

JYU DISSERTATIONS 162

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**Kati Kivisaari**

# The Effects of Ionizing radiation on bank vole in Chernobyl Exclusion Zone

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UNIVERSITY OF JYVÄSKYLÄ  
FACULTY OF MATHEMATICS  
AND SCIENCE

JYU DISSERTATIONS 162

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**Kati Kivisaari**

**The Effects of Ionizing radiation  
on bank vole in Chernobyl  
Exclusion Zone**

Esitetään Jyväskylän yliopiston matemaattis-luonnontieteellisen tiedekunnan suostumuksella  
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## ABSTRACT

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Yhteenveto: Ionisoivan säteilyn vaikutukset metsämyyriin Tšernobylistä  
Diss.

The Chernobyl disaster in 1986 created an environment that provides unique opportunity to explore the eco-evolutionary impacts of chronic exposure to low-dose radioactive contaminants on wild animals. Studying the key species in the area could help determine the effects of radiation on species interactions and thus improve the radiation impact assessment. The bank vole (*Myodes glareolus*) was chosen as study species as it combines ecological relevance with laboratory tractability. Multi-generational experiments were conducted to test the wild populations exposed to long-term low-level ionizing radiation by analysing: the impact of added food sources on population sizes, the effects of radiation on reproduction of males and females, and radiation induced effects on organs. In the first study, bank vole abundances decreased with increasing radiation. Adding food sources to the field increased vole abundances only in low radiation environments. To find out whether lower abundances result from problems in male reproduction, in the second study, properties of the bank vole sperm were investigated. Sperm mid-pieces got shorter with increasing radiation. In addition, the proportion of static sperm increased in higher radiation areas. Thus, there were some negative effects on male reproduction. In the third study, reciprocal transplant experimental design was used to find study female reproduction. Females tended to survive better when moved to similar type of radiation environment indicating a possible adaptation to radiation in the environments. However, their pups survived better if the female was pregnant when living in the clean sites. Finally, in the fourth study, differences in various organ masses between the contaminated and clean sites were investigated. Results revealed smaller brain mass in the contaminated sites, and increased heart and spleen masses in the contaminated sites. In this thesis many correlative radiation effects were found, however the strength of the radiation in large areas of Chernobyl are very low. It is still unclear whether these effects are really radiation induced or do they come from combined stress effects in the area.

Keywords: Growth; ionizing radiation; *Myodes glareolus*; radioresistance; reproduction; survival.

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# TIIVISTELMÄ

Kivisaari, Kati

Ionisoivan säteilyn vaikutukset metsämyyriin Tšernobylässä

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Diss.

Tšernobylin ydinkatastrofi vuonna 1986 on luonut ainutlaatuisen ympäristön kroonisen säteilyaltistuksen tutkimiselle. Tutkimalla alueen avainlajeja on mahdollista selvittää säteilyvaikutuksia myös eliöryhmätasolla ja täten kehittää ympäristövaikutusten arviointia. Tutkimuslajina oli metsämyyrä (*Myodes glareolus*), joka on yksi runsaslukuisimmista lajeista alueella ja sietää lisäksi hyvin laboratorio-oloja. Matalatasoisen ionisoivan säteilyn vaikutusta metsämyyräpopulaatioon testattiin useilla sukupolvilla. Tutkin säteilyn vaikutusta populaatiokokoon, lisääntymistä molemmilla sukupuolilla ja vaikutusta ruumiinkokoon ja sisäelinten kokoon. Ensimmäisessä osakokeessa havaittiin negatiivinen korrelaatio säteilyn ja yksilömäärän välillä. Edes ravinnon lisääminen alueelle ei auttanut kasvattamaan populaatiokokoa korkean säteilyn alueella. Väitöskirjassa tutkittiin yksilömäärän harventumista korkean säteilyn alueella, sekä säteilyn vaikutusta molempien sukupuolten lisääntymisominaisuuksiin. Toisessa osakokeessa havaittiin, että metsämyyräkoiraiden siittiöiden keskikappale pienentyi säteilyn kasvaessa. Lisäksi säteilymäärän kasvaessa liikkumattomien siittiöiden määrä kasvoi. Tämä saattaa vaikuttaa koiraiden lisääntymiskykyyn, mutta tässä väitöskirjassa asiaa ei olla tarkemmin tutkittu. Kolmannessa osakokeessa naaraita siirrettiin poikasten kanssa joko matalan tai korkean säteilyn alueelle ja niiden havaittiin selviytyvän paremmin, jos siirtyminen tapahtui samankaltaiselle alueelle, jolta yksilöt oli alun perin pyydetty. Toisaalta poikasten selviytyminen parani, jos emo oli pyydetty alun perin puhtaalta alueelta. Viimeisessä osakokeessa tutkittiin metsämyyrien ruumiinkokoa ja sisäelinten massoja. Tulokset paljastivat aivojen massan pienentyvän säteilyn kasvaessa. Lisäksi sydän ja perna olivat suurempia korkean säteilyn alueella. Tutkimuksessa löydettiin useita negatiivisia säteilyvaikutuksia, mutta koska säteilyn voimakkuus alueella on paikoin suhteellisen alhainen, ei ole varmaa johtuvatko tulokset suorasta säteilyvaikutuksesta vai useammasta kroonisesta stressitekijästä alueella.

Avainsanat: Ionisoiva säteily; kasvu; lisääntyminen; *Myodes glareolus*; radioresistentti; selviytyminen.

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## LIST OF ORIGINAL PUBLICATIONS

The thesis is based on the following original papers, which will be referred to in the text by their Roman numerals I-IV.

