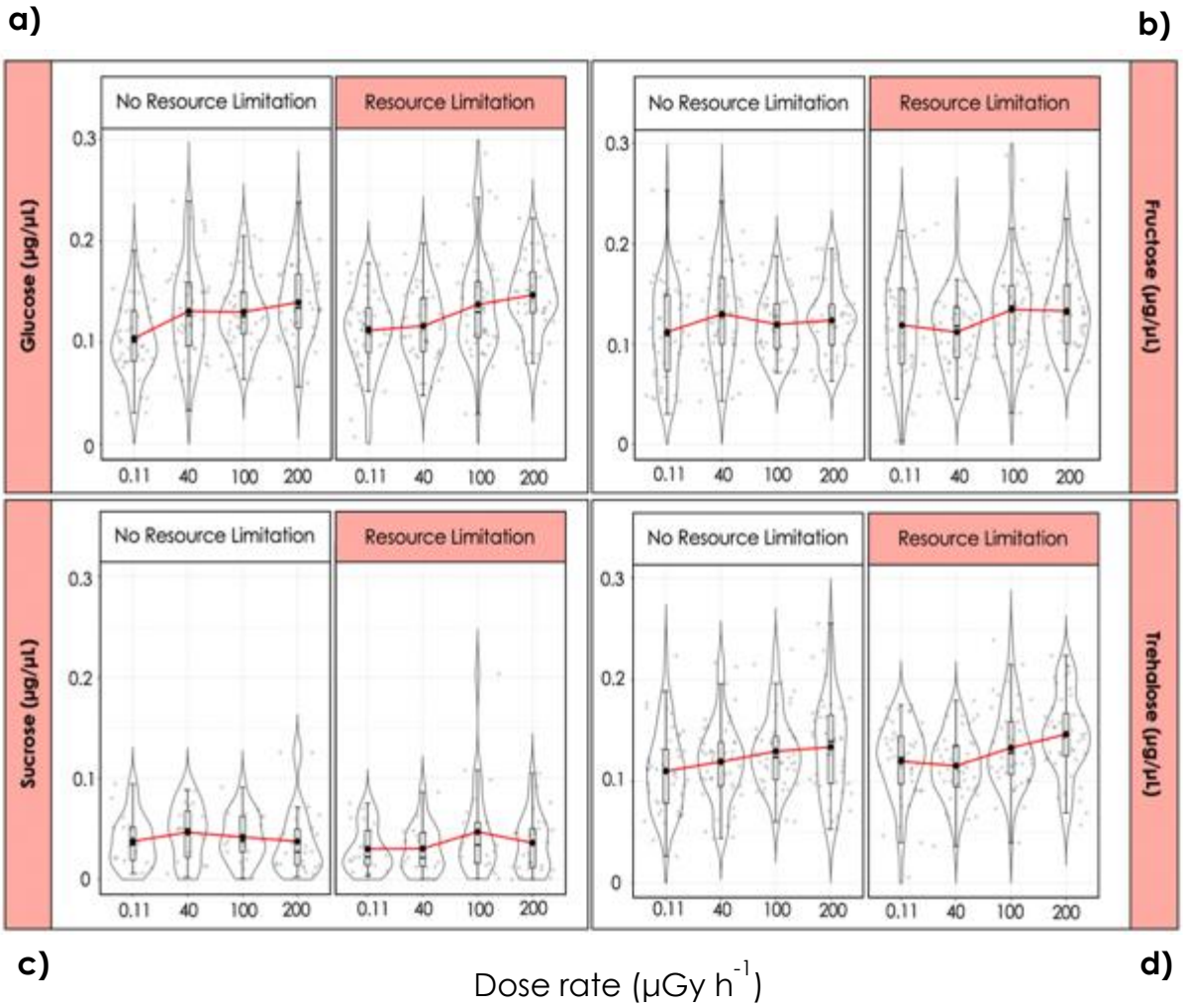

Figure 3.8a). The amount of glucose within bumblebee haemolymph increased with dose rate of radiation received but was not significantly affected by resource limitation (right panel). The model analysing these data is shown in Table S3.2c. b) The concentration of fructose within bumblebee haemolymph increased with dose rate of radiation received but was not affected by resource limitation (right panel). The model analysing these data is shown in Table S3.2d. c) The volume of sucrose within bumblebee haemolymph was not affected by dose rate or resource limitation (right panel). The model analysing these data is shown in Table S3.2f. d) Trehalose content within bumblebee haemolymph increased with the dose rate of radiation received but not with the reduction in resource (right panel). The model analysing these data is shown in Table S3.2e. For each panel of the four panel plots the red line and black diamonds show differences between dose rates in each of the treatments. Points on each graph show mean of four repeat measures of each sugars volume in 1µl of haemolymph per bumblebee. n = 359 bumblebees.

As well as these biochemical measures I then undertook physiological measurements on the gut, in order to understand if the increase in volume of liquid ingested affected this aspect of digestion. The weight of the bumblebee gut, when emptied of all contents, was unchanged either by radiation exposure (Table S3.2l, dose rate of 200 µGy hr^{-1}, $t_{(1)} = -1.427$, $P = 0.155$) or by the resource limitation treatment (Figure 3.9a; Table S3.2l, , $t_{(1)} = 1.218$, $P = 0.225$). There was additionally no significant interaction between dose rate and resource limitation (Table

S3.2l, $t_{(1)}$ = -1.109, *P* = 0.276). The weight of the gut contents showed a small increase with radiation exposure in both the resource abundant and limited treatments, however neither measures were significant (Figure 9b, Table S3.2m, $t_{(1)}$ = 0.686, *P* = 0.494). The interaction between dose rate and resource limitation treatment was also not significant (Table S3.2m).

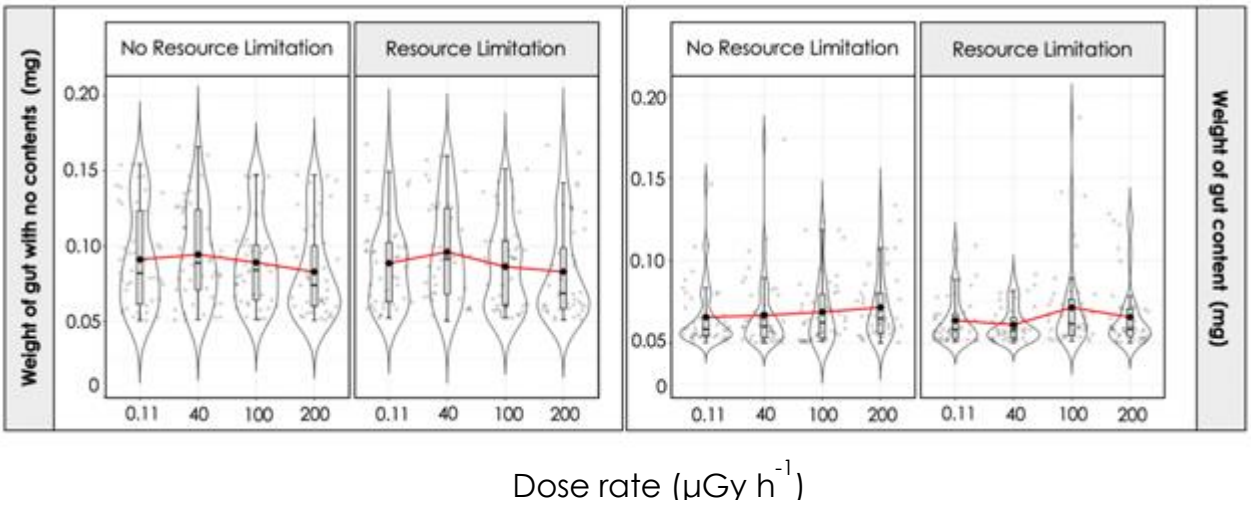

Figure 3.9a). The weight of the bumblebee gut when emptied of its contents was lower following radiation exposure but not significantly so. This effect is seen in both resource abundant (left panel) and resource limited treatments (right panel). Points on each graph show mean of gut weight per bumblebee. The model analysing these data is shown in Table S3.2l. b) The weight of the contents of the bumblebee gut was not affected by radiation exposure or resource limitation (right panel). Points on each graph show mean of gut contents weight per bumblebee. The model analysing these data is shown in Table S3.2m.

I observed gut colour variation between individual bumblebees when extracting their guts. There appears to be a small reduction in the probability of having the darkest colour gut (colour 5) with radiation exposure for bumblebees in the resource limited treatment (Figure 3.10). However, this reduction in probability was not significant for the dose rate of radiation received (Table S3.3, $t_{(1)}$ = -0.213, *P* = 0.831) nor for the interaction between dose rate and the resource limitation treatment (Table S3.3, $t_{(1)}$ = 0.368, *P* = 0.712).

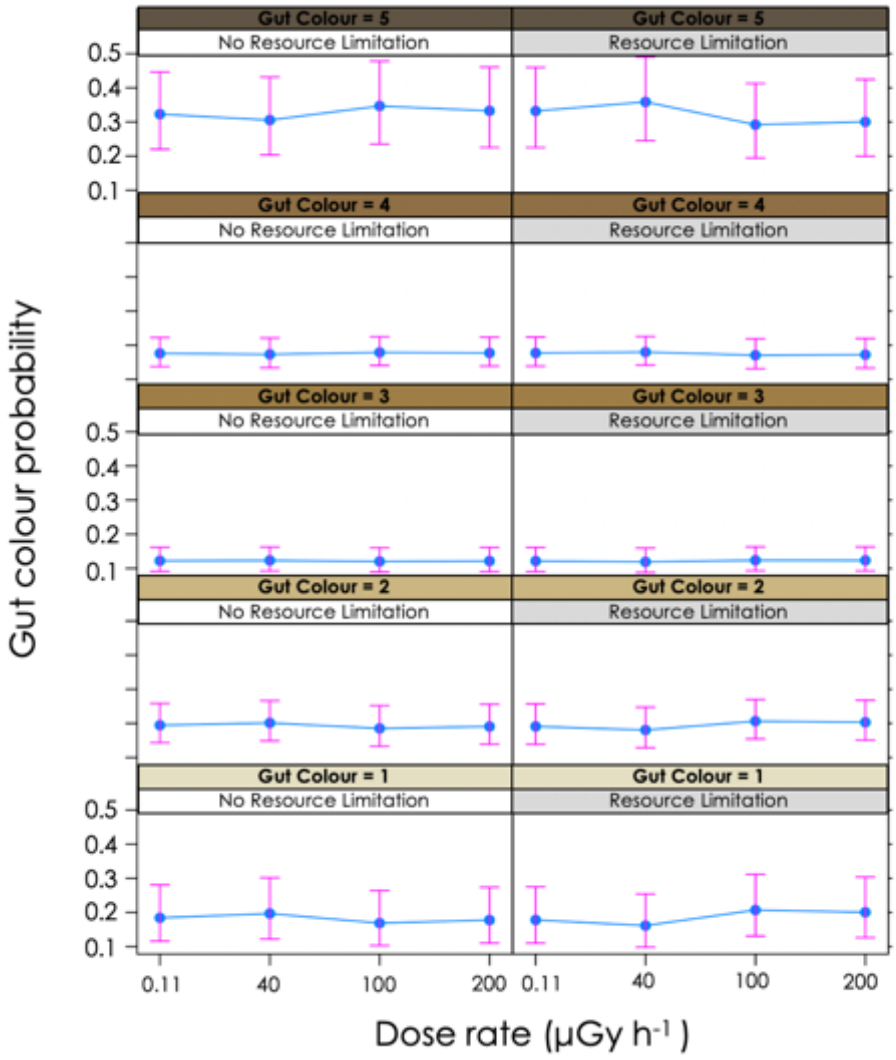

Figure 3.10. The probability of a darker colour of bumblebee gut was not correlated with the dose rate of radiation received or the resource limitation treatment. The top set of graphs are for the darkest colour gut (colour = 5), descending to the lightest colour gut (colour = 1). The left panels each represent bumblebees fed a plentiful resource diet and the right panels the resource limitation treatment. Blue points on each graph represent the mean probability of a bumblebee having that colour gut and the blue lines show mean change in these probabilities. The pink bars show the standard error of these means. n = 359 bumblebees.

3.5. Discussion

This study supports previous findings that low, environmentally relevant, dose rates of ionising radiation significantly affect bumblebee energy budgets. Here I show that the increase in sucrose consumed by irradiated bumblebees is biochemically converted and is likely used through increased metabolism. This is supported by the finding that radiation exposure has led to an increase in nearly all sugar concentrations I measured in bumblebee haemolymph. I recorded no change in bumblebee tissue for carbohydrate and fat storage under my radiation treatments, which supports my previous hypothesis that the excess nectar consumed is being used to fuel a metabolically costly response to radiation exposure (Burrows et al, 2022). These effects on bumblebee biochemistry were recorded at dose rates as low as 40 μGy hr^{-1}; as a result I hypothesise that bumblebees in radiologically contaminated landscapes could experience this biochemical response.

Bumblebee nectar consumption increased by 26.6% and metabolic rate increased by 37.2% at dose rates of 200 μGy hr^{-1} (accumulated dose of 67200 μGy) in comparison to controls (0.11 μGy hr^{-1}), which verifies previous findings of a metabolic syndrome occurring as a result of radiation exposure (Burrows et al, 2022). In this study I introduced a nutritional limitation treatment for four days. I hypothesised that if energy intensive recovery processes are activated as a result of radiation exposure, then this would be evidenced by resource reserves depleting faster in bumblebees with limited nutritional access. In insects, lipid reserves are often mobilised during times of nutritional stress through increased lipid oxidation measured by UV absorption (McCue et al, 2015). However, I did not record any changes in biochemical or physiological measures for bumblebees in the nutritional limitation treatment, apart from a significant reduction of carbohydrates within tissue. This is consistent with existing knowledge of insect physiology as during times of stress carbohydrates are often broken down as a key source of energy (Gaxiola et al, 2005). I predicted that there would be an interaction between dose rate of radiation received and the resource limitation treatment, such that the effect of dose on nutrient levels would change under limited resource. The absence of this interaction means I have no support for the hypothesis that an energy intensive process is occurring. It could be argued that the low nectar concentration that I provided in the nutrient limitation treatment was not low enough or not provided for long

enough to cause symptoms of starvation or effect storage of lipids. However, I found evidence of a reduction in bumblebee weight over the four-day period as well as a reduction in carbohydrate storage. This is consistent with findings in queen bumblebees for which starvation has negative impacts on carbohydrate storage (Woodard et al, 2019). Also, larger worker bees have been found to die within two days of starvation when only given access to water (Couvillon & Dornhaus, 2010). Therefore, the reduction in resource over four days was effective enough to cause a physiological effect on bumblebees, however there is no evidence that the extent of resource loss recorded in the resource limitation treatment, either through weight loss or in carbohydrate storage, was any greater in the high dose treatment than it was in the control or lower dose (40 μGy hr^{-1}) treatments. Another explanation is that when bumblebees have limited food they alter their response based on the energy available to them. Therefore, if energy is not available to support a radiation recovery mechanism, perhaps bumblebees adjust their energy supply and as a result down regulate their investment in this recovery response. I suggest further studies should explore conducting both dose rate and resource limitation treatments for longer period to see if a recovery response is activated.

This study does however find novel evidence of a change in bumblebee biochemistry in response to radiation. I demonstrate a significant increase in the concentration of glucose and trehalose within bumblebee hemolymph at dose rates as low as 40 μGy hr^{-1} and as low as 100 μGy hr^{-1} for fructose. There was no significant increase in sucrose within the haemolymph in response to radiation and its levels within haemolymph were less than other sugars; I hypothesise this is due to sucrose being rapidly converted into glucose and fructose, within the crop, midgut and when it initially enters the haemolymph (Even et al, 2012). The findings of an increase in haemolymph sugars indicates that when radiation triggers more sucrose to be consumed, this sucrose is then converted in the mid-gut to glucose and fructose, then transported into the haemolymph. Therefore, it seems likely that of the excess sucrose that was being consumed relatively little was being stored, but it is indeed being rapidly used by bumblebee metabolism. This is supported by my findings of no effect of radiation on the amount of lipid and glycogen being stored within bumblebee tissues. I have previously found evidence of increased bumblebee movement in response to radiation exposure (Burrows et al, 2022); therefore, this increase in haemolymph sugar could be being used to fuel this

activity. However, increased sugars within the gut can have negative effects on bumblebees because excess sugar has been recorded to enhance growth of the common gut parasite *Crithidia bombi* within cell culture medium (simulating the gut environment) (Palmer-Young & Thursfield, 2017). This suggests that the presence of gut parasites could lead to further changes in sugar concentrations in bumblebees. Therefore, in radiologically contaminated landscapes such as Chernobyl, this excess sugar could be detrimental for bumblebee parasite loads. This is again supported by laboratory work that has shown increased parasite loads in bumblebees exposed to radiation (Raines, thesis). The finding of this study of an increase in haemolymph sugars in response to radiation could therefore provide a mechanism for responses recorded in bumblebees previously (Raines, thesis; Burrows et al, 2022).

The concentration of sucrose within the haemolymph was not significantly affected by radiation or resource limitation. This result is in keeping with bumblebee biology due to high levels of efficiency of converting sucrose into glucose and fructose. During foraging flights bumblebees will seek out higher concentrations of sucrose and metabolise it quickly just to maintain the energy required for flight (Pattrick et al, 2020). As the breakdown products of sucrose are glucose and fructose, I recorded a significant increase in both sugars in response to radiation, which supports this rapid conversion of sucrose to supply energy. I additionally recorded a 14.3% increase in trehalose during radiation exposure. Trehalose is a disaccharide haemolymph sugar used for short-term storage and transport (Shukla et al, 2015). Within the fat body of insects, glucose is converted into trehalose via the trehalose biosynthetic pathway, in order to be effectively transported into the haemolymph (Satake et al, 2000). During times of stress such as during drought or high temperatures, trehalose will often accumulate in order to support survival (Yu et al, 2008 ; Tang et al, 2014). Despite this large increase in haemolymph sugars in response to radiation, I recorded no effect of dose rate on the amount of lipid and glycogen being stored. This suggests these sugars are being used by the bumblebee to fuel a physiological response, which requires large amounts of energy.

I provide evidence that there is an energetically costly response to radiation. This is due to an increase in metabolic rate in response to radiation, which occurred whether bumblebees were subjected to low resource or high resource feeding regimes. One suggested mechanism is that radiation is activating a biological system such as the immune response as a result of

this stressor, which can lead to increased nectar consumption (Tyler et al, 2006). My findings suggest that the mechanism that is driving this increase in energy consumption is still occurring even when food is limited. However, this has not manifested in the depletion of stores in the manner predicted. I hypothesised that an increase in metabolic rate would result in the depletion of lipid and glycogen storage in tissues, as bumblebees would need to use these reserves to maintain life processes. In insects, glycogen is often broken-down during starvation stress (Parkash et al, 2012; Rovenko et al, 2015). My data clearly demonstrate that the additional sucrose consumed following radiation exposure is not just simply passing through the gut, as I detected elevated sugar within the haemolymph. This additional energy within the haemolymph could be simply being used to fuel higher metabolic rate. My findings are consistent with the hypothesis that this additional sugar is being metabolised to fuel movement, as previous work has shown increased activity levels in response to radiation exposure (Burrows et al, 2022). However, this study cannot identify the exact aspect of metabolism that is being fuelled.

Most of the nutrition obtained through nectar is digested and absorbed within the midgut, which is home to a diverse gut microbiome. This microbiome supports digestion and also has a variety of metabolic capabilities (Flint et al, 2012). I hypothesised that the increase in nectar consumed is driving a change in sugar metabolism; bumblebees might simply consume more to feed an altered flora of microbes in the gut. The sugars glucose and fructose pass over the microbiome and then through the gut membrane in order to enter the haemolymph stream. During this time, bacteria in the gut are key for fermenting dietary carbohydrates (Engel et al, 2012). However, the microbiome could also equally be negatively affected by this increase in sucrose consumption: when food is abundant key bacteria in the microbiome can be washed out by large volumes of liquid, leading to key bacteria to be expelled (Powell et al, 2016). The bumblebee gut microbiota has evolved to effectively utilise glucose and fructose (Kwong & Moran 2016), so if key bacteria are washed out, then less sugar is fermented and more potentially harmful sugars will pass through into the haemolymph. Additionally, it is possible that CO_2 is produced by the microbiome which could contribute to the increase in recorded metabolic rate following radiation exposure, as CO_2 is produced when the gut microbiome metabolises sugar. The bacterium taxon *Bifidobacterium* is highly abundant within the bee gut, this bacterium promotes uptake of sugar by the bee as well as respiring aerobically

(Kwong et al, 2014). Therefore, more carbohydrates within the diet could lead to more carbon dioxide being produced by the gut microbiome.

It should be noted that at the highest dose rate used in this study, the total accumulated dose is 67,200 μGy (0.672 Gy). Whilst this is a very high accumulated dose, it is still not in the region of dose rates that are known to cause mortality in adult flies; for adult flies 100% mortality has been reported at 1500 Gy (Pathinkar et al, 2017). However, for bumblebees present in the CEZ at these dose rates for several weeks this accumulated dose is closer to the 4 Gy that is known to cause 50% mortality in humans (Nuclear Commission, 2020). As individuals in the zone are exposed to gamma radiation it should also be noted that gamma has higher energies than other forms of radiation such as x-rays. A conceptual model on the responses of organisms to accumulated doses of radiation in the environment has found that there are four zones in which organisms are exposed (Polikarpov et al, 1998). In areas of reduced background radiation, the dose uncertainty is less than 0.00004 Gy per year. In this model the accumulated doses in this study fall in to the zone of damage to ecosystems where there is potential for wider damage from 4 Gy per year (Polikarpov et al, 1998).

This study set out to identify the mechanism driving the metabolically costly response that occurs as a result of radiation exposure. I provided further experimental evidence that this costly response does occur through the verification of an increase in metabolic rate and nectar consumption in response to radiation exposure. I additionally provided novel evidence of this increase in energy budget through the identification of an increase in energy in the form of sugars within haemolymph. This shows that the excess nectar consumed is being biochemically converted and used by bumblebees to fuel a response. My prediction of a faster running down of nutrient reserves in bumblebees when they had limited food resources was not supported by my results. However, I suggest that bumblebees are prioritising a mechanism that is driving this extra energy expenditure due to an increase in metabolic rate regardless of the sucrose provided. Whilst this study fails to identify a mechanism or the organ that is using this energy, it does successfully show sugar moving through the insect body, being metabolised and used in the context of metabolic rate. This is a vital first step in identifying what is driving this key impact of radiation on bumblebee life history.

Chapter 4: The impacts of ecologically relevant radiation exposure on gut microbial community composition in bumblebees

Key Words: Ionising radiation, Microbiome, Insects, Bacterial community, 16S Sequencing, Energy budget, Eco-toxicology, Radiological contamination.

4.1 Abstract

(1) The gut microbiome is essential for bumblebee health and is usually robust when faced with environmental stressors. However, few studies have recorded how the stressor of low dose radiation could impact the insect microbiome. As the microbiota of bumblebees is associated with critical functions such as the digestion of food, any stressors affecting it could have consequences for bumblebee health.

(2) I studied the impacts of radiation on the bumblebee microbiome to better understand any potential mechanism driving changes in nutrient acquisition and metabolic rate previously recorded in bumblebees. I investigated whether radiation could be affecting the gut microbiome, either due to direct damage, or indirectly due to increased nectar consumption. My results will provide a better assessment of the metabolic syndrome that occurs as a result of low dose radiation exposure.

(3) I investigated the effect of radiation on gut microbial community composition by irradiating bumblebees at dose rates of 0.11, 40 and 200 μGy h^{-1}. I then conducted 16S amplicon sequencing of the V4 region to identify and compare bacteria in each sample. I included a temporal treatment to understand how bacterial community composition may change over time: 45 bumblebees were irradiated for 3 days, and another 45 bumblebees for 10 days. The bumblebees irradiated for 3 days were the temporal control.

(4) When exposed to dose rates of 200 $\mu Gy\ h^{-1}$ there was a significant increase in species richness of the gut bacterial community. This increase in richness was recorded in the 'core' microbiome, but was less pronounced when including rarer bacterial taxa in the analysis. Interestingly there was no change in beta diversity (similarity or dissimilarity between gut microbiome communities) associated with radiation exposure.

(5) I speculate that these changes in gut microbial community composition might either be due to direct or indirect effects of radiation, or could be driven indirectly through other radiation effects of bumblebee physiology. Changes in microbiome composition can lead to physiological and even behavioural changes within an individual. Therefore, irradiation could influence host development and key life history processes.

4.2. Introduction

Bumblebees harbour a distinct gut microbiota (Martinson et al, 2011); typically only a few host-specific bacterial symbionts dominate this highly specialised microbial community (Martinson et al, 2011 ; Koch & Schmid-Hempel, 2011 ; Meeus et al, 2015). Cells found within the bumblebee gut divide rapidly and as a result the community composition has the potential to turnover in response to environmental change. Diet has been found to influence community richness and diversity, with the consumption of diets rich in fructose associated with decreased colonisation by some bacteria (Billet et al, 2015). My previous work showed that bumblebees exhibit a metabolic syndrome when exposed to the stressor of low doses of radiation: as dose rates increased, individuals consumed significantly more nectar and elevated their metabolic rate (Burrows et al, 2022). The gut microbiota has a variety of metabolic capabilities (Flint et al, 2012), therefore it is important to understand if changes in the microbiome could be driving these radiation-induced metabolic shifts or if radiation is driving changes in the microbiome directly. It is unclear whether radiation causes damage to bacteria within the bumblebee microbiome, however it has been speculated that irradiation can directly impact bacterial DNA (Kim et al, 2015). Alternatively, radiation-induced increases in nectar flowing through the gut could be drivers of changes in gut microbial composition. Studies using radiation for aseptic sterilisation demonstrate that high dose exposures (10 – 70 kGy) are necessary to kill most microbes, exposures that are greatly in excess of those relevant to radiologically contaminated environments (McNamara et al, 2003). Nevertheless, high dose radiation exposure of the human gut has been shown to alter its microbial community composition (Packey & Ciorba, 2011).

Previous studies have shown that irradiation significantly alters bacterial compositions to genus level in the small and large intestines of mammals, whilst a mechanism was not found it was speculated this was due to DNA damage (Kim et al, 2015). However, dose rates studied are often orders of magnitude higher than those that are considered relevant to our environment. At the lower dose rates found in contaminated landscapes such as the Chernobyl Exclusion Zone (CEZ), studies have reported alterations in the abundance of some bacterial species found within the microbiome. For example, there are significant changes in abundance of cultivatable bacteria associated with the feathers of birds as a result of radiation exposure in the CEZ (Czirjak et al, 2010). Furthermore, there is some evidence that

the radiosensitivity of feather-associated bacterial communities from birds caught in the CEZ may have been shaped by the radiation levels in the environment the birds inhabited (Gonzalez et al, 2016). There are fewer studies that examine the effects of radiation on gut microbial communities at environmentally-relevant dose rates. One field study from the CEZ examined the effects of radiation on the bank vole gut microbial community: it found no effect on overall gut community species richness, but revealed a substantial increase in the ratio of *Firmicutes* to *Bacteriodetes* when voles were taken from a study site with dose rates of approximately 30 μGy h^{-1} (Lavrinenko et al, 2018). Another study on small mammals in the CEZ reported associations between radiation and altered abundance of some gut bacterial families (Lachnospiraceae and Muribaculaceae), abundance alterations that could be used as biomarkers for future studies of radiation exposure (Antwis et al, 2020). Biomarkers are biological markers which can be used to predict or monitor radiological effects. To identify whether the microbiome could indeed be a potential biomarker for radiation exposure, I need to study a wider range of species. This study on bumblebees will begin this species-diversification, whilst also allowing us to assess potential linkages between the metabolic syndrome that occurs as a result of radiation exposure and the community composition of the microbiome.

In this study, I focussed on the bumblebee *Bombus terrestris*, a species commonly found within the CEZ. As bumblebees are social insects, their microbiota can be transferred directly or indirectly between generations, which generally results in microbial community stability (Koch & Schmid-Hempel, 2011). Studies using 16S rRNA sequencing have shown that in a single gut, bacteria equate to roughly ~ 30 million cells (Li et al, 2015). A few specific bacterial taxa dominate the bumblebee microbiome, including *Snodgrassella*, *Gilliamella* and *Lactobacillaceae.* These bacteria are essential to maintain bumblebee health as they contribute to carbohydrate digestion (Zheng et al, 2018) and pathogen defence (Bosmans et al, 2018). Approximately 99% of bacteria can be found within the hindgut (Martinson et al, 2012), which is dominated by *Snodgrassella* (a non-sugar fermenter that forms a layer on the gut epithelium) and *Gilliamella* (which forms a dense biofilm on top of *Snodgrassella*). *Gilliamella* is a sugar fermenter which digests complex carbohydrates that would otherwise be difficult or even harmful for an individual to digest (Zheng et al, 2016). *Lactobacillaceae* also play a role in the metabolism of lipids, amino acids and carbohydrates within the host;

interestingly, an increase in their abundance is associated with an increase in bumblebee memory retention (Li et al, 2021). Other bacteria found within the gut are more sporadic and associated with the environment in which the bumblebee is found (Meeus et al, 2015). A better understanding of changes in composition of these key bacterial communities would shed light on the complex interplay between microbiota and health, especially during times of stress.

As the gut microbiome of bumblebees is associated with critical functions, such as food digestion and immune responses, any stressors affecting these microbes could potentially affect bumblebee health and threaten colony survival. Nevertheless, the microbiome of bumblebees can help mitigate stressors; for example, in response to resource-limitation *Gilliamella* and *Snodgrassella* synthesise essential amino acids (Zheng et al, 2019) which can help stabilise the microbial community. Additionally, when food is abundant, the microbiome can be threatened via washout due to high feeding rates, which can lead to bacteria being expelled from the gut. To prevent this from occurring, core symbionts form a biofilm to prevent clearance from the gut (Powell et al, 2016). The microbiota also exhibits other traits to assist with stress responses; for example, bee symbionts can continue to grow even when exposed to heat stress up to 52^{o}C (Hammer et al, 2021). Nevertheless, some external environmental stresses do negatively affect the microbiome. For example, exposure to pesticides alters the gut microbiome composition and leads to dysbiosis (Rothman et al, 2020). Additionally, in times of cold stress *Snodgrassella* is unable to consistently colonise the gut (Hammer et al, 2021). Therefore, it is important to understand the effect of external stressors on community abundance and diversity, as the microbiome is key to fundamental biological processes.

As previous work has found a significant increase in nectar consumption in response to radiation exposure (Burrows et al, 2022), I hypothesised that this increase in consumption is associated with changes in the gut microbiome. In this study, I therefore focus on the buff-tailed bumblebee *Bombus terrestris* and perform 16S sequencing of the V4 region to investigate changes in gut microbial community composition in response to experimental radiation exposure.

4.3. Materials and Methods

I investigated the effect of radiation on the gut microbial community composition of bumblebees. For this study I used 90 bumblebees and exposed some of these to radiation dose rates similar to those currently found in the Chernobyl Exclusion Zone. I then investigated differences in gut microbial community composition between dose rate groups.

4.3.1 Study system husbandry

For this experiment a total of 10 *Bombus terrestris audax* colonies were purchased from Biobest®. Four days after arrival, each colony was anaesthetised with CO_2 and all individuals marked with commercial bumblebee paints. The following day, all colonies were again anesthetised and newly emerged bees marked with coloured paints. All colonies were negative for the presence of the parasite *Crithidia bombi* (a total of 15 bumblebees were removed from each colony prior to the marking process and their faeces checked using a microscope). To ensure the normal development of a gut microbial community, newly emerged bumblebees were then left in their colonies for a further 4 days to enable exposure to the colony microbiome. All of these bumblebees of the same age were then removed and individually housed in clear plastic containers (55mm (l) x 55mm (w) x 60mm (h)). Each bumblebee had access to *ad libitum* pollen and 40% (w/v) sucrose mixed with distilled water (nectar solution) provided in a 12ml falcon tube with an access hole punctured in the side for feeding. All bumblebees received cotton wool for nesting material; filter paper was used to line each container to assist cleaning. Each bumblebee remained in their container for the experiment's duration, with containers cleaned and nectar solution changed on day 5.

4.3.2 Irradiation Treatment

In order to investigate the effects of radiation on gut microbial community composition, 90 bumblebees were placed in the radiation facility at the University of Stirling. This is an environmentally controlled facility (12 hr light: dark cycle (07h – 19h)), for which data loggers recorded temperature (mean = 25.9^{o}C, range ± 0.8) and humidity (mean = 35.3%, range ± 8.8) to monitor consistency. In this facility a ^{137}Cs source delivers controlled doses of gamma radiation, with a control area present beside the source in which individuals are exposed to

the same environmental conditions but not any radiation. In the control area background radiation levels are 0.11 ± 0.01 μGy h^{-1} (Raines *et al.*, 2020). Within each dose rate treatment all containers were kept on a narrow shelving unit (110mm width) to minimise spatial and dose rate variability.

For this experiment bumblebees were placed at two distances from the radiation source: 30 bumblebees received a dose of 200 μGy h^{-1} (+/- 9 μGy h^{-1}), another 30 bumblebees received a dose of 40 μGy h^{-1} (+/- 1 μGy h^{-1}); a final 30 bumblebees were placed in the control area (0.11 μGy h^{-1}). To assess temporal changes in bacterial community composition, half of the bumblebees at each dose rate were removed from the experiment after these 3 days (n=45). Those bumblebees experienced accumulated doses of 14400 μGy (200 μGy h^{-1}) and 2880 μGy (40 μGy h^{-1}). The remaining bumblebees stayed in their treatments until day 10 of the experiment (n=45). These bumblebees received accumulated doses of 48000 μGy (200 μGy h^{-1}) and 9600 μGy (40 μGy h^{-1}). Throughout the experiment no bumblebees died.

Once removed from the facility bumblebees were anesthetised with CO_2. The mid and hindgut regions of each bumblebee's gut were then dissected and removed using disinfected dissection equipment. Each gut was individually placed in a 1.5ml microcentrifuge tube and stored at -20°C for later analysis.

4.3.3 DNA extraction and 16S Amplicon sequencing

In order to quantify the microbial community of bumblebee intestinal tissue, DNA was extracted from guts and purified using the DNeasy® Tissue kit (Qiagen). To prepare each sample, bumblebee guts were frozen in a 1.5ml microcentrifuge tube by placing it in liquid nitrogen. Guts were then crushed for 3 minutes using a sterile micro pestle to a fine consistency and 180 μl of proprietary buffer ATL was then added. The prepared samples were processed according to the total DNA from animal tissues instructions provided by the manufacturer (Qiagen). To maximise DNA yield, the final step of this purification protocol involving the elution of DNA into proprietary buffer AE was carried out twice. DNA was then quantified using a Qubit Fluorometer with a Qubit® dsDNA BR assay kit (Invitrogen, Waltham,

Massachusetts, USA). All samples had a minimum DNA concentration of 12.5 ng/µl. All samples of DNA were then stored in aliquots of 50 µl at -80^{o}C until sequencing.

All bacterial 16S gene amplification and sequencing was performed on the V4 region at BGI (Huada Gene Institute) Genomics (Shenzhen, China). For the polymerase chain reaction (PCR), 30ng of DNA template and 16S rRNA fusion primers were added. All products were then purified using Agencourt AMPure XP beads, dissolved in elution buffer and labelled to aid library construction. The Agilent 2100 Bioanalyser was used to detect library size and concentration. Qualified libraries were then sequenced on the HiSeq platform according to their insert size.

4.4.4 Statistical analysis

The low-quality reads were filtered from the raw data by removing reads whose length was <75% of their original length after truncation. All reads that were contaminated by adaptor sequences, ambiguous bases and low complexity reads were also removed. Paired-end reads were then added to tags using the Fast Length Adjustment of Short reads programme (FLASH, v1.2.11). Tags were clustered into operational taxonomic units (OTUs) with a 97% cut off value using UPARSE software (v7.0.1090) and chimera sequences were compared with the Gold database using UCHIME (V4.2.40). Representative OTU sequences were classified using Ribosomal Database Project Classifier (v2.2) to obtain OTU taxonomy. A minimum confidence threshold of 0.6 was set and sequences were then verified on the Greengenes database (v2.01305) in QIIME (v1.8.0). USEARCH_global was used to compare all tags to OTUs to obtain abundance statistics for each bumblebee sample. All OTU sequences were verified using BLAST.

For most analysis I retained only the OTUs that were represented by more than 0.1% of the reads per sample, as I aimed to focus on the core bacteria in the bumblebee gut. This resulted in 12 OTUs covering 99.7% of the reads of the total 324 OTUs in my sequencing dataset. To ensure I also considered the effects of radiation on rarer taxa I additionally ran analysis on the OTUs that were represented by more than 0.0015% of the reads per sample, which resulted in the selection of the top 49 OTUs.