- I Mappes, T., Z. Boratynski, K. Kivisaari, A. Lavrinienko, G. Milinievsky, T.A. Mousseau, A.P. Møller, E. Tukalenko, P.C. Watts. 2019. Ecological mechanisms can modify radiation effects in a key forest mammal of Chernobyl. *Ecosphere* 10, e02667.
- II Kivisaari K., S. Calhim, P. Lehmann, Z. Boratyński, T. A. Mousseau, A. P. Møller, T. Mappes. 2019. Changes in sperm size and reduction in sperm swimming endurance in Chernobyl bank voles. Manuscript.
- III Kivisaari, K., S. Sanches, A. Lavrinienko, E. Tukalenko, P. Lehmann, T. Mappes. 2019. Radiation effects on bank vole female reproduction and survival in the Chernobyl Exclusion Zone. A reciprocal transplant experiment. Manuscript.
- IV Kivisaari, K., Z. Boratynski, P. Lehmann, A. Lavrinienko, J. Kesäniemi, T. Mappes. 2019. The effect of chronic low-dose environmental radiation on organ mass of bank voles. Manuscript.

The table shows the contributions to the original papers.

	I	II	III	IV
Original idea	TAM, ZB, TM, KK	KK, TAM, SC	KK, TAM	KK, TAM
Data	TAM, KK, ZB, KK, AL, GM, TM, AM, PW	KK, PL, TAM	KK, SS, AL, ET	KK, AL, JK, PL, ZB, TAM
Analyses	TAM	KK, SC, ZB	KK, SS, PL	KK, ZB, PL
Writing	TAM, KK, ZB, KK, AL, GM, TM, AM, PW	KK, PL, SC, ZB, TAM, AP, TM	KK, SS, AL, ET, PL, TAM	KK, AL, JK, PL, ZB, TAM

KK = Kati Kivisaari, TAM = Tapio Mappes, PL = Philipp Lehmann, ZB = Zbyszek Boratyński, GM=Gennadi Milinievsky, PW=Phillip Watts, SC = Sara Calhim, TM = Timothy Mousseau, AM = Anders Møller, AL= Anton Lavrinienko, JK= Jenni Kesäniemi, SS=Sofia Sanches, ET = Eugene Tukalenko

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## ABBREVIATIONS

$\alpha$	alpha (particulate radiation)
ALH	amplitude of lateral head displacement (sperm measurement: $\mu\text{m}$ )
$\beta$	beta (particulate radiation)
BCF	beat-cross frequency (sperm measurement: Hz)
BSA	bovine serum albumin
CASA	computer assisted sperm analysis
CEZ	Chernobyl Exclusion Zone
CNPP	Chernobyl nuclear power plant
$\gamma$ -rays	gamma rays (electromagnetic radiation)
Gy	Grey (SI unit for absorbed radiation)
HA	sperm head area (HL $\times$ HW, $\mu\text{m}^2$ )
HL	sperm head length ( $\mu\text{m}$ )
HW	sperm head width ( $\mu\text{m}$ )
IAEA	International Atomic Energy Agency
INES	International Nuclear and Radiological Event Scale
IR	ionizing radiation
LDIR	low dose ionizing radiation
LDRIR	low-dose-rate ionizing radiation
LET	linear Energy Transfer
LIN	linearity (VSL/VCL ratio, sperm measurement)
LNT-model	linear no threshold -model
MPL	sperm midpiece length ( $\mu\text{m}$ )
MPV	sperm midpiece volume ( $\pi \times \text{MPL} \times \text{MPW}$ , $\mu\text{m}^3$ )
MPW	sperm midpiece width ( $\mu\text{m}$ )
PBS	phosphate buffered saline
PCA	principal component analysis
RT	reciprocal transplant
RTS	relative testis size
Sv	Sievert (SI unit for equivalent dose)
VAP	average path velocity (sperm measurement: $\mu\text{m/s}$ ),
VCL	curvilinear velocity (sperm measurement: $\mu\text{m/sec}$ )
VSL	straight line velocity (sperm measurement: $\mu\text{m/sec}$ )

# 1 INTRODUCTION

## 1.1 Chernobyl disaster (Chornobyl, Prypiat)

The Chernobyl disaster remains on the top of the INES (International Nuclear and Radiological Event) scale due to its widespread impact. The accident at the Chernobyl Nuclear power plant (CNPP) reactor 4 released more than 9 million terabecquerels (TBq) of fission products, mostly cesium-137 ( $\text{Cs}^{137}$ ) and strontium-90 ( $\text{Sr}^{90}$ ), and unspent nuclear fuel, in this case plutonium-239. These radionuclides were distributed across a vast geographic landscape including large parts of northern Ukraine, Belarus, and Russia, as well as some areas in Scandinavia and Central Europe (Fig. 1). Contamination levels near Chernobyl vary highly in regional scale, with ambient radiation levels varying as much as two orders of magnitude in places just a few hundred meters apart. This variation creates a possibility to study the effects of varying concentrations of radioactive contaminants in an ecologically similar environment. Due to half-lives of approximately 30 years, both  $\text{Cs}^{137}$  and  $\text{Sr}^{90}$  are very persistent contaminants in Chernobyl area.

To protect humans from overexposure to radiation, the Chernobyl Exclusion Zone (CEZ) was established and it covers approximately 30 km radius surrounding the destroyed reactor (Fig. 2). Wildlife in the CNPP area one of the best models for studying the biological impacts of radiation in the environment, being exposed to radioisotopes such as strontium-90 ( $\text{Sr}^{90}$ ), cesium-137 ( $\text{Cs}^{137}$ ), and plutonium-239 ( $\text{Pu}^{239}$ ) (UNSCEAR 2000). Despite the potential, Chernobyl ecosystem's biological health has been largely based on anecdotal observations rather than systematic, population-based scientific studies. Only few long-term ecological studies have been conducted within the area during past three decades. Although long term studies are still scarce, these have shown that contaminants in the CEZ have wide-ranging impacts, with increased mutation rates and morphological abnormalities, reduced population abundances, sperm aberrations, and local species extinctions leading to loss of biodiversity in the most contaminated regions (see e.g. Møller and Mousseau 2011, Møller *et al.* 2014)

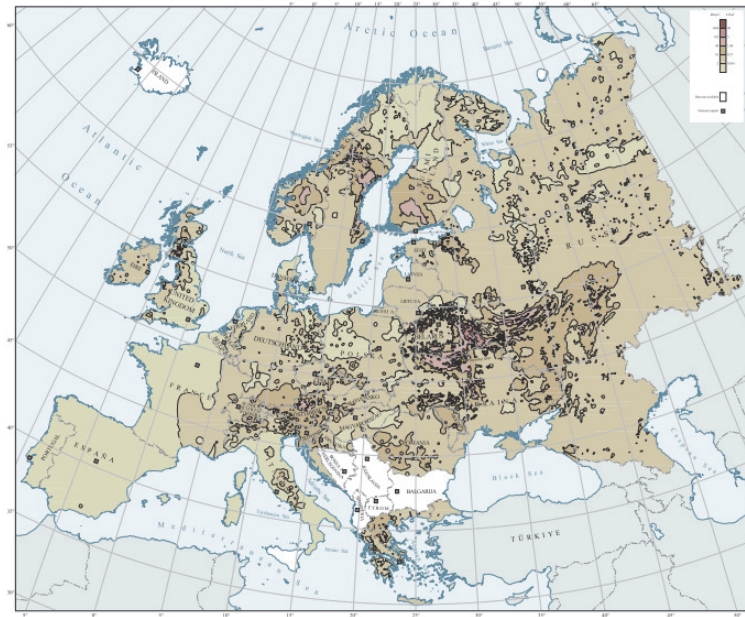


FIGURE 1 The deposition of cesium-137 on surface ground in Europe after the Chernobyl disaster. White areas have missing data. Adapted from (UNSCEAR 2000).

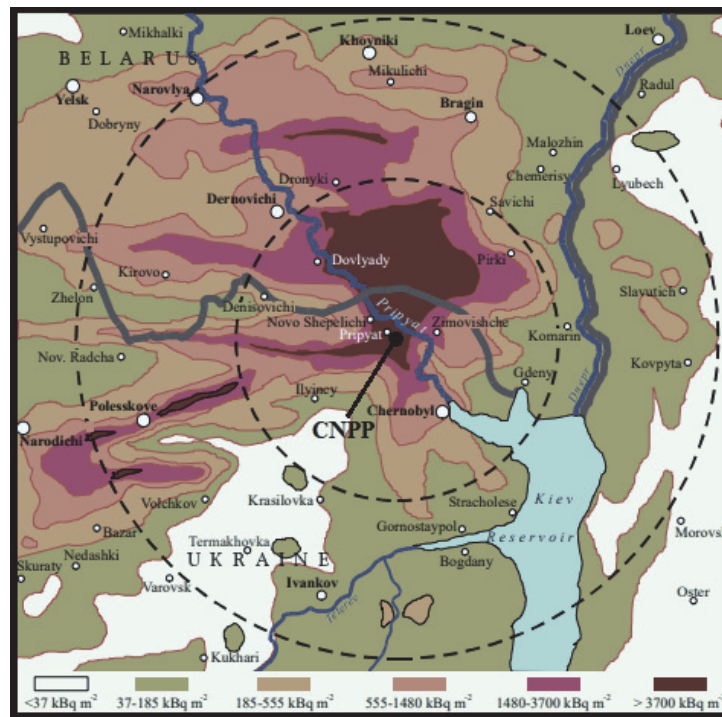


FIGURE 2 The deposition of cesium-137 on the ground surface in the close vicinity of Chernobyl nuclear power plant (CNPP). Distances of 30 km and 60 km are indicated with lines. Adapted from (UNSCEAR 2000). The Chernobyl Exclusion Zone (CEZ) covers the area within 30 km radius (inner circle) from the CNPP in both Ukraine and Belarus side of the border.