I conducted all analysis in R version 4.2.2 (R Development Core Team, 2020), with community diversity estimation and ordination methods implemented in the R Package 'vegan' (Oksanen *et al.*, 2015). Alpha diversity (Species richness, Shannon diversity and Simpsons diversity) and Beta diversity (Bray Curtis) were calculated using vegan. Radiation effects on these diversity measures were assessed using linear models. Predictors included the categorical variables of dose rate and days within the experiment. The interaction between dose rate and days were also included. This analysis was conducted on the top 12 OTUs to investigate the effects on the core microbiome and then repeated for the top 49 OTUs to account for rarer taxa.

Non-metric multidimensional scaling through the R package 'vegan' was used to visualise the level of similarity in community composition between the different samples when grouped by the categorical variables 'dose rate' and 'days within the experiment' (Oksanen *et al.*, 2015). This analysis was conducted only on the top 49 OTUs (0.0015% reads per sample), as more than 20 OTUs are needed to give meaningful comparison. This analysis was based on Bray-Curtis similarities (relative abundance data) and goodness of fit was assessed through Shepard plots.

To assess differential abundance between treatments of specific OTUs in the core bumblebee microbiome contributing to compositional differences, I conducted an analysis of compositions of microbiome with bias correction (ANCOM-BC) using the R package 'ancombc' (Lin & Peddada, 2020). This recently developed method for analysis was conducted on the top 12 OTUs only for simplicity and data were grouped according to dose rates of radiation received in order to determine effects on abundance. Due to an inability to include interaction term in ANCOM-BC, data from different days were analysed separately.

4.4. Results

4.4.1 Descriptive assessment of changes in microbial abundance in response to radiation exposure.

A total of 6,574,278 reads were obtained from the 90 bumblebees sampled, with an average of 73,048 reads per bumblebee. Following data filtering 324 OTUs were detected, of which 12 were identified as the core microbiome as they each individually represented more than 0.1% of reads in every sample and, when these 12 were combined, they comprised more than 90% of the total reads in the data set. This 'core' microbiome was itself heavily dominated by a small number of highly abundant OTUs; the vast majority of reads were from only five different OTUs (Figure 4.1).

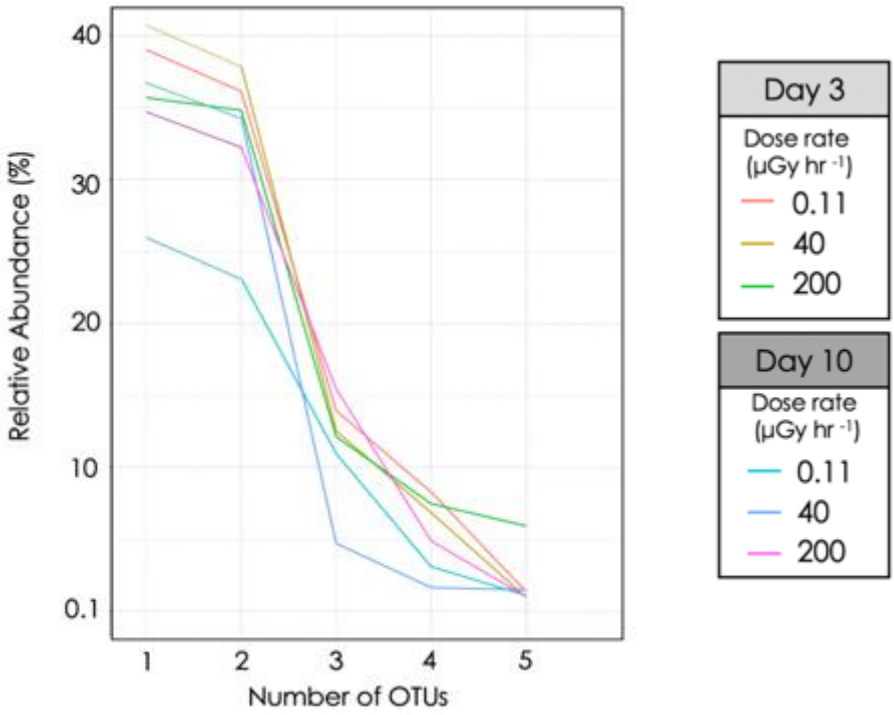

Figure 4.1. The relative abundance of OTUs is consistent for the top three OTUs before dropping dramatically. Each trend line represents one of the 6 treatments, with the two temporal treatments (3 days and 10 days) split in to the three dose rate treatments (0.11, 40 and 200 µGy hr $^{-1}$); n= 15 bumblebees per treatment, n = 90 bumblebees.

Differences in relative abundance of microbes making up the core microbiome were assessed visually for the 5 most common OTUs within the core (greater than 1.1% of reads per sample) and the 7 rarer OTUs within the core (between 1.1% and 0.1% reads per sample). Amongst

the top five OTUs *Gilliamella apicola* and *Snodgrassella alvi* were the most dominant. These two species had roughly similar abundances in all three radiation treatments at day 3 (Figure 4.2). In the temporal contrast between day 3 and day 10, *Gilliamella apicola* became more dominant in all radiation treatment groups by the later timepoint (Figure 4.2). However, this increase in relative dominance of *Gilliamella apicola* was strongest in the control treatment, and with a smaller increase in the two radiation treatments (Figure 4.2). By day 10, *Snodgrassella alvi* had lower relative abundance in bees exposed to 200 µGy hr^{-1} compared to either the controls or bees at 40 µGy hr^{-1} (Figure 4.2). For rarer OTUs, it was notable that *Bombiscardovia sp* was at considerably lower relative abundance after 3 days of exposure to 200 µGy hr^{-1} of radiation. However, this trend was not replicated in the bees that had been exposed to radiation for after 10 days (Figure 4.2).

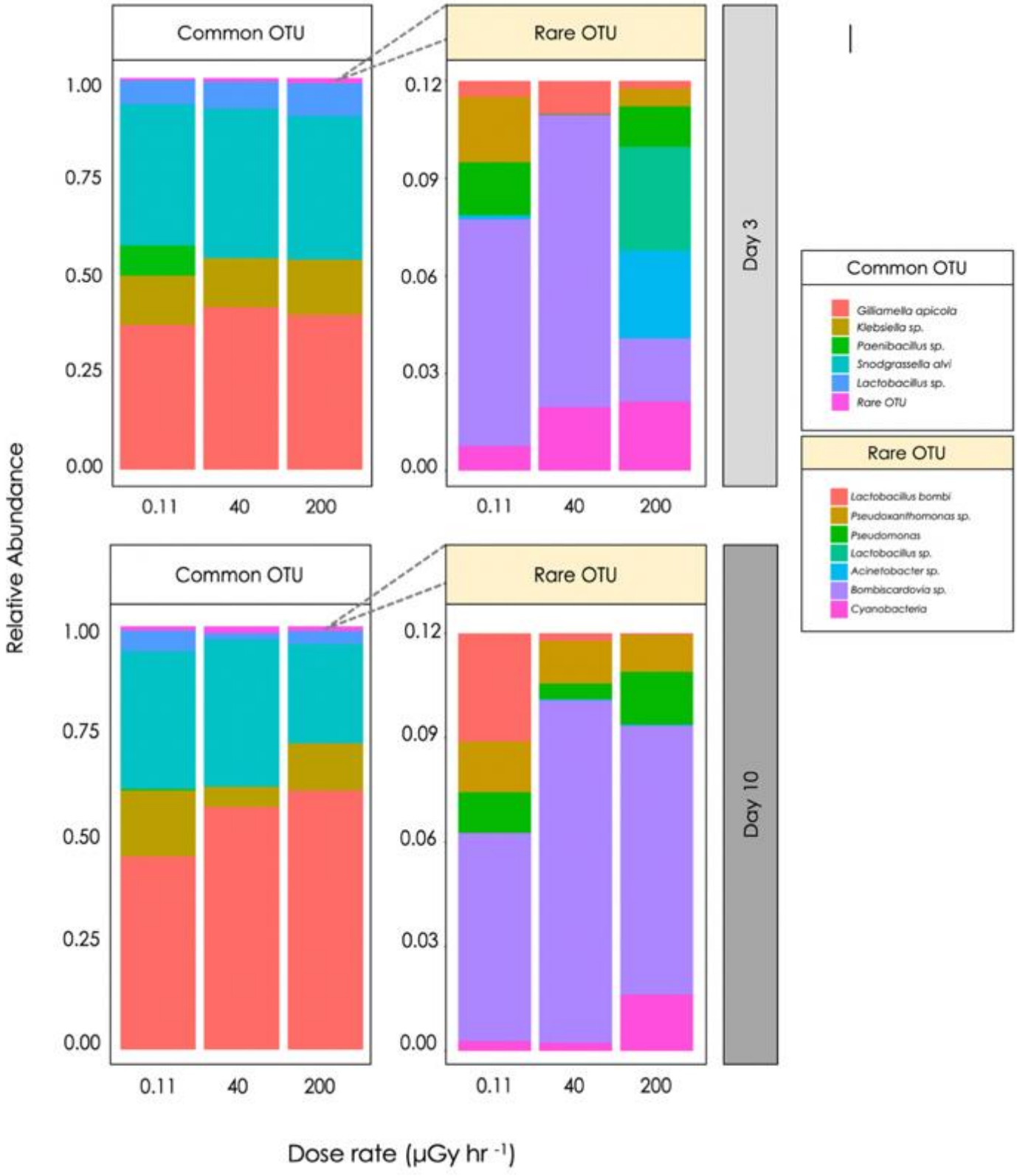

Figure 4.2. The relative abundance of OTUs changed with exposure to radiation and days within the experiment. Data are presented (left panels) for the most common OTUs (over 1.1% of reads per sample) and (right panels) for the rarer OTUs (between 0.1% and 1.1% of reads per sample) from the core microbiome. Relative abundance was calculated by summing all reads for all OTUs from bumblebees and then calculated percent relative abundance. Each colour represents a different bacterial OTU identified in the key. n = 90 bumblebees.

4.4.2 Impacts of radiation exposure on microbial community diversity.

I then undertook investigations to understand how species richness of these 12 core OTUs changed in the bumblebee gut during radiation exposure. Whilst there was a marked elevation of species richness in the 200 μGy hr^{-1} treatment after 3 days, this increase was less pronounced after 10 days exposure; this temporal change is supported by a significant dose rate by time interaction (Figure 4.3; Table 4.1; dose rate by days interaction, $F_{(2,83)} = 4.990$, $P = 0.009$).

Table 4.1. Parameter estimates for models investigating the effect of radiation on bumblebee gut microbiome species richness for the 'core' microbiome. This analysis includes the 12 most common OTUs (1.1% of reads per sample). Dose rates included were 200, 40 μGy hr^{-1} and controls. Model was linear with normally distributed errors. Table S4.1a describes the minimal model used. Table S4.1b contains terms removed from the model in reverse order of deletion during model simplification.

Species Richness					
a. Minimal Model					
Predictors		**Estimate**	**SE**	***F***	**P Value**
(Intercept)		8.667	0.442	-	-
Dose rate (μGy hr^{-1})		-	-	3.386	0.038
	40 μGy hr^{-1}	0.333	0.462	-	-
	200 μGy hr^{-1}	1.167	0.461	-	-
Days within experiment (day 10)		-0.067	0.377	0.031	0.860
Dose rate (μGy hr^{-1}) by days within the experiment (day 10)		-	-	4.990	0.009
	40 μGy hr^{-1}	1.867	0.885	-	-
	200 μGy hr^{-1}	-0.866	0.885	-	-

When just looking at differences between dose rate treatments after 3 days exposure, there was a 18.5 % increase in species richness following exposure to 200 μGy hr^{-1} compared to the control treatment (Table S4.2; $F_{(2,87)} = 6.836$, $P = 0.003$). In an analysis of species richness differences between dose rate treatments after 10 days, whilst both radiation exposure treatments had higher species richness than the control group, the effect of radiation was not significant (Table S4.3; $F_{(2,87)} = 2.012$, $P = 0.147$).

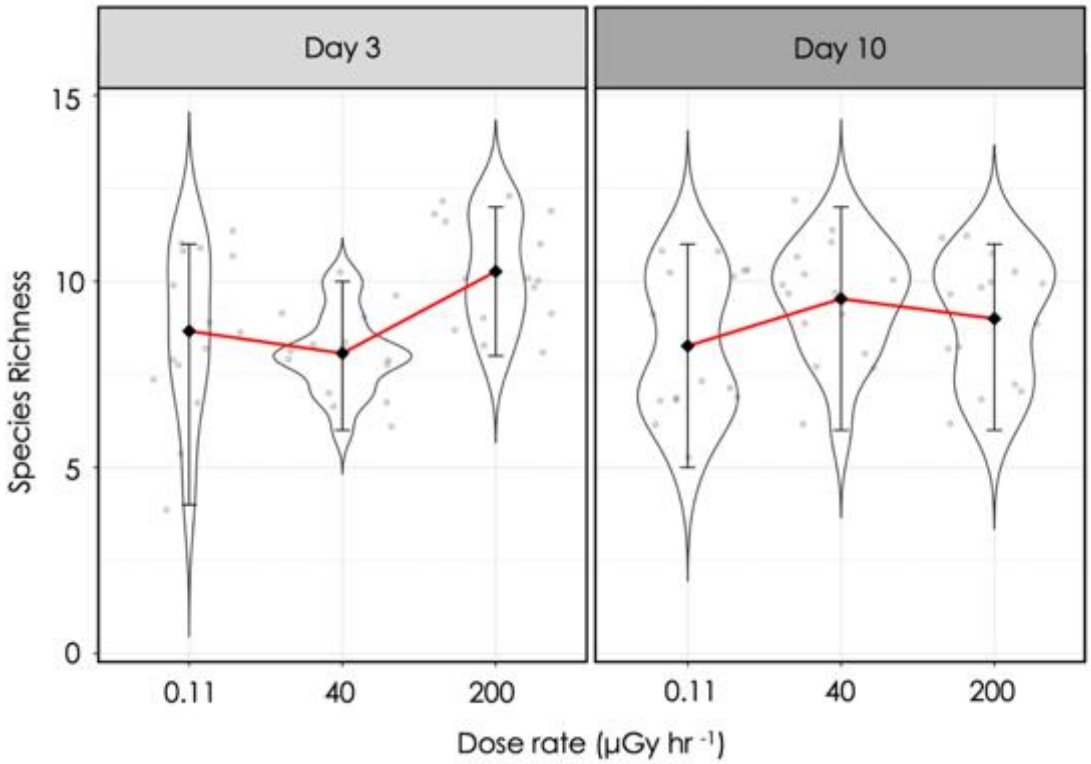

Figure 4.3. Species richness of the core gut microbiome (the 12 most common OTUs) elevated with dose rate during the first 3 days of radiation exposure, a difference that remained but was not statistically significant after 10 days of exposure. Graphs show differences in mean species richness for the 'core' OTUs on day 3 of radiation (left) and day 10 of radiation (right). Points on each graph show species richness in each bumblebee. The model analysing these data is shown in Table S4.1; the fit is represented by the red line and black diamonds, highlighting differences between dose rates. n = 90 bumblebees.

I then repeated this analysis to investigate if the trends in species richness found above were restricted to the 'core' microbiome or whether rarer OTUs were also affected by radiation exposure. I examined the 49 most common OTUs, which were selected because they each represented over 0.0015% of the reads in every sample. When considering this wider microbiome, the differences in species richness that existed between radiation treatment groups again varied between the two time points (Figure 4.4; Table 4.2; dose rate by days interaction, $F_{(2,83)} = 3.289$, $P = 0.042$).

Table 4.2. Parameter estimates for models investigating the effect of radiation on bumblebee gut microbiome species richness which includes rarer taxa in the microbiome of bees exposed irradiation. This analysis includes the 49 most common OTUs (0.0015% of reads per sample). Dose rates included were 200, 40 μGy hr^{-1} and controls. Model was linear with normally distributed errors. Table S4.4a describes the minimal model used. Table S4.4b contains terms removed from the model in reverse order of deletion during model simplification.

Species Richness					
a. Minimal Model					
Predictors		**Estimate**	**SE**	***F***	**P Value**
(Intercept)		20.111	1.638	-	-
Dose rate (μGy hr^{-1})		-	-	0.641	0.529
	40 μGy hr^{-1}	0.067	2.006	-	-
	200 μGy hr^{-1}	2.000	2.006	-	-
Days within experiment (day 10)		-0.289	1.638	0.031	0.860
Dose rate (μGy hr^{-1}) by days within the experiment (day 10)		-	-	3.289	0.042
	40 μGy hr^{-1}	9.467	3.910	-	-
	200 μGy hr^{-1}	1.867	3.910	-	-

For this wider microbiome, the trends were less distinct than for the core: after 3 days treatment exposure species richness was low at 40μGy hr^{-1} compared to the other three treatments, whereas by day 10 both irradiated treatments showed slightly elevated species richness compared to the controls.

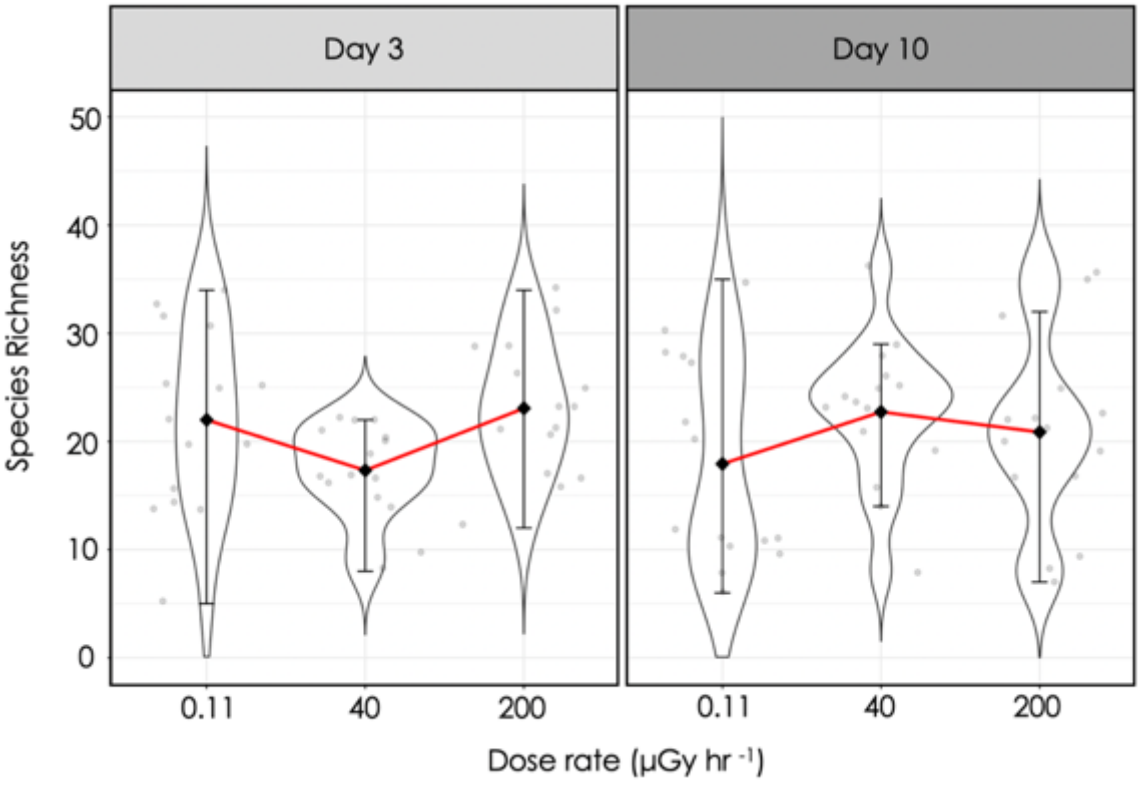

Figure 4.4. Bumblebee gut microbiome species richness of rarer OTUs was low at 40 µGy hr^{-1} after 3 days of treatment in comparison to controls. By day 10 of the experiment both 40 and 200 µGy hr^{-1} show elevated species richness compared to controls. Graphs show differences in mean species richness for the 'core' OTUs on day 3 of radiation (left) and day 10 of radiation (right). Points on each graph show mean species richness per bumblebee. The model analysing these data is shown in Table S4.1; the fit is represented by the red line and black diamonds, highlighting differences between dose rates. n= 12 OTUs, n = 90 bumblebees.

I used Shannon's diversity index to investigate how diversity of the microbial community was influenced by radiation exposure and time. I first looked at the core microbiome consisting of 12 OTUs: whilst diversity significantly decreased with time through the experiment (Table S4.5; days within experiment, $F_{(1,83)}$ = 7.140, P = 0.020), this decrease was not influenced by radiation exposure (Table S4.5, days within experiment by dose rate interaction: $F_{(2,83)}$ = 0.175, P = 0.985). When expanding this analysis to include rarer bacterial taxa (as above), the decrease in diversity over the time remained (Table S4.6; days within experiment, $F_{(1,83)}$ = 9.528, P = 0.003) but was again not influenced by radiation exposure (Table S4.7, days within experiment by dose rate interaction: $F_{(2,83)}$ = 0.702, P = 0.499).

To further investigate changes in alpha diversity I used the Simpson's index to account for the number of OTUs present as well as relative abundance. I again found a decrease in diversity with time the bumblebees were within the experiment for the most common OTUs (Table S4.7, days within experiment, $F_{(1,83)}$ = 12.876, P = 0.001) and also when including the rarer taxa (Table S4.8, days within experiment, $F_{(1,83)}$ = 9.715, P = 0.028). However, again these diversity trends were not significantly affected by radiation exposure for either group of microbes.

4.4.3 Statistical analysis of abundance variation in response to radiation exposure for individual microbial taxa.

As microbiome data is often subject to sample-specific and taxon-specific biases, I used ANCOM-BC (Lin & Peddada, 2020), to assess differences in abundance of individual OTUs between my radiation treatment groups. This analysis of gut microbiome composition assessed differential abundance of OTUs in each of the irradiation treatments (40 and 200 µGy hr^{-1}) relative to the control treatment for the core microbiome (12 taxa) (Figure 4.5). After 3 days of radiation *Pseudomonas* and *Pseudoxanthomonas sp* were significantly less abundant in bumblebees exposed to 200 µGy hr^{-1} in comparison to the control treatment

(Table S4.9). In comparison to controls *Paenibacillus sp* was twice as abundant when exposed to 40 µGy hr^{-1}, however at 200 µGy hr^{-1} there was no significant difference relative to the control treatment (Table S4.9). In contrast *Lactobacillus bombi* was significantly more abundant in bees at the highest dose rate of 200 µGy hr^{-1} than in controls (Table S4.9). After 10 days of irradiation, two of the OTUs studied in this analysis showed a significant change in abundance in comparison to the control treatment. The OTUs *Gilliamella apicola* and *Lactobacillus bombi* both decreased in abundance at 200 µGy hr^{-1} in comparison to control treatments (Table S4.10). When the data from the two radiation treatments were pooled to compare irradiated (40 and 200 µGy hr^{-1}) with non-radiated bumblebees, none of the OTUs present within the core microbiome were found to differ in abundance at either timepoint (Table S4.11; Table S4.12). When data were pooled to compare low and no radiation (0 and 40 µGy hr^{-1} combined) with the highest dose of radiation studied (200 µGy hr^{-1}), after 3 days within the experiment there was a significant decrease in *Snodgrassella alvi* abundance and a significant increase in *Paenibacillus sp* within the higher-dose group in comparison to the other two treatments (Table S4.13). However, when analysing this pooled dataset for day 10 there was no significant change in any OTUs with radiation exposure (Table S4.14).

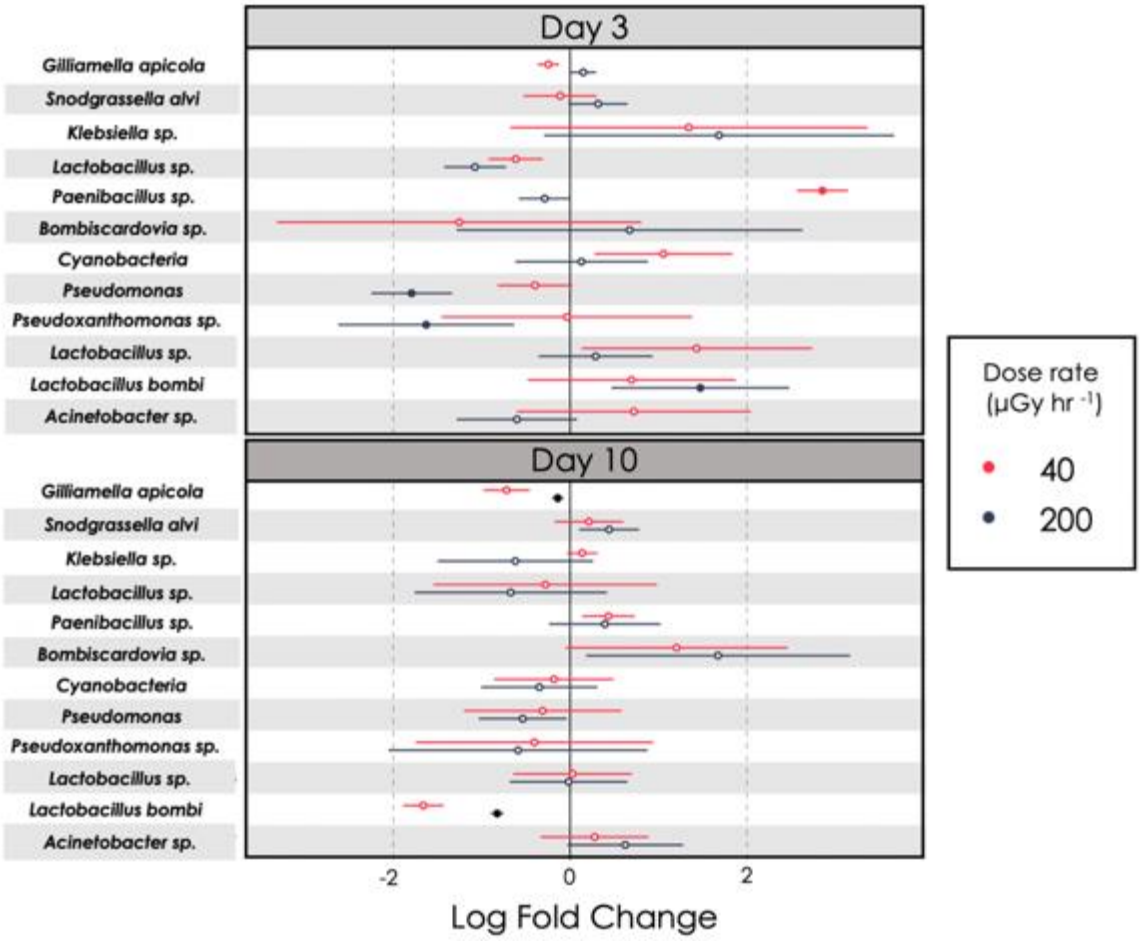

Figure 4.5. The effect of radiation on the relative abundance of the core taxa in the bumblebee gut microbiome. The abundance of each OTU in the two radiation treatments is plotted with reference to the control treatment, for the 'core' OTUs on day 3 of radiation (top) and day 10 of radiation (bottom). The x axis is the log fold change to the base e, therefore a score of 1 indicates the microbe is 2x more abundant in that treatment than it was in the previous treatment. Filled points represent OTUs that show significant differential abundance relative to control bumblebees. The parameter estimates (and SE) are represented by the red (40 µGy hr $^{-1}$) and black (200 µGy hr $^{-1}$) lines. The ANCOM-BC model analysing data for day 3 is S4.9 and day 10 is s4.10. n = 90 bumblebees. Note that confidence intervals of parameter estimates are calculated before the P values are adjusted for multiple tests.

4.4.4 Assessment of gut microbial community dissimilarity between radiation treatment groups.

I then investigated beta diversity metrics in order to assess the similarity or dissimilarity between the gut microbiome community of bumblebees in the different treatment groups. I used non-metric multidimensional scaling (NMDS) to define community similarity based on abundance of all 12 core OTUs; I found no evidence for overall community differentiation based on either radiation dose rate or time within the experiment (Figure 4.6; Table S4.15).

In Figure 4.6, points are clustered within ordination space with ellipses overlapping for all of the treatment groups: this demonstrates no significant bumblebee gut microbiome community differences between the dose rates.

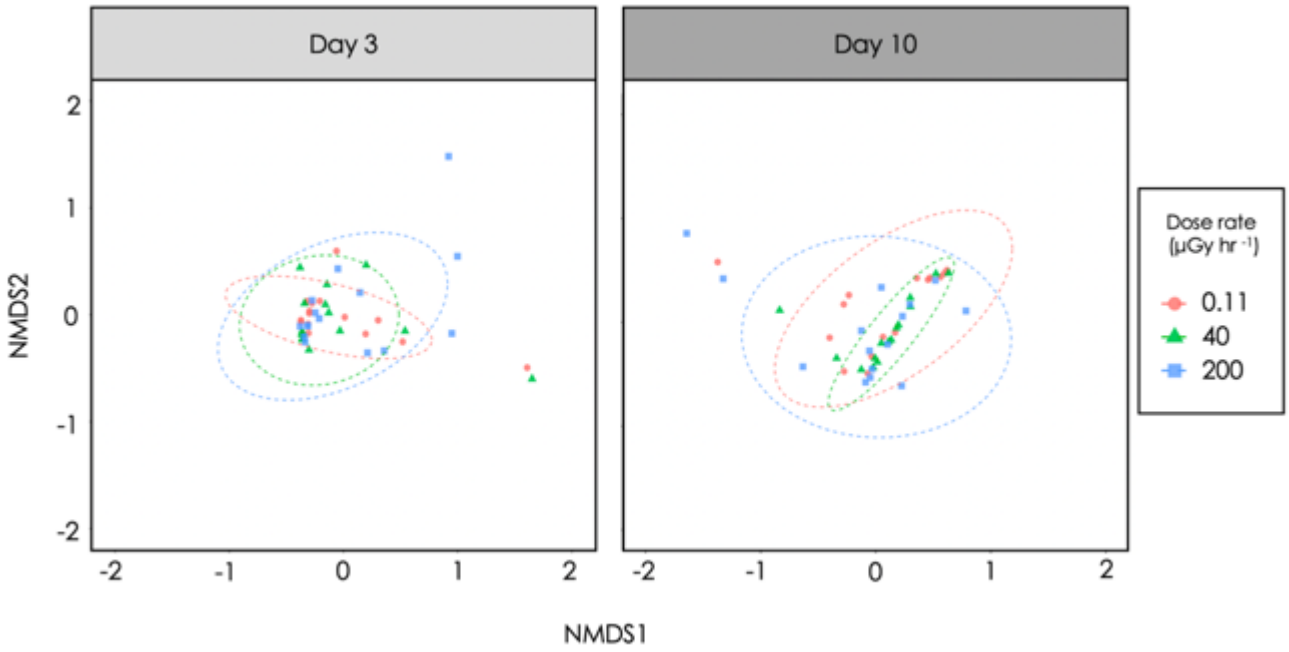

Figure 4.6. Bumblebee gut microbiota shows no distinct separation between dose rate treatment groups after either 3 or 10 days of radiation exposure. Non-metric multidimensional scaling (NMDS) based on the Steinhaus dissimilarity between dose rate groups was used to investigate changes in beta diversity. Metric scaling was used as the starting solution. Data are represented for bumblebees within the experiment for three days (left) and ten days (right). Each ellipse plotted represents 95% confidence intervals around centroids of each dose rate group. Ellipses represent bumblebees in each dose rate category of 0.11 (n = 30), 40 (n =30) and 200 (n=30). Goodness of fit was verified using stress and plotted in a Shepard diagram (Figure S4.1).

To further explore changes in beta diversity of gut microbial communities I then calculated Bray-Curtis dissimilarity for comparisons between different treatment groups. I did this both for the 'core' microbiome (12 OTUs) and for the OTU set including rarer taxa (the top 49 OTUs). These analyses revealed no differences in beta community diversity between the dose rate treatments for the core microbiome (Table S4.16, $t_{(2,83)}$ = -0.368, P = 0.183) or for the data set including rarer taxa (Table S4.17; $t_{(2,83)}$ = 0.317 ; P = 0.432).

4.5 Discussion

This study investigated the dynamics of bacterial gut communities in bumblebees exposed to radiation. I did not find evidence of a large shift in the composition of the microbiome in response to radiation exposure. I however did identify trends at some dose rates at different time points that suggest there are changes in diversity metrics; nevertheless, these results were not always consistent. There was a clear effect of radiation on species richness of bacteria, however again this was not always consistent across dose rates and time points. I present evidence of a potential early effect of radiation on species richness at 200 μGy hr^{-1} and a late effect of species richness at 40 μGy hr^{-1}. I additionally present evidence that some taxa vary in abundance between irradiated and control groups, however again these changes are variable.

In this study the 'core' taxa within the bumblebee microbiome were considered to be the 12 most common OTUs recorded in my analysis. I recorded an increase in bumblebee microbiome richness of these core OTUs with exposure to dose rates of 200 μGy hr^{-1} and 40 μGy hr^{-1}. This increase in richness reflects that sequencing has detected more of the core taxa in bumblebees that are exposed to radiation than bumblebees that are not exposed. The size of this effect is that 2 extra taxa were detected at dose rates of 200 μGy hr^{-1} (accumulated dose of 14400 μGy) relative to controls, whereas an average 0.5 extra taxa were recorded at 40 μGy hr^{-1} (accumulated dose of 9600 μGy). This increase in species richness was significant after just 3 days within the experiment, however whilst the effect remains after 10 days, the effect was no longer significant. The gut microbiota is critical for the health of bumblebees and richness is considered to be an important part of gut function. It has been previously shown that radiation exposure causes a significant increase in the amount of nectar eaten (Burrows et al, 2022). An increase in the volume of food consumed could therefore have created changes the nutrient environment in the gut affecting bacterial growth. A higher productivity of bacteria could increase the number of microbial taxa able to coexist in the gut (Horner-Devine et al, 2003). I identified more core taxa in some irradiated treatments, therefore some of these taxa must have become relatively less abundant; this change could be indicative of competitive dominance of some of the more common taxa reducing slightly compared to control treatments. Whilst I are unable to identify the consequences of this

physiologically, it is likely the control treatment signifies an ideal microbiome composition and therefore changes under irradiation are unlikely to be positive for bumblebee gut health. Radiation could be directly affecting bacteria within the gut which is leading to this alteration in species richness. However, I also acknowledge that this effect may be driven by radiation impacting the microbiome indirectly through changes I have previously recorded in metabolic rate and nectar consumption. This study did not explicitly investigate the potential mechanism driving this effect, therefore I are unable to discern whether radiation is driving these changes directly or indirectly.

Whilst the increase in the richness of the microbiome that I observed when considering just the core 12 microbial taxa, also occurred when I expanded the analysis to include rarer taxa (the 49 most abundant OTUs in my data set), this effect was not significant after 10 days within the experiment. The community richness increased by 14.6 % at 40 µGy hr^{-1} and 8.5 % at 200 µGy hr^{-1}. I hypothesised that as a result of irradiation the core microbiota could be shrinking in abundance, which is providing opportunity for rarer microbe taxa to grow. When exposed to stress, bumblebees have shown increased bacterial richness in their gut microbiome, for example, when they are exposed to the common gut parasite *Crithidia bombi* (Koch et al, 2012). Therefore, it could be that radiation is acting in a similar manner and causing a stress response that reduces the domination of core bacteria. Another potential explanation, is that an increase in the volume of nectar travelling through the gut is causing the 'wash' out of dominant members of the core bacterial community. However, I did not measure the volume of nectar that was excreted from the bumblebee during the experiment, so I cannot test this hypothesis directly with my data.

I tested differential abundances of specific OTUs in the core bumblebee microbiome through ANCOM-BC analysis to see how they contributed to compositional differences. Of all 12 OTUs investigated, only two OTUs showed a change in abundance after 10 days of irradiation exposure. After 10 days *Gilliamella and Lactobacillus sp.* significantly increased in abundance at 200 µGy hr^{-1} in comparison to control treatments. As a bumblebee ages, *Gilliamella* has been found to increase in relative abundance, which is hypothesised to be due to it being a better competitor and often excluding other core microbes over time (Hammer et al, 2022). This is further confirmed through visual inspection of relative abundance (Figure 4.2):

Gilliamella did increase in all of the treatment groups between day 3 and day 10 of the experiment. However, this increase is less pronounced in radiation treatments compared to controls. Therefore, a decrease in this bacterium may suggest that another microbe is outcompeting it within the gut and causing a reduction in relative abundance. This competitor bacterium could potentially be *Snodgrassella*, which was at higher relative abundance at 40 and 200 µGy hr^{-1} compared to controls at the day 10 timepoint (Figure 4.2). It has been recorded that *Snodgrassella* affects honeybee immune gene expression by leading to the expression of host antimicrobial peptides in response to pathogen infection which benefitted individual health through faster recovery (Horak, 2020). Therefore, exposure to radiation stress could be triggering an immune response in which *Snodgrassella* increases in abundance to trigger an immune response and prevent overgrowth of other bacteria.