## 1.2 Ionizing radiation and its sources

Radiation in the environment comes both from non-ionizing and ionizing sources. Non-ionizing radiation such as visible light and infrared are not especially harmful to biota. Ionizing radiation (IR) sources such as particulate radiation, gamma ( $\gamma$ ) rays and X-rays on the other hand have enough energy to detach electrons from atoms or molecules, thereby ionizing them (Council 2006). Particulate radiation are fast-moving, small particles that have energy and mass. When an unstable atom disintegrates, it produces particulate radiation, like alpha ( $\alpha$ ) and beta ( $\beta$ ) particles. When uranium decays it releases radioactive  $\alpha$ -particles (made up of protons and neutrons), these are large and can only travel a short distance (IAEA 2010). However, these can be dangerous when inhaled or ingested.  $\beta$  -particles are fast-moving electrons or positrons emitted by the radioactive decay of an atomic nucleus during the process of  $\beta$ -decay. They can travel further and penetrate more than  $\alpha$ -particles and are also hazardous if inhaled or ingested (IAEA 2010). Both alpha and beta decays are often accompanied by gamma radiation. Gamma ( $\gamma$ ) rays are a type of electromagnetic radiation, but are also ionizing because of their high energy (Council 2006). They are extremely penetrating and several inches of lead or even a few feet of concrete are required to stop them. Gamma ( $\gamma$ ) rays are a radiation hazard for the entire body, because although they will pass through the body, tissues will absorb some rays (IAEA 2010). Doses in areas with increased radiation levels from man-made sources (fallout zones, nuclear fuel production plants, nuclear bomb testing sites) can increase the risk of many radiation related effects in local biota. X-rays are essentially similar to gamma rays, where  $\gamma$ -rays come from inside the nucleus of an atom, X-rays come from processes outside the nucleus (IAEA 2010). X-rays aren't quite as penetrating as  $\gamma$ -rays, and just a few millimeters of lead can stop them (IAEA 2010). Protection against occupational X-ray radiation doses is particularly important in medical science.

Biological effects of radiation arise when ionizing radiation interacts with tissue (or organism) and leaves energy behind (IAEA 2010). When travelling through matter (e.g. tissue) ionizing radiation gradually loses energy depending on the type of radiation and the density of the material (IAEA 2010). The Linear Energy Transfer (LET) is the density of energy deposition in a material and its unit is  $\text{keV}/\mu\text{m}$  (IAEA 2010). The biological effect of radiation depends on its average LET: particulate radiation is usually high LET radiation, whereas X- and  $\gamma$ -rays are low LET radiations due to their sparse ionizations (IAEA 2010). In radiation biology radiation is usually measured as absorbed dose and its unit is gray ( $\text{Gy} = \text{energy (J) absorbed per unit mass of tissue}$ ) (IAEA 2010). As biological effectiveness depends largely on LET, in radiation protection term 'equivalent dose' (unit Sievert) is used (IAEA 2010). Sievert (Sv) is calculated by multiplying absorbed dose by a radiation weighting factor. (IAEA 2010). The unit of effective dose is also Sv, however this is sum of the

products of equivalent doses to each tissue and the tissue weighting factor (IAEA 2010). For example, 1 Gy of gamma radiation on the skin would result an equivalent dose of 1 Sv (radiation weighting factor for gamma radiation is 1) and 0.01 Sv of effective dose. In case of  $\alpha$ -particles effective dose would be 0.2 Sv due to high LET and if ingested more sensitive organs (such as ovaries) can suffer from effective doses as high as 4 Sv (Council 2006, IAEA 2010).

In the CEZ the most abundant radioactive isotopes are  $\text{Cs}^{137}$  and  $\text{Sr}^{90}$  (Beresford *et al.*, 2018). Externally, effects of radioactive cesium ( $\text{Cs}^{134}$  and  $\text{Cs}^{137}$ ) result from gamma radiation. However, if ingested, it is both a beta and gamma radiation source (Ashraf *et al.* 2014). In rodent models, cesium does not bioaccumulate in any significant degree, and single doses are eventually excreted from the body once the source is removed (Nelson *et al.* 1961). Biological half-life (time it takes for half of the contaminant to be removed by biological processes) of  $\text{Cs}^{137}$  has not been previously estimated on bank voles, but on the mice it has been estimated to be around 6 days (Sato *et al.* 1997) and our estimates from the laboratory measurements are close to this (5-6 days). On the other hand,  $\text{Sr}^{90}$  bioaccumulates to the bone, but because  $\text{Sr}^{90}$  is beta active, separating it from the gamma radiation in the field conditions was impossible with available machinery. The contribution of different radionuclides to the bank vole internal dose have been estimated to be a bit under 50% in both  $\text{Cs}^{137}$  and  $\text{Sr}^{90}$  (Beresford *et al.* 2019). However, these estimates are only from the Red Forest area, which is the most contaminated area in the CEZ.

The effects caused by ionizing radiation thus depend on the type of radiation, how it is absorbed and whether the dose is gained as acute or chronic dose. IR can break chemical bonds through ionization or excitation which forms free radicals (unpaired electrons) that are very reactive with other molecules (e.g. DNA, proteins) (Wang *et al.* 2012). These direct effects are more common with high LET radiation (e.g.  $\alpha$ -particles). Indirect effects come through water radiolysis: radiation is absorbed by cellular water and this interaction produces unstable  $\text{H}_2\text{O}^+$  ions that again decompose to other highly reactive radicals ( $\text{OH}^\cdot$ ,  $\text{H}^\cdot$ ) (IAEA 2010). Radiolysis leads to cell stress, and induces the production of cellular signaling chemicals that trigger an antioxidant response in attempts to alleviate the stress (Azzam *et al.* 2012). Cell protection mechanisms can scavenge radicals from water radiolysis, thus direct radiation effects are in more prominent role in biological damage (IAEA 2010). If the signaling chemicals are produced faster than the antioxidant response can cope, e.g. when exposed to higher levels of radiation, oxidative damage occurs (Feinendegen 2005, Einor *et al.* 2016). Cells “die” when they lose the reproductive integrity (by apoptosis, necrosis, mitotic catastrophe or induced senescence) and both direct and indirect effects of IR can induce these (IAEA 2010). Radiation induced bystander effects on the other hand result from unirradiated cells responding signals from unstable irradiated cells which increases genomic instability (Lorimore and Wright 2003).

### 1.3 Life history strategies

Base assumption in life history trait theory is that there are trade-offs between growth, reproduction and longevity and that these are subject to natural selection (Cole 1954, Congdon *et al.* 2001). The pace-of-life syndrome hypothesis suggests that certain traits co-evolved along with particular life history strategies in response to environmental conditions (Réale *et al.* 2010, Montiglio *et al.* 2018). Several factors can affect life history traits, namely species-specific factors (metabolic rates and mating systems), abiotic factors (limitation of resources and density-independent factors) and biotic factors (predation, competition), and whether the environment is predictable or stochastic (Stearns 1989, Montiglio *et al.* 2018). These factors can be affected by external contaminants (Congdon *et al.* 2001). Indirect contaminant effects alter operative environments where individual lives, whereas direct contaminant effects alter the physiological performance of an individual organisms and thereby affect the resource allocation (Fig. 3; Congdon *et al.* 2001). Both types of contaminant effects have the potential to alter the reproductive rates and/or the mortality rates of a population and may consequently determine population density and survival (Congdon *et al.* 2001).

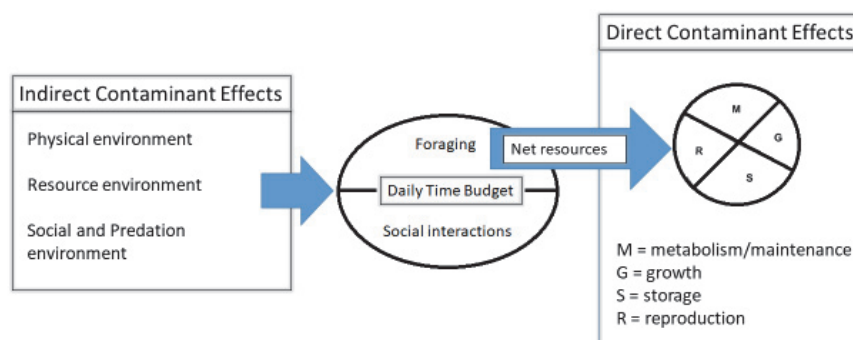


FIGURE 3 Congdon and colleagues summarized the factors affecting the daily activity and resource budgets of individual organisms. On the left are the operative environments that potentially influence the daily time budget available for social interaction and foraging. Daily time-activity budget is divided between foraging and social interactions, and each function potentially includes a risk of mortality. Foraging determines the amount of net assimilated resource available for allocating to growth (G), storage (S), maintenance and metabolism (M), and reproduction (R). This allocation determines the mass-energy budget, and each different allocation determines the age-specific growth and reproductive rates. Indirect contaminant effects alter the operative environments, for example abundance of food sources or predators. Direct contaminant effects alter the physiological performance of an individual and thereby affect its resource allocation. Adapted from (Congdon *et al.* 2001).

Trade-offs play a great role in biodiversity, since it creates variation in life history strategies. Many traits are heritable and the individuals with the most suitable traits in a given environment benefit the most. Trade-offs can involve one or multiple traits (Agrawal *et al.* 2010). A one-trait trade-off is caused by opposing selection resulting from different selective agents, environments or fitness components, whereas a two-trait tradeoff happens when selection on two traits share a limiting resource (Agrawal *et al.* 2010). Offspring size is often linked with offspring survival and the number of offspring increases the fitness of the parent (Oksanen *et al.* 2001). Another commonly used trade-off example in mammals is litter size versus pup size (Speakman 2008). However, limiting factor for all of these are the environmental resources, ability to utilize the resources and female body size (Speakman 2008). Stearns (1989) proposes that at least 45 possible trade-offs among 10 major life history traits exist. The trade-offs that have received the most attention are (1) current reproduction vs. survival; (2) current vs. future reproduction; (3) current reproduction vs. parental growth; (4) current reproduction vs. parental condition; and (5) number of offspring vs. offspring size (Stearns 1989).

The evolution of phenotypic and genotypic variation of an organism and its life history traits are affected by the environment they experience. Both abiotic (temperature, space, precipitation, etc.) and biotic factors (competition, predation, parasites, diseases) are part of this variation (Costantini *et al.* 2010). Sudden changes in the environment increase the stress level and can affect fitness (Costantini *et al.* 2010). There are many models that aim to describe how fitness can be affected by stress level, such as the linear no-threshold model, the threshold model and the hormetic model (Fig. 4). In the linear no-threshold (LNT) model fitness decreases with increasing levels of stress. In the threshold model fitness does not change until a threshold value is reached after which fitness starts to decrease with increasing stress level. In the hormetic model, low levels of stress increase fitness, but decrease it at high levels when compared to the control (Costantini *et al.* 2010). The LNT -model has been widely tested in laboratory animals and also in epidemiological studies of humans (Land 2002, Council 2006). Population growth is determined by birth and mortality rates and age structure in population (Rockwood 2006). In addition, both abiotic and biotic environmental impacts can affect population size. These are categorized into density-dependent (decrease with increasing densities e.g. nests, food) and density-independent (weather, contaminants etc.) factors (Rockwood 2006). For instance, higher densities attract predators and allow easier transfer for parasites and diseases.