More OTUs showed changes in abundance after just 3 days of radiation exposure. After 3 days of radiation *Pseudomonas* and *Pseudoxanthomonas sp* decreased in abundance when exposed to 200 µGy hr^{-1} in comparison to the control treatment. *Pseudomonas* species within insects has been shown to be involved in detoxification (Ceja-Navarro et al, 2015). It has also been shown to be involved in digestion activities contributing to nutritional supplementation (Briones-Roblero et al, 2017), therefore a reduction in its abundance could negatively affect digestion. *Pseudoxanthomonas sp* is a bacterium that has been shown to degrade microorganisms and neonicotinoid insecticides (Pang et al, 2020), therefore again its reduction could have negative impacts if bumblebees were exposed to opportunistic microorganisms. However, both these species were no longer more abundant after 10 days of radiation, suggesting this may be a short-term effect in response to initial radiation exposure. The OTU *Lactobacillus bombi* increased in relative abundance in comparison to controls after 3 days irradiation at 200 µGy hr^{-1}. This bacterium has been recorded to improve overall bee health and stimulate egg production (Audisio, 2017). Interestingly, the bacterial taxon *Paenibacillus* showed the largest change in response to radiation after 3 days within the experiment with a large increase in relative abundance when exposed to 40 µGy hr^{-1} in comparison to the control treatment. *Paenibacillus* is often a pathogenic taxon within bees, however there are non-pathogenic forms which can have antimicrobial properties (Keller et al, 2018).

I additionally speculated that increased nectar consumption as a result of radiation exposure, could also positively influence other aspects of bumblebee health, which could then alter the community of gut bacteria (Ryu et al, 2008). However, I found no significant effect of radiation on the diversity of species found within the bumblebee gut when using both alpha and beta metrics. I did however observe changes in diversity with the time a bumblebee was within the experiment, suggesting a potential effect of ageing on the gut microbiota. An effect of ageing has been recorded at the colony level with older colonies exhibiting fewer core symbionts and more opportunistic bacteria (Li et al, 2015). However less is known about individual-level senescence of the gut microbiome and studies suggest that the microbiome is generally stable in older bees with little evidence of disruption (Hammer at al, 2022). This suggests that the consequences of radiation or the metabolic syndrome occurring as a result of radiation exposure could have greater negative impacts as a bumblebee ages. This result could have been affected by bumblebees being reared individually in a laboratory environment. Whilst studying bumblebees in a laboratory environment provided the advantage of being able to study disturbance in the microbiome without any environmental impacts. Environmental exposure to microbes in the wild results in more heterogeneity within the microbiome. Worker bumblebees recorded in the field often lack several core bacteria and instead demonstrate increased colonisation from opportunistic bacteria (Koch et al, 2012). The microbiome of queen bumblebees and workers are largely identical, as the queen provides workers with their core microbiome within the nest. However, as queens rarely forage outside of the nest after cohort formation they are less likely to pick up opportunistic bacteria than workers (Hammer et al, 2022). It should also be noted that bumblebees within the natural environment will have different microbiome compositions to those reared in laboratory settings. In this experiment bumblebees were also fed ‘sterile’ nectar solutions, whilst in the wild nectar will often contain a variety of bacteria. Therefore any effects recorded on the microbiome as a result of radiation exposure could be significantly different in contaminated landscapes.

Overall, I observed several changes in the microbial community in the gut of bumblebees exposed to 10 days of environmentally relevant ionising radiation. I present evidence that radiation causes changes in species richness in bumblebees which could be as a result of direct or indirect effects of radiation. The microbiota of bumblebees is closely linked with

physiological and even behavioural changes within the individual. Therefore, gut bacteria often respond to both direct and indirect changes within the host. Any changes in microbial communities as a result of irradiation could therefore influence host development and key life history processes. I suggest that further research should focus on discerning the exact mechanism driving the changes presented.

Chapter 5: Environmentally relevant radiation exposure impacts fecundity and development in *Drosophila melanogaster*

Key Words: Ionising radiation, Life history, Insects, *Drosophila melanogaster*, fecundity, development success, Eco-toxicology, Radiological contamination.

5.1 Abstract

(1) The impacts of current levels of radiation found in the Chernobyl Exclusion Zone are extensively debated. A wide variety of species have been studied there but findings are often hard to generalise due to the large range of species and exposure pathways studied. The model organism *Drosophila melanogaster* is naturally widespread in this environment but the impacts of radiation on this species are often only investigated at acute high dose rates.

(2) I studied the impacts of radiation on the key life history trait of fecundity in *D. melanogaster*. Also, to understand if radiation exposure has fitness impacts even when an organism is no longer exposed, I studied the fecundity of flies that had developed under irradiation when they subsequently mated at background dose rates. I focused on how radiation exposure might impact the ageing process by studying a substantial portion of the fly lifespan.

(3) I monitored fly fecundity (egg production), egg to adult viability, and the adult offspring sex ratio for fly pairs under irradiation for 28 days. I then took flies that successfully developed under 200 µGy h^{-1} and mated them under control conditions to mates from a separate constant density fly stock. I subsequently monitored fecundity of these pairs over 10 days.

(4) When pairs of mated flies were exposed to radiation, fecundity declined significantly over time. However, in the first 18 hours of radiation exposure the number of eggs produced briefly increased markedly. Radiation also influenced the sex ratio of offspring, causing an increase in the number of male offspring produced. Female flies that were offspring of irradiated parents and which developed under radiation exposure, still suffered fecundity impairment when radiation exposure stopped.

(5) I show that environmentally relevant radiation exposure significantly reduced fecundity and development success in flies. I hypothesise that this is likely due to a direct effect of radiation rather than accelerated senescence. My study also shows a change in sex ratio during irradiation, which could have significant consequences for fly populations in contaminated landscapes. Previous studies found that bumblebees suffer reproductive impairment at the dose rates I studied, but it has remained unclear whether these effects in bumblebees are atypical of insects in general. My data on *D. melanogaster* suggest that effects of radiation on animal reproduction may be more widespread than previously appreciated.

5.2. Introduction

Human use of radiation is rapidly growing, from services such as power generation to nuclear medicine. Yet, whilst radiation's impact on humans is well characterised, there is considerable debate about its impacts on our environment. This is particularly true for the Chernobyl Exclusion Zone (CEZ). The 1986 disaster deposited $1.85x10^{18}$ Bq of radionuclides heterogeneously across a large landscape (IAEA, 2006). The dose rates over 30 years later are much lower in the CEZ, ranging from near background level (<0.1 µGy hr^{-1}) to 250 µGy hr^{-1} (Beresford, 2020), however the biological impacts of these dose rates on wildlife living in the exclusion zone are debated. Experimental work on bumblebees at dose rates similar to those found in the CEZ has shown that radiation influences life history traits via negative impacts on metabolic processes (Burrows et al, 2022). Laboratory studies at field-realistic doses have also shown that lower dose-rates of radiation have negative impacts on the reproduction of bumblebees (Raines et al, 2020). However, the eusocial biology of bumblebees and their unique life history can make it difficult to generalise effects to other insects and it might potentially be argued that bumblebees may be especially unusual in their susceptibility to radiation. I therefore studied *Drosophila melanogaster*, the most well characterised laboratory model organism, for which there are well established methods for investigating life history traits. This allows better assessment of the generality of effects of radiation on insects, such as reduced reproduction, that have been previously recorded in bees. I additionally studied how radiation may affect key life history traits within this fly species by recording effects on fecundity over a substantial portion of the fly life-span. My aim was that this study would provide an experimental test of the reproductive effects a fly is likely to experience when living within a radiologically contaminated environment.

The model organism *D. melanogaster* is naturally widespread within the CEZ and has been studied to understand the effects of radioactive contamination in our environment (Mosse et al, 2006). For example, it has been found that flies exhibit sensitivity to radiation through features such as a shorter lifespan and higher frequencies of lethal mutations (Yushkova, 2022). However, this previous work involved exposing offspring from flies taken from the CEZ to high acute doses in order to be able to see these effects, so it is not directly applicable to realistic scenarios for environmental exposure. Further studies have found an increase in

lethal mutations in *D. melanogaster* in the CEZ (Zainullin, 1992); however, dose rates were considerably higher at the time of this study than they are today. Additionally, work from the CEZ often uses methodologies which are complex and findings are therefore difficult to extrapolate to ecological effects (e.g. taking individuals from contaminated landscapes and exposing them to acute dose rates). Other studies largely examine molecular effects of exposure. For example, transgenerational effects have been observed in CEZ flies in the form of chromosomal rearrangements that have led to a decrease in survival rate of offspring after 160 generations of lab breeding (Yushkova & Bashylkova, 2021). These chromosomal rearrangements affect development at early stages of embryogenesis and therefore can lead to death of fertilised eggs (Attia et al, 2015). Further investigations on sex-linked recessive lethals found descendant generations of flies develop a radio-adaptive response (Hancock et al, 2019).

For the study organism *D. melanogaster* there is debate within the literature surrounding the effects of radiation on lifespan. Some studies report that high doses of radiation accelerate functional senescence (Lamb, 1964; Giess and Planel, 1977). Lifespan of both sexes has also been recorded to decrease when individuals are irradiated as eggs at doses starting from 250 mGy (Vaiserman et al, 2021). However, others have reported that accumulated doses as high as 600,000 to 800,000 μGy result in an increase in the lifespan of flies (Marples and Collis, 2008; Moskalvev et al, 2006); some of these effects may be mediated by heat shock protein genes, particularly in flies preconditioned by radiation exposure (Moskalev et al, 2009). Some studies report a lifespan extension in the offspring of irradiated males and females (Shameer et al, 2015), whilst others only find this effect in males (Zainullin & Moskalev, 2001). The commonality between all of these studies is however that the levels of radiation considered are far above the highest dose rate currently found in the CEZ (250 μGy hr^{-1}). To better assess the impacts of low dose radiation relevant to contaminated environments, I require a more comprehensive understanding of direct effects on insect fitness and life history.

I therefore used *D. melanogaster* to provide generality to studying effects of radiation by focussing on the key life history trait of fecundity. I consider fecundity a good general measure, as it provides an understanding of reproduction over time. I also focused on reproductive activity as it enabled us to investigate the effects of low dose radiation on

functional senescence. It is well characterised that reproduction declines with age in flies (Mueller, 1987; Partridge et al, 1999) and that increases in reproductive activity of *D. melanogaster* are associated with a reduced lifespan (Flatt, 2011; Semaniuk et al, 2018). This is especially true for female flies as continuous mating impacts lifespan as males harm females due to sexual conflict which is when two sexes have conflicting optimal fitness strategies (Partridge et al, 1987; Chapman et al, 1995). In times of stress, such as in times of reduced food provision, females may have to invest more in egg laying which then trades off against lifespan. Exposure to the stress of low doses from radon exposure starting from 30 µGy have been shown to reduce fecundity and increase viability in *D. melanogaster*, hypothesised to be radon exposure causing lethal damage during the production of gametes (Pimentel et al, 2003). In my experiment, I aimed to study how radiation influences the profile of reproductive senescence.

It has been well established that when an organism is exposed to stress it can lead to impacts on longevity (Partidge & Barton, 1996; Sgro et al, 2013). It is therefore important to study the impacts of stressors, such as radiation throughout a large proportion of the lifespan of a species to understand its long-term effects. This will allow for a better understanding of any alterations in reproductive timing and whether there is any change in its regulation in response to low dose radiation. Higher acute doses of radiation (0.25 – 1 Gy) have been shown to lead to decreased body weight and increased loco-motive activities during the lifespan of *D. melanogaster* when irradiated as an egg (Vaiserman et al, 2004). Other work additionally primarily examines the effect of short-term exposure of radiation, with effects then monitored within controlled conditions. I took a novel approach by exposing flies to low dose rates over several weeks, therefore uniquely allowing for the study of radiation on life history during long term exposure.

Due to large spatial heterogeneity in radionuclide contamination in environments such as the Chernobyl Exclusion Zone, organisms often experience variable dose rates if they move around the environment. Depending on their movement pattern, individuals may be exposed to radiation only briefly or may be exposed as juveniles but not as adults. Therefore, there is a real need to understand more generally the extent to which the effects of radiation are short term or whether they persist after an individual moves out of a contaminated site. It is

well known that environments that are experienced during early-life events have the potential to lead to life stage sensitivity (Monaghan, 2008). I therefore suggest it is important to not only consider fitness consequences when an organism is exposed as an adult, but also to examine effects that could occur in adults if they experienced radiation during their pre-adult development. In *D. melanogaster* the thermal and nutritional environments during development can exert different effects on adult reproductive success, for example females raised in the stressor of a cold environment had reduced reproductive success (Min et al, 2020). I therefore investigated the effect of radiation on adult flies that developed under irradiation exposure from irradiated parents. I also suggest that it is equally as important to consider both the short term and long-term impacts of low dose radiation. In bumblebees, exposure leads to an increase in metabolic rate but when a bumblebee is no longer exposed, metabolic rate returns to normal (Burrows et al, 2022).

My hypothesis for this study was that fecundity in *D. melanogaster* is affected by radiation levels found at 'post disaster' sites. Previous studies found that exposure to environmentally relevant radiation dose rates not only reduces reproduction in bumblebees but also causes a metabolic syndrome. Therefore, I hypothesised that this type of life history response would be recorded in a second species. Studies involving acute exposure to high dose rates have found that radiation reduces fecundity and increases viability in *D. melanogaster*. I therefore investigated the fecundity profile of this scientifically important species over a substantial proportion of its lifespan at environmentally relevant dose rates. It has been shown that reproductive output changes with senescence, I therefore hypothesised that this process might be influenced by radiation exposure. I additionally investigated whether an individual has the ability to recover after developing in an irradiated environment and whether this recovery differs depending on sex.

5.3. Materials and Methods

5.3.1 Fly Culturing

I used *Drosophila melanogaster* originating from wild caught female flies collected in the Chernobyl Exclusion Zone in the summer of 2016. These flies were used due to availability of the stocks. These flies were however genetically investigated and it was found there was no clear population genetic structuring between flies sampled inside and outside of the Chernobyl Exclusion Zone. Any effects on Chernobyl flies are anticipated to be potentially be greater in naive flies. These flies were maintained at the University of Stirling as iso-female lines for four years at a constant temperature of 18^{o}C. After ~40 generations, 100 of these iso-female lines were used to create an outcrossed population by selecting two inseminated females per line and splitting them across 20 fly bottles to oviposit. This recombinant population was subsequently maintained at a large population size, with 200 inseminated females used to found each new generation. To found each new generation the 20 females were added to a bottle and left to lay eggs, females were then removed and the subsequent offspring formed the next generation. A female will lay approximately 800 eggs in one lifetime therefore 20 flies were sufficient per bottle.

Throughout the experiment flies were kept at the University of Stirling environmentally-controlled radiation facility (12 hr light: dark cycle (07h – 19h)). A ^{137}Cs source at one end of the facility room emits gamma radiation. Flies in vials were placed on shelving units at varied distances from the radiation source to deliver different dose rates of radiation (200 to 40 µGy h^{-1}). Dose rates were verified before the start of the experiment using dosimeters (Model 23-1 Electronic Personal Dosimeter, EKO-TEKNIK). To minimise any within-treatment variation in dose rate, fly vials were placed on a tilted shelving; uniformity was verified by dosimeters. The temperature (mean = 25.3^{o}C, range ± 3.9) and humidity (mean = 33.8%, range ± 8.7) at each shelving unit was recorded throughout the experiment every 2 minutes (three loggers per dose rate treatment).

All rearing and experimentation was conducted using Lewis food medium (Lewis, 1960). The flies used to start this experiment were the sixth generation after the recombinant population was established (see above). These flies were collected three days after they emerged from

pupae; 10 inseminated females were added to each of 20 bottles containing food medium and allowed to lay eggs for three days before being removed. I used the resulting adults to generate an age-matched population of flies for experimentation that had been bred at controlled density following the techniques of Clancy and Kennington (Clancy & Kennington, 2001). I placed the seventh-generation adults into a laying cage and collected eggs for 18 hours on apple juice agar plates seeded with yeast. Plates were then flooded with PBS buffer and a paintbrush used to dislodge eggs into a 50ml collection tube. Eggs were rinsed with PBS and left to settle, before a 13µl volume of packed eggs was transferred to the food medium in each of 25 fly bottles using a pipette. Offspring then developed under control conditions in the radiation facility, with the radiation source shielded so that flies were not exposed to radiation during development. Offspring were subsequently harvested as virgins within four hours of eclosion. For 24 hours following collection, female flies were kept individually to allow them to sexually mature, whilst males were housed in groups of 10, in fly food vials. Then, single male and female flies were paired in individual vials, and kept for four days under control conditions to mate and age.

5.3.2 Experiment 1: The effect of radiation on *D. melanogaster* fecundity

I assessed the effects of radiation on *D. melanogaster* by recording three life-history metrics: fecundity (egg production), egg to adult viability, and the offspring sex ratio. Fly pairs were exposed to doses of 200 or 40 µGy h^{-1}, or to control conditions. Dose rates were selected as previous chapters identified significant effects down to 40 µGy h^{-1} and 200 µGy h^{-1} is one of the highest dose rates found in the CEZ. The control treatment was housed in the same facility so that environmental conditions remained consistent with irradiated groups, but flies were placed outside the radiation field so that they were not exposed. The levels of background radiation at the site of the facility are 0.11 ± 0.01 µGy h^{-1} (Raines *et al.*, 2020). For all analysis and reporting I refer to the radiation levels in the control area as 0.11 µGy h^{-1}.

To measure the effects of radiation on egg production, I placed 60 female-male fly pairs at each of the three dose rates (200, 40 and 0.11 µGy h^{-1}) for 28 days (see Figure 5). Initially, fly pairs were left on food in the radiation field for 18 hours, then taken from the radiation facility to an adjacent room, tipped into a fresh vial of food and then returned to the experiment. All

eggs counts were for an 18-hour period, with counting always beginning between 13:00pm and 14:00pm. These 18-hour egg counts were repeated every 3 days for 28 days. To ease egg counting, all food that eggs were laid upon was dyed with blue food colouring (PME Brand, Royal Blue). Accumulated dose rates for flies at the end of the 28-day period were: 134400 µGy (200 µGy h^{-1}), 67200 µGy (100 µGy h^{-1}) and 26880 µGy (40 µGy h^{-1}). To assess egg-to-adult development success, the eggs in all vials were counted using a stereo microscope within two hours of removal from their dose rate, and then returned to their dose rate treatment. Egg vials were left for 16 days until all adults had eclosed; then offspring flies were counted and their sex recorded.

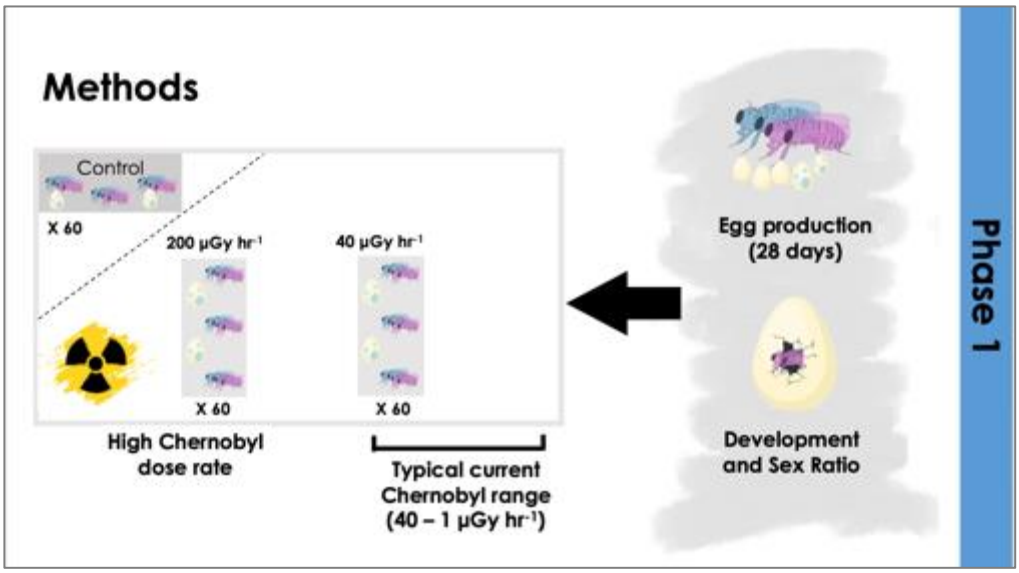

Figure 5. The experimental design for Experiment 1: The effect of radiation on *D. melanogaster* fecundity. For this experiment 60 male/female pairs were kept at the dose rates of 200 and 40 µGy h^{-1}. These pairs remained in the experiment for 28 days. Every 3 days an egg count took place for these pairs. At the end of each count, the pairs were tipped in to a fresh vile and the eggs placed infront of the parents to allow them to develop under radiation exposure. The vials were then monitored and checked fro development success and sex ratio.

During this experiment I observed substantial effects of radiation on fecundity during the first day after exposure commenced. To verify these rapid radiation effects on egg production, a further 60 pairs of flies (prepared in the same manner) were placed at 200 (accumulated dose 3600 µGy) and 0.11 µGy h^{-1}. The number of eggs produced by each pair was recorded after 18 hours, after which this additional test stopped.

5.3.3 Experiment 2: Legacy effects of prior radiation exposure on adult reproductive success in *D. melanogaster*

To determine if adults that developed as larvae under irradiation suffered fitness consequences when they were no longer irradiated, I studied a group of flies that came from eggs that were laid after their parents had experienced 10 days or irradiation at 200 µGy h^{-1} or under control conditions (see Figure 5.1). These eggs hatched and developed to adulthood under the same treatment conditions as their parents; then I took one female and one male fly from each vial of these 'day 10' eggs. These flies were collected as virgins from the two dose rates (n=120 individuals per dose rate). To provide virgin females and males that were of the same age as those used in this experiment a new constant density population was established by pipetting eggs (see above) into 20 food bottles, which were then reared under control conditions. The emergence of these flies coincided with the emergence of adults from the 'day 10' egg vials. For this experiment all experimental flies were then paired with a virgin fly taken from this constant density generation.

The experimental treatments for this experiment comprised:

(1) A male fly that developed under 200 µGy h^{-1} paired with a stock female (n = 60).

(2) A female fly that developed under 200 µGy h^{-1} paired with a stock male (n = 60).

(3) A male fly that developed in the control area of the facility at 0.11 µGy h^{-1} paired with a stock female (n = 60).

(4) A female fly that developed in the control area of the facility at 0.11 µGy h^{-1} paired with a stock male (n = 60).

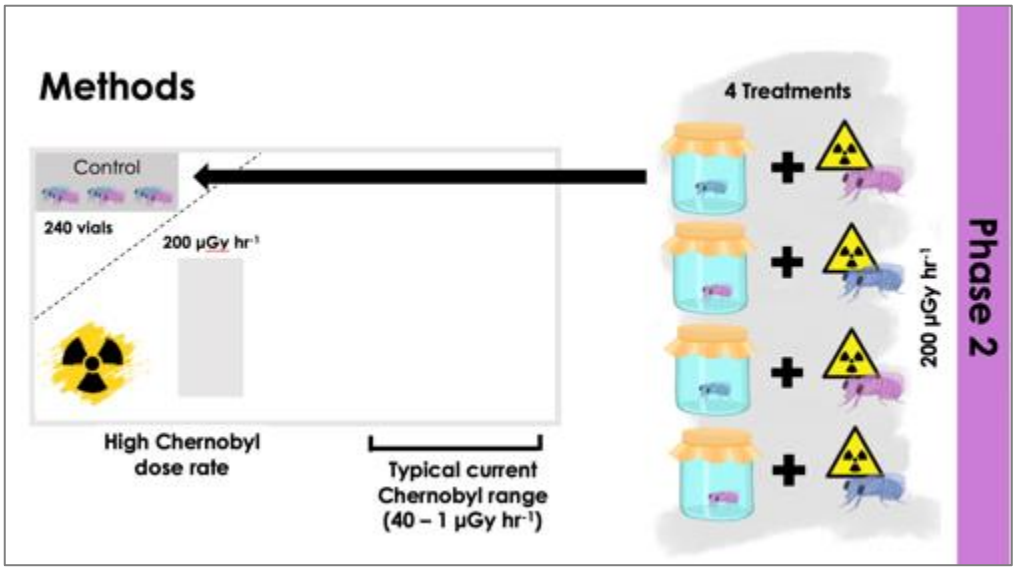

Figure 5.1. The experimental design for Experiment 2: Legacy effects of prior radiation exposure on adult reproductive success in *D. melanogaster*. For this experiment male and female flies were taken from day 10 egg vials that were left to develop and paired with a constant density stock fly. These were then kept in the control area to monitor egg production.

The flies in these four different treatments were housed in the control area of the radiation facility (at 0.11 µGy h^{-1}) to study fecundity. Adult flies in the 200 µGy h^{-1} treatments originated from eggs laid by parents that had experienced this dose rate for 10 days; the eggs developed through larval and pupal stages for a further 10 days; then adults emerged from pupae and were removed from the radiation treatment within 4 hours of eclosion. To measure the impacts of this previous radiation history on egg production, 18-hour egg counts were again conducted on blue food medium as per the method employed in experiment 1. This experiment occurred over 10 days with a total of 4 egg counts taken during this time on days 1, 4, 7 and 10.

5.3.4 Statistical analysis

I conducted analysis in R version 4.2.2 (R Development Core Team, 2022). The only predictors that were mean centred and scaled were the environmental variables of temperature and humidity as recorded by the nearest data logger to any given fly vial. The mean of these environmental variables was calculated for the 24-hour period prior to any measurement. All analyses that involved repeated measures on fly pairs contained individual-level random effects. As initial graphs of raw data demonstrated a non-linear relationship between fecundity and time in the experiment, a polynomial term for days within the experiment was

fitted for some models. The order for the polynomial terms was selected based on the Akaike information criterion (AIC). Model simplification was performed where appropriate by eliminating terms from the full model using likelihood-ratio tests, these tests then generated p-values. All models were validated through the use of Q-Q plots and residual histograms.

Experiment 1: The effect of radiation on D. melanogaster fecundity

The effects of radiation on the number of eggs produced by pairs of *D. melanogaster* were analysed using linear mixed effects models in lmer from package lme4 (Bates et al, 2015). The dose of radiation that fly pairs received was included in the analysis as a categorical factor as there was three dose rates. When drawing initial graphs of raw data fecundity trends, there appeared to be an initial increase in egg output over the first few days of radiation exposure, followed by a strong decline. I therefore fitted a third-order polynomial term for days within the experiment; in order to test if dose rate altered this curved relationship, I included its two-way interaction with dose rate treatment. I also included covariates for the environmental variables temperature and humidity, and their interaction. To verify that temperature and humidity did not influence the impacts of radiation on fecundity, the interaction of these environmental variables with dose rate was also included in models. As this model included repeated measures on the same fly pairs, I included a random effect for individual pair.

To investigate whether the effect of radiation on fecundity of breeding pairs occurred within a few days, I analysed the number of eggs laid on only days 1 and 3 of the experiment with a linear mixed effects model. Dose rate of radiation was again included as a factor. As only two measurements were included in this analysis, environmental variables of temperature and humidity were included with no interaction term. The number of days within the experiment was included as a factor. A random effect was included in this analysis as multiple measures were made on the same fly pair. To verify whether an effect of radiation could be recorded after just 18 hours of exposure, a further cohort of 60 fly pairs were added to the experiment at 200 and 0.11 $\mu Gy\ h^{-1}$: I used a linear model to test differences in fecundity between these two dose rates. I then used a similar model structure to explore whether radiation impacted the total fecundity of all 180 fly pairs over the 28-day period of the main experiment, with the dose rate each pair received included as a fixed factor.

To test whether radiation affected the success of fly egg development to adulthood I used a generalised linear mixed effects model with a binomial error distribution. Models had a two-vector response variable using the cbind function to group the number of eggs that successfully developed into adults with those that failed to reach adulthood for each egg vial. The dose of radiation that the flies received was included as a factor. As the relationship between development success and time within the experiment was non-linear, I included a second order polynomial term for days within the experiment and for its interaction with dose rate. Again, environmental variables of temperature and humidity were included, as well as their interaction with each other and dose rate. As I again conducted multiple measures on the same fly pairs I included a random effect for individual. To investigate the effect of radiation on the sex ratio of flies that reached adulthood an identical model was used, again with a second order polynomial for day and its interaction with dose based on AIC selection criteria. For this analysis the sex ratio response variable was specified using the cbind function to group the number of male and female offspring.

Experiment 2: ***Legacy effects of prior radiation exposure on adult reproductive success in D. melanogaster***

The effects of radiation on the number of eggs produced after irradiation during juvenile development stages was analysed using linear mixed effects models. The treatment a parental fly received was included as a factor. There were four treatments in this experiment which included: a male that developed under 200 µGy h^{-1} paired with a stock female, a female that developed under 200 µGy h^{-1} paired with a stock male, a male that developed under 0.11 µGy h^{-1} paired with a stock female and a female that developed under 0.11 µGy h^{-1} paired with a stock male. Fecundity was assessed at four timepoints over 10 days: the covariate of time within the experiment was included. I also included the environmental variables temperature and humidity, recorded from the nearest data logger 24 hours before the egg counting measure was taken. I included these environmental variables, their interactions with each other and with treatment. I tested if treatment effects varied through time by adding an interaction between treatment and days in the experiment. I included a random effect for the parental vial, which represents the parents of each focal fly within this experiment. I additionally included a random effect for individuals, as this model included repeated

measures on the same fly pairs. To test for sex-specific effects of previous radiation exposure, I ran two separate analyses, one for the male data and one for female data. To investigate the effect of radiation on total fecundity, the total number of eggs produced by each fly pair in each of the four treatments was calculated: for this data set the effect of radiation was assessed using a linear model with just the radiation treatment as a factor.

Table 5. A list of predictors included in all models conducted on data collected for Experiment 2. In these models a female that developed under control conditions that was paired with a stock density male was used as the reference.

Predictors
(Intercept)
Treatment Female 200 µGy h^{-1} Male 200 µGy h^{-1} Male Control
Days within the experiment
Treatment by days in the experiment Female 200 µGy h^{-1} Male 200 µGy h^{-1} Male Control
Average humidity during the days when the egg counting measurements were made (%)
Average temperature during the days when the egg counting measurements were made (°C)
Treatment by humidity during the days when the sex ratio measurements were made (%) Female 200 µGy h^{-1} Male 200 µGy h^{-1} Male Control
Treatment by average temperature during the days when the sex ratio measurements were made (°C) Female 200 µGy h^{-1} Male 200 µGy h^{-1} Male Control
Average temperature (°C) by humidity (%) during the days when the sex ratio measurements were made

5.4 Results

5.4.1 Experiment 1: The effect of radiation on D. melanogaster fecundity

In order to study fecundity through the majority of the life course *of D. melanogaster* I counted the number of eggs produced by fly pairs over an 18-hour period, every three days. When fly pairs were kept within the control area (0.11 µGy h^{-1}), there was a small decline in fecundity with age over the 28-day experimental period (Figure 5.2). However, when pairs of mated flies were exposed to dose rates of 200 µGy h^{-1} there was a dramatic impact on the age dependant profile of fecundity compared to the controls. After 28 days of radiation exposure, the 18-hour fecundity of flies exposed to 200 µGy h^{-1} was an average of 8 eggs produced compared to 27 eggs produced in the control treatment (Figure 5.2; Table 5.1; radiation exposure by day interaction, $\chi^2_{(6)} = 172.02$, $P = 2.2 \times 10^{-16}$).

Table 5.1. Parameter estimates for models investigating the effect of radiation dose rate on the number of eggs produced by *Drosophila melanogaster* breeding pairs over 30 days. Dose rates were 200, 40 µGy hr^{-1} and controls (0.11 µGy hr^{-1}). The environmental variables, temperature and humidity were mean centered and scaled by the standard deviation. For this model one standard deviation of temperature is 0.25°C and humidity is 4.75%. Model was linear mixed effects with normally distributed errors, it included a third order polynomial for the day variable both singly and in its interaction with dose rate. Model was selected using AIC model selection (Table S5.1c). Multiple measures were made on 180 breeding pairs during these observations. Table S5.1a describes the minimal model used. Table S5.1b contains terms removed from the model in reverse order of deletion during model simplification. In the minimal model the P value of single variables was calculated by removing any interaction term it was also found within.

a. Minimal Model				
Predictors	**Estimate**	**SE**	χ^2	**P Value**
(Intercept)	29.10	1.02	-	-
Dose rate (µGy hr^{-1})	-		80.33	2.2×10^{-16}
40 µGy hr^{-1}	-4.89	1.44	-	-
200 µGy hr^{-1}	-6.36	1.44	-	-
Days within the experiment	-	-	298.8	2.2×10^{-16}
(Poly 1)	16.97	19.26	-	-
(Poly 2)	-52.32	6.30	-	-
(Poly 3)	9.19	8.15	-	-
Average humidity during the days when the egg counting measurements were made (%)	-	-	20.82	5.03×10^{-6}
	-0.06	0.08	-	-
Dose rate (µGy hr^{-1}) by days in the experiment	-	-	172.02	2.2×10^{-16}
40 µGy hr^{-1} (Poly 1)	29.23	7.16	-	-
40 µGy hr^{-1} (Poly 2)	-9.87	8.97	-	-
40 µGy hr^{-1} (Poly 3)	8.20	10.67	-	-
200 µGy hr^{-1} (Poly 1)	-4.39	0.27	-	-
200 µGy hr^{-1} (Poly 2)	16.67	9.00	-	-
200 µGy hr^{-1} (Poly 3)	7.43	0.21	-	-
Dose rate (µGy hr^{-1}) by humidity during the days when the egg counting measurements were made (%)	-	-	6.01	0.01
	-	-	-	-
	-	-	-	-

200 µGy hr^{-1}	-0.21	0.12	-	-
40 µGy hr^{-1}	-0.28	0.12	-	-
b. Terms removed from model in reverse order of deletion				
Average temperature during the days when the egg counting measurements were made (°C)	- 0.26	- 0.20	1.74 -	0.19 -
Average temperature (°C) by humidity (%) during the days when the egg counting measurements were made	- -0.09	- 0.06	2.53 -	0.11 -
Dose rate (µGy hr^{-1}) by average temperature during the days when the egg counting measurements were made (°C)	- - -	- - -	0.32 - -	0.85 - -
40 µGy hr^{-1}	-0.32	0.57	-	-
200 µGy hr^{-1}	-0.25	0.60	-	-

This dramatic effect of radiation on fecundity was not only driven by the highest dose rate I studied; when pairs were exposed to 40 µGy h^{-1} the decline remained, with a 51.4% reduction in egg output between day 1 and day 28 of the experiment. I included the environmental variables of humidity and temperature within my analysis and found that humidity moderately modified the effect of radiation (Table 5.1; radiation exposure by humidity interaction, $\chi^2_{(2)} = 6.01$, $P = 0.01$). However, the effect size of humidity in comparison to the size of the effect of radiation was negligible.