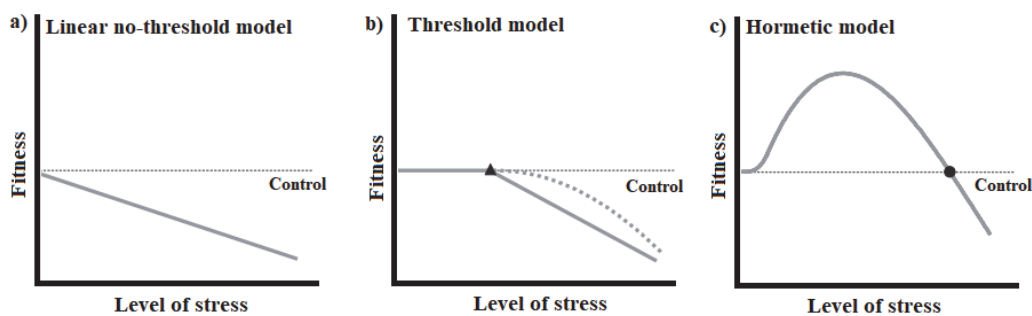


FIGURE 4 Three ways how increasing stress level can affect fitness. a) In the linear no-threshold model fitness decreases with increasing levels of stress compared to the control; b) in the threshold model fitness does not change until a threshold value after which fitness starts to decline with increasing levels of stress when compared to the control; c) in the hormetic model, low levels of stress increase fitness, but decrease it at high levels when compared to the control. A dashed line represents the situation with no stress exposure. Adapted from (Costantini *et al.* 2010).

## 1.4 The effect of ionizing radiation on biota

The detrimental effects of high doses of ionizing radiation are well known. Nuclear disaster survivors as well as medically radiated subjects have established the limits for acute and chronic radiation doses in humans (Guskova *et al.* 1987). Laboratory experiments have been used also to establish lethal acute doses of gamma radiation as well as recorded the effects of low dose rate irradiation on mice (Ryu *et al.* 2016). However, results from wildlife studies dealing with low dose radiation are not as straightforward. Exposure to radionuclides in the wild has increased due to human activities such as: uranium mining, nuclear energy production, nuclear waste treatment and nuclear bomb testing, and these have left many areas contaminated worldwide (Lourenço *et al.* 2016). The biological effects of low-dose (usually defined as single dose of <100 mGy) radiation exposure are still under debate (Bonner 2003, Calabrese and O'Connor 2014). Also, a little is known about the radiation dose response curves for natural ecosystems (although see Møller and Mousseau 2011, Garnier-Laplace *et al.* 2015).

The effects of chronic radiation span from lower community diversity of soil bacteria (Romanovskaya *et al.* 1998) to lowered abundance of invertebrates (Møller and Mousseau 2009) and to lower densities of mammals (Møller and Mousseau 2013). Also some phenotypic effects such as reduction in pheomelanin (red to pink color) based coloration (Boratyński *et al.* 2014) and increased cataract frequency (Lehmann *et al.* 2016) have been found in rodents inhabiting the CEZ. Experiments, where the source of external radiation was manipulated, suggest that small rodents can be very resistant to external radiation doses (Mihok *et al.* 1985, Mihok 2004). However, these experiments



did not take ingested contaminated food sources or ecosystem effects into account.

Most studies on radiation and tissue damage are conducted in laboratory conditions and are not necessarily ecologically relevant. Studies on radiation induced organ damage and oxidative stress effects, are mostly conducted on invertebrates, plants and birds, instead of mammals, the more suitable model for human radiosensitivity tests (Garnier-Laplace *et al.* 2013). For example, wild birds have been shown to have smaller relative brain mass when environmental radiation increases (Møller *et al.* 2011). A recent review suggests that wild populations are more radiosensitive than those used in laboratory studies, most likely since they are exposed to multiple stressors in the natural environment (Garnier-Laplace *et al.* 2013). Extrapolating the effects of radiation observed in the laboratory on a wild population is not simple, due to the complexity of natural systems (Mothersill *et al.* 2018), and this highlights the importance of wildlife studies in addition to controlled laboratory experiments.

Damage to DNA stimulates DNA repair mechanisms, and increases resistance to oxidative stress (Feinendegen 2005). A few hours of acute exposure to low-level radiation has been shown to stimulate these repair mechanisms (Feinendegen 2005). However, if the dose is large enough, apoptosis of damaged cells can happen even several months after subjection (Feinendegen 2005). These results form the basis for cancer treatments (Feinendegen 2005). It is notable, that most of radiation hormesis studies have been based on exposure to fixed single doses of radiation, not on accumulated exposure (Feinendegen 2005).

Low dose ionizing radiation (LDIR) very often means single doses of less than 100 mSv and low-dose-rate ionizing radiation (LDRIR) is defined <6 mSv/h (Tang *et al.* 2017). Giving accurate doses like this is easy in laboratory studies, but in wildlife studies, doses need to be estimated and are not as accurate. In Chernobyl, the radiation burden from external sources for bank voles has been estimated to be 65-70% of the total dose and the rest comes from respiration and diet (Beresford *et al.* 2018). Maximum radiation doses within some areas of the CEZ have been estimated to be around 86 mGy/day (Geras'kin *et al.* 2008), but radiation level in the area varies a lot.

In this thesis, the term background radiation refers to the natural radiation level occurring all over the planet from natural radiation sources, such as space and bedrock. For increased radiation level in the contaminated areas of Chernobyl, the term ionizing radiation (IR) or just radiation is used.

## 1.5 Aims of the thesis

The aim of my PhD project was to take a more experimental approach to Chernobyl wildlife studies. This thesis builds on the preceding research on selection mechanisms maintaining genetic variance in wild mammalian populations (Haapakoski and Ylönen 2010, Mappes *et al.* 2012, Morkkonen *et al.*

2012, Schroderus *et al.* 2012). In my research, I used a small wild mammal, the bank vole (*Myodes glareolus*), since it combines ecological relevance with laboratory tractability. Small mammals hold a key spot in forest ecosystems and serve as important indicators of the functioning ecosystem. Bank voles are one of the most common mammals in Chernobyl, which ensured an adequate number of individuals for the experiments. Multi-generational experiments were conducted to test the effects of the long-term low-level ionizing radiation on wild bank vole population. The goal of the thesis was to better understand the effects of low-level ionizing radiation on wild bank vole populations inhabiting the CEZ. To understand these effects, focus was on three key life history traits: growth, reproduction and maintenance.