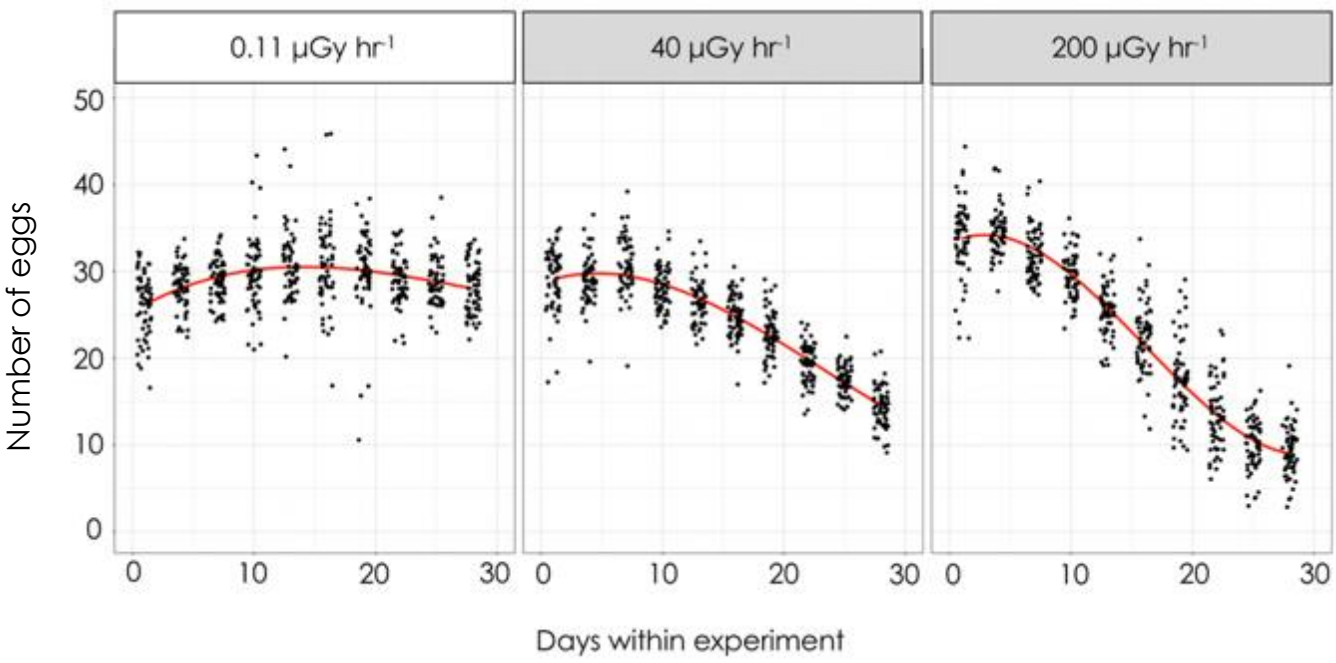

Figure 5.2. Exposure to increasing radiation dose rates decreased the number of eggs produced by mating pairs of *D. melanogaster* over 28 days. Data are presented for the number of eggs laid by pairs during an 18-hour window, flies were exposed to 200 µGy h^{-1}, 40 µGy h^{-1} or control (0.11 µGy h^{-1}). The trend lines were calculated

from a mixed effects model (Table S5.1). The figure was generated from an analysis of all data and includes a third order polynomial term for day and its interaction with dose rate. Plotted points represent the model residuals and were jittered; n= 180 fly pairs (60 per treatment), n= 1800 observations.

Whilst late life fecundity collapsed in flies exposed to radiation, there appeared to be an initial increase in early life fecundity during radiation exposure (Figure 5.2). I therefore took data from just the first two fecundity observations (days 1 and 3) and found a 24.2% increase in egg production at 200 μGy h^{-1} compared to controls (Table S5.2; dose rate, $\chi^2_{(2)} = 10.96$, $P = 0.004$). For pairs exposed to 40 μGy h^{-1} this significant initial increase remained; in this case with a 10.74% increase in eggs produced. Temperature and humidity of the radiation facility did have an effect on the number of eggs produced, however their effects were relatively small and independent of radiation dose rate (Table S5.2). In order to verify this initial increase in fecundity with radiation exposure, and to narrow down the time in which this effect begins to occur, I repeated my experiment with 60 new pairs of flies studying just the first 18 hours of radiation exposure. These flies were kept in the control area (0.11 μGy h^{-1}) and at 200 μGy h^{-1}. I again found a significant increase in fecundity, with an increase in egg production of flies exposed to 200 μGy h^{-1} compared to controls during the first 18-hours of radiation exposure (Table 5.2; dose rate, $\chi^2_{(2)} = 10.68$, $P = 4.15 \times 10^{-5}$).

Table 5.2. Parameter estimates for models investigating the effect of radiation dose rate on the number of eggs produced by a cohort of 60 *Drosophila melanogaster* breeding pairs entered in to radiation for 18 hours. Dose rates were 200 μGy hr^{-1} and controls (0.11 μGy hr^{-1}). Model was linear with normally distributed errors. A total of two counts were made for each of the 60 breeding pairs. Table S5.3a describes the minimal model used.

a. Minimal Model					
Predictors		**Estimate**	**SE**	χ^2	**P Value**
(Intercept)		27.77	1.29	-	-
Dose rate (μGy hr^{-1})		-	-	5.96	0.02
	200 μGy hr^{-1}	0.02	0.01	-	-

To investigate the effect of radiation on total fecundity during the course of the experiment, I assessed the total number of eggs recorded over the 28-day experimental period (the total counted during the ten 18-hour fecundity assessments). Despite the initial early increase in fecundity, over the whole 28 days, egg production was significantly reduced in the radiation treatments (Figure 5.3; dose rate, Table S5.4; $\chi^2_{(2)} = 5.96$, $P = 0.02$). In comparison to the

control treatment there was a 21.8% fecundity reduction for flies exposed to 200 µGy h^{-1} and a 16.9% decline for flies exposed to 40 µGy h^{-1}.

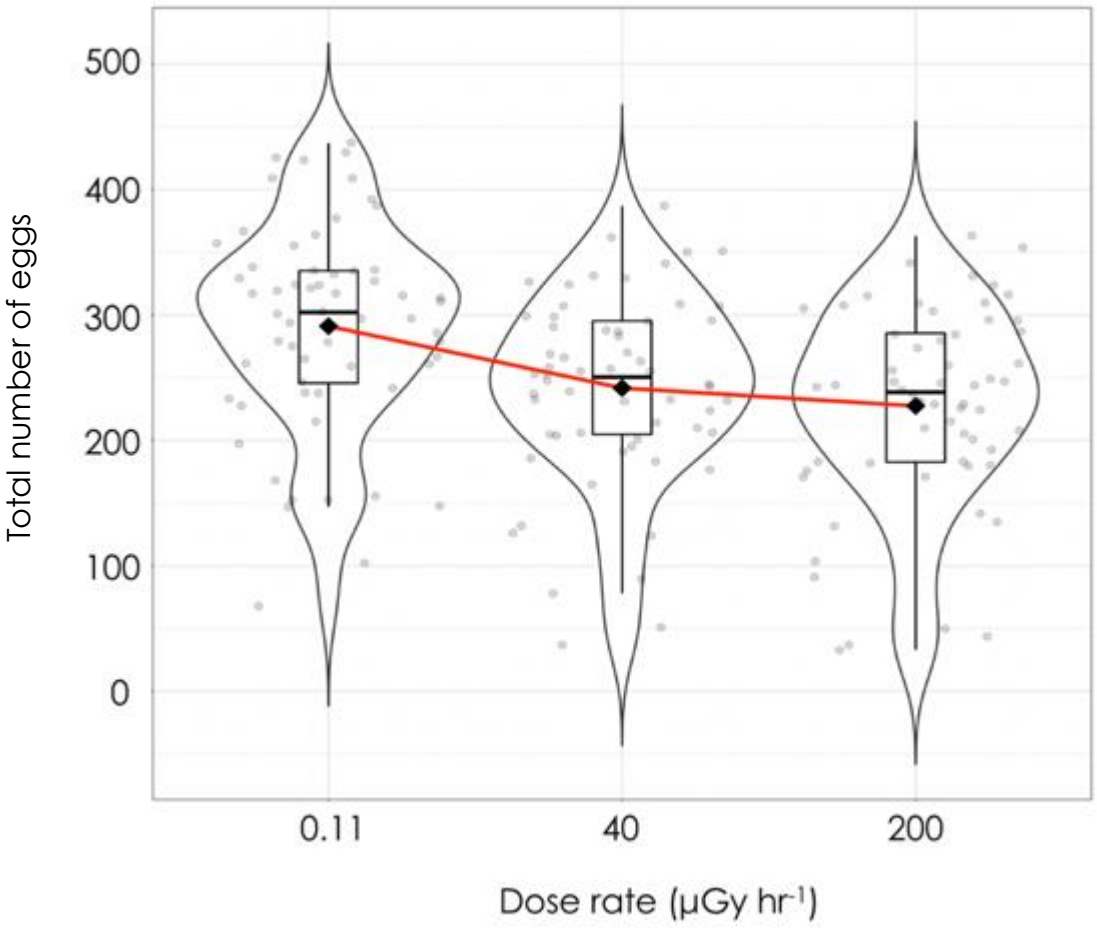

Figure 5.3. *Drosophila melanogaster* total fecundity decreased with exposure to increasing dose rates. Data are presented for the total number of eggs recorded from 180 breeding pairs in during 18-hour observation windows. These 18-hour windows were recorded on ten occasions over 28 days within the experiment. Points represent mean number of eggs produced by each pair. The model analysing these data is shown in Table S5.3. The fit is represented by the red line and black diamonds which highlight differences between dose rates for the total number of eggs produced. n = 180 breeding pairs (60 per treatment), means calculated from n= 1800 observations.

To understand the effects of radiation upon the eggs that were laid in this experiment, I assessed the proportion of eggs that successfully developed to adult flies. The manner in which fly age affected developmental success differed significantly between the three dose rate treatments (Table S5.5; radiation exposure by day interaction, $\chi^2_{(4)} = 41.79$, $P = 1.83 \times 10^{-8}$). In the control group, development success remains largely constant with fly age; whereas for flies exposed to 200 µGy h^{-1} there was a significantly non-linear decrease through time (Figure 5.4). The effect of radiation on development success of flies at 40 µGy h^{-1} also shows

a similar decline over time (Figure 5.4). The average temperature of the radiation facility had an effect on development success but effects were independent of dose rate (Table S5.5).

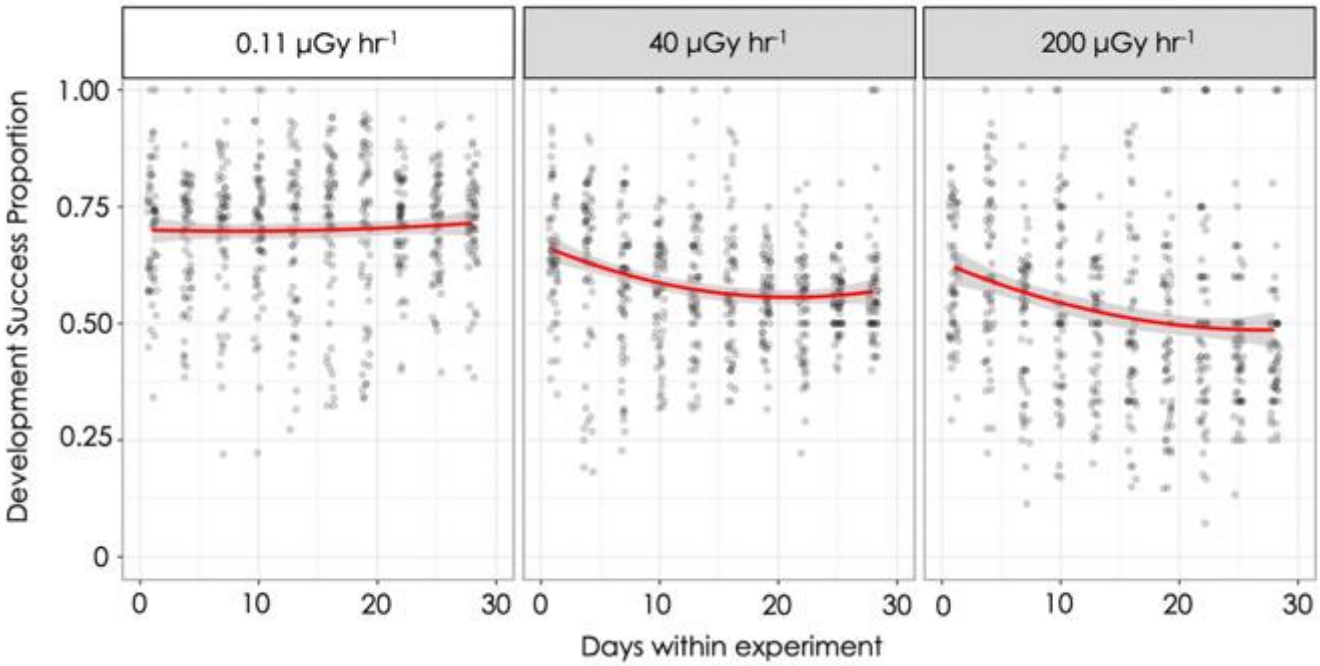

Figure 5.4. Exposure to increasing radiation dose rates led to a decrease in the development success of *D. melanogaster* eggs growing to adulthood over 28 days. Data are presented for the number of eggs laid in each 18 hour window that developed in to adults when exposed to 200 µGy h^{-1}, 40 µGy h^{-1} and control (0.11 µGy h^{-1}). The development success proportion ranges from 0 (no eggs successfully developed to adults) to 1 (all eggs successfully developed to adults). The trend lines were calculated from a mixed effects model (Table S5.5). The figure was generated from an analysis of all data and includes a third order polynomial term for day and its interaction with dose rate. Plotted points represent the model residuals and were jittered; n= 180 fly pairs, n= 1800 observations.

As well as assessing development success of flies from eggs that were laid under radiation exposure, I also analysed the sex ratio of those flies. The proportion of offspring that were male increased by 11% with irradiation at 200 µGy h^{-1} relative to controls (Table S5.6; dose rate, $\chi^2_{(2)} = 67.79$, $P = 1.85 \times 10^{-15}$). At 200 µGy h^{-1} the proportion of males produced increased steadily throughout the experiment, in comparison to the control treatment, for which the sex ratio remained constant as the flies aged (Figure 5.5; Table S5.6; dose rate by days in the experiment $\chi^2_{(4)} = 48.68$, $P = 6.81 \times 10^{-10}$). (Figure 5.5). At 40 µGy h^{-1}, whilst the proportion of male offspring increased initially, this rate of increase flattened off after 20 days. The impact of environmental variables was again significant but negligible in comparison to the size of the effect of radiation (Table S5.6).

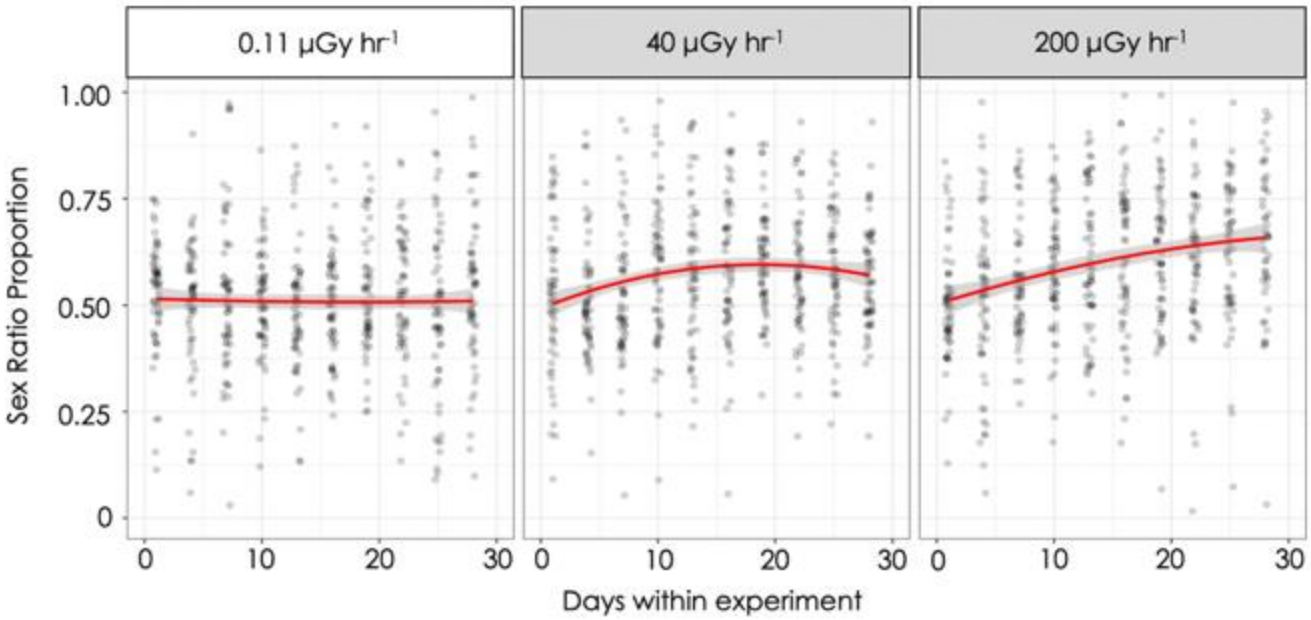

Figure 5.5. Exposure to increasing radiation dose rates led to an increase in the proportion of male *D. melanogaster* adults produced over 28 days. Data are presented for the offspring sex ratio (proportion males) when exposed to 200 µGy h^{-1}, 40 µGy h^{-1} and control (0.11 µGy h^{-1}). The sex ratio proportion ranges from 0 (all males produced) to 1 (all females produced). The trend lines were calculated from a mixed effects model (Table S5.6). The figure was generated from an analysis of all data and includes a second order polynomial term for day and its interaction with dose rate. Plotted points represent the model residuals and were jittered; n= 180 fly pairs, n= 1800 observations.

5.4.2 Experiment 2: Legacy effects of prior radiation exposure on adult reproductive success in *D. melanogaster*

I then undertook a new experiment to investigate the effect of radiation on the reproductive success of both males and females that experienced radiation at 200 µGy h^{-1} pre-reproduction. A total of 240 flies reared under control (n =120) and irradiated conditions (n = 120), were mated to virgin males/females from a constant density stock and kept in a control area for subsequent fecundity measurements. I then monitored the number of eggs produced by these pairs at four time points over 10 days.

My estimate of total fecundity of females originating from the 200 µGy h^{-1} treatment was 10.45% lower than that of females reared in control conditions, and was similarly lower than the male treatments (Figure 5.6; Table 5.3; $\chi^2_{(3)} = 29.07$, $P = 2.23 \times 10^{-6}$).

Table 5.3. Parameter estimates for models investigating the effect of radiation dose rate on the total fecundity of male and female flies that came from eggs that were laid and developed under irradiation (200 µGy h^{-1}) and control conditions (0.11 µGy hr^{-1}). Model was linear with normally distributed errors. This model investigated the total of all egg counting measures from 240 breeding pairs over 10 days of observations. Four treatments are described which include one parent taken from the 200 µGy h^{-1} and control treatments which were mated to a virgin male/female from a constant density stock. Model is in comparison to females that came from eggs that developed in control conditions. Table S5.7a describes the minimal model used.

a. Minimal Model					
Predictors		**Estimate**	**SE**	χ^2	**P Value**
(Intercept)		183.44	2.91	-	-
Treatment		-	-	29.07	$2.23x10^{-6}$
	Female 200 µGy h^{-1}	-19.18	4.11	-	-
	Male 200 µGy h^{-1}	-0.98	4.06	-	-
	Male Control	1.13	1.16	-	-

The total number of eggs recorded from pairs where the male was previously exposed to 200 µGy h^{-1} was no different to that in which males developed under control conditions (Figure 5.6).

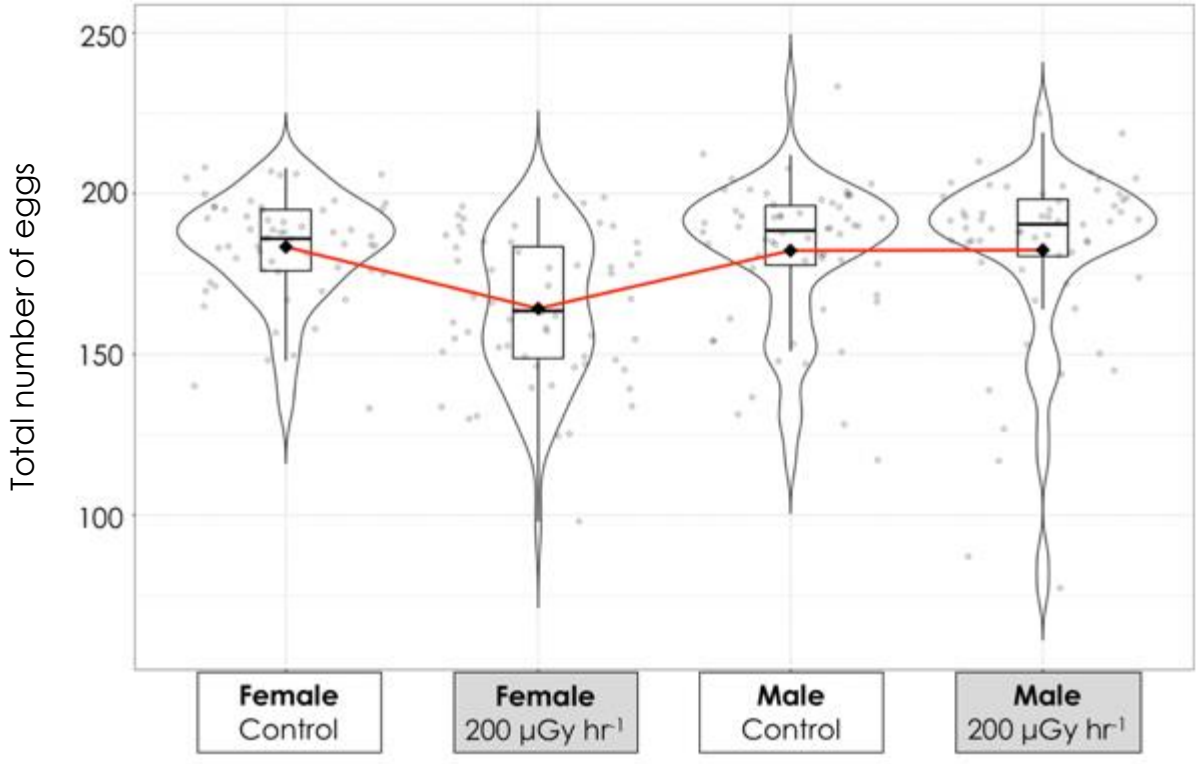

Figure 5.6 The total number of eggs produced by female flies that were exposed to radiation during their development was reduced relative to controls when mated under un-irradiated conditions. Data are presented for the total number of eggs produced by 240 breeding pairs in four 18-hour windows over 10 days in the control area of the radiation facility. Points represent the mean number of eggs produced by flies each treatment group. The graph shows four treatment groups, which include one parent either taken from the 200 µGy h^{-1} treatment,

or from the control treatment, and which were mated to a virgin male/female from a constant density stock. The model analysing these data is shown in Table S5.7. The fit is represented by the red line and black diamonds which highlight differences between treatment groups for the total number of eggs produced. n = 240 breeding pairs.

To further investigate these legacy effects of pre-reproduction radiation exposure, I assessed how the fecundity of these flies changed though time and analysed data from male and female flies separately. First, I compared the number of eggs produced by females exposed to 200 µGy h^{-1} pre-reproduction to females that were reared under control conditions. I conducted 18-hour fecundity estimates over 10-days, with four egg counts being taken during this experimental period. For females that experienced pre-reproduction radiation exposure the slope of the relationship between time and fecundity is significantly negative, whereas the females that were in the control treatment during their early adulthood development showed a positive trend (Table S5.9; $\chi^2_{(1)} = 43.13$, $P = 5.1x10^{-11}$; Figure 5.7). Second, in the case of males, whilst the direction of the trends were similar as for females, there was no significant effect of prior radiation exposure on fecundity: the mean fecundity was only 1% higher in the radiation treatment (Figure 5.7; Table S5.10; $\chi^2_{(1)} = 0.11$, $P = 0.15$).

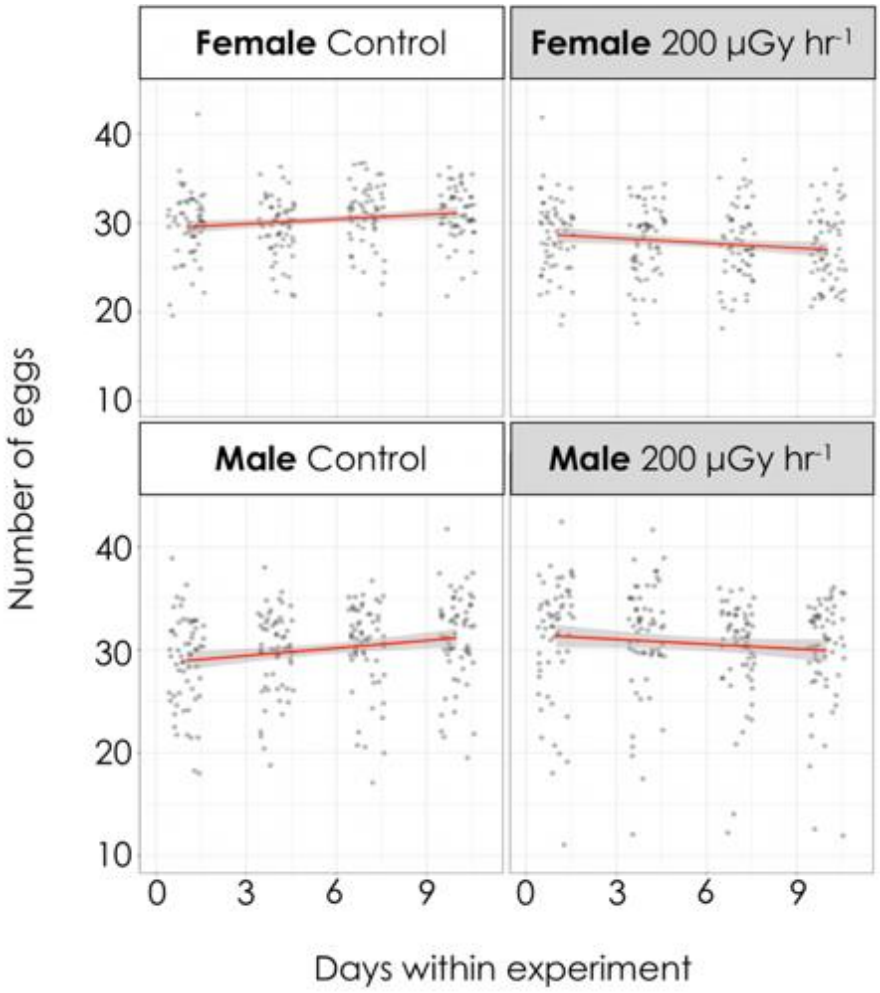

Figure 5.7. The number of eggs produced by *Drosophila melanogaster* females that were previously irradiated during their development decreased significantly over time in comparison to flies that developed under control conditions. The number of eggs produced by previously irradiated males also decreased over time but this was not significant. Data are presented for 18-hour fecundity estimates as measured at 4 time points 10 days after mating. The top left and right panels show the number of eggs produced by females that came from eggs that were laid and developed under 200 µGy h^{-1} and control conditions (0.11 µGy hr^{-1}), n =120 breeding pairs. The model analysing these data is shown in Table S5.9. The bottom left and right panel show the number of eggs produced by males that came from eggs that were laid and developed under 200 µGy h^{-1} and control conditions (0.11 µGy hr^{-1}), n = 120 breeding pairs. The model analysing these data is shown in Table S5.10. Plotted points represent raw data values and were jittered. The trend lines and shaded 95% confidence intervals were calculated from a mixed effects model with the same terms as shown in Table S5.9 for females and Table S5.10 for males.

5.5. Discussion

I studied the impact of Chernobyl-level radiation exposure on fecundity of *Drosophila melanogaster*. When breeding pairs were exposed to radiation at both 200 and 40 µGy h^{-1}, reproductive activity collapsed as flies aged up to 28 days, in comparison to fly pairs kept under control conditions. I also observed a significant decrease in the ability of those eggs to develop to adulthood, again at both the dose rates studied. I additionally present evidence

that ionising radiation significantly affects future reproduction of flies that develop under radiation exposure, with females being most effected. These findings support previous studies that found lower doses of radiation also impact reproduction in bumblebees (Raines et al, 2020).

One striking impact of radiation on *D. melanogaster* was an immediate significant increase in fecundity, which was initially seen to occur during the first three days of the experiment, when fecundity was measured over 28 days. My repeat study narrowed down the timescale for this effect, with fecundity upregulation found to occur within just 18 hours of exposure. The increase in egg production over the first 3 days of the experiment was a 16.6% increase compared to controls, indicating that fly physiology rapidly responds to radiation exposure. The mechanism driving this dramatic short-term effect is unclear, as spermatogenesis and oogenesis occur within 5 days in flies. However I hypothesise that radiation is triggering a stress response that causes flies to lay eggs quickly. Another explanation for these findings could be terminal investment, this hypothesis suggests that in response to impending mortality individuals will increase investment in current reproduction (Clutton-Brock,1984 ; Minchella & Loverde, 1981). In *D. melanogaster*, exposure to stressors such as cold shock triggers an increase in egg laying (Gulyas & Powell, 2022). Radiation-induced increased investment at a detriment to lifespan however seems unlikely as no flies died during exposure. The immediate increase in fecundity could be linked to 'egg dumping behaviour', which has been recorded when eggs are laid by older parents (Mossman et al, 2019). It takes one week during oogenesis for eggs to transit along the ovariole and mature (King, 1968 ; Cuevas, 2015). This initial upregulation in egg laying behaviour occurred after just 18 hours, therefore the eggs laid during this period were created prior to radiation exposure. The total accumulated dose over those 18 hours of irradiation would be 3.6 mGy; whilst this is a marked dose of radiation, it is equivalent to spending 14.4 hours at the highest dose rate (250 µGy h^{-1}) in the Chernobyl Exclusion Zone. This therefore highlights that lower doses that can be found in contaminated environments can generate marked physiological changes. The increase in egg production lasts for 3 days of irradiation, suggesting this isn't just an immediate dumping of eggs but they are exhibiting an ongoing physiological response to radiation. I studied fecundity of mating fly pairs, therefore this stimulation of egg production could either be driven by an effect of radiation on male physiology (e.g. seminal fluid, or

courtship behaviour) or alternatively an effect on female reproduction (e.g. oogenesis). Proteins within male seminal fluid can trigger an upregulation in egg production (Herndon and Wolfner, 1995; Heifetz et al, 2000): within the first 24 hours after mating the male seminal fluid molecule 'sex peptide' stimulates oocyte progression (Soller et al, 1997). Whilst it could be argued that this increase in egg production is a male effect due to the similar short time frame, my study did not allow us to determine whether this upregulation in egg production was due to impacts of radiation on the male, on the female, or on both.

After the first three days of irradiation, the number of eggs produced by *D. melanogaster* and their development success dropped dramatically. At 200 μGy hr^{-1} fly fecundity dropped by 73.52% between day 4 and 28. Reproductive activity is generally considered costly in flies due to it placing demands on energy resources in often dynamic environmental conditions. When stressed, an organism must make a life history decision on whether to invest resources into reproduction or survival (Schwenke et al, 2016). When exposed to stressors such as heat stress, it has been found that oogenesis is impaired in *D. melanogaster* with fewer eggs produced in later stages of synthesis, as well as a greater number of cells undergoing programmed cell death (Gruntenko et al, 2003). Therefore, when flies are irradiated it could be inferred that this stress is driving a re-allocation of resources away from reproduction and into other important functions such as acquiring food. This effect has been suggested based on data from bumblebees, which under low doses of radiation consume more nectar (Burrows et al, 2022). In nature, this decrease in fecundity could have large impacts on the fitness of populations as it could cause a decrease in population sizes. However, the magnitude of this fitness loss would depend on how long flies normally live within a natural environment, whilst radiation-induced fecundity loss in this experiment began early in the experiment, the majority of the loss only occurred at late ages (after ~15 days). In populations where fly lifespan is naturally shorter, the fecundity loss caused by radiation exposure might be smaller. For example, in the CEZ flies may be predated and therefore only live for a few days when effects of radiation on fecundity are minimal in comparison to flies that live for their usual lifespan of 60 days.

Often within nature, stressors experienced by parents and during individual development can lead to effects within adulthood. I therefore also investigated the legacy effects of radiation

on adults that came from eggs which were laid and developed under radiation exposure. These adults developed from eggs that were laid by parents who had themselves experienced 10 days of radiation at 200 μGy hr^{-1}. I tested whether these adults suffered fitness consequences when they were no longer being irradiated, by measuring their egg output when they were mated with a control fly that developed under control conditions. Surprisingly, females that were irradiated at 200 μGy hr^{-1} as larvae produced significantly fewer eggs in adulthood than control females. In contrast, males did not suffer a significant cost of pre-adult radiation exposure. It is possible that this sex-specific effect could be driven by changes in behaviour and I suggest this should be explored in future work. The effect of radiation has been found to carry through generations, with descendant populations of *D. melanogaster* in the Chernobyl Exclusion Zone developing radio-adaptive like responses through changes in sex-linked recessive lethal frequency (Hancock et al. 2019). These lethals are mutagenic effects that are proven to increase with radiation exposure and so are used as an indicator of damage. The cumulative impacts of radiation found in this study could have detrimental impacts on flies found within the Chernobyl Exclusion Zone through the reduction of population sizes. I do also acknowledge that environmental effects could also influence this effect in wild populations. I conducted my experiment in a controlled environmental facility in order to study the impacts of radiation with minimal confounding variables but I did still measure temperature and humidity. There was a small but significant effect of environmental covariates on the number of eggs produced under irradiation.

I suggest that there are two broad mechanisms in which radiation may be driving these decreases in reproduction and development success: directly through an aspect of damage from irradiation or via accelerated ageing. For *D. melanogaster*, fertility peaks within the first week of adulthood and then declines with age until a female is no longer fertile (Sgro et al, 2000). This study did examine effects on hallmarks of aging, however it only did so for the early to mid-stages of the fly lifecycle. Therefore, whilst I did not assess fecundity in the very late stages of life, the reduction in fecundity over time that I recorded is consistent with an acceleration of senescent processes in irradiated flies. Accelerated senescence can occur when a fly is exposed to a new stressor, for example blue spectrum LED exposure accelerates senescence in flies (Nash et al, 2019). It could be argued however this experiment is not an example of senescence, as the flies that were kept under control conditions showed no

substantial age-related reduction in egg production over this time. This suggests the effect seen in this study is more likely to be a direct effect of damage through irradiation.

I provide experimental evidence that sex ratios can shift in response to radiation at different doses, as the sex ratio of offspring produced by adults under irradiation became male-biased during the experiment. Evolutionary models of sex allocation state that when an organism is exposed to stress it should produce more female offspring, as females have more guaranteed reproductive returns than males (Trivers & Willard, 1973). Therefore, this shift is not in the right direction to be consistent with the Trivers-Willard hypothesis. I hypothesise this effect is a symptom of radiation exposure rather than an adaptive response. It is possible that this effect is due to alterations in sex determination mechanisms, changes in post fertilisation mortality (during egg, larval, or pupal stages) or due to differential susceptibility of X and Y sperm. I do provide evidence that egg-to-adult viability is reduced under radiation exposure; therefore, it is possible a biased sex ratio could be produced if impaired viability affects females more strongly than males. Whilst sex determination mechanisms are very different between organisms, this study provides experimental proof that sex ratios can shift in response to radiation exposure; therefore, it is not inconceivable that the same response could be seen in humans. The effect of radiation on offspring sex ratio has indeed been studied widely in humans with an unclear consensus of effects. Whilst many report no effects of radiation on sex ratio (Winther et al, 2003 ; Choi et al, 2007), others suggest effects could occur. For example, for men employed at the Sellafield nuclear installation, there was a significant increase in the number of males produced by fathers exposed to doses exceeding 10,000 µSv in the 90 days before conception (Dickinson et al, 1996). I therefore suggest that sex ratio change should be investigated in more organisms at these dose rates.

Within the Chernobyl Exclusion Zone ambient external dose rates range from typical background level up to 250 µGy hr^{-1} (Beresford, Scott and Copplestone, 2020). Several studies from Chernobyl report negative impacts of radiation on organisms (Møller *et al.*, 2007; Møller and Mousseau, 2009; Møller, Barnier and Mousseau, 2012; Hermosell *et al.*, 2013; Kesäniemi *et al.*, 2019). Studies undertaken in a laboratory setting with dose rates similar to Chernobyl also record negative effects on life history traits (Raines et al, 2020; Burrows et al, 2022). Whilst I observed a significant increase in fly egg production within the first 4 days of radiation

exposure, there was a significant collapse of egg production over more extended time periods under radiation exposure. The effect of this collapse, combined with reduced development success, shows that radiation clearly has costly impacts on reproductive fitness. My study therefore identifies a direct effect of radiation that could be costly for flies living in the Chernobyl Exclusion Zone. My findings also have policy implications for the International Commission on Radiological Protection, which have an environmental protection framework that is used to protect both humans and wildlife. For wildlife, a eusocial bee is used as a reference animal (RAP) to determine the dose rate band within which deleterious effects are predicted to begin to occur. By choosing eusocial bees to represent all insects in dose rate assessments. this system could be criticised as bees have an atypical eusocial ecology. However, I argue that current data give no indication that bees are a special case, having recorded very similar effects in *D. melanogaster*.

I find evidence of significant impacts of ecologically relevant radiation exposure on fecundity in *D. melanogaster*. This study's results could be extrapolated to argue that the previously recorded effects on reproductive life history of bumblebees are direct effects of radiation on reproduction (Raines et al, 2020), rather than a more complex mechanism associated with eusocial brood care. My observation of an impact of radiation on the successful development of eggs into adults also suggests the previous observations of reproductive impairment in bumblebees could be due to egg viability effects. It has been suggested that bumblebees could be unique in their response to radiation due to their somewhat unique life history (Burrows et al, 2022). However, the findings presented here categorically demonstrate that in a completely unrelated invertebrate species comparable effects on life history occur. This study therefore provides unifying evidence that effects of these radiation dose rates on life history traits are not unique to bumblebees.