First, in order to pinpoint possible changes in population dynamics, the ecology of the species in its environment must be known. In chapter I, the question was whether food supplementation would change the population densities of bank voles within the CEZ. Previous research on insects shows that population sizes are declining with radiation within the CEZ (Møller and Mousseau 2009), which suggest that at least one potential food source could be declining.

In the second part of the thesis, I focused on reproduction. Bank voles give birth to up to four litters per breeding season (Oksanen *et al.* 2001). I wanted to test the fitness of males and females in both contaminated and clean (near background level radiation) environments. In chapter II, sperm morphology and motility from individuals captured from both clean and contaminated sites of the CEZ was studied. In chapter III, I further studied the fitness of females in a reciprocal transplant setting, by moving females with their newborn litters to new environment and recording the survival of both.

Finally, in chapter IV, the body condition of bank voles by morphometrically measuring the relative organ size was studied. The phenotypic responses to radiation in individual level under different radioactive burden was assessed. I recorded the morphological data from a large group of individuals captured between years 2011-2017. These data included the masses of organs as well as the full body measurements from all the animals.

Because of the relatively low number of studies performed on non-human species, the true environmental impact of the Chernobyl disaster is yet unknown. However, there is a pressing need for more information, because it has been estimated that there is a 50% chance for another Chernobyl-sized accident will happen by 2042 (Wheatley *et al.* 2016).

## 2 MATERIALS AND METHODS

### 2.1 Study species *Myodes glareolus*

#### 2.1.1 Species information

Bank voles (*Myodes* [=*Clethrionomys*] *glareolus*, Schreber 1780) are widely abundant throughout Europe in both coniferous and deciduous forests (Fig. 5; Ledevin *et al.* 2010). Also in the CEZ bank voles are the most abundant rodent species and can be found even in the most contaminated locations (Chesser *et al.* 2000). Around 50 generations of bank voles have inhabited the CEZ after the explosion, thus potential mutations and local adaptation could be expected (Baker *et al.* 2017). In the wild, bank voles can live up to two years, but due to high predation rates, estimated survival is one breeding season (Ostfeld 1985, Macdonald 2001).



FIGURE 5 Bank voles are found through Europe and has spread over Ural Mountains to east. Adapted from (Hutterer *et al.* 2016).

Bank voles inhabit mixed forests, thickets, meadows and forest gaps, and also forage in fields, but prefer locations with enough ground level coverage from predators (Mazurkiewicz 1994, Torre and Arrizabalaga 2008). Bank voles have very variable diet from roots, plant stems and leaves, to seeds, lichens and insects (Canova 1993). Bank voles get their diet from the surface layer of their habitat, where radiation levels have the greatest impact (Riise *et al.* 1990, Boratyński *et al.* 2016). Adult bank vole body length measures up to 11 cm and the average body mass is less than 20 g in both sexes (Fig. 6; Lundrigan and Mueller 2003).

Bank voles are one of the key species in the CEZ ecosystem due to mixed diet and being an important food source to many predatory species inhabiting the zone. However, in this thesis it was not possible to study the effects of predation to the populations.



FIGURE 6 *Myodes glareolus* ©Kati Kivisaari

### 2.1.2 Sex differences

Fast reproduction allows bank voles to have multiple litters per year. Bank vole females mature quickly at 2-3 weeks and males mature at 6-8 weeks (Bujalska 1990, Macdonald 2001). The age of maturation can however be delayed depending on the number of mature individuals in the habitat. Females can delay maturation until they occupy an exclusive territory (Bujalska 1985). Newly weaned female pups establish home ranges between those of reproductive females (Bujalska 1985). If a breeding female dies, weaned female may occupy her place and attain maturity. Home ranges of immature females and males are smallest, whereas mature females have slightly larger and mature males have the largest home ranges (Bujalska 1990, Koskela *et al.* 1997, Koskela *et al.* 1998, Jonsson *et al.* 2002, Haapakoski and Ylönen 2010). Males mark their home ranges with urine and secretions of the preputial gland and these urinary markings are assumed to be related to a dominance hierarchy among males (Bujalska 1985). Population density decreases the home range size for mature males, and home range sizes seem to vary in relation to food conditions (Mazurkiewicz 1971, Bondrup-Nielsen and Karlsson 1985, Bujalska 1990, Haapakoski and Ylönen 2010).

Bank vole females mate with multiple males and are considered promiscuous (Miska-Schramm *et al.* 2016). In the wild, reproductive season lasts from April to late September (Oksanen *et al.* 2001), however in the laboratory it can reproduce all year long (Miska-Schramm *et al.* 2016). The estrous cycle lasts for 5 to 8 days and fertile period is on average 52.4 h (Bujalska 1990). Females give birth to 1 to 10 pups, pregnancy last 18-19 days and pups are weaned in approximately 20 days (Koivula *et al.* 2003, Oksanen *et al.* 2007, Schroderus *et al.* 2012). Females also go through post-partum estrus and if mates are present, they can mate shortly after giving birth (Ostfeld 1985, Horne and Ylönen 1996). Litter is weaned after 20-25 days and weanlings forage within female territory until they mature (Bujalska 1990). During breeding season, females reduce their territory size and aggressively defend them; also chances of infanticide towards other females pups increase (Koskela *et al.* 1997, Oksanen *et al.* 2001).