Chapter 6: General Discussion

6.1 Summary

The work from this PhD thesis investigated whether low doses of radiation, at a level currently found in some radiologically contaminated environments, could impact key life history traits within bumblebees. It also aimed to identify the mechanisms that could be driving any effects. Identifying either the mechanisms underlying radiation 'stress' or conserved phenotypes that consistently respond to radiation exposure could provide a generalisable metric that might be used in the field for understanding how radiation impacts diverse invertebrate species in areas such as the Chernobyl Exclusion Zone (CEZ). This thesis found an unprecedented upregulation in feeding, metabolic rate and movement within bumblebees in response to low dose rates (in the 40-200 µGy hr^{-1} range). This is some of the first evidence to show that radiation exposure affects fundamental metabolic processes and the energy budget of bumblebees. Additionally, I found that when radiation exposure stops, bumblebee metabolic rate returned to normal levels, but that feeding remained elevated post-exposure. This finding could have wider impacts for bumblebees living in contaminated environments and moving through areas of varying dose rates.

Subsequent chapters studied how this radiation-induced increase in nutrient acquisition impacts bumblebee nutritional systems. Elevated consumption in response to radiation led to significant increase in sugars within bumblebee haemolymph. I conducted an array of other biochemical measurements on bumblebee tissue, finding that bumblebees did not store the excess nectar consumed as a result of low dose exposure. This suggests that the extra sugar that was consumed was being actively used in bumblebee metabolism. I also found small changes in the bumblebee microbiome as a result of increasing radiation exposure. Species richness of bacteria residing within the bumblebee gut increased, whilst this demonstrated that radiation exposure does affect the microbiome, my study could not identify whether radiation exposure had either direct or indirect impacts on the bumblebee microbiome.

In order to understand if the effects I recorded were unique to bumblebees (perhaps as a result of their eusocial biology), I then investigated the effects of radiation on *Drosophila*

melanogaster. This work followed on from previous studies conducted on bumblebees at low dose rates, which found impacts on reproduction (Raines et al, 2020). I found dramatic decreases in fecundity and development success in flies as a result of exposure, as well as a change in sex ratio. This work clearly highlighted that low doses of radiation have significant impacts on invertebrates other than bumblebees. This discussion chapter will now explore some of the questions posed by this thesis and explore the wider implications of my findings.

6.2 What potential mechanisms could be driving physiological changes in response to radiation?

This thesis identified dramatic effects of low dose radiation on a variety of physiological measurements recorded in bumblebees. Whilst the chapters in this thesis were unable to identify a specific mechanism driving these effects, I hypothesise that low dose radiation exposure is triggering metabolically costly recovery mechanisms, which result in the increase in metabolic rate and nutrient acquisition (Chapter 2). For this hypothesised recovery mechanism to occur, biomolecules would need to be damaged as a result of radiation exposure. I cannot speculate on whether membranes, proteins or DNA would sustain any damage at these dose rates. Previous work suggests that low dose rates in the range studied in this thesis are not high enough to trigger molecular damage (Smith et al, 2012), however further work is required to better understand if damage could be driving effects recorded in this thesis. However, whilst this thesis did not identify the exact mechanism, it did determine extra energy was being consumed and being used by bumblebees in response to radiation. A similar effect to this has been recorded in bacteria exposed to a stressor, these bacteria adapted to confer antibiotic resistance and enable survival through metabolically costly mechanisms (Handel et al, 2016). I observed an increase in bumblebee movement as a result of radiation exposure (Chapter 2), which could be as a result of increased appetite causing individuals to search for food. It could also have been the result of a direct effect of radiation on behaviour. Effects on behaviour have been recorded on birds living in the Chernobyl Exclusion Zone, where radiation has been suggested to modify the selection of nesting sites (Moller & Mousseau, 2007 ; Gagnaire et al, 2011). Therefore, it is possible that behavioural mechanisms mediate some of the effects I observed.

I speculate that radiation could be triggering the immune response as a result of radiation stress, which might then lead to changes in energy usage (Chapter 3). In bumblebees, the triggering of the immune system in response to non-pathogenic stimulation has been found to be metabolically costly, driving a significant increase in nectar consumption (Tyler et al, 2006). It is possible that radiation exposure could alter the interactions between insects and their symbiotic microbes, which could trigger costly immune system activation. By investigating effects of radiation on bumblebee microbiome (Chapter 4), I was able to speculate further that radiation may be triggering a similar costly response. This thesis identified a variety of metrics that change in response to radiation and are relevant to fitness e.g. metabolic rate and food consumption and reproductive output. These metrics could be used as universal signatures of radiation exposure within the field. However, my measures are less easily measured in contaminated environments than biomarker-based metrics, such as comet assays or micronucleus assays (Beresford et al, 2020). Despite this, future assays could be designed to measure my metrics within the CEZ. For example, appetite could be measured in field collected invertebrates in the CEZ to compare different sites. Animals could also be collected to take measurements on their metabolic rate, or potentially for insects that reproduce frequently instantaneous measures could be taken on fecundity and hatch rate. However, this work would need to be supported with laboratory studies in order to account for potential environmental confounding factors. This is especially relevant for measures taken in Chapter 5 on *D. melanogaster,* for which 18-hour egg collections could be recreated from females caught within the CEZ. Additionally, haemolymph could be collected from a range of organisms across contaminated environments to look for elevated haemolymph sugar. This thesis has provided an excellent foundation for future studies within the CEZ to further investigate whether these measures could become core biomarkers of radiation. From all the measures on biochemistry, reproduction and feeding, I suggest that haemolymph sugar could be the most effective universal biomarker of radiation stress due to its high correlation with exposure. This increase in sugar shows that individuals are fuelling a response and not directly storing or excreting additional nutrition. This sugar, might then be used to fuel energy required for foraging (Pattrick et al, 2020). However, despite studying the flow of this extra food through the organism's digestive tract (Chapter 3 and Chapter 4), further work is still required in order to determine how what the metabolic processes are that this additional sugar is supporting.

This thesis did not set out to investigate the molecular effects of radiation exposure at lower dose rates. However, it is important to consider them as ionising radiation can have some novel effects on cells. For example, ionising radiation can cause by-stander effects are effects that arise after irradiation by cells whose nuclei have not been directly affected and this includes DNA damage as well as epigenetic effects in which gene expression is altered without altering the DNA (Zhou, 2005). These effects have been shown to occur in human tissue cultures (Zhou et al, 2001). Radiation has also been shown to induce variation within germline cells, especially in mice (Bridges, 2001). Other areas which should be explored to understand the effects of radiation and potential mechanisms driving them also include induced genomic instability, which was highlighted by the CERRIE committee as an area that requires more investigation with regards to external and internal doses (CERRIE, 2004). This effect has been recorded in mice taken from Fukushima with changed recorded in hematopetic cells (Aryoshi, 2022). When examining potential mechanisms driving effects at lower doses it is important that these mechanisms are investigated in more detail.

6.3 Dose rates: At what dose rates do I consider the effects of radiation to start occurring in invertebrates?

One of the aims set out throughout the chapters of this thesis was to identify the dose rates at which individuals start to suffer appreciable fitness loss and physiological change. This is due to the International Commission on Radiological Protection (ICRP) setting Derived Consideration Reference Levels (DCRL) to predict when adverse effects of radiation are likely to occur for a range of organisms within the environment. There is a DCRL set for the Reference bee, which states that bees are unaffected by radiation below dose rates of 417 µGy hr^{-1} (Zinger et al, 2008). However, there is now a growing body of evidence that suggests that this DCRL should be lowered (Raines et al, 2020). Whilst these chapters were not designed to test the exact dose rates at which fitness loss begins to occur, Chapter 2 investigated the effect of a gradient of radiation exposures beginning from dose rates as low as 14 µGy hr^{-1}, up to 200 µGy hr^{-1}. I found that the effect of radiation on nectar consumption per unit of exposure remained constant down to dose rates of 50 µGy hr^{-1}. In the introductory chapter of this thesis I described the linear no threshold model, which assumes there is a linear relationship between the total dose an organism receives and the risk of impacts on lifespan (Tubiana et al, 2009). The experiment conducted in Chapter 2, whilst exploring a gradient of exposures, could not test the LNT hypothesis which suggests that effects should persist to almost zero dose as it was relatively low powered at lower dose rates; therefore, this thesis did not explicitly test the linearity or threshold of radiation impacts on feeding. However, my other studies do identify that effects of radiation can occur rapidly following exposure. For example, I identify an effect of 200 µGy hr^{-1} on egg production in *D. melanogaster* after just 18 hours of radiation exposure (Chapter 5). In bumblebee experiments for which I had relatively larger sample sizes, I identified effects on the microbiome and levels of sugars within the haemolymph at dose rates as low as 40 µGy hr^{-1} (Chapter 3 and Chapter 4). Therefore, I do identify that there are marked responses to radiation at much lower doses than currently deemed safe.

Many studies of radiation focus on risks of cancer in relation to total dose an individual received as most exposures, especially in humans, are short term and high dose e.g. medical testing. Therefore, a key focus of studies using higher dose rates is stochastic damage related

to cancer risk. As a result, there is a stronger expectation that the effects observed in this thesis will be proportional to the total dose received rather than the dose rate. I acknowledge for my studies that I often refer to dose rates and not the total accumulated dose an individual received. I consider my studies to use low dose rates; in contrast much previous work conducted on insects has used very high dose rate acute exposures (Dyck et al, 2005 ; Bakri et al, 2005) in the context of triggering reproductive sterility for crop protection technologies (Bakri et al, 2005). However, for my fly study I saw an increase in egg production within just 18 hours of exposure to 200 μGy hr^{-1}, which is a very rapid effect considering the relatively low dose rates. Nevertheless, these flies still did receive a total accumulated dose of 4000 μGy during this 18-hour window. Additionally, in Chapter 2 I tested for how fast the effect of radiation on bumblebee feeding occurred, whilst this effect was not quite significant after 24 hours, it was strongly significant after 5 days of exposure when bumblebees had received a total accumulated dose of 24,000 μGy. For the dramatic effects seen on the bumblebee energy budget, the total accumulated dose was closer to 48,000 μGy by the end of a 10-day irradiation period for the bumblebees exposed to the highest dose rate. Therefore, these studies cannot effectively discern to what extent that effects of radiation are principally driven by total accumulated dose or dose rate. I suggest the extent to which this is important is largely dependent on organism lifespan. For a bacterium that divides every 20 minutes (Allen & Waclaw, 2019), both dose rate and accumulated dose may be of similar magnitudes. However, for longer lived organisms, such as for bumblebees which the queens live for around one year (Goulson, 2010), there is the opportunity for organisms to accumulate a very substantial total dose. I suggest that longer lived organisms found in heterogeneously contaminated environments, such as bumblebees, may live for longer periods of time whilst being exposed to radiation continuously. Therefore, the total dose of radiation they will receive will be much higher than the 30-days of exposure bumblebees commonly experienced within this thesis. Alternatively, animals may move in and out of contaminated patches whilst foraging or dispersing, during which they may actually only experience high dose rates briefly and for a fraction of their lifespan. The length of exposure an organism receives in the CEZ will as a result depend on the study species' specific ecology and mobility. It should however be noted than in particular for insects, larval stages are generally quite long and larvae tend to be immobile. Therefore, there is potential for larvae to accumulate quite high doses of radiation prior to adulthood if the organism is residing in a high dose area.

Whilst the total accumulated doses in my studies are high, I show that effects of radiation on bumblebees occur below dose rates considered safe by the international commission on radiological protection (ICRP, 2008). The current DCRL predicts no effects for all bee species between 400 – 4000 μGy hr^{-1}. Previous studies on bumblebees have suggested the DCRL should be lowered to between 40 – 400 μGy hr^{-1} due to a 6% reduction in reproductive output of bumblebees found at 100 μGy hr^{-1} (Raines, 2020). I therefore produce more evidence for the re-designation of insect DCRLs by work in Chapter 2, which identifies effects on the bumblebee energy budget at dose rates as low as 50 μGy hr^{-1} and effects at a cellular level in the bumblebee microbiome from 100 μGy hr^{-1} in Chapter 4. These dramatic effects for endpoints that are not as binary as mortality, add to this growing body of evidence to reduce the DCRL, which was mostly set by extrapolating from studies of high dose rate acute exposures (Copplestone et al, 2015 ; ICRP, 2008). I suggest that future work to investigate effects of radiation on organisms should focus on chronic exposure rather than acute exposure, as plenty of work has been conducted to determine the acute lethal dose for non-human biota (Gad, 2014). There needs to be further work on the sublethal effects of chronic low dose exposure which can be extrapolated to organisms living in contaminated landscapes, rather than just testing doses at which sterility occurs (Larsson, 2012; Mothersill et al, 2018).

6.4 How could bumblebees living in the Chernobyl Exclusion Zone be affected?

One of the main aims of this thesis was to investigate the effects of dose rates that are currently found in the CEZ in order to understand if radiation could be damaging bumblebees living there. Previous controversial studies conducted in the CEZ reported finding effects on bumblebee population abundance at very low dose rates (0.01 – 0.1μGy hr^{-1}), particularly at close to background level (Moller et al, 2012 ; Moller & Mousseau, 2009). This work has been widely criticised, with the suggestion that the dosimetry used was inaccurate. This is based on known radiation levels in the areas that were surveyed and due to effects recorded at dose rates lower than those of UK background (Beresford et al, 2008). Furthermore, this previous work did not account for habitat quality as a factor that could cause variation in bumblebee population sizes. Nevertheless, these studies from over 10 years ago were the first to suggest

that bumblebees might be sensitive to Chernobyl-level dose rates; observations that were built upon when effects on bumblebee reproduction were identified in the laboratory at low dose rates (Raines, 2020).

Current dose rates in the CEZ are heterogenous, ranging from <0.1 – 250 μGy hr^{-1} across 2600 km^2 (Beresford et al, 2020). Dose rates across large parts of the exclusion zone are commonly low (<0.1 – 5) with the highest doses of 250 μGy hr^{-1} found in isolated areas, such as the area known as the Red Forest (Beresford et al, 2020). The results of this thesis are strongly relevant to the higher dose rates found within the CEZ. However, the studies in this thesis do not claim that all organisms in the whole of the CEZ will be similarly affected due to there being many areas with these lower dose rates. Yet even for organisms such as bumblebees living in relatively uncontaminated areas of the CEZ, there is still a strong possibility they may visit areas with much higher dose rates even if just briefly, for example during foraging flights. Bumblebees can forage across large distances, with workers foraging on average 1.5km from their colonies (Osbourne, 2008). Therefore, even bumblebees with nests in places of low exposure, may still encounter the higher dose rates that I investigated, even if just briefly whilst foraging. I found significant upregulation in fecundity of *D. melanogaster* (Chapter 5) after just 18 hours of radiation exposure. This observation suggests that large physiological effects as a result of low dose rates are possible in relatively short timescales, even if the evidence I have is for timescales longer than a foraging trip by other species such as bumblebees.

The dose rates at which I found the majority of my substantial effects of radiation on feeding, metabolic rate and haemolymph sugar ranged from 100 – 200 μGy hr^{-1}, which could be experienced by bumblebees nesting and foraging in areas of the CEZ with high dose rates. Bumblebees often build their nest in the subsurface of soil (Pugesek & Crone, 2022); the distribution of radionuclides in the soil can mean that these locations receive dose rates considerably higher than is experienced in the air above the land surface. This could mean that bumblebee queens are particularly vulnerable to the effects of radiation recorded within this thesis, as they spend the majority of their life-cycle underground. Bumblebee queens overwinter within holes in the ground and only emerge from February to June (Lye et al, 2012). When the queen emerges, she must replenish all of the fat reserves that were lost

during her hibernation period before searching for a new suitable nest area (Goulson, 2010). If a queen hibernates at a site where the soil delivers higher dose rates of radiation, she may experience the increased energetic demands I observed in this thesis throughout the whole over-winter period; this could have significant impacts on hibernation mortality rates, as well as subsequent reproductive fitness.

6.5 What impact does low dose radiation have on bumblebee fitness?

This thesis identifies that low dose radiation exposure influences fundamental metabolic processes in individual bumblebees, which has the potential to affect key life history traits. I additionally took a suite of measurements on biochemistry and the microbiome and found impacts on these essential physiological systems. However, it is important to consider the extent that this matters for bumblebee fitness and debate what sensitive life stages could be impacted. Whilst this research was conducted on worker bumblebees, queen bumblebees could be the most affected as queens often live for nearly a year in contrast to workers that live for approximately a month. Therefore, the total accumulated dose experienced by queen bumblebees will be much higher. For the work in this thesis it should be noted that reproductive success in bumblebees is largely dependent on queen function and my work did not study effects of radiation on queens. Furthermore, reproductive success is also dependent on communal nest behaviour which I also did not study. It is however important to consider effects on workers as they are responsible for brood care and foraging (Free, 1955). It is within the first few weeks of colony establishment that nest weight can increase with the volume of nectar provided to the developing brood determining the size of the eventual colony (Rotheray et al, 2017). Therefore, in radiologically contaminated areas queen bumblebees may need to invest more resource in themselves, leaving less available to the developing brood and leading to decreased colony sizes. This could also affect larvae developing within the colony as they may also have increased resource requirements, therefore they may release more pheromones to stimulate foraging activity which leads to more foraging flights (Le Conte et al, 2001; Costa et al, 2021). A reduction in larval resources as a result of individuals having to invest more in their own fitness can influence the determination of castes (Chole et al, 2019). Equally, increased foraging flights could lead to increased risk of individuals being exposed to parasites that reside on flowers (Shykoff and

Schmid-Hempel, 1991). It will also increase the chances of bumblebees being predated if they must leave the nest more often. This could additionally affect Darwinian fitness which includes fitness components such as life-span.

I found a large increase in nectar consumption at 200 µGy hr^{-1}; even at lower radiation dose rates (50 µGy hr^{-1}) individual bumblebees were affected (Chapter 2). Therefore, even at the lower doses I studied, bumblebees will experience fitness consequences. However, will of course be a lower dose rate 'threshold' at which the effects of radiation are trivial in comparison to other ecological stressors that bumblebees experience. This thesis did not investigate where this 'threshold' lies, but I did repeatedly identify important impacts on individual fitness, on biochemistry and on the microbiome in a dose rate band between 40 - 100 µGy hr^{-1}. The effects I observed at these dose rates were often sizeable, therefore I suggest that effects on individual bumblebee fitness are likely to persist at lower doses. However, these lower dose rates need to be studied in more detail with greater statistical power to understand when effects begin to occur.

There are however some radiological limitations to the studies presented in this thesis, as I did not assess potential radiation recovery mechanisms. Ionising radiation can damage cells through the generation of reactive oxygen species (ROS), however in response to this the cell responds by producing natural antioxidants such as glutathione, superoxide dismutases, and catalase (Zhou et al, 2014). Future studies should investigate antioxidant production to understand if ROS production is overwhelming cellular defences and causing damage (Pizzino et al, 2017). Additionally, this thesis just used external radiation sources to deliver a radiation dose rate. Whilst this is indicative of a radiologically contaminated environment, results could have been different if internal emitters were used. The UK Government Committee Explaining Radiation Risks from Internal Emitters suggests that more work needs to be done to understand the uncertainties surrounding the inhalation and ingestion of radionuclides (CERRIE, 2004). This could be relevant for bumblebees consuming contaminated nectar within the CEZ. However, work from the CEZ does show that 95% of radiation exposure comes from external gamma and therefore this work could be more relevant to other contaminated landscapes (Beresford et al, 2020). Some work has been done on this such as examining the effects of oral doses of radioactive caesium in butterflies found at Fukushima (Gurung et al,

2019). However, much more work is needed to understand the effects of ingestion of contaminated material with consideration of confounding factors. Another limitation is that this study used a high LET radiation in order to mimic radiation levels within the CEZ. Higher LET results in lower cell survival per absorbed dose as it induces dense ionisation for localised DNA damage. Where as, lower LET radiation is sparse resulting in more diffuse dose rates, e.g. X-rays (Kim et al, 2017). It would be interesting to repeat studies in this thesis with a low LET emitter to understand if effects are unique to high LET radiation.

Whilst this thesis effectively identifies impacts on individual bumblebee workers at low dose rates, previous work has identified key fitness effects at the colony level: low dose radiation exposure impairs colony reproduction and delays colony growth (Raines et al, 2020). The work in this thesis has built upon this finding by using a diverse suite of metrics to identify more detailed effects on individual worker bumblebees. This thesis therefore adds more evidence that it is likely that bumblebee fitness will be affected by radiation exposure. This is especially true in contaminated environments, where effects recorded in this thesis will affect whole colonies. For example, when resources are scarce bumblebee reproductive activity is reduced and therefore the size of a colony is smaller (Requier et al, 2019). However, this could be further investigated in future work by examining colony foraging flights to look at whole colony competency in order to understand in more detail how colonies compensate and adapt to increased nutritional requirements, especially in combination with other stressors.

6.6 To what extent are bumblebees special?

The first three data chapters of this thesis focused on bumblebees, which are ecologically important organisms found within the CEZ. However, bumblebees have a unique life-history and exhibit unique behaviours as a result of living eusocially. Therefore, it must be questioned whether bumblebees are just simply unique in their response to low dose rates of radiation and to what extent the effects recorded can be generalized to other species. This thesis however addressed this in Chapter 5 by investigating the effect of ecologically relevant radiation exposure on the fecundity of *D. melanogaster.* In this study on flies I found equally as dramatic effects as in bumblebees, with late life fecundity collapsing during radiation exposure. Furthermore, a similar dramatic reduction occurred in ability of fly eggs to develop

successfully into adults. This suggests that bumblebees are not just simply unusual in their response to radiation but that effects are also likely to occur in other invertebrates. Future work however should compare both bumblebee and *D. melanogaster* on a molecular level, such as through efficiency of DNA repair processes. This will provide key information on factors which could account for species differences.

6.7 What further work could follow on from this thesis?

This thesis identified that under low radiation dose rates bumblebees experience increased nutritional demands and feed more. It then successfully followed the fate of this consumed sugar through the bumblebee, via metrics such as biochemical measures and microbiome analysis. However, this thesis often focused on dose rates between 100 – 200 μGy hr^{-1}. As my studies now show interesting effects below these dose rates, it is clear that assessing effects at even lower dose rates is important. Therefore, any future work should invest more replication in to dose rates between 5-50 μGy hr^{-1}. This would ensure that any future decisions by the International Commission on Radiological Protection to reduce the current DCRL for insects can be done with the most complete information. Additionally, any future work should investigate the effects of radiation on species other than just bumblebees. This thesis began this important work by investigating the effects of radiation on *D. melanogaster* reproduction (Chapter 5), which added to evidence that low dose radiation exposure effects reproduction in a species other than just bumblebees (Raines et al, 2020). It is therefore important to identify if the nutritional effects recorded in bumblebees in this thesis could be recorded in other invertebrates found in the Chernobyl Exclusion Zone. Especially as the general life-history metrics I studied are likely to be transferrable to many other species.

A key aspect for future research is investigation of the mechanisms driving the physiological responses discussed in this thesis (Chapter 2 ; Chapter 3 ; Chapter 4). Whilst this study did not identify an exact mechanism, I suggest that identifying the underlying mechanisms will be important to fully understand the effects of radiation across species. It could be important to pair up my observations with molecular measures assessing DNA damage, to determine the extent to which such damage occurs at these dose rates. In previous work on frogs at contaminated sites in Fukushima, mitochondrial DNA damage was observed, which increased in a dose dependent manner (Gombeau et al, 2020). Furthermore, *Arabadopsis thaliana*

taken from the Chernobyl Exclusion Zone exhibited changes in the genome when taken from sites with the highest dose rates (Horemans et al, 2018). Therefore, further work is required to investigate mechanisms that may be driving effects recorded in this thesis. However improved sampling is required in contaminated environments to control for potentially confounding variables.

Radiation-induced DNA damage can lead to novel mutations in the gene pool of exposed populations; this generation of genomic variation could potentially fuel evolutionary change through natural selection. It would therefore be interesting to understand the extent to which the changes in physiological measures that I found are associated with mutation or other genomic changes. This has been investigated in *Daphnia* populations taken from lakes within the CEZ. It was found that measures of genetic diversity were significantly higher in areas with the highest dose rates (Goodman et al, 2022). Therefore, it would be interesting to investigate if similar effects occur in bumblebee populations sampled from the CEZ.

The opening introduction chapter of this thesis explored the consequences for humans of being exposed to radiation; human studies have been where most of our epidemiological knowledge on radiation effects originates. Whilst bumblebees and humans are very different in their biology, it should be considered whether the physiological changes recorded in this study could be identified within humans exposed to similar dose rates. This is especially relevant to Chapter 5 which recorded shifts in sex ratio in response to radiation exposure. Whether radiation causes shifts in sex ratio on offspring has been discussed in literature, with findings often being conflicted (Winther et al, 2003; Choi et al, 2007; Dickinson et al, 1996). My findings on effects of radiation on metabolic rate could also be applicable to humans, with cellular metabolism already being shown to increase in mice in response to high acute doses of radiation (Kim et al, 2019). I suggest that further work on these dose rates within humans could be important, especially as humans can often experience similar dose rates to the organisms within the study, such as the exposure of humans to radiation as part of medical testing. For example, during a CT scan of the human head an individual will receive a total accumulated dose of 2,100 µSv (Bindman et al, 2009). I therefore speculate that this work could provide some useful information on the effects of radiation on humans; however further high-powered studies would be needed to gain any evidence.

6.8 Conclusions

This thesis provides substantial evidence of the effects of low dose radiation on bumblebees, identifying that irradiation influences fundamental metabolic processes. Laboratory based studies found a dramatic increase in feeding, metabolic rate, bumblebee movement and the concentration of sugars in the haemolymph. Whilst the exact mechanism driving this effect was not identified, these results show that bumblebees are biochemically using the nectar they are consuming to fuel a response and not storing or immediately excreting it. This increase in nectar consumption also has influence on the bumblebee microbiome. I hypothesise that bumblebees are fueling a metabolically costly recovery response to low doses of radiation.

The change in energy budget of bumblebees in response to radiation could have policy implications as it highlights that bees are sensitive to the effects of radiation at levels currently considered safe by ICRP. I support other work that suggests the ICRP should substantially reduce the DCRL for bees, in order to protect bumblebees effectively. This suggestion is strengthened by my work highlighting strong reproductive effects in *D. melanogaster*, which indicates that other invertebrates also require lower radiation protection thresholds to protect wild populations.

I acknowledge that this thesis was conducted within a laboratory environment, therefore I cannot state what the effects would be in the CEZ with combined actions of other co-stressors. However, I suggest that under field conditions effects of radiation may be greater as insects would not be under 'ideal' conditions. Whilst I find strong effects of radiation at dose rates between 40 – 200 μGy hr^{-1}, I suggest that future studies should focus on much lower dose rates and use larger sample sizes between 0 – 50 μGy hr^{-1} to effectively identify the exact dose rate at which these effects occur and alter policy accurately. By completing high powered experiments in this dose rate range, this work could be used to better inform government when building new nuclear power stations or a geological disposal facility (BEIS, 2018).

In conclusion, these laboratory experiments suggest that bumblebees residing in the CEZ are very likely to suffer fitness loss as a result of low dose radiation exposure effecting their energy budgets. This effect will lead to workers foraging more often and which could lead to detrimental impacts on not only the individual but also on the colony.

Chapter 7: References

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Chapter 8: Appendix

8.1 Appendix Chapter 2: Ecologically relevant radiation exposure triggers elevated metabolic rate and nectar consumption in bumblebees

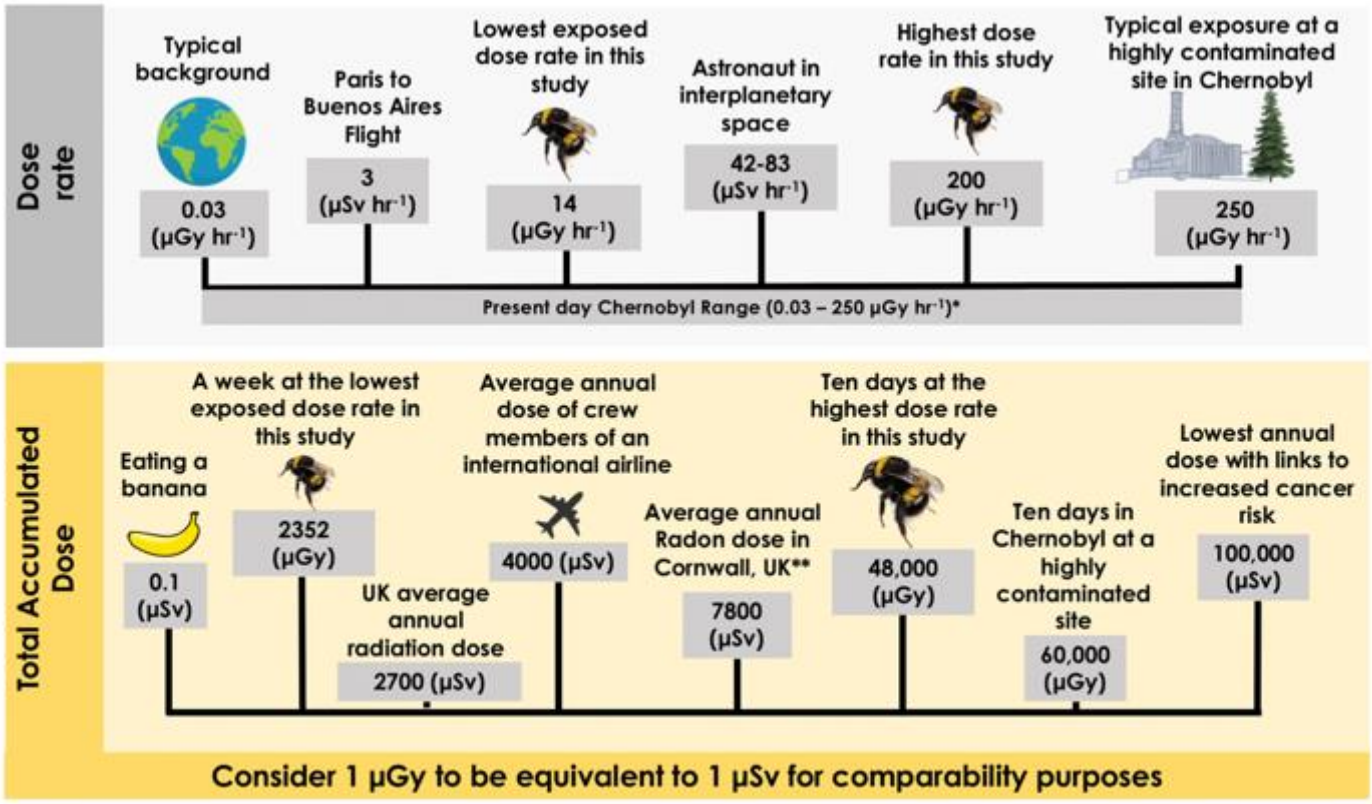
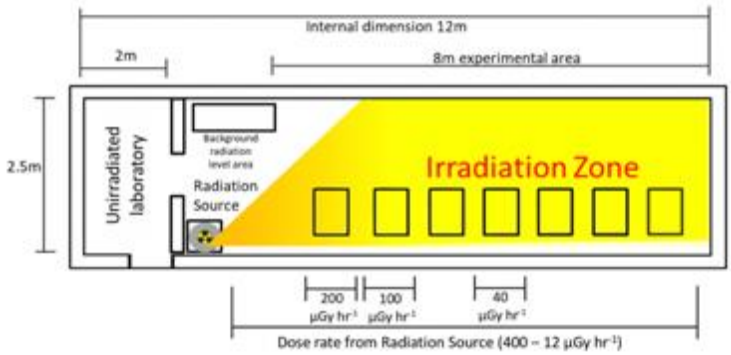


Figure S2.1: A diagram of various dose rates experienced in our natural environment in comparison with dose rates used in experiment 1 and 2 (ANSTO., 2022 ; Beresford *et al*., 2020 ; Chancellor *et al*., 2018)



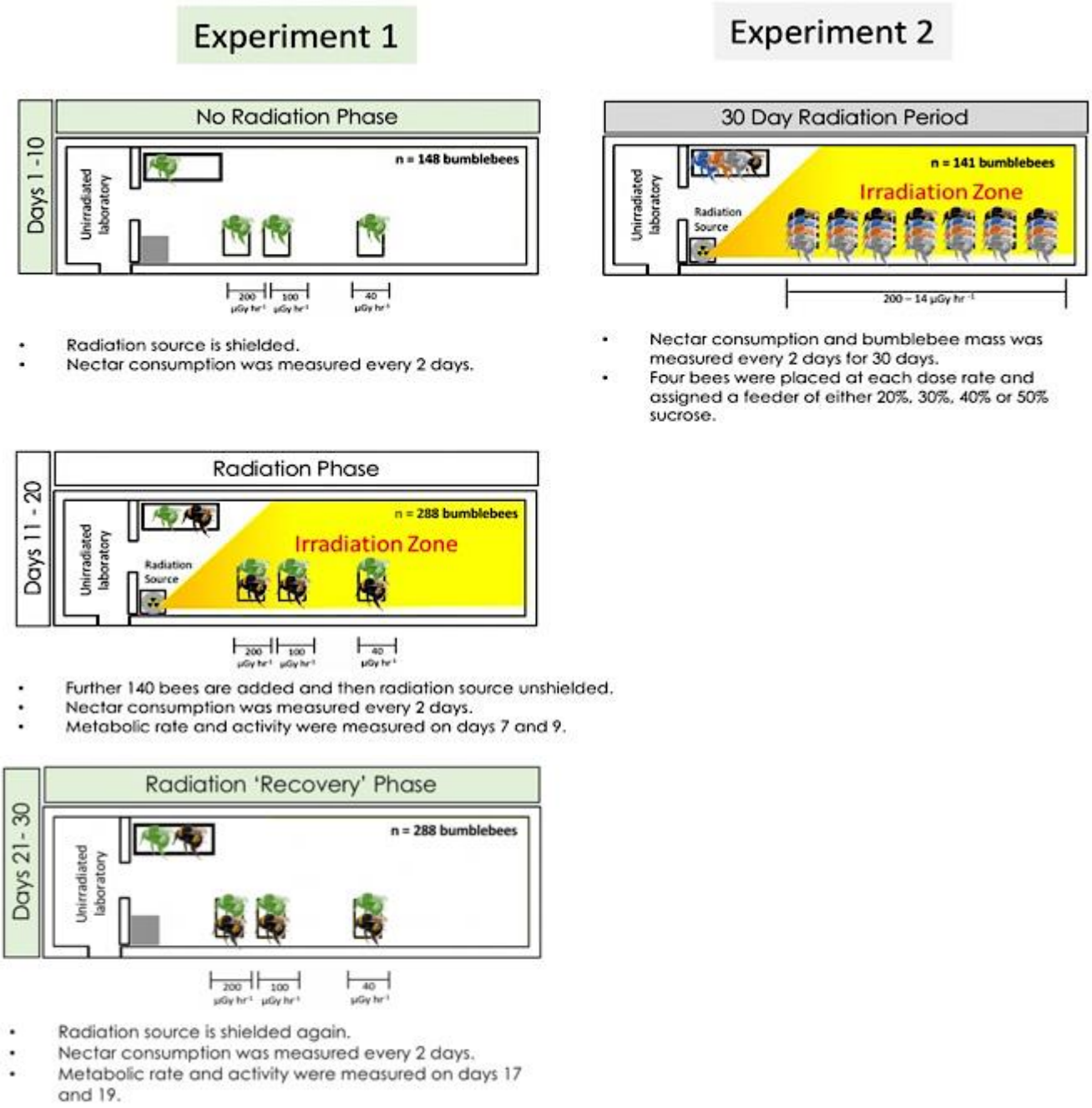


Figure S2.2: A diagram of the radiation facility at the University of Stirling**.** The top image represents the radiation facility with dimensions. The subsequent diagrams represent the two experiments and their design. For experiment 1: black boxes represent shelving units on which bumblebees were placed. Green bumblebees represent those that entered the experiment on day 1 and black bumblebees represent those that entered in the radiation phase. For experiment 2: the different coloured bees represent each of the four sucrose feeding treatments of 20%, 30%, 40% and 50%.

Experiment 1: The effect of radiation on bumblebee nectar consumption

Table S2.1. Parameter estimates for models investigating the effect of the position of a bumblebee in the experimental facility for 10 days prior to radiation exposure (pre-radiation phase) on consumption of 40%

sucrose nectar solution. Future dose rates were 200, 100, 40 μGy hr^{-1} and controls. The response variable (ml) was square root transformed. All variables except dose rate and days were mean centered and scaled by the standard deviation. For this model one standard deviation of temperature is 0.07°C, humidity is 3.41% and bumblebee mass of bee at start of the experiment 1.02 g. Model was linear mixed effects with normally distributed errors. Multiple measures were made on 148 bumblebees during these observations. Table S2.1a describes the minimal model used. Table S2.1b contains terms removed from the model in reverse order of deletion during model simplification.

a. Minimal Model				
Predictors	**Estimate**	**SE**	χ^2	**P Value**
(Intercept)	0.55	0.01	-	-
Days within the experiment	-0.01	$1.37x10^{-3}$	21.19	$4.17x10^{-6}$
Mass of bee at start of experiment (g)	0.02	0.01	4.93	0.03
Average temperature during the days when the nectar measurements were made (°C)	0.28	0.07	14.38	$1.50x10^{-4}$
Average humidity during the days when the nectar measurements were made (%)	0.01	$2.42x10^{-3}$	11.18	$8.26x10^{-4}$
b. Terms removed from model in reverse order of deletion				
Future dose rate (μGy hr^{-1})	$-1.16x10^{-4}$	$9.81x10^{-5}$	1.44	0.23
Access to a second low nectar concentration feeder	$1.23x10^{-2}$	$1.38x10^{-2}$	0.82	0.36
Age of bee at start of experiment (days)	$3.54x10^{-3}$	$6.05x10^{-3}$	0.35	0.55
Average temperature (°C) by humidity (%) during the days when the nectar measurements were made	$4.98x10^{-2}$	$4.01x10^{-2}$	1.56	0.21
Future dose rate (μGy hr^{-1}) by age of bee at start of experiment (days)	$8.30x10^{-5}$	$8.91x10^{-5}$	0.91	0.34
Future dose rate (μGy hr^{-1}) by mass of bee (g)	$-1.30x10^{-4}$	$9.70x10^{-5}$	1.89	0.17
Future dose rate (μGy hr^{-1}) by days in the phase	$-5.35x10^{-6}$	$1.80x10^{-5}$	0.09	0.77

Table S2.2. Parameter estimates for models investigating the effect of radiation dose rate on bumblebee nectar consumption (of 40% sucrose solution) during the 10-day radiation phase of the experiment. Dose rates were 200, 100, 40 μGy hr^{-1} and controls. The response variable (ml) was square root transformed. All variables except dose rate and days were mean centered and scaled by the standard deviation. For this model one standard deviation of temperature is 0.07°C, humidity is 3.41% and bumblebee mass of bee at start of the experiment 1.02 g. Model was linear mixed effects with normally distributed errors. Multiple measures were made on 288 bumblebees during these observations. Table S2.2a describes the minimal model used. Table S2.2b contains terms removed from the model in reverse order of deletion during model simplification.

a. Minimal Model				
Predictors	**Estimate**	**SE**	χ^2	**P Value**
(Intercept)	13.30	0.43	-	-
Days within the experiment	2.20	0.06	23.66	$1.15x10^{-6}$
Dose rate (μGy hr^{-1})	0.10	0.15	39.74	$2.90x10^{-10}$
Mass of bee at start of experiment (g)	7.34	1.47	16.94	$3.85x10^{-5}$
Average temperature during the days when the nectar measurements were made (°C)	118.00	2.16	9.07	$2.00x10^{-3}$
Average humidity during the days when the nectar measurements were made (%)	2.38	0.03	7.36	$7.00x10^{-3}$
Dose rate (μGy hr^{-1}) by days within the experiment	0.02	0.01	38.25	$6.22x10^{-10}$
Average temperature (°C) by humidity (%) during the days when the nectar measurements were made	45.500	10.02	3.97	0.05
b. Terms removed from model in reverse order of deletion				
Access to a second low nectar concentration feeder	$-1.97x10^{-2}$	$1.48x10^{-2}$	1.78	0.18
Age of bee at start of experiment (days)	$-1.10x10^{-3}$	$6.33x10^{-3}$	0.03	0.86

Dose rate (μGy hr^{-1}) by age of bee at start of experiment (days)	$5.65x10^{-5}$	$8.62x10^{-5}$	0.45	0.50
Dose rate (μGy hr^{-1}) by mass of bee (g)	$-3.84x10^{-5}$	$1.00x10^{-4}$	0.14	0.70
Dose rate (μGy hr^{-1}) by average temperature during the days when the nectar measurements were made (°C)	$3.28x10^{-3}$	$1.40x10^{-3}$	0.55	0.19
Dose rate (μGy hr^{-1}) by average humidity during the days when the nectar measurements were made (%)	$-6.61x10^{-6}$	$4.32x10^{-5}$	0.02	0.89

Table S2.3. Parameter estimates for models investigating the effect of radiation dose rate on bumblebee nectar consumption (of 40% sucrose solution) during the 10-day radiation phase of the experiment excluding the top dose rate of 200 μGy hr^{-1}. This model is for dose rates of 100, 40 μGy hr^{-1} and controls. The response variable (ml) was square root transformed. All variables except dose rate and days were mean centered and scaled by the standard deviation. For this model one standard deviation of temperature is 0.07°C, humidity is 3.41% and bumblebee mass of bee at start of the experiment 1.02 g. Model was linear mixed effects with normally distributed errors. Multiple measures were made on 213 bumblebees during these observations. Table S2.3a describes the minimal model used. Table S2.3b contains terms removed from the model in reverse order of deletion during model simplification.

a. Minimal Model				
Predictors	**Estimate**	**SE**	χ^2	**P Value**
(Intercept)	$4.93x10^{-1}$	$1.55x10^{-2}$	-	-
Dose rate (μGy hr^{-1})	$7.15x10^{-4}$	$2.32x10^{-4}$	12.27	$1.00x10^{-3}$
Mass of bee at start of experiment (g)	$3.36x10^{-2}$	$9.14x10^{-3}$	13.26	$2.00x10^{-3}$
Average humidity during the days when the nectar measurements were made (%)	$-1.06x10^{-2}$	$2.39x10^{-3}$	19.52	$9.93x10^{-6}$
b. Terms removed from model in reverse order of deletion				
Access to a second low nectar concentration feeder	$-1.76x10^{-2}$	$1.80x10^{-2}$	0.97	0.33
Days within the experiment	$-9.88x10^{-4}$	$1.32x10^{-3}$	0.52	0.47
Age of bee at start of experiment (days)	$-6.02x10^{-3}$	$7.56x10^{-3}$	0.66	0.42
Average temperature during the days when the nectar measurements were made (°C)	$6.59x10^{-2}$	$1.23x10^{-1}$	0.30	0.58
Dose rate (μGy hr^{-1}) by days within the experiment	$-1.57x10^{-4}$	$1.20x10^{-4}$	0.63	0.43
Average temperature (°C) by humidity (%) during the days when the nectar measurements were made	$3.11x10^{-2}$	$5.13x10^{-2}$	0.38	0.54
Dose rate (μGy hr^{-1}) by mass of bee (g)	$-1.23x10^{-4}$	$2.17x10^{-4}$	0.34	0.56
Dose rate (μGy hr^{-1}) by age of bee at start of experiment (days)	$-8.30x10^{-6}$	$3.34x10^{-5}$	0.07	0.79

Table S2.4. Parameter estimates for models investigating the effect of radiation dose rate on bumblebee nectar consumption (of 40% sucrose solution) during the 10-day radiation phase of the experiment at a doses rate of 40 μGy hr^{-1} and controls. Dose rates of 200 and 100 μGy hr^{-1} were removed. The response variable (ml) was square root transformed. All variables except dose rate and days were mean centered and scaled by the standard deviation. For this model one standard deviation of temperature is 0.07°C, humidity is 3.41% and bumblebee mass of bee at start of the experiment 1.02 g. Model was linear mixed effects with normally distributed errors. Multiple measures were made on 146 bumblebees during these observations. Table S2.4a describes the minimal model used. Table S2.4b contains terms removed from the model in reverse order of deletion during model simplification.

a. Minimal Model				
Predictors	**Estimate**	**SE**	χ^2	**P Value**
(Intercept)	0.51	0.01	-	-
Days within the experiment	$-3.00x10^{-3}$	$1.00x10^{-3}$	4.93	0.03
Mass of bee at start of experiment (g)	0.03	0.01	6.51	0.01

Average humidity during the days when the nectar measurements were made (%)	-0.01	$3.00x10^{-3}$	4.23	$3.00x10^{-3}$
b. Terms removed from model in reverse order of deletion				
Average temperature during the days when the nectar measurements were made (°C)	-0.24	0.14	2.76	0.10
Dose rate (μGy hr^{-1})	0.03	0.02	1.51	0.22
Age of bee at start of experiment (days)	$-6.00x10^{-3}$	$8.00x10^{-3}$	0.57	0.45
Access to a second low nectar concentration feeder	$-7.00x10^{-3}$	0.02	0.13	0.72
Dose rate (μGy hr^{-1}) by days within the experiment	-0.03	0.02	2.81	0.09
Dose rate (μGy hr^{-1}) by mass of bee (g)	-0.02	0.02	0.49	0.48
Average temperature (°C) by humidity (%) during the days when the nectar measurements were made	0.03	0.07	0.18	0.67
Dose rate (μGy hr^{-1}) by age of bee at start of experiment (days)	$- 5.94x10^{-4}$	$3.55x10^{-3}$	0.009	0.92

Table S2.5. Paired t-tests conducted to assess changes in bumblebee nectar consumption (40% sucrose) between day 10 at the end of the no radiation phase and two days later, after two days of exposure during the radiation phase. A total of 295 bees were measured on day 10 and 12.

Dose rate (μGy hr^{-1}) group	Mean difference	df	T	P value
200	$-4.00x10^{-3}$	74	-0.18	0.86
100	-0.03	73	-1.24	0.23
40	0.04	71	1.89	0.07
0.11	0.03	73	1.44	0.16

Table S2.6. Parameter estimates for models investigating the effect of radiation dose rate on bumblebee nectar consumption from the 5% nectar solution during the 10-day radiation phase of the experiment. Dose rates were 200, 100, 40 μGy hr^{-1} and controls. The response variable (ml) was square root transformed. All variables except dose rate and days were mean centered and scaled by the standard deviation. For this model one standard deviation of temperature is 0.07°C, humidity is 3.41% and bumblebee mass of bee at start of the experiment 1.02 g. Model was linear mixed effects with normally distributed errors. Multiple measures were made on 144 bumblebees during these observations. Table 2.6a describes the minimal model used. Table S2.6b contains terms removed from the model in reverse order of deletion during model simplification.

a. Minimal Model				
Predictors	**Estimate**	**SE**	χ^2	**P Value**
(Intercept)	0.60	0.04	-	-
b. Terms removed from model in reverse order of deletion				
Days within the experiment	0.02	0.01	1.61	0.21
Average temperature during the days when the nectar measurements were made (°C)	-1.54	0.96	1.61	0.20
Mass of bee at start of experiment (g)	0.04	0.04	0.86	0.35
Dose rate (μGy hr^{-1})	$-2.23x10^{-4}$	$3.98x10^{-4}$	0.37	0.54
Average humidity during the days when the nectar measurements were made (%)	$3.27x10^{-3}$	$1.33x10^{-2}$	0.06	0.80
Age of bee at start of experiment (days)	$-4.17x10^{-4}$	0.04	0.01	0.94
Dose rate (μGy hr^{-1}) by mass of bee (g)	$-5.77x10^{-4}$	$4.50x10^{-4}$	1.98	0.16
Dose rate (μGy hr^{-1}) by average humidity during the days when the nectar measurements were made (%)	$-1.55x10^{-4}$	$1.32x10^{-4}$	1.96	0.16
Average temperature (°C) by humidity (%) during the days when the nectar measurements were made	0.33	0.25	2.26	0.13

Dose rate (μGy hr^{-1}) by days in the phase	$9.90x10^{-5}$	$1.26x10^{-4}$	0.76	0.38
Dose rate (μGy hr^{-1}) by average temperature during the days when the nectar measurements were made (°C)	-0.01	0.01	0.34	0.56
Dose rate (μGy hr^{-1}) by age of bee at start of experiment (days)	$-3.9x10^{-7}$	$3.98x10^{-4}$	0.01	0.90
Access to a second-high nectar concentration feeder	$1.57x10^{-2}$	$4.48x10^{-2}$	0.03	0.10

Table S2.7. Parameter estimates for models investigating the effect of previous radiation dose rate on bumblebee nectar consumption (40%) during the recovery phase of the experiment. The response variable (ml) was square root transformed. All variables except dose rate and days were mean centered and scaled by the standard deviation. For this model one standard deviation of temperature is 0.07°C, humidity is 3.41% and bumblebee mass of bee at start of the experiment 1.02 g. Model was linear mixed effects with normally distributed errors. Multiple measures were made on 288 bumblebees during these observations. Table S2.7a describes minimal model used. Table S2.7b describes terms removed from the model in reverse order of deletion during model simplification.

a. Minimal Model				
Predictors	**Estimate**	**SE**	χ^2	**P Value**
(Intercept)	404.00	16.10	-	-
Days within the experiment	15.00	3.00	24.44	$7.66x10^{-7}$
Prior dose rate received (μGy hr^{-1})	0.66	0.14	21.35	$3.84x10^{-6}$
Mass of bee at start of experiment (g)	28.00	9.75	8.24	$4.00x10^{-3}$
Average temperature for days leading up to nectar measurements at the data logger closest to the bumblebee (°C)	-53.50	67.70	0.63	0.43
Average humidity for days leading up to nectar measurements at the data logger closest to the bumblebee (%)	-15.50	3.68	17.70	$2.59x10^{-5}$
Prior dose rate received (μGy hr^{-1}) by days within the experiment	0.08	0.02	12.48	$4.11x10^{-4}$
Average temperature (°C) by humidity levels (%) for days leading up to nectar measurements at the data logger closest to the bumblebee	-61.50	30.20	4.17	0.04
b. Terms removed from model in reverse order of deletion				
Access to a second low nectar concentration feeder	-23.5	19.9	1.42	0.23
Age of bee at start of experiment at start of experiment (days)	3.93	8.39	0.23	0.63
Prior dose rate received (μGy hr^{-1}) by mass of bee (g)	0.04	0.14	0.09	0.77
Prior dose rate received (μGy hr^{-1}) by age of bee at start of experiment (days)	-0.02	0.12	0.03	0.86

Table 2.8. Parameter estimates for models investigating whether the effect of radiation on bumblebee nectar consumption (40%) changed across the radiation and recovery phases of the experiment. The response variable (ml) was square root transformed. All variables except dose rate and days were mean centered and scaled by the standard deviation. For this model one standard deviation of temperature is 0.07°C, humidity is 3.41% and bumblebee mass of bee at start of the experiment 1.02 g. Model was linear mixed effects with normally distributed errors. Multiple measures were made on 288 bumblebees during these observations. Table S2.8a describes minimal model used. Table S2.8b describes terms removed from the model in reverse order of deletion during model simplification.

a. Minimal Model				
Predictors	**Estimate**	**SE**	χ^2	**P Value**
(Intercept)	539.00	26.00	-	-
Days within the phase of the experiment	8.92	1.31	45.67	1.40×10^{-11}
Dose rate / Prior dose rate (μGy hr^{-1})	0.64	0.10	39.45	3.37×10^{-10}
Mass of bee at start of experiment (g)	31.70	7.33	18.25	1.94×10^{-5}
Average temperature during the days when the nectar measurements were made (°C)	121.00	55.40	4.77	0.03
Change from radiation to recovery phase	-108.00	12.00	91.93	$<2.2 \times 10^{-16}$
Average humidity during the days when the nectar measurements were made (%)	-8.82	1.68	27.31	1.74×10^{-7}
Dose rate / Prior dose rate (μGy hr^{-1}) by days within phase	0.12	0.02	58.95	1.62×10^{-14}
Average temperature (°C) by humidity (%) during the days when the nectar measurements were made	46.90	16.00	8.59	3.00×10^{-3}
Days within the phase by recovery phase	6.98	3.00	5.35	0.02
b. Terms removed from model in reverse order of deletion				
Access to a second low nectar concentration feeder	-25.40	14.70	3.04	0.08
Age of bee at start of experiment (days)	2.40	6.27	0.15	0.70
Dose rate / Prior dose rate (μGy hr^{-1}) by age of bee at start of experiment (days)	0.04	0.09	0.19	0.66
Dose rate / Prior dose rate (μGy hr^{-1}) by removal of radiation in the recovery phase	0.05	0.10	0.22	0.64
Dose rate / Prior dose rate (μGy hr^{-1}) by mass of bee (g)	-0.03	0.10	0.09	0.76

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

Table S2.9. Parameter estimates for models investigating the effect of radiation exposure (0.11 vs 200 μGy hr^{-1}) on mean bumblebee metabolic rate over a 5-minute observation period during the radiation phase of the experiment. The response variable (μmol min^{-1} CO_2) was square root transformed. All variables except dose rate and days were mean centered and scaled by the standard deviation. For this model one standard deviation of temperature is 0.07°C, humidity is 3.41% and bumblebee mass of bee at start of the experiment 1.02 g. Model was linear mixed effects with normally distributed errors. Multiple measures were made on 60 bumblebees for each time point. Table S2.9a describes minimal model used. Table S2.9b describes terms removed from the model in reverse order of deletion during model simplification.

a. Minimal Model				
Predictors	**Estimate**	**SE**	χ^2	**P Value**
(Intercept)	3.82	0.12	-	-
Day	-0.29	0.06	20.70	5.36×10^{-6}
Radiation exposure	0.34	0.15	4.80	0.03
Temperature of air drawn over bee (°C)	3.94	1.93	4.26	0.04
b. Terms removed from model in reverse order of deletion				
Time bee spent moving during measurement (s)	1.00×10^{-3}	6.92×10^{-4}	2.33	0.13
Humidity levels of air drawn over bee (%)	0.04	0.03	1.46	0.23
Age of bee at start of experiment at start of experiment (days)	-0.04	0.07	0.38	0.54
Access to a second low nectar concentration feeder	-0.08	0.16	0.30	0.59
Mass of bee at start of experiment (g)	-0.54	1.47	0.13	0.72
Radiation exposure by mass of bee (g)	-0.12	0.16	0.68	0.41
Radiation exposure by age of bee at start of experiment (days)	-0.10	0.16	0.42	0.52
Temperature levels (°C) by humidity levels (%) of air drawn over bee	0.27	0.51	0.28	0.60

Radiation exposure by days in experiment	0.03	0.08	0.11	0.75
Radiation exposure by humidity levels of air drawn over bee (%)	-7.96×10^{-3}	7.73×10^{-2}	0.014	0.91
Radiation exposure by temperature of air drawn over bee (°C)	-3.84×10^{-2}	1.76×10^{-1}	4.0×10^{-3}	0.99

Table S2.10. Parameter estimates for models investigating the effect of the previous radiation exposure (0.11 vs 200 μGy hr^{-1}) dose rate on mean bumblebee metabolic rate over a 5-minute observation period during the recovery phase of the experiment. The response variable (μmol min^{-1} CO_2) was square root transformed. All variables except dose rate and days were mean centered and scaled by the standard deviation. For this model one standard deviation of temperature is 0.07°C, humidity is 3.41% and bumblebee mass of bee at start of the experiment 1.02 g Model was linear mixed effects with normally distributed errors. Multiple measures were made on 60 bumblebees for each timepoint. Table S2.10a describes minimal model used. Table S2.10b describes terms removed from the model in reverse order of deletion during model simplification.

a. Minimal Model				
Predictors	**Estimate**	**SE**	χ^2	**P Value**
(Intercept)	3.90	0.07	-	-
Humidity levels of air drawn over bee (%)	-0.05	0.02	5.37	0.02
b. Terms removed from model in reverse order of deletion				
Mass of bee at start of experiment (g)	1.60	1.28	1.58	0.21
Prior radiation exposure	-0.17	0.14	1.66	0.20
Temperature levels of air drawn over bee (°C)	1.81	1.68	1.20	0.27
Time bee spent moving during measurement (s)	3.62×10^{-4}	6.79×10^{-4}	0.30	0.58
Access to a second low nectar concentration feeder	-0.06	0.14	0.17	0.68
Days within the experiment	-0.01	0.06	7.0×10^{-3}	0.93
Age of bee at start of experiment at start of experiment (days)	4.0×10^{-3}	0.06	4.0×10^{-3}	0.95
Prior radiation exposure by days within the experiment	-0.12	0.09	3.46	0.06
Temperature levels (°C) by humidity levels (%) of air drawn over bee	1.09	1.18	0.92	0.34
Prior radiation exposure by age of bee at start of experiment (days)	0.09	0.12	0.60	0.44
Prior radiation exposure by mass of bee (g)	-0.08	0.15	0.36	0.55

Table S2.11. Parameter estimates for models investigating changes in the effect of radiation dose rate (0.11 vs 200 μGy hr^{-1}) on mean bumblebee metabolic rate over a 5-minute observation period across the radiation and recovery phases of the experiment. The response variable (μmol min^{-1} CO_2) was square root transformed. All variables except dose rate and days were mean centered and scaled by the standard deviation. For this model one standard deviation of temperature is 0.07°C, humidity is 3.41% and bumblebee mass of bee at start of the experiment 1.02 g. Model was linear mixed effects with normally distributed errors. Multiple measures were made on 60 bumblebees during these observations. Table S2.11a describes minimal model used. Table S2.11b describes terms removed from the model in reverse order of deletion during model simplification.

a. Minimal Model				
Predictors	**Estimate**	**SE**	χ^2	**P Value**
(Intercept)	3.83	0.11	-	-
Days within the experiment	-0.29	0.06	16.50	4.88×10^{-5}
Radiation exposure	0.35	0.14	1.66	0.20
Phase of the experiment	0.03	0.15	1.69	0.19
Temperature of air drawn over bee (°C)	3.96	1.36	5.05	0.03
Radiation exposure by phase	-0.44	0.19	5.54	0.02

Days within experiment by phase	0.21	0.06	10.93	$1.00x10^{-3}$
b. Terms removed from model in reverse order of deletion				
Time bee spent moving during measurement (s)	$7.15x10^{-4}$	$4.95x10^{-4}$	2.14	0.14
Access to a second low nectar concentration feeder	-0.07	0.11	0.39	0.53
Mass of bee at start of experiment (g)	0.02	0.05	0.22	0.64
Humidity levels of air drawn over bee (%)	-0.01	0.02	0.26	0.61
Age of bee at start of experiment at start of experiment (days)	$2.00x10^{-3}$	0.05	0.01	0.91
Radiation exposure by days in experiment	-0.07	0.06	1.26	0.26
Radiation exposure by mass of bee (g)	0.07	0.11	0.43	0.51
Radiation exposure by age of bee at start of experiment (days)	-0.01	0.07	0.10	0.76
Temperature levels (°C) by humidity levels (%) of air drawn over bee	-0.02	0.47	$3.0x10^{-3}$	0.96
Phase by radiation exposure by days within the experiment	-0.16	0.13	1.70	0.19

Table S2.12. Parameter estimates for zero inflated model investigating the effect of radiation dose rate (0.11 vs 200 μGy hr^{-1}) on the time a bee spent active during the radiation phase of the experiment. The response variable (s) was not square root transformed. All variables except dose rate and days were mean centered and scaled by the standard deviation. For this model one standard deviation of temperature is 0.07°C, humidity is 3.41% and bumblebee mass of bee at start of the experiment 1.02 gModel is zero inflated which assumed errors had a Gaussian distribution. Multiple measures were made on 60 bumblebees for each timepoint. Table S2.12a describes minimal model used. Table S2.12b describes terms removed from the model in reverse order of deletion during model simplification. Table S2.12c describes terms removed from the zero inflated part of the model in reverse order of deletion during model simplification. No terms were significant for the zero inflated part of the model.

a. Minimal Model				
Predictors	**Estimate**	**SE**	χ^2	**P Value**
(Intercept)	127.50	12.51	-	-
Radiation exposure	37.80	18.01	2.10	0.04
b. Terms removed from model in reverse order of deletion				
Days within the experiment	8.40	5.94	1.41	0.16
Age of bee at start of experiment (days)	-9.65	8.35	-1.16	0.25
Temperature levels of air drawn over bee (°C)	139.07	246.22	0.57	0.57
Mass of bee at start of experiment (g)	0.82	9.12	0.09	0.93
Radiation exposure by age of bee at start of experiment (days)	15.59	11.88	1.31	0.19
c. Terms removed from the Zero-inflated part of the model in reverse order of deletion				
Radiation exposure	0.55	0.46	1.19	0.24
Mass of bee at start of experiment (g)	-0.24	0.22	-1.05	0.30
Days within the experiment	-0.05	0.15	-0.34	0.74
Age of bee at start of experiment (days)	-0.02	0.22	-0.07	0.94
Temperature levels of air drawn over bee (°C)	0.90	6.82	0.13	0.90
Radiation exposure by age of bee at start of experiment (days)	0.23	0.31	0.72	0.47

Table S2.13. Parameter estimates for zero inflated model investigating the effect of radiation dose rate (0.11 vs 200 μGy hr^{-1}) on the time a bee spent active during the recovery phase of the experiment. All variables except dose rate and days were mean centered and scaled by the standard deviation. For this model one standard deviation of temperature is 0.07°C, humidity is 3.41% and bumblebee mass of bee at start of the experiment 1.02 g. Model investigating time a bee was active is zero inflated which assumed errors had a Gaussian

distribution. Multiple measures were made on 60 bumblebees for each timepoint. Table S2.13a describes minimal model used. Table S2.13b describes terms removed from the model in reverse order of deletion during model simplification. Table S2.13c describes terms removed from the zero inflated part of the model in reverse order of deletion during model simplification. No terms were significant for the zero inflated part of the model.

a. Minimal Model				
Predictors	**Estimate**	**SE**	χ^2	**P Value**
(Intercept)	130.32	10.53	-	-
b. Terms removed from model in reverse order of deletion				
Temperature levels of air drawn over bee (°C)	260.49	276.96	0.94	0.35
Days within the experiment	2.27	7.65	0.30	0.77
Mass of bee at start of experiment (g)	-2.42	11.18	-0.22	0.83
Radiation exposure	4.61	21.21	0.22	0.83
Age of bee at start of experiment (days)	-1.44	9.03	-0.16	0.88
Radiation exposure by age of bee at start of experiment (days)	3.11	14.15	0.22	0.83
c. Terms removed from the Zero-inflated part of the model in reverse order of deletion				
Radiation exposure	0.04	0.42	0.91	0.36
Days within the experiment	-0.22	0.14	-1.51	0.13
Mass of bee at start of experiment (g)	-0.16	0.22	-0.74	0.46
Temperature levels of air drawn over bee (°C)	-2.37	5.49	-0.43	0.67
Age of bee at start of experiment (days)	0.01	0.19	0.05	0.96
Radiation exposure by age of bee at start of experiment (days)	-0.53	0.29	-1.81	0.07

Table S2.14. Parameter estimates for zero inflated model investigating the effect of radiation dose rate (0.11 vs 200 μGy hr^{-1}) on the distance a bee moved during the radiation phase of the experiment. To investigate distance a bee moved, all variables except dose rate and days were mean centered and scaled by the standard deviation. For this model one standard deviation of temperature is 0.07°C, humidity is 3.41% and bumblebee mass of bee at start of the experiment 1.02 g. Model is zero inflated which assumed errors had a Gaussian distribution. Multiple measures were made on 60 bumblebees for each timepoint. Table S2.14a describes minimal model used. Table S2.14b describes terms removed from the model in reverse order of deletion during model simplification. Table S2.14c describes terms removed from the zero inflated part of the model in reverse order of deletion during model simplification. No terms were significant for the zero inflated part of the model.

a. Minimal Model				
Predictors	**Estimate**	**SE**	χ^2	**P Value**
(Intercept)	100.78	9.79	-	-
Radiation exposure	25.21	14.29	1.76	0.05
b. Terms removed from model in reverse order of deletion				
Temperature levels of air drawn over bee (°C)	225.06	140.86	1.60	0.11
Age of bee at start of experiment (days)	-4.03	6.56	-0.62	0.54
Mass of bee at start of experiment (g)	3.35	7.01	0.48	0.63
Days within the experiment	0.05	6.73	7.0×10^{-3}	0.99
Radiation exposure by age of bee at start of experiment (days)	9.77	9.51	1.03	0.30
c. Terms removed from the Zero-inflated part of the model in reverse order of deletion				
Radiation exposure	0.72	0.45	1.58	0.12
Mass of bee at start of experiment (g)	-0.15	0.22	-0.67	0.50
Days within the experiment	-0.04	0.15	-0.28	0.78
Age of bee at start of experiment (days)	-0.04	0.22	-0.19	0.85
Temperature levels of air drawn over bee (°C)	0.59	6.57	0.09	0.93
Radiation exposure by age of bee at start of experiment (days)	0.22	0.31	0.72	0.47

Table S2.15. Parameter estimates for zero inflated model investigating whether the effect of radiation dose rate (0.11 vs 200 μGy hr^{-1}) on the time a bee spent active changed between the radiation and recovery phases of the experiment. Multiple measures were made on 60 bumblebees for each timepoint. Table S15a describes minimal model used. For time a bee spent active all variables except dose rate and days were mean centered and scaled by the standard deviation. For this model one standard deviation of temperature is 0.07°C, humidity is 3.41% and bumblebee mass of bee at start of the experiment 1.02 g. Model is zero inflated which assumed errors had a Gaussian distribution. Table S2.15b describes terms removed from the model in reverse order of deletion during model simplification. Table S2.15c describes terms removed from the zero inflated part of the model in reverse order of deletion during model simplification. No terms were significant for the zero inflated part of the model.

a. Minimal Model				
Predictors	**Estimate**	**SE**	χ^2	**P Value**
(Intercept)	138.21	7.01	-	-
b. Terms removed from model in reverse order of deletion				
Radiation exposure	21.87	13.92	1.57	0.12
Phase of the experiment (Recovery)	-15.43	13.87	-1.11	0.27
Temperature levels of air drawn over bee (°C)	246.11	155.10	1.59	0.11
Age of bee at start of experiment (days)	-5.45	6.19	-0.88	0.38
Days within the experiment	2.42	5.58	0.43	0.67
Mass of bee at start of experiment (g)	-1.64	7.08	-0.23	0.82
Radiation exposure) by age of bee at start of experiment (days)	9.38	9.21	1.02	0.31
Days within the experiment by phase (recovery)	-1.53	9.56	-0.16	0.87
Radiation exposure by phase (Recovery)	-1.16	9.51	-0.12	0.90
c. Terms removed from the Zero-inflated part of the model in reverse order of deletion				
Radiation exposure	0.45	0.31	1.47	0.14
Days within the experiment	-0.14	0.10	-1.30	0.19
Mass of bee at start of experiment (g)	-0.20	0.16	-1.25	0.21
Phase of the experiment (Recovery)	0.32	0.31	1.04	0.30
Temperature levels of air drawn over bee (°C)	-0.50	4.16	-0.12	0.91
Age of bee at start of experiment (days)	1.00×10^{-3}	0.14	0.01	0.99
Days within the experiment by phase (recovery)	-0.18	0.22	-0.86	0.39
Radiation exposure by age of bee at start of experiment (days)	-0.18	0.21	-0.87	0.39
Radiation exposure by phase (Recovery)	-0.14	0.64	-0.22	0.83

Experiment 2: The dose-rate threshold of the effect of radiation on bumblebee nectar consumption

Table S2.16. Parameter estimates for model investigating the effect of radiation dose rate on bumblebee nectar consumption (ml). Multiple measures were made on 141 bumblebees during these observations. Table S16a describes minimal model used. The response variable (ml) was square root transformed. All variables except dose rate and days were mean centered and scaled by the standard deviation. For this model one standard deviation of temperature is 0.36°C, humidity is 2.68% and bumblebee mass of bee at start of the experiment 1.04 g. Model was linear mixed effects with normally distributed errors. Table S2.16b describes terms removed from the model in reverse order of deletion during model simplification.

a. Minimal Model				
Predictors	**Estimate**	**SE**	χ^2	**P Value**
(Intercept)	727.00	12.82	-	-
Concentration of nectar	-3.63	1.16	6.79	0.01
Days within the experiment	3.98	1.00	27.57	1.5×10^{-7}
Dose rate (μGy hr^{-1})	0.39	0.16	4.89	0.03

Average temperature during the days when the nectar measurements were made (°C)	-0.07	0.01	21.21	4.12×10^{-6}
Concentration of nectar by days within experiment	744.00	8.97	10.86	9.80×10^{-4}
Concentration of nectar by dose rate (μGy hr^{-1})	-2.36	0.84	4.39	0.04
Concentration of nectar by dose rate (μGy hr^{-1}) by days within the experiment	-4.88	1.97	6.03	0.01
b. Terms removed from model in reverse order of deletion				
Dose rate (μGy hr^{-1}) by days within experiment	-0.08	0.01	2.0×10^{-3}	0.96
Mass of bee at start of experiment (g)	-1.49	9.48	2.85	0.08
Age of bee at start of experiment at start of experiment (days)	-2.16	2.41	1.02	0.32
Average humidity during the days when the nectar measurements were made (%)	0.04	0.03	0.91	0.34
Dose rate (μGy hr^{-1}) by mass of bee (g)	0.39	0.16	4.74	0.29
Dose rate (μGy hr^{-1}) by age of bee at start of experiment (days)	-0.07	0.05	3.25	0.06
Average temperature (°C) by humidity (%) during the days when the nectar measurements were made	8.73×10^{-3}	6.49×10^{-3}	1.80	0.18
Dose rate (μGy hr^{-1}) by average temperature during the days when the nectar measurements were made (°C)	-1.9×10^{-4}	2.66×10^{-4}	0.53	0.47
Dose rate (μGy hr^{-1}) by average humidity during the days when the nectar measurements were made (%)	0.01	0.01	0.18	0.67

Table S2.17. A breakdown of parameter estimates for model S16 when dose rates are removed in increments to investigates a potential threshold effect of radiation dose rate on bumblebee nectar consumption (ml). For each line of the table, data from dose rates above the threshold stated were removed (in 10 μGy hr^{-1} increments) and the parameter estimate for the effect of radiation was recalculated.

Dose rates	Estimate	SE	χ^2	P Value
192	3.53×10^{-4}	1.61×10^{-4}	2.16	0.03
180	2.42×10^{-4}	1.83×10^{-4}	1.85	0.18
170	1.36×10^{-4}	2.08×10^{-4}	0.32	0.57
160	1.35×10^{-4}	2.06×10^{-4}	0.31	0.56
150	2.64×10^{-4}	2.34×10^{-4}	4.51	0.03
140	2.66×10^{-4}	2.51×10^{-4}	1.41	0.25
130	2.17×10^{-4}	3.08×10^{-4}	0.59	0.44
120	2.17×10^{-4}	3.09×10^{-4}	3.80	0.05
110	5.15×10^{-4}	3.54×10^{-4}	2.92	0.08
100	5.43×10^{-4}	3.74×10^{-4}	1.63	0.20
90	2.75×10^{-4}	4.37×10^{-4}	0.58	0.44
80	4.77×10^{-4}	5.08×10^{-4}	2.11	0.15
70	5.56×10^{-4}	6.22×10^{-4}	4.51	0.03
60	5.97×10^{-4}	6.79×10^{-4}	0.57	0.45
50	2.54×10^{-4}	7.70×10^{-4}	0.09	0.76
40	1.72×10^{-3}	1.10×10^{-3}	4.29	0.04
30	1.50×10^{-3}	1.33×10^{-3}	0.23	0.63
20	2.82×10^{-3}	2.11×10^{-3}	0.35	0.56

Table S2.18. Parameter estimates for model investigating the effect of radiation dose rate on bumblebee (wet) mass. Multiple measures were made on 141 bumblebees during these observations. Table S18a describes minimal model used. The response variable (grams) was square root transformed. All variables except dose rate and days were mean centered and scaled by the standard deviation. For this model one standard deviation of

temperature is 0.36°C, humidity is 2.68% and bumblebee mass of bee at start of the experiment 1.04 g. Model was linear mixed effects with normally distributed errors. Table S2.18b describes terms removed from the model in reverse order of deletion during model simplification.

a. Minimal Model				
Predictors	**Estimate**	**SE**	χ^2	**P Value**
(Intercept)	0.14	2.86×10^{-3}	-	-
Days within the experiment	-56.69×10^{-4}	1.22×10^{-4}	18.09	2.17×10^{-5}
Mass of bee at start of experiment (g)	2.61×10^{-2}	2.64×10^{-3}	71.99	$< 2.2 \times 10^{-16}$
b. Terms removed from model in reverse order of deletion				
Average humidity during the days when the nectar measurements were made (%)	8.34×10^{-4}	6.66×10^{-4}	2.96	0.09
Concentration of nectar	1.37×10^{-4}	3.39×10^{-4}	1.09	0.31
Dose rate (μGy hr^{-1})	2.68×10^{-5}	4.27×10^{-5}	0.53	0.47
Average temperature during the days when the nectar measurements were made (°C)	1.55×10^{-4}	2.75×10^{-3}	0.02	0.88
Dose (μGy hr^{-1}) by days within the experiment	2.82×10^{-6}	2.47×10^{-6}	1.34	0.25
Concentration of nectar by days within the experiment	9.17×10^{-6}	1.15×10^{-5}	0.82	0.38
Concentration of nectar by dose rate (μGy hr^{-1})	2.24×10^{-6}	3.78×10^{-6}	0.40	0.53
Average temperature (°C) by humidity (%) during the days when the nectar measurements were made	4.71×10^{-4}	1.20×10^{-3}	0.12	0.38
Dose rate (μGy hr^{-1}) by average humidity during the days when the nectar measurements were made (%)	-1.98×10^{-6}	6.92×10^{-6}	0.08	0.35
Dose rate (μGy/hr^{-1}) by mass of bee at start of experiment (g)	9.69×10^{-6}	4.69×10^{-5}	0.08	0.73
Dose rate (μGy hr^{-1}) by average temperature during the days when the nectar measurements were made (°C)	7.11×10^{-6}	4.88×10^{-5}	0.02	0.88

Table S2.19. Parameter estimates for model investigating the effect of radiation dose rate on bumblebee dry weight. The response variable (grams) was square root transformed. All variables except dose rate and days were mean centered and scaled by the standard deviation. For this model one standard deviation of bumblebee mass of bee at start of the experiment is 1.04 g. Model was linear mixed effects with normally distributed errors. Multiple measures were made on 141 bumblebees during these observations. Table S2.19a describes minimal model used. Table S2.19b describes terms removed from the model in reverse order of deletion during model simplification.

a. Minimal Model				
Predictors	**Estimate**	**SE**	χ^2	**P Value**
(Intercept)	4.24×10^{-2}	5.51×10^{-4}	-	-
Concentration of nectar	1.56×10^{-4}	4.87×10^{-5}	19.77	9.93×10^{-6}
Dose rate (μGy hr^{-1})	1.13×10^{-5}	6.98×10^{-6}	2.11	0.11
Mass of bee at start of experiment (g)	7.22×10^{-3}	5.32×10^{-4}	470.57	$< 2.2 \times 10^{-16}$
Dose rate (μGy hr^{-1}) by mass of bee at start of experiment (g)	3.03×10^{-5}	7.02×10^{-6}	18.71	1.76×10^{-5}
b. Terms removed from model in reverse order of deletion				
Concentration of nectar by dose rate (μGy hr^{-1})	-1.89×0^{-7}	5.96×10^{-7}	0.10	0.75

Table S2.20. Parameter estimates for model investigating the effect of radiation dose rate on bumblebee thorax temperature. The response variable (degrees centigrade) was square root transformed. All variables except dose rate and days were mean centered and scaled by the standard deviation. For this model one standard deviation

of bumblebee mass of bee at start of the experiment 1.04 g. Model was linear mixed effects with normally distributed errors. Multiple measures were made on 141 bumblebees during these observations. Table S2.20a describes minimal model used. Table S2.20b describes terms removed from the model in reverse order of deletion during model simplification.

a. Minimal Model				
Predictors	**Estimate**	**SE**	χ^2	**P Value**
(Intercept)	26.22	0.05	-	-
b. Terms removed from model in reverse order of deletion				
Dose rate (μGy hr^{-1})	$1.57x10^{-3}$	$2.04x10^{-5}$	2.54	0.11
Mass of bee (g)	0.06	0.04	2.28	0.13
Concentration of nectar	$4.00x10^{-3}$	$3.00x10^{-3}$	1.39	0.24
Days within the experiment	$3.00x10^{-3}$	0.01	0.47	0.50
Thorax width of bee (mm)	$-1.00x10^{-3}$	0.05	0.05	0.83
Dose rate (μGy hr^{-1}) by thorax width of bee (mm)	$9.59x10^{-4}$	$7.15x10^{-4}$	1.64	0.24
Dose rate (μGy hr^{-1}) by bee mass (g)	$-1.00x10^{-3}$	$8.71x10^{-4}$	2.23	0.14
Concentration of nectar by days in experiment	$5.33x10^{-4}$	$4.11x10^{-4}$	1.71	0.19
Concentration of nectar by dose rate (μGy hr^{-1})	$-5.70x10^{-5}$	$5.69x10^{-5}$	0.80	0.37
Days in experiment by dose rate (μGy hr^{-1})	$5.46x10^{-5}$	$8.16x10^{-5}$	0.46	0.50
Mass of bee (g) by thorax width (mm)	$3.00x10^{-3}$	0.04	0.01	0.95
Days in experiment by dose rate (μGy hr^{-1}) by concentration of nectar	$5.46x10^{-6}$	$8.16x10^{-6}$	0.97	0.49

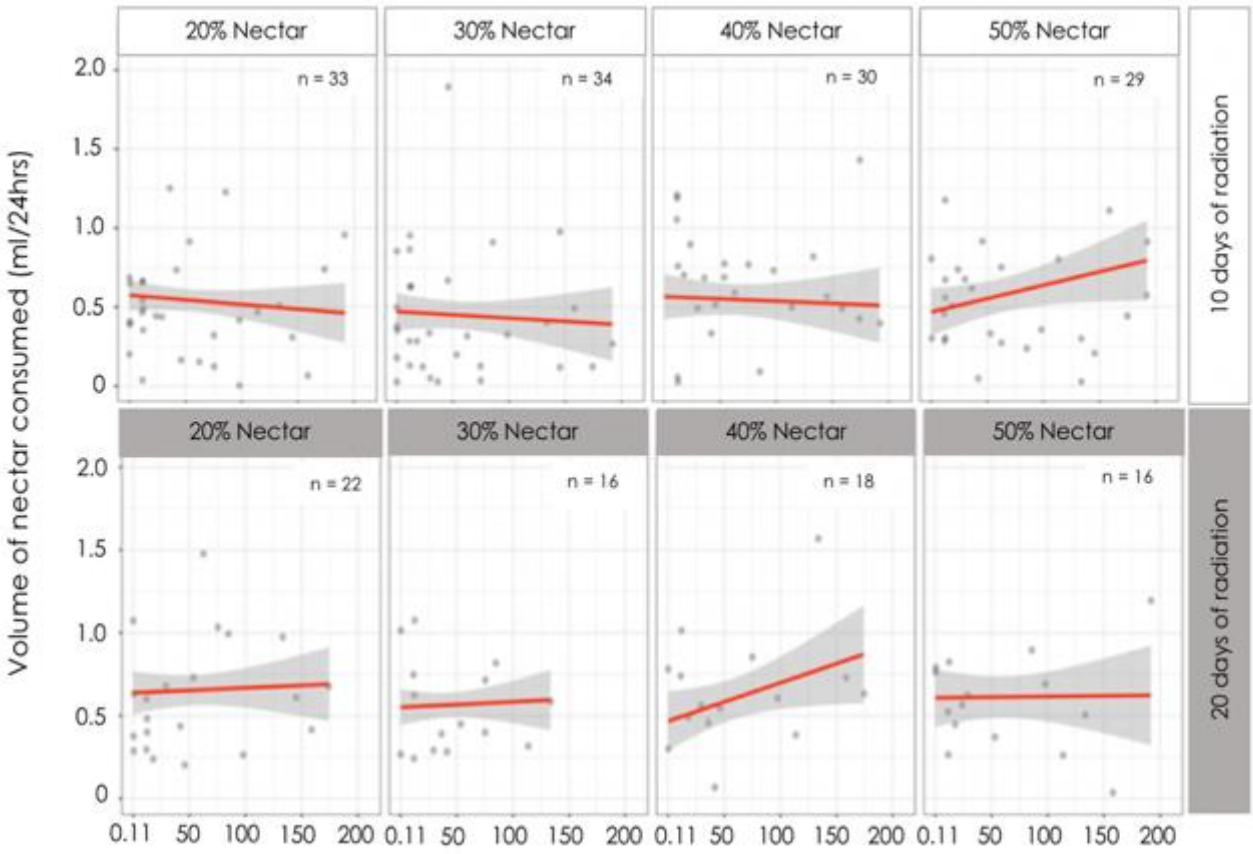


Figure S2.3 The volume of nectar consumed for bees provided with a nectar concentration of either 20%, 30%, 40% or 50% whilst exposed to a gradient of radiation exposure. The top four panels represent the model estimates for volume of nectar consumed on day 10, following 10 days of radiation exposure. The bottom four panels represent the model estimates for volume of nectar consumed at day 20 after a further 10 days of radiation exposure. Lines denote model fit. Points on each graph represent raw data values from each bee measured. The full model from which this was calculated was the minimal model presented in Table S16. However, for this figure nectar concentration was fitted as a fixed factor rather than a covariate to enable independent estimates of the radiation effect for each panel.

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8.2 Appendix Chapter 3: The biochemical and nutritional consequences of increased metabolic activity as a result of radiation exposure in bumblebees

Table S3.1. Parameter estimates for models investigating the effect of radiation dose rate and resource limitation treatment on the weight change of bumblebees between day 10 and day 14 of the experiment. Dose rates were 200, 100, 40 μGy hr^{-1} and controls (0.11 μGy hr^{-1}). Model was linear mixed effects with normally distributed errors. Model was selected using AIC model selection. Table S3.1a describes the minimal model used. Table S3.1b contains terms removed from the model in reverse order of deletion during model simplification. In the minimal model the P value of single variables was calculated by removing any interaction term it was also found within.

a. Minimal Model				
Predictors	**Estimate**	**SE**	χ^2	**P Value**
(Intercept)	0.027	0.004	-	-
Resource limitation treatment	-0.007	0.003	5.356	0.021
b. Terms removed from model in reverse order of deletion				
Dose rate (μGy hr^{-1})	-	-	0.359	0.783
40 μGy hr^{-1}	-0.004	0.004	-	-
100 μGy hr^{-1}	-0.003	0.004	-	-
200 μGy hr^{-1}	-0.002	0.005	-	-
Dose rate (μGy hr^{-1}) by resource limitation treatment	-	-	0.232	0.630
40 μGy hr^{-1}	-0.007	0.008	-	-
100 μGy hr^{-1}	-0.014	0.008	-	-
200 μGy hr^{-1}	-0.003	0.009	-	-
Average humidity during the days when the weight measurements were made (%)	0.003	0.001	0.175	0.798
Average temperature during the days when the weight measurements were made (°C)	0.001	0.001	0.198	0.801

Table S3.2. Parameter estimates for a MANOVA models investigating the effect of radiation dose rate and resource limitation treatment on physiological and biochemical changes in bumblebees. Dose rates were 200, 100, 40 μGy hr^{-1} and controls (0.11 μGy hr^{-1}). All predictors were mean centered and scaled by the standard deviation. Model was multivariant analysis of variance with normally distributed errors. The interaction between dose rate and resource acquisition, whether a bee was in a resource limited or abundant treatment group, as well as the temperature and humidity of the experimental facility were removed during model simplification. Table Sa describes the minimal model used. Table Sb contains terms removed from the model in reverse order of deletion during model simplification. Each below table is generated from one MANOVA test for each predictor.

Table S3.2a. Parameter estimates for a MANOVA models investigating the effect of radiation dose rate and resource limitation treatment on the volume of sucrose (ml) consumed by bumblebees on day 14 of the experiment.

a. MANOVA Model				
Predictors	**Estimate**	**SE**	**t Value**	**P Value**
(Intercept)	-0.090	0.064	-1.404	0.162
Dose rate (μGy hr^{-1})	-	-	-	-
40 μGy hr^{-1}	0.074	0.034	2.188	0.029
100 μGy hr^{-1}	0.155	0.034	4.554	9.77×10^{-6}
200 μGy hr^{-1}	0.337	0.032	10.381	$< 2 \times 10^{-16}$
Resource limitation treatment	-0.047	0.024	-1.941	0.054
Mass of bee at start of experiment (g)	0.106	0.187	0.569	0.570
Terms removed from model in reverse order of deletion				
Dose rate (μGy hr^{-1}) by resource limitation treatment	-	-	-	-

40 μGy hr^{-1}	-0.079	0.067	-1.176	0.241
100 μGy hr^{-1}	-0.119	0.067	-1.765	0.093
200 μGy hr^{-1}	-0.054	0.064	-0.833	0.406
Average humidity of the experimental facility (%)	0.011	0.044	0.250	0.803
Average temperature of the experimental facility (°C)	0.126	0.187	0.675	0.500

Table S3.2b. Parameter estimates for a MANOVA models investigating the effect of radiation dose rate and resource limitation treatment on the metabolic rate of bumblebees.

b. MANOVA Model				
Predictors	**Estimate**	**SE**	**t Value**	**P Value**
(Intercept)	-5.752	4.813	-1.195	0.234
Dose rate (μGy hr^{-1})	-	-	-	-
40 μGy hr^{-1}	-1.691	2.537	-0.666	0.506
100 μGy hr^{-1}	-0.129	2.558	-0.050	0.959
200 μGy hr^{-1}	2.143	2.447	1.693	0.002
Resource limitation treatment	2.489	1.834	1.357	0.176
Mass of bee at start of experiment (g)	7.123	4.115	0.505	0.614
Terms removed from model in reverse order of deletion				
Dose rate (μGy hr^{-1}) by resource limitation treatment	-	-	-	-
40 μGy hr^{-1}	-1.971	1.347	-0.775	0.439
100 μGy hr^{-1}	-1.366	1.115	-0.654	0.514
200 μGy hr^{-1}	-0.585	0.985	-1.137	0.257
Average humidity of the experimental facility (%)	0.089	0.046	0.321	0.538
Average temperature of the experimental facility (°C)	0.543	0.198	0.611	0.627

Table S3.2c. Parameter estimates for a MANOVA models investigating the effect of radiation dose rate and resource limitation treatment on the standard mass index (SMI) of bumblebees.

c.MANOVA Model				
Predictors	**Estimate**	**SE**	**t Value**	**P Value**
(Intercept)	-0.551	0.096	-5.740	$4.04x10^{-8}$
Dose rate (μGy hr^{-1})	-	-	-	-
40 μGy hr^{-1}	0.010	0.051	0.123	0.902
100 μGy hr^{-1}	-0.016	0.051	-0.310	0.757
200 μGy hr^{-1}	-0.010	0.048	-0.202	0.840
Resource limitation treatment	0.023	0.037	0.620	0.536
Mass of bee at start of experiment (g)	2.282	0.282	8.102	$8.53x10^{-14}$
Terms removed from model in reverse order of deletion				
Dose rate (μGy hr^{-1}) by resource limitation treatment	-	-	-	-
40 μGy hr^{-1}	0.084	0.102	0.828	0.410
100 μGy hr^{-1}	0.049	0.103	0.477	0.634
200 μGy hr^{-1}	0.035	0.098	0.363	0.717
Average humidity of the experimental facility (%)	0.051	0.031	0.516	0.535
Average temperature of the experimental facility (°C)	-0.003	0.012	0.456	0.793

Table S3.2d. Parameter estimates for a MANOVA models investigating the effect of radiation dose rate and resource limitation treatment on the amount of lipids in bumblebee tissue.

d. MANOVA Model				
Predictors	**Estimate**	**SE**	**t Value**	**P Value**
(Intercept)	0.004	0.027	0.161	0.872

Dose rate (μGy hr^{-1})	-	-	-	-
40 μGy hr^{-1}	0.003	0.014	0.188	0.851
100 μGy hr^{-1}	0.016	0.014	1.155	0.250
200 μGy hr^{-1}	0.024	0.013	1.842	0.067
Resource limitation treatment	-0.001	0.011	-0.064	0.949
Mass of bee at start of experiment (g)	-0.068	0.078	-0.875	0.383
Terms removed from model in reverse order of deletion				
Dose rate (μGy hr^{-1}) by resource limitation treatment	-	-	-	-
40 μGy hr^{-1}	-0.002	0.028	-0.060	0.952
100 μGy hr^{-1}	-0.010	0.028	-0.360	0.719
200 μGy hr^{-1}	0.022	0.027	0.810	0.419
Average humidity of the experimental facility (%)	0.005	0.045	0.112	0.911
Average temperature of the experimental facility (°C)	0.031	0.045	0.692	0.490

Table S3.2e. Parameter estimates for a MANOVA models investigating the effect of radiation dose rate and resource limitation treatment on the amount of carbohydrates in bumblebee tissue.

e. MANOVA Model				
Predictors	**Estimate**	**SE**	**t Value**	**P Value**
(Intercept)	-0.158	0.169	-0.934	0.351
Dose rate (μGy hr^{-1})	-	-	-	-
40 μGy hr^{-1}	0.095	0.089	1.064	0.289
100 μGy hr^{-1}	0.155	0.090	1.727	0.086
200 μGy hr^{-1}	0.050	0.086	0.584	0.560
Resource limitation treatment	-0.134	0.065	2.071	0.040
Mass of bee at start of experiment (g)	-0.107	0.497	-0.214	0.831
Terms removed from model in reverse order of deletion				
Dose rate (μGy hr^{-1}) by resource limitation treatment	-	-	-	-
40 μGy hr^{-1}	-0.057	0.181	-0.317	0.752
100 μGy hr^{-1}	-0.095	0.182	-0.522	0.603
200 μGy hr^{-1}	-0.139	0.173	-0.804	0.422
Average humidity of the experimental facility (%)	0.262	0.277	0.947	0.345
Average temperature of the experimental facility (°C)	0.183	0.289	0.635	0.526

Table S3.2f. Parameter estimates for a MANOVA models investigating the effect of radiation dose rate and resource limitation treatment on the amount of glycogen in bumblebee tissue.

f. MANOVA Model				
Predictors	**Estimate**	**SE**	**t Value**	**P Value**
(Intercept)	-0.144	0.226	-0.639	0.524
Dose rate (μGy hr^{-1})	-	-	-	-
40 μGy hr^{-1}	0.161	0.119	1.355	0.177
100 μGy hr^{-1}	0.233	0.120	1.941	0.054
200 μGy hr^{-1}	0.202	0.115	1.758	0.081
Resource limitation treatment	0.136	0.086	1.578	0.116
Mass of bee at start of experiment (g)	-0.212	0.663	-0.320	0.749
Terms removed from model in reverse order of deletion				
Dose rate (μGy hr^{-1}) by resource limitation treatment	-	-	-	-
40 μGy hr^{-1}	-0.068	0.241	-0.286	0.775
100 μGy hr^{-1}	0.061	0.242	0.252	0.801
200 μGy hr^{-1}	0.059	0.231	0.258	0.797
Average humidity of the experimental facility (%)	0.123	0.159	0.776	0.439
Average temperature of the experimental facility (°C)	0.142	0.384	0.369	0.712

Table S3.2g. Parameter estimates for a MANOVA models investigating the effect of radiation dose rate and resource limitation treatment on the amount of protein in bumblebee tissue.

g. MANOVA Model				
Predictors	**Estimate**	**SE**	**t Value**	**P Value**
(Intercept)	-0.003	0.070	-0.047	0.963
Dose rate (μGy hr^{-1})	-	-	-	-
40 μGy hr^{-1}	-0.001	0.037	0.010	0.992
100 μGy hr^{-1}	-0.030	0.037	-0.813	0.417
200 μGy hr^{-1}	-0.041	0.036	-1.148	0.253
Resource limitation treatment	0.011	0.027	0.426	0.671
Mass of bee at start of experiment (g)	-0.082	0.206	-0.399	0.691
Terms removed from model in reverse order of deletion				
Dose rate (μGy hr^{-1}) by resource limitation treatment	-	-	-	-
40 μGy hr^{-1}	-0.129	0.074	-1.740	0.094
100 μGy hr^{-1}	-0.038	0.074	-0.518	0.605
200 μGy hr^{-1}	-0.066	0.071	-0.940	0.348
Average humidity of the experimental facility (%)	0.062	0.114	0.547	0.585
Average temperature of the experimental facility (°C)	0.030	0.118	0.257	0.797

Table S3.2h. Parameter estimates for a MANOVA models investigating the effect of radiation dose rate and resource limitation treatment on the amount of glucose in bumblebee haemolymph.

h. MANOVA Model				
Predictors	**Estimate**	**SE**	**t Value**	**P Value**
(Intercept)	-0.137	0.009	-15.912	$< 2x10^{-16}$
Dose rate (μGy hr^{-1})	-	-	-	-
40 μGy hr^{-1}	0.012	0.005	2.711	0.007
100 μGy hr^{-1}	0.026	0.005	5.799	$3.01x10^{-8}$
200 μGy hr^{-1}	0.039	0.004	8.866	$8.00x10^{-16}$
Resource limitation treatment	-0.003	0.003	-0.100	0.921
Mass of bee at start of experiment (g)	0.518	0.025	8.547	$< 2x10^{-16}$
Terms removed from model in reverse order of deletion				
Dose rate (μGy hr^{-1}) by resource limitation treatment	-	-	-	-
40 μGy hr^{-1}	0.004	0.009	0.451	0.653
100 μGy hr^{-1}	-0.003	0.009	-0.386	0.700
200 μGy hr^{-1}	-0.011	0.008	-1.254	0.212
Average humidity of the experimental facility (%)	0.014	0.056	0.321	0.567
Average temperature of the experimental facility (°C)	0.005	0.014	0.147	0.598

Table S3.2i. Parameter estimates for a MANOVA models investigating the effect of radiation dose rate and resource limitation treatment on the amount of sucrose in bumblebee haemolymph.

i. MANOVA Model				
Predictors	**Estimate**	**SE**	**t Value**	**P Value**
(Intercept)	-0.146	0.021	-14.995	$< 2x10^{-16}$
Dose rate (μGy hr^{-1})	-	-	-	-
40 μGy hr^{-1}	0.015	0.005	0.217	0.611
100 μGy hr^{-1}	0.017	0.003	0.311	0.433
200 μGy hr^{-1}	0.034	0.004	0.758	0.105
Resource limitation treatment	0.006	0.011	1.407	0.163
Mass of bee at start of experiment (g)	0.565	0.039	19.611	$< 2x10^{-16}$
Terms removed from model in reverse order of deletion				
Dose rate (μGy hr^{-1}) by resource limitation treatment	-	-	-	-
40 μGy hr^{-1}	-0.013	0.010	-1.260	0.209

100 μGy hr^{-1}	-0.018	0.010	-1.795	0.094
200 μGy hr^{-1}	-0.006	0.011	-0.598	0.550
Average humidity of the experimental facility (%)	0.134	0.059	0.159	0.432
Average temperature of the experimental facility (°C)	0.543	0.085	0.235	0.654

Table S3.2j. Parameter estimates for a MANOVA models investigating the effect of radiation dose rate and resource limitation treatment on the amount of fructose in bumblebee haemolymph.

j. MANOVA Model				
Predictors	**Estimate**	**SE**	**t Value**	**P Value**
(Intercept)	-0.135	0.010	-13.393	$< 2x10^{-16}$
Dose rate (μGy hr^{-1})	-	-	-	-
40 μGy hr^{-1}	0.010	0.005	1.859	0.065
100 μGy hr^{-1}	0.012	0.005	2.318	0.022
200 μGy hr^{-1}	0.022	0.005	4.264	$3.26x10^{-5}$
Resource limitation treatment	-0.001	0.004	-0.229	0.819
Mass of bee at start of experiment (g)	0.551	0.030	18.709	$< 2x10^{-16}$
Terms removed from model in reverse order of deletion				
Dose rate (μGy hr^{-1}) by resource limitation treatment	-	-	-	-
40 μGy hr^{-1}	0.008	0.011	0.764	0.446
100 μGy hr^{-1}	0.005	0.011	0.424	0.672
200 μGy hr^{-1}	0.013	0.010	1.314	0.191
Average humidity of the experimental facility (%)	0.019	0.016	1.139	0.256
Average temperature of the experimental facility (°C)	-0.002	0.017	-0.136	0.892

Table S3.2k. Parameter estimates for a MANOVA models investigating the effect of radiation dose rate and resource limitation treatment on the amount of trehalose in bumblebee haemolymph.

k. MANOVA Model				
Predictors	**Estimate**	**SE**	**t Value**	**P Value**
(Intercept)	-0.123	0.0106	-11.630	$< 2x10^{-16}$
Dose rate (μGy hr^{-1})	-	-	-	-
40 μGy hr^{-1}	0.001	0.005	0.157	0.876
100 μGy hr^{-1}	0.017	0.006	3.116	0.002
200 μGy hr^{-1}	0.024	0.005	4.403	$1.84x10^{-5}$
Resource limitation treatment	-0.001	0.004	-0.220	0.826
Mass of bee at start of experiment (g)	0.507	0.031	16.284	$< 2x10^{-16}$
Terms removed from model in reverse order of deletion				
Dose rate (μGy hr^{-1}) by resource limitation treatment	-	-	-	-
40 μGy hr^{-1}	-0.001	0.011	-0.110	0.913
100 μGy hr^{-1}	0.003	0.010	0.261	0.795
200 μGy hr^{-1}	-0.013	0.010	-1.260	0.209
Average humidity of the experimental facility (%)	0.145	0.059	0.125	0.578
Average temperature of the experimental facility (°C)	0.111	0.099	0.259	0.614

Table S3.2l. Parameter estimates for a MANOVA models investigating the effect of radiation dose rate and resource limitation treatment on the weight (g) of the bumblebee gut when its contents are removed.

l. MANOVA Model				
Predictors	**Estimate**	**SE**	**t Value**	**P Value**
(Intercept)	-0.017	0.012	-1.417	0.158
Dose rate (μGy hr^{-1})	-	-	-	-
40 μGy hr^{-1}	0.004	0.006	0.559	0.577
100 μGy hr^{-1}	-0.004	0.007	-0.666	0.506
200 μGy hr^{-1}	-0.009	0.006	-1.427	0.155

Resource limitation treatment	0.006	0.005	1.218	0.225
Mass of bee at start of experiment (g)	0.051	0.036	1.422	0.157
Terms removed from model in reverse order of deletion				
Dose rate (μGy hr^{-1}) by resource limitation treatment	-	-	-	-
40 μGy hr^{-1}	-0.019	0.013	-1.460	0.146
100 μGy hr^{-1}	-0.009	0.013	-0.677	0.499
200 μGy hr^{-1}	-0.013	0.012	-1.094	0.276
Average humidity of the experimental facility (%)	0.032	0.020	1.564	0.119
Average temperature of the experimental facility (°C)	0.009	0.020	0.452	0.651

Table S3.2m. Parameter estimates for a MANOVA models investigating the effect of radiation dose rate and resource limitation treatment on the weight (g) of the bumblebee gut contents.

m. MANOVA Model				
Predictors	**Estimate**	**SE**	**t Value**	**P Value**
(Intercept)	0.005	0.008	0.564	0.573
Dose rate (μGy hr^{-1})	-	-	-	-
40 μGy hr^{-1}	-0.005	0.004	-1.171	0.243
100 μGy hr^{-1}	0.005	0.005	1.126	0.262
200 μGy hr^{-1}	0.003	0.004	0.686	0.494
Resource limitation treatment	0.001	0.003	0.169	0.866
Mass of bee at start of experiment (g)	-0.023	0.025	-0.930	0.354
Terms removed from model in reverse order of deletion				
Dose rate (μGy hr^{-1}) by resource limitation treatment	-	-	-	-
40 μGy hr^{-1}	0.007	0.008	0.753	0.453
100 μGy hr^{-1}	0.004	0.009	0.418	0.676
200 μGy hr^{-1}	0.011	0.009	1.222	0.223
Average humidity of the experimental facility (%)	0.010	0.014	0.727	0.468
Average temperature of the experimental facility (°C)	-0.003	0.005	-0.487	0.627

Table S3.3. Parameter estimates for models investigating the effect of radiation dose rate and resource limitation treatment on the colour of bumblebee guts. The gut colour was graded visually on a scale of 1-5, with 1 being the lightest colour and 5 being the darkest colour. Dose rates were 200, 100, 40 μGy hr^{-1} and controls (0.11 μGy hr^{-1}). Model was an ordinal logistic regression model. Table S3.3a describes the minimal model used.

a. Minimal Model				
Predictors	**Estimate**	**SE**	**t Value**	**P Value**
(Intercept)	-0.573	0.475	-	-
b. Terms removed from model in reverse order of deletion				
Dose rate (μGy hr^{-1})	-	-	-0.213	0.831
40 μGy hr^{-1}	-0.081	0.377		
100 μGy hr^{-1}	0.107	0.375		
200 μGy hr^{-1}	0.044	0.372		
Resource limitation treatment	0.0418	0.374	0.116	0.911
Dose rate (μGy hr^{-1}) by resource limitation treatment	-	-	0.368	0.712
40 μGy hr^{-1}	0.199	0.538		
100 μGy hr^{-1}	-0.294	0.531	0.368	0.712
200 μGy hr^{-1}	-0.191	0.530	-0.553	0.579
			-0.359	0.719
Mass of bee at start of experiment (g)	4.052	1.630	2.485	0.012
Average humidity during the experimental period (%)	0.419	0.472	0.888	0.374
Average temperature during the experimental period (°C)	-0.573	0.475	-1.206	0.227

8.3 Appendix Chapter 4: The impacts of ecologically relevant radiation exposure on gut microbial community composition in bumblebees

Table S4.2. Parameter estimates for models investigating the effect of radiation on bumblebee gut microbiome species richness for the 'core' microbiome of bees exposed to 3 days of irradiation. This analysis includes the 12 most common OTUs (1.1% of reads per sample). Dose rates included were 200, 40 μGy hr^{-1} and controls. Model was linear with normally distributed errors. Table S4.2a describes the minimal model used. Table S4.2b contains terms removed from the model in reverse order of deletion during model simplification.

Species Richness					
a. Minimal Model					
Predictors		**Estimate**	**SE**	***F***	**P Value**
(Intercept)		8.664	0.435	-	-
Dose rate (μGy hr^{-1})		-	-	6.836	0.003
	40 μGy hr^{-1}	-0.600	0.615	-	-
	200 μGy hr^{-1}	1.600	0.615	-	-

Table S4.3. Parameter estimates for models investigating the effect of radiation on bumblebee gut microbiome species richness for the 'core' microbiome of bees exposed to 10 days of irradiation. This analysis includes the 12 'core' OTUs (1.1% of reads per sample). Dose rates included were 200, 40 μGy hr^{-1} and controls. Model was linear with normally distributed errors. Table S4.3a describes the minimal model used. Table S4.3b contains terms removed from the model in reverse order of deletion during model simplification.

Species Richness					
a. Minimal Model					
Predictors		**Estimate**	**SE**	***F***	**P Value**
(Intercept)		8.667	0.448	-	-
Dose rate (μGy hr^{-1})		-	-	2.012	0.147
	40 μGy hr^{-1}	1.267	0.634	-	-
	200 μGy hr^{-1}	0.733	0.634	-	-

Table S4.5. Parameter estimates for models investigating the effect of radiation on bumblebee gut microbiome using the Shannon diversity index. Each model analyses the 'core' bumblebee microbiome over 10 days within the experiment (1.1% of reads per sample). Dose rates included were 200, 40 μGy hr^{-1} and controls. Model was linear with normally distributed errors. Table S4.5a describes the minimal model used. Table S4.5b contains terms removed from the model in reverse order of deletion during model simplification.

Shannon Diversity					
a. Minimal Model					
Predictors		**Estimate**	**SE**	***F***	**P Value**
(Intercept)		1.022	0.080	-	-
Days within experiment (day 10)		-0.268	0.113	7.14	0.020
b. Terms removed from model in reverse order of deletion					
Dose rate (μGy hr^{-1})		-	-	0.315	0.672
	40 μGy hr^{-1}	-0.113	0.113	-	-
	200 μGy hr^{-1}	0.048	0.113	-	-
Dose rate (μGy hr^{-1}) by days within the experiment (day 10)		-	-	0.175	0.985
		0.153	0.160	-	-
	40 μGy hr^{-1}	0.002	0.160	-	-
	200 μGy hr^{-1}				

Table S4.6. Parameter estimates for models investigating the effect of radiation on bumblebee gut microbiome using the Shannon diversity index for rarer taxa. This analysis includes the 49 most common OTUs (0.0015% of reads per sample). Dose rates included were 200, 40 μGy hr^{-1} and controls. Model was linear with normally distributed errors. Table S4.6a describes the minimal model used. Table S4.6b contains terms removed from the model in reverse order of deletion during model simplification.

Simpsons Diversity				
a. Minimal Model				
Predictors	**Estimate**	**SE**	***F***	**P Value**
(Intercept)	1.018	0.048	-	-
Days within experiment (day 10)	-0.211	0.068	9.528	0.003
b. Terms removed from model in reverse order of deletion				
Dose rate (μGy hr^{-1})	-	-	0.459	0.633
40 μGy hr^{-1}	-0.028	0.084	-	-
200 μGy hr^{-1}	0.051	0.084	-	-
Dose rate (μGy hr^{-1}) by days within the experiment (day 10)	-	-	0.702	0.499
	0.171	0.169	-	-
40 μGy hr^{-1}	-0.004	0.169	-	-
200 μGy hr^{-1}				

Table S4.7. Parameter estimates for models investigating the effect of radiation on bumblebee gut microbiome using the Simpsons diversity index. Each model analyses the 'core' bumblebee microbiome over 10 days within the experiment (1.1% of reads per sample). Dose rates included were 200, 40 μGy hr^{-1} and controls. Model was linear with normally distributed errors. Table S4.7a describes the minimal model used. Table S4.7b contains terms removed from the model in reverse order of deletion during model simplification.

Simpsons Diversity				
a. Minimal Model				
Predictors	**Estimate**	**SE**	***F***	**P Value**
(Intercept)	0.568	0.025	-	-
Days within experiment (day 10)	-0.125	0.035	12.876	0.001
b. Terms removed from model in reverse order of deletion				
Dose rate (μGy hr^{-1})	-	-	0.368	0.693
40 μGy hr^{-1}	0.007	0.043	-	-
200 μGy hr^{-1}	0.035	0.043	-	-
Dose rate (μGy hr^{-1}) by days within the experiment (day 10)	-	-	0.804	0.451
	0.108	0.086	-	-
40 μGy hr^{-1}	0.039	0.086	-	-
200 μGy hr^{-1}				

Table S4.8. Parameter estimates for models investigating the effect of radiation on bumblebee gut microbiome using the Simpsons diversity index for rarer taxa. This analysis includes the 49 most common OTUs (0.0015% of reads per sample). Dose rates included were 200, 40 μGy hr^{-1} and controls. Model was linear with normally distributed errors. Table S4.8a describes the minimal model used. Table S4.8b contains terms removed from the model in reverse order of deletion during model simplification.

Simpsons Diversity				
a. Minimal Model				
Predictors	**Estimate**	**SE**	***F***	**P Value**
(Intercept)	1.038	0.084	-	-
Days within experiment (day 10)	-0.267	0.119	9.715	0.028
b. Terms removed from model in reverse order of deletion				
Dose rate (μGy hr^{-1})	-	-	0.446	0.656
40 μGy hr^{-1}	-0.114	0.119	-	-
200 μGy hr^{-1}	0.053	0.119	-	-

Dose rate (μGy hr^{-1}) by days within the experiment (day 10)	-	-	0.023	0.982
	0.171	0.169	-	-
40 μGy hr^{-1}	-0.004	0.169	-	-
200 μGy hr^{-1}				

Table S4.9. Parameter estimates for ANCOMBC analysis of the effect of radiation exposure on the differential abundance of the 12 most common OTUs in the bumblebee microbiome in comparison to controls. ANCOMBC was conducted for bumblebees that were within the experiment for 3 days. OTUs were selecyed as they represent the core microbiome of the bumblebees studied (1.1% of reads per sample). Analysis compares dose rate treatments of 40 μGy hr^{-1} (40) and 200 μGy hr^{-1} (200) to control bumblebees. Model output includes model coefficient (Coef), standard error (Se), test statistic (W), P value and adjusted P value. The differential abundance (Diff abund) column indicates whether the taxon is differentially abundant (TRUE) or not (FALSE). Any OTUs identified as significantly differentially abundant are highlighted in yellow.

OTU	Coef (200)	Coef (40)	Se (200)	Se (40)	W (200)	W (40)	P Value (200)	P Value (40)	Adj. P Value (200)	Adj. P Value (40)	Diff abund (200)	Diff abund (40)
Gilliamella apicola	0.149	-0.247	0.075	0.062	0.313	-0.534	0.755	0.593	1	1	FALSE	FALSE
Snodgrassella alvi	0.316	-0.113	0.171	0.212	0.850	-0.273	0.395	0.784	1	1	FALSE	FALSE
Klebsiella sp.	1.685	1.343	1.011	1.031	1.667	1.301	0.096	0.193	0.765	1	FALSE	FALSE
Lactobacillus	-1.076	-0.615	0.179	0.156	-1.580	-1.107	0.113	0.268	0.792	1	FALSE	FALSE
Paenibacillus sp.	-0.287	2.855	0.150	0.148	-0.637	3.815	0.524	1.36×10^{-4}	1	0.002	FALSE	TRUE
Bombiscardovia sp.	0.674	-1.254	0.998	1.052	0.676	-1.192	0.499	0.233	1	1	FALSE	FALSE
Cyanobacteria	0.129	1.056	0.383	0.399	0.189	1.320	0.850	0.187	1	1	FALSE	FALSE
Pseudomonas	-1.793	-0.396	0.233	0.216	-4.143	-0.553	3.42×10^{-5}	0.580	4.10×10^{-4}	1	TRUE	FALSE
Pseudoxanthomonas sp.	-1.628	-0.035	0.508	0.725	-3.208	-0.048	0.001	0.961	0.015	1	TRUE	FALSE
Lactobacillaceae sp.	0.287	1.433	0.330	0.666	0.871	2.151	0.384	0.0314	1	0.346	FALSE	FALSE
Lactobacillus bombi	1.474	0.695	0.513	0.601	2.876	1.157	0.004	0.247	0.040	1	TRUE	FALSE
Acinetobacter sp.	-0.602	0.721	0.347	0.676	-1.734	1.067	0.083	0.286	0.745	1	FALSE	FALSE

Table S4.10. Parameter estimates for ANCOMBC analysis of the effect of radiation exposure on the differential abundance of the 12 most common OTUs in the bumblebee microbiome in comparison to controls. ANCOMBC was conducted for bumblebees that were within the experiment for 10 days. OTUs were selecyed as they represent the core microbiome of the bumblebees studied (1.1% of reads per sample). Analysis compares dose rate treatments of 40 μGy hr^{-1} (40) and 200 μGy hr^{-1} (200) to control bumblebees. Model output includes model coefficient (Coef), standard error (Se), test statistic (W), P value and adjusted P value. The differential abundance (Diff abund) column indicates whether the taxon is differentially abundant (TRUE) or not (FALSE). Any OTUs identified as significantly differentially abundant are highlighted in yellow.

OTU	Coef (200)	Coef (40)	Se (200)	Se (40)	W (200)	W (40)	P Value (200)	P Value (40)	Adj. P Value (200)	Adj. P Value (40)	Diff abund (200)	Diff abund (40)
Gilliamella apicola	-0.141	-0.719	0.034	0.134	-0.325	-1.347	0.746	0.178	0.004	1.000	TRUE	FALSE
Snodgrassella alvi	0.441	0.212	0.173	0.199	1.183	0.530	0.237	0.596	1.000	1.000	FALSE	FALSE
Klebsiella sp.	-0.619	0.136	0.449	0.090	-0.729	0.137	0.466	0.891	1.000	1.000	FALSE	FALSE
Lactobacillus	-0.672	-0.280	0.555	0.646	-1.212	-0.433	0.226	0.665	1.000	1.000	FALSE	FALSE
Paenibacillus sp.	0.393	0.434	0.322	0.152	0.931	0.785	0.352	0.432	1.000	1.000	FALSE	FALSE
Bombiscardovia sp.	1.674	1.206	0.763	0.641	1.739	1.281	0.082	0.200	0.903	1.000	FALSE	FALSE
Cyanobacteria	-0.348	-0.183	0.336	0.345	-0.798	-0.336	0.425	0.737	1.000	1.000	FALSE	FALSE
Pseudomonas	-0.536	-0.311	0.254	0.455	-0.820	-0.475	0.412	0.635	1.000	1.000	FALSE	FALSE
Pseudoxanthomonas sp.	-0.589	-0.405	0.748	0.686	-0.788	-0.590	0.431	0.555	1.000	1.000	FALSE	FALSE
Lactobacillaceae sp.	-0.017	0.029	0.340	0.343	-0.050	0.086	0.960	0.932	1.000	1.000	FALSE	FALSE
Lactobacillus bombi	-0.826	-1.659	0.037	0.117	-1.122	-2.313	0.262	0.021	0.032	0.249	TRUE	FALSE
Acinetobacter sp.	0.623	0.277	0.335	0.312	1.864	0.886	0.062	0.376	0.749	1.000	FALSE	FALSE

Table S4.11. Parameter estimates for ANCOMBC analysis of the effect of radiation exposure on the bumblebee microbiome when pooling data to compare radiated (40 μGy hr^{-1} and 200 μGy hr^{-1}) to non-irradiated (controls). Analysis was conducted on the differential abundance of the 12 most common OTUs in the bumblebee microbiome for bumblebees that were within the experiment for 3 days. OTUs were selected as they represent the core microbiome of the bumblebees studied (1.1% of reads per sample). Analysis compares dose rate treatments of 40 μGy hr^{-1} (40) and 200 μGy hr^{-1} (200) to control bumblebees. Model output includes model coefficient (Coef), standard error (Se), test statistic (W), P value and adjusted P value. The differential abundance (Diff abund) column indicates whether the taxon is differentially abundant (TRUE) or not (FALSE). Any OTUs identified as significantly differentially abundant are highlighted in yellow.

OTU	Coef (Radiated)	Se (Radiated)	W (Radiated)	P Value (Radiated)	Adj. P Value (Radiated)	Diff abund (Radiated)
Gilliamella apicola	-0.085	0.412	-0.206	0.837	1.000	FALSE
Snodgrassella alvi	0.066	0.364	0.181	0.857	1.000	FALSE
Klebsiella sp.	1.478	0.944	1.566	0.117	0.821	FALSE
Lactobacillus	-0.881	0.508	-1.734	0.083	0.663	FALSE
Paenibacillus sp.	1.249	0.556	2.246	0.025	0.296	FALSE
Bombiscardovia sp.	-0.326	0.906	-0.359	0.719	1.000	FALSE
Cyanobacteria	0.557	0.613	0.908	0.364	1.000	FALSE
Pseudomonas	-1.130	0.510	-2.218	0.026	0.296	FALSE
Pseudoxanthomonas sp.	-0.867	0.566	-1.531	0.126	0.821	FALSE
Lactobacillaceae sp.	0.825	0.435	1.897	0.057	0.520	FALSE
Lactobacillus bombi	1.049	0.486	2.156	0.031	0.311	FALSE
Acinetobacter sp.	0.0241	0.449	0.054	0.957	1.000	FALSE

Table S4.12. Parameter estimates for ANCOMBC analysis of the effect of radiation exposure on the bumblebee microbiome when pooling data to compare radiated (40 μGy hr^{-1} and 200 μGy hr^{-1}) to non-irradiated (controls). Analysis was conducted on the differential abundance of the 12 most common OTUs in the bumblebee microbiome for bumblebees that were within the experiment for 10 days. OTUs were selected as they represent the core microbiome of the bumblebees studied (1.1% of reads per sample). Analysis compares dose rate treatments of 40 μGy hr^{-1} (40) and 200 μGy hr^{-1} (200) to control bumblebees. Model output includes model coefficient (Coef), standard error (Se), test statistic (W), P value and adjusted P value. The differential abundance (Diff abund) column indicates whether the taxon is differentially abundant (TRUE) or not (FALSE). Any OTUs identified as significantly differentially abundant are highlighted in yellow.

OTU	Coef (Radiated)	Se (Radiated)	W (Radiated)	P Value (Radiated)	Adj. P Value (Radiated)	Diff abund (Radiated)
Gilliamella apicola	-0.497	0.435	-1.142	0.254	1.000	FALSE
Snodgrassella alvi	0.259	0.333	0.779	0.436	1.000	FALSE
Klebsiella sp.	-0.308	0.814	-0.379	0.705	1.000	FALSE
Lactobacillus	-0.543	0.527	-1.029	0.303	1.000	FALSE
Paenibacillus sp.	0.347	0.391	0.886	0.376	1.000	FALSE
Bombiscardovia sp.	1.373	0.801	1.714	0.086	0.951	FALSE
Cyanobacteria	-0.332	0.406	-0.818	0.413	1.000	FALSE
Pseudomonas	-0.490	0.563	-0.869	0.385	1.000	FALSE
Pseudoxanthomonas sp.	-0.564	0.613	-0.919	0.358	1.000	FALSE
Lactobacillaceae sp.	-0.061	0.318	-0.190	0.849	1.000	FALSE
Lactobacillus bombi	-1.309	0.701	-1.866	0.062	0.744	FALSE
Acinetobacter sp.	0.383	0.291	1.316	0.188	1.000	FALSE

Table S4.13. Parameter estimates for ANCOMBC analysis of the effect of radiation exposure on the bumblebee microbiome when pooling data for controls and the lowest rate studied to compare non-irradiated (0.11 μGy hr^{-1} and 40 μGy hr^{-1}) to radiated (200 μGy hr^{-1}) bumblebees. Analysis was conducted on the differential abundance of the 12 most common OTUs in the bumblebee microbiome for bumblebees that were within the experiment for 3 days. OTUs were selected as they represent the core microbiome of the bumblebees studied (1.1% of reads per sample). Analysis compares dose rate treatments of 40 μGy hr^{-1} (40) and 200 μGy hr^{-1} (200) to control bumblebees. Model output includes model coefficient (Coef), standard error (Se), test statistic (W), P value and adjusted P value. The differential abundance (Diff abund) column indicates whether the taxon is differentially abundant (TRUE) or not (FALSE). Any OTUs identified as significantly differentially abundant are highlighted in yellow.

OTU	Coef (Radiated)	Se (Radiated)	W (Radiated)	P Value (Radiated)	Adj. P Value (Radiated)	Diff abund (Radiated)
Gilliamella apicola	-1.138	0.405	-2.805	0.005	0.050	FALSE
Snodgrassella alvi	-1.087	0.328	-3.307	0.001	0.011	TRUE
Klebsiella sp.	-0.315	0.787	-0.401	0.688	1.000	FALSE
Lactobacillus	-0.893	0.550	-1.627	0.104	0.830	FALSE
Paenibacillus sp.	2.182	0.700	3.118	0.002	0.020	TRUE
Bombiscardovia sp.	-2.408	0.932	-2.583	0.010	0.088	FALSE
Cyanobacteria	0.175	0.747	0.233	0.815	1.000	FALSE
Pseudomonas	-0.316	0.654	-0.483	0.629	1.000	FALSE
Pseudoxanthomonas sp.	-0.037	0.617	-0.060	0.952	1.000	FALSE
Lactobacillaceae sp.	0.473	0.614	0.770	0.441	1.000	FALSE
Lactobacillus bombi	-0.859	0.559	-1.537	0.124	0.869	FALSE
Acinetobacter sp.	0.206	0.621	0.331	0.740	1.000	FALSE

Table S4.14. Parameter estimates for ANCOMBC analysis of the effect of radiation exposure on the bumblebee microbiome when pooling data for controls and the lowest rate studied to compare non-irradiated (0.11 μGy hr^{-1} and 40 μGy hr^{-1}) to radiated (200 μGy hr^{-1}) bumblebees. Analysis was conducted on the differential abundance of the 12 most common OTUs in the bumblebee microbiome for bumblebees that were within the experiment for 10 days. OTUs were selected as they represent the core microbiome of the bumblebees studied (1.1% of reads per sample). Analysis compares dose rate treatments of 40 μGy hr^{-1} (40) and 200 μGy hr^{-1} (200) to control bumblebees. Model output includes model coefficient (Coef), standard error (Se), test statistic (W), P value and adjusted P value. The differential abundance (Diff abund) column indicates whether the taxon is differentially abundant (TRUE) or not (FALSE). Any OTUs identified as significantly differentially abundant are highlighted in yellow.

OTU	Coef (Rad)	Se (Rad)	W (Rad)	P Value (Rad)	Adj. P Value (Rad)	Diff abund (Rad)
Gilliamella apicola	-0.603	0.442	-1.364	0.172	1.000	FALSE
Snodgrassella alvi	0.037	0.352	0.106	0.915	1.000	FALSE
Klebsiella sp.	0.492	0.835	0.589	0.556	1.000	FALSE
Lactobacillus	0.103	0.555	0.186	0.853	1.000	FALSE
Paenibacillus sp.	0.284	0.536	0.530	0.596	1.000	FALSE
Bombiscardovia sp.	0.415	0.876	0.473	0.636	1.000	FALSE
Cyanobacteria	0.037	0.510	0.073	0.942	1.000	FALSE
Pseudomonas	0.004	0.575	0.006	0.995	1.000	FALSE
Pseudoxanthomonas sp.	-0.064	0.614	-0.104	0.917	1.000	FALSE
Lactobacillaceae sp.	0.084	0.247	0.341	0.732	1.000	FALSE
Lactobacillus bombi	-1.199	0.457	-2.621	0.009	0.105	FALSE
Acinetobacter sp.	0.011	0.257	0.044	0.965	1.000	FALSE

Table S4.15. Parameter estimates for the goodness of fit from NMDS analysis of the effect of radiation exposure on bumblebee microbiome diversity. The left table represents data recorded for bumblebees exposed to 3 days of radiation. The right table represents data recorded for bumblebees exposed to 10 days of radiation.

Goodness of Fit						
Day 3				**Day 10**		
Predictors	**R^2**	**P Value**	**Stress**	**R^2**	**P Value**	**Stress**
Dose rate (μGy hr^{-1})	0.0315	0.542	0.104	0.0442	0.38	0.168

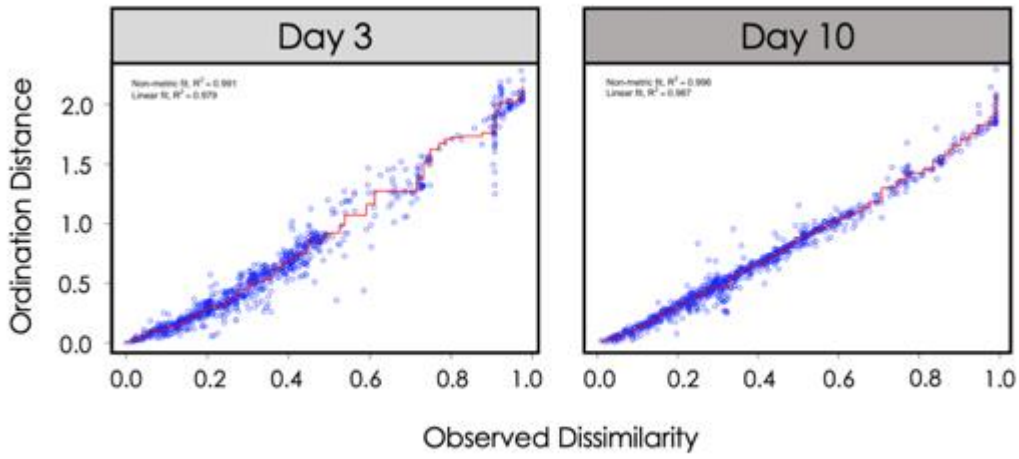

Figure S4.1. Shepard diagram revealing stress of bumblebee gut microbiota diversity when inputted in to NMDS analysis. Minimum stress= 0.03 and maximum stress= 0.20 from 20 random starts. Data is represented for bumblebees within the experiment for three days (left) and ten days (right).

Table S4.16. Parameter estimates for models investigating the effect of radiation on bumblebee gut microbiome using the Bray Curtis index. Each model analyses the 'core' bumblebee microbiome over 10 days within the experiment (1.1% of reads per sample). Dose rates included were 200, 40 μGy hr^{-1} and controls. Model was linear with normally distributed errors. Table S4.16a describes the minimal model used. Table S4.16b contains terms removed from the model in reverse order of deletion during model simplification.

Bray Curtis				
a. Minimal Model				
Predictors	**Estimate**	**SE**	***t***	**P Value**
(Intercept)	0.295	0.66	-	-
b. Terms removed from model in reverse order of deletion				
Days within experiment (day 10)	0.106	0.035	1.844	0.543
Dose rate (μGy hr^{-1})	-	-	-0.368	0.183
40 μGy hr^{-1}	-0.002	0.035	-	-
200 μGy hr^{-1}	0.001	0.035	-	-
Dose rate (μGy hr^{-1}) by days within the experiment (day 10)	-	-	0.804	0.546
	0.521	0.099	-	-
40 μGy hr^{-1}	0.533	0.099	-	-
200 μGy hr^{-1}				

Table S4.17. Parameter estimates for models investigating the effect of radiation on bumblebee gut microbiome using the Bray Curtis Index for rarer taxa. This analysis includes the 49 most common OTUs (0.0015% of reads per sample). Dose rates included were 200, 40 μGy hr^{-1} and controls. Model was linear with normally distributed errors. Table S4.17a describes the minimal model used. Table S4.17b contains terms removed from the model in reverse order of deletion during model simplification

Bray Curtis				
a. Minimal Model				
Predictors	**Estimate**	**SE**	***t***	**P Value**
(Intercept)	0.165	0.065	-	-
b. Terms removed from model in reverse order of deletion				
Days within experiment (day 10)	-0.24	0.067	-1.957	0.643
Dose rate (μGy hr^{-1})	-	-	0.317	0.432
40 μGy hr^{-1}	0.351	0.098	-	-
200 μGy hr^{-1}	0.614	0.098	-	-
Dose rate (μGy hr^{-1}) by days within the experiment (day 10)	-	-	0.165	0.858
	0.787	0.121	-	-
40 μGy hr^{-1}	0.366	0.121	-	-
200 μGy hr^{-1}				

8.4 Appendix Chapter 5: Levels of radiation exposure similar to those found in the Chernobyl Exclusion Zone cause reduced fecundity and developmental success in *Drosophila melanogaster*

Experiment 1: The effect of radiation on D. melanogaster fecundity

Table S5.1c. A comparison for models investigating the effect of radiation dose rate on the number of eggs produced by *Drosophila melanogaster* breeding pairs over 30 days. Models were selected using AIC model selection. The model with the lowest AIC value was selected (highlighted in green). All models had the same random effects which include days within the experiment and the identifying code of each set of fly pairs.

Model Selection	**AIC**
Number of Eggs ~ dose + poly(day,1) + temperature +humidity + dose : day + temperature : humidity + temperature : dose + humidity : dose	
Random Effects: (Day | ID)	11018.29
Number of Eggs ~ dose + poly(day,2) + temperature +humidity + dose : day + temperature : humidity + temperature : dose + humidity : dose	
Random Effects: (Day | ID)	10848.11
Number of Eggs ~ dose + poly(day,3) + temperature +humidity + dose : day + temperature : humidity + temperature : dose + humidity : dose	
Random Effects: (Day | ID)	10805.55
Number of Eggs ~ dose + poly(day,1) + temperature +humidity + dose : poly(day,1) + temperature : humidity + temperature : dose + humidity : dose	
Random Effects: (Day | ID)	10994.68
Number of Eggs ~ dose + poly(day,2) + temperature +humidity + dose : poly(day,2) + temperature : humidity + temperature : dose + humidity : dose	
Random Effects: (Day | ID)	10813.38
Number of Eggs ~ dose + poly(day,3) + temperature +humidity + dose : poly(day,3) + temperature : humidity + temperature : dose + humidity : dose	
Random Effects: (Day | ID)	10741.57

Table S5.2. Parameter estimates for models investigating the effect of radiation dose rate on the number of eggs produced by *Drosophila melanogaster* breeding pairs for the first three days of radiation exposure. Dose rates were 200, 40 μGy hr^{-1} and controls (0.11 μGy hr^{-1}). The environmental variables, temperature and humidity were mean centered and scaled by the standard deviation. For this model one standard deviation of temperature is 0.04°C and humidity is 3.25%. Model was linear mixed effects with normally distributed errors. A total of two measures were made on the 180 breeding pairs during these observations. Table S5.2a describes the minimal model used. Table S5.2b contains terms removed from the model in reverse order of deletion during model simplification. In the minimal model the P value of single variables was calculated by removing any interaction term it was also found within.

a. Minimal Model					
Predictors		**Estimate**	**SE**	χ^2	**P Value**
(Intercept)		26.89	1.39	-	-
Dose rate (μGy hr^{-1})		-	-	10.96	0.004
	40 μGy hr^{-1}	2.89	1.99	-	-
	200 μGy hr^{-1}	6.53	1.96	-	-

Average humidity during the days when the egg counting measurements were made (%)	- 0.33	- 0.11	8.57 -	0.003 -
Average temperature during the days when the egg counting measurements were made (°C)	- -0.65	- 0.23	7.47 -	0.005 -
b. Terms removed from model in reverse order of deletion				
Days within the experiment	-0.46	0.44	1.07	0.30

Table S5.4. Parameter estimates for models investigating the effect of radiation dose rate on the total fecundity of *Drosophila melanogaster* breeding pairs for 30 days. Dose rates were 200, 40 μGy hr^{-1} and controls (0.11 μGy hr^{-1}). Model was linear with normally distributed errors. This model investigated the total of all egg counting measures from 180 breeding pairs during these observations. Table S5.4a describes the minimal model used.

a. Minimal Model					
Predictors		**Estimate**	**SE**	χ^2	**P Value**
(Intercept)		291.05	10.20	-	-
Dose rate (μGy hr^{-1})		-	-	10.68	$4.15x10^{-5}$
	40 μGy hr^{-1}	-49.27	14.24	-	-
	200 μGy hr^{-1}	-63.55	14.23	-	-

Table S5.5. Parameter estimates for models investigating the effect of radiation dose rate on the proportion of eggs produced by *Drosophila melanogaster* breeding pairs that developed successfully in to adults. Dose rates were 200, 40 μGy hr^{-1} and controls (0.11 μGy hr^{-1}). The environmental variables, temperature and humidity were mean centered and scaled by the standard deviation. For this model one standard deviation of temperature is 0.95°C and humidity is 4.73%. Model was binomial general linear mixed effects with normally distributed errors, it included a second order polynomial for the day variable both singly and in its interaction with dose rate. Model was selected using AIC model selection (Table S5.5c). The response variable was fitted with a cbind function (cbind(Success, Failure)), with "success" representing the number of eggs that successfully developed in to adults and "failure" the number of eggs that did not develop. Negative parameter estimates represent a decrease in development success. Multiple measures were made on 180 breeding pairs during these observations and then all eggs from these pairs monitored for 16 days until all adults had eclosed. Table S5.5a describes the minimal model used. Table S5.5b contains terms removed from the model in reverse order of deletion during model simplification. In the minimal model the P value of single variables was calculated by removing any interaction term it was also found within.

a. Minimal Model					
Predictors		**Estimate**	**SE**	χ^2	**P Value**
(Intercept)		0.95	0.06	-	-
Dose rate (μGy hr^{-1})		-	-	80.94	$2.2x10^{-16}$
	40 μGy hr^{-1}	-0.62	0.08	-	-
	200 μGy hr^{-1}	-0.99	0.09	-	-
Days within the experiment		-	-	95.38	$2.2x10^{-16}$
	(Poly 1)	-4.87	0.80	-	-
	(Poly 2)	3.10	0.43	-	-
Average temperature during the days when the adult counting measurements were made (°C)		- 0.03	- 0.01	3.79 -	0.05 -
Dose rate (μGy hr^{-1}) by days in the experiment		-	-	41.79	$1.83x10^{-8}$
	40 μGy hr^{-1} (Poly 1)	-5.87	1.68	-	-
	40 μGy hr^{-1} (Poly 2)	1.92	1.04	-	-
	200 μGy hr^{-1} (Poly 1)	-11.13	1.71	-	-
	200 μGy hr^{-1} (Poly 2)	1.10	1.08	-	-

b. Terms removed from model in reverse order of deletion				
Average humidity during the days when the adult counting measurements were made (%)	0.01 -	0.01 -	0.04 -	0.84 -
Dose rate (μGy hr^{-1}) by humidity during the days when the adult counting measurements were made (%)	- -	- -	0.27 -	0.96 -
40 μGy hr^{-1}	$-2.23x10^2$	$1.22x10^2$	-	-
200 μGy hr^{-1}	$1.37x10^2$	$1.05x10^2$	-	-
Dose rate (μGy hr^{-1}) by average temperature during the days when the adult counting measurements were made (°C)	- - -	- - -	0.56 - -	0.76 - -
40 μGy hr^{-1}	$1.14x10^2$	$5.32x10^2$	-	-
200 μGy hr^{-1}	$-2.86x10^2$	$6.73x10^2$	-	-
Average temperature (°C) by humidity (%) during the days when the adult counting measurements were made	-0.01 - -	0.001 - -	0.01 - -	0.94 - -

Table S5.5c. A comparison for models investigating the effect of radiation dose rate on the proportion of eggs produced by *Drosophila melanogaster* breeding pairs that developed successfully in to adults. Models were selected using AIC model selection. The model with the lowest AIC value was selected (highlighted in green). All models had the same random effects which include days within the experiment and the identifying code of each set of fly pairs.

Model Selection	AIC
Successful offspring, Failed offspring ~ dose + poly(day,1) + temperature +humidity + dose : day + temperature : humidity + temperature : dose + humidity : dose **Random Effects:** (Day | ID)	9159.54
Successful offspring, Failed offspring ~ dose + poly(day,2) + temperature +humidity + dose : day + temperature : humidity + temperature : dose + humidity : dose **Random Effects:** (Day | ID)	9124.01
Successful offspring, Failed offspring ~ dose + poly(day,3) + temperature +humidity + dose : day + temperature : humidity + temperature : dose + humidity : dose **Random Effects:** (Day | ID)	9185.56
Successful offspring, Failed offspring ~ dose + poly(day,1) + temperature +humidity + dose : poly(day,1) + temperature : humidity + temperature : dose + humidity : dose **Random Effects:** (Day | ID)	9159.85
Successful offspring, Failed offspring ~ dose + poly(day,2) + temperature +humidity + dose : poly(day,2) + temperature : humidity + temperature : dose + humidity : dose **Random Effects:** (Day | ID)	9106.15
Successful offspring, Failed offspring ~ dose + poly(day,3) + temperature +humidity + dose : poly(day,3) + temperature : humidity + temperature : dose + humidity : dose **Random Effects:** (Day | ID)	9129.36

Table S5.6. Parameter estimates for models investigating the effect of radiation dose rate on the sex ratio of adults that successfully developed from *Drosophila melanogaster* breeding pairs under radiation exposure. Dose rates were 200, 40 μGy hr^{-1} and controls (0.11 μGy hr^{-1}). The environmental variables, temperature and humidity were mean centered and scaled by the standard deviation. For this model one standard deviation of temperature is 0.25°C and humidity is 4.75%. Model was binomial general linear mixed effects with normally distributed errors, it included a second order polynomial for the day variable both singly and in its interaction with dose rate. Model was selected using AIC model selection (Table S6c). The response variable was fitted with a cbind function (cbind(Male, Female)), with "male" representing the number of eggs that successfully

developed in to males and "females" the number of eggs that successfully developed in to females. A positive parameter estimate represents an increase in the number of males that developed. Multiple measures were made on 180 breeding pairs during these observations and then all eggs from these pairs monitored for 16 days until all adults had eclosed. Table S5.6a describes the minimal model used. Table S5.6b contains terms removed from the model in reverse order of deletion during model simplification. In the minimal model the P value of single variables was calculated by removing any interaction term it was also found within.

a. Minimal Model				
Predictors	**Estimate**	**SE**	χ^2	**P Value**
(Intercept)	0.027	0.02	-	-
Dose rate (μGy hr^{-1})	-	-	67.79	$1.89x10^{-15}$
40 μGy hr^{-1}	0.26	0.04	-	-
200 μGy hr^{-1}	0.30	0.04	-	-
Days within the experiment	-	-	28.75	$5.72x10^{-7}$
(Poly 1)	2.59	0.59	-	-
(Poly 2)	-1.43	0.55	-	-
Average temperature during the days when the sex ratio	-	-	4.06	0.04
measurements were made (°C)	0.02	0.01	-	-
Average humidity during the days when the sex ratio	-	-	2.62	0.11
measurements were made (%)	-0.02	0.02	-	-
Dose rate (μGy hr^{-1}) by days in the experiment	-	-	48.68	$6.81x10^{-10}$
40 μGy hr^{-1} (Poly 1)	3.91	1.40	-	-
40 μGy hr^{-1} (Poly 2)	-3.66	1.33	-	-
200 μGy hr^{-1} (Poly 1)	9.43	1.59	-	-
200 μGy hr^{-1} (Poly 2)	-1.26	1.51	-	-
Average temperature (°C) by humidity (%) during the	-	-	6.51	0.01
days when the sex ratio measurements were made	0.04	0.01	-	-
Dose rate (μGy hr^{-1}) by average temperature during the	-	-	6.87	0.03
days when the sex ratio measurements were made (°C)	-	-	-	-
40 μGy hr^{-1}	0.11	0.04	-	-
200 μGy hr^{-1}	0.05	0.05	-	-
b. Terms removed from model in reverse order of deletion				
Dose rate (μGy hr^{-1}) by humidity during the days when	-	-	0.47	0.79
the sex ratio measurements were made (%)	-	-	-	-
	-	-	-	-
40 μGy hr^{-1}	0.03	0.04	-	-
200 μGy hr^{-1}	0.01	0.03	-	-

Table S5.6c. A comparison for models investigating the effect of radiation dose rate on the sex ratio of adults that successfully developed from *Drosophila melanogaster* breeding pairs under radiation exposure. Models were selected using AIC model selection. The model with the lowest AIC value was selected (highlighted in green). All models had the same random effects which include days within the experiment and the identifying code of each set of fly pairs.

Model Selection	**AIC**
Males , Females ~ dose + poly(day,1) + temperature +humidity + dose : day + temperature : humidity + temperature : dose + humidity : dose **Random Effects:** (Day | ID)	7754.08
Males, Females ~ dose + poly(day,2) + temperature +humidity + dose : day + temperature : humidity + temperature : dose + humidity : dose **Random Effects:** (Day | ID)	7845.87
Males, Females ~ dose + poly(day,3) + temperature +humidity + dose : day + temperature : humidity + temperature : dose + humidity : dose	7953.04

Random Effects: (Day | ID)	
Males, Females ~ dose + poly(day,1) + temperature +humidity + dose : poly(day,1) + temperature : humidity + temperature : dose + humidity : dose	
Random Effects: (Day | ID)	7632.14
Males, Females ~ dose + poly(day,2) + temperature +humidity + dose : poly(day,2) + temperature : humidity + temperature : dose + humidity : dose	
Random Effects: (Day | ID)	7601.69
Males, Females ~ dose + poly(day,3) + temperature +humidity + dose : poly(day,3) + temperature : humidity + temperature : dose + humidity : dose	
Random Effects: (Day | ID)	7751.65

Experiment 2: The effect of radiation on reproductive success of *D. melanogaster* after irradiation during juvenile development stages

Table S5.8. Parameter estimates for models investigating the effect of radiation on the number of eggs produced by *Drosophila melanogaster* male and females that came from eggs that were laid and developed under 200 µGy h^{-1} and control conditions (0.11 µGy hr^{-1}). The environmental variables, temperature and humidity were mean centered and scaled by the standard deviation. For this model one standard deviation of temperature is 0.43°C and humidity is 3.9%. Model was linear mixed effects with normally distributed errors. Multiple measures were made on 240 breeding pairs during these observations. Four treatments are described which include one parent taken from the 200 µGy h^{-1} and control treatments which were mated to a virgin male/female from a constant density stock. Model is in comparison to females that came from eggs that developed in control conditions. Table S5.8a describes the minimal model used. Table S5.8b contains terms removed from the model in reverse order of deletion during model simplification. In the minimal model the P value of single variables was calculated by removing any interaction term it was also found within.

a. Minimal Model				
Predictors	**Estimate**	**SE**	χ^2	**P Value**
(Intercept)	30.56	0.49	-	-
Treatment	-	-	29.07	2.32×10^{-6}
Female 200 µGy h^{-1}	-3.19	0.68	-	-
Male 200 µGy h^{-1}	-0.16	0.46	-	-
Male Control	-0.18	0.16	-	-
Days within the experiment	0.02	0.01	0.07	0.92
Treatment by days in the experiment	-	-	86.67	2.2×10^{-16}
Female 200 µGy h^{-1}	-0.25	0.03	-	-
Male 200 µGy h^{-1}	-0.19	0.06	-	-
Male Control	0.03	0.04	-	-
b. Terms removed from model in reverse order of deletion				
Average humidity during the days when the egg counting measurements were made (%)	-0.05	0.03	1.97	0.16
Average temperature during the days when the egg counting measurements were made (°C)	-0.02	0.40	0.04	0.94
Treatment by humidity during the days when the sex ratio measurements were made (%)	-	-	5.98	0.11
	-	-	-	-
	-	-	-	-
Female 200 µGy h^{-1}	0.02	0.09	-	-
Male 200 µGy h^{-1}	0.21	0.07	-	-

Male Control	0.13	0.04		
Treatment by average temperature during the days when the sex ratio measurements were made (°C)	- -	- -	5.25	0.15
Female 200 µGy h^{-1}	0.44	0.14		
Male 200 µGy h^{-1}	0.03	0.10		
Male Control	0.19	0.12		
Average temperature (°C) by humidity (%) during the days when the sex ratio measurements were made	-0.08	0.12	0.46	0.49

Table S5.9. Parameter estimates for models investigating the effect of radiation on the number of eggs produced by *Drosophila melanogaster* females that came from eggs that were laid and developed under 200 µGy h^{-1} and control conditions (0.11 µGy hr^{-1}). The environmental variables, temperature and humidity were mean centered and scaled by the standard deviation. For this model one standard deviation of temperature is 0.43°C and humidity is 3.9%. Model was linear mixed effects with normally distributed errors. Multiple measures were made on 240 breeding pairs during these observations. Two treatments are described which include one parent taken from the 200 µGy h^{-1} and control treatments which were mated to a virgin male/female from a constant density stock. Model is in comparison to females that came from eggs that developed in control conditions. Table S5.9a describes the minimal model used. Table S5.9b contains terms removed from the model in reverse order of deletion during model simplification. In the minimal model the P value of single variables was calculated by removing any interaction term it was also found within.

a. Minimal Model				
Predictors	**Estimate**	**SE**	χ^2	**P Value**
(Intercept)	29.49	0.48	-	-
Treatment	-	-	29.19	3.08×10^{-7}
Female 200 µGy h^{-1}	-1.06	0.68	-	-
Days within the experiment	-	-	0.02	0.96
	0.13	0.03	-	-
Average humidity during the days when the egg counting measurements were made (%)	- -0.13	- 0.05	6.10 -	0.01 -
Treatment by days in the experiment	-	-	43.13	5.1×10^{-11}
Female 200 µGy h^{-1}	-0.25	0.04	-	-
b. Terms removed from model in reverse order of deletion				
Average temperature during the days when the egg counting measurements were made (°C)	- 0.55	- 0.58	0.92 -	0.34 -
Average temperature (°C) by humidity (%) during the days when the egg counting measurements were made	- -0.09	- 0.17	0.31 -	0.57
Treatment rate by average temperature during the days when the egg counting measurements were made (°C)	- -	- -	0.13 -	0.71 -
Female 200 µGy h^{-1}	0.24	0.65	-	-
Treatment by humidity during the days when the egg counting measurements were made (%)	- -	- -	0.05 -	0.83 -
Female 200 µGy h^{-1}	-0.04	0.18	-	-

Table S5.10. Parameter estimates for models investigating the effect of radiation on the number of eggs produced by *Drosophila melanogaster* males that came from eggs that were laid and developed under 200 µGy h^{-1} and control conditions (0.11 µGy hr^{-1}). The environmental variables, temperature and humidity were mean centered and scaled by the standard deviation. For this model one standard deviation of temperature is 0.43°C and humidity is 3.9%. Model was linear mixed effects with normally distributed errors. Multiple measures were made on 240 breeding pairs during these observations. Two treatments are compared which include one parent taken from the 200 µGy h^{-1} and control treatments which were mated to a virgin male/female from a constant density stock. Table S5.10a describes the minimal model used. Table S5.10b contains terms removed from the model in reverse order of deletion during model simplification. In the minimal model the P value of single variables was calculated by removing any interaction term it was also found within.

a. Minimal Model				
Predictors	**Estimate**	**SE**	χ^2	**P Value**
(Intercept)	30.18	0.56	-	-
b. Terms removed from model in reverse order of deletion				
Treatment	-	-	0.11	0.15
Male 200 µGy h^{-1}	0.03	0.76	-	-
Days within the experiment	-	-	1.73	0.56
	0.02	0.02	-	-
Treatment by days in the experiment	-	-	1.44	0.52
Male 200 µGy h^{-1}	0.24	0.04	-	-
Average humidity during the days when the egg	-	-	0.41	0.52
counting measurements were made (%)	0.03	0.04	-	-
Average temperature during the days when the egg	-	-	1.21	0.27
counting measurements were made (°C)	-0.61	0.55	-	-
Treatment by average temperature during the days	-	-	2.85	0.09
when the egg counting measurements were made (°C)	-	-	-	-
Male 200 µGy h^{-1}	1.04	0.62	-	-
Treatment by humidity during the days when the egg	-	-	0.98	0.32
counting measurements were made (%)	-	-	-	-
Male 200 µGy h^{-1}	-0.17	0.17	-	-
Average temperature (°C) by humidity (%) during the	-	-	0.16	0.69
days when the egg counting measurements were made	-0.06	0.17	-	-