## **2.2 Field studies in Chernobyl**

### **2.2.1 Chernobyl field conditions and sampling scheme (Studies I - IV)**

Individuals for the experiments discussed in this thesis were captured from 120 trapping sites within and just outside the CEZ in summers 2011, 2015, 2016 and 2017 and winter 2016 (Fig. 7). Among years 2015-2017 many of the sites overlapped due to experimental setups and new sites were added as areas with more abundant bank vole populations were found. More detailed number of areas and captured individuals for each trapping are described in the manuscripts (I-IV). Sites were selected to cover environments with variable radiation levels and distances from the Chernobyl Nuclear Power Plant (CNPP). All areas had optimal conditions for bank voles with enough ground level coverage. Trapping was conducted using Ugglan Special2 live traps (Grahnb, Sweden) baited with potato and sunflower seeds and distance between neighboring trapping locations were always minimum 500 m. During winter trapping 2016, individuals were captured using snap traps baited with bread and peanut oil. Number of traps in each location varied among years depending on the ongoing experiments and number of traps available, aiming to maximize capture rate. Grid form trapping was used in experimental areas and line trapping was used when capturing experimental animals.

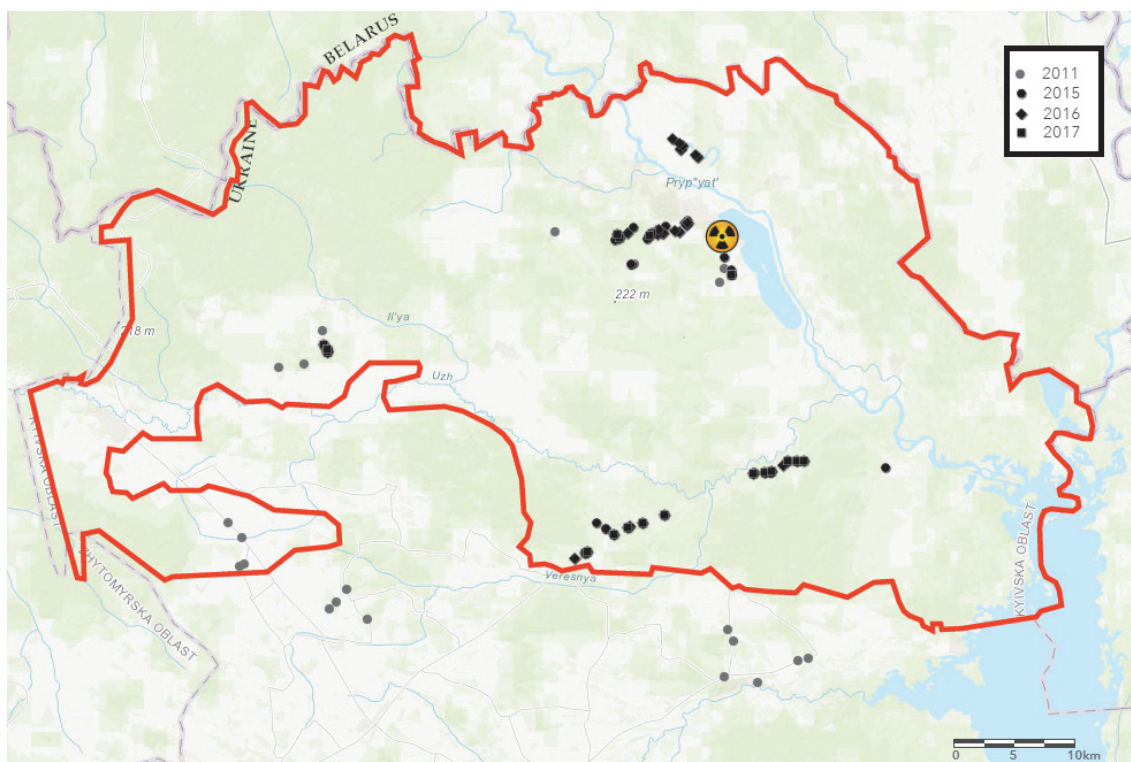


FIGURE 7 Bank vole trapping sites for the experiment in years 2011, 2015, 2016 and 2017. Year 2011 few trapping sites were chosen from outside the zone, but due to risk of possible disturbance of experimental areas, trapping sites were chosen within the CEZ in following years. Many of the sites overlap among years 2015-2017 due to experiments and patchiness of the radiation in the area.

Trapping sites were considered to be clean if averaged ambient radiation level was less than  $1 \mu\text{Gy/h}$ . Locations with higher radiation were considered to be contaminated. Depending on experimental setup traps were set for 1-3 days in each trapping location, traps were checked daily and captured animals were transported into the laboratory located within the CEZ. All studies except chapter III were conducted using adult bank voles. Juvenile individuals were excluded based on visual traits (grey juvenile fur) and body mass ( $< 14 \text{ g}$ ).

To quantify variation in habitat among locations, percentage vegetation cover was estimated within a 1 m radius around each trap at three layers: forest litter (vegetation of 0–50 cm high), bushes (0.5– 2 m), and in the tree canopy. These were selected to estimate whether contaminated and clean areas are different in key components of habitat structure. Litter cover and bushes the main habitat in which bank voles burrow and take refuge (Mazurkiewicz 1994), and a cover of tree canopy determines a general forest type (Cajander 1949).

## 2.2.2 Radiation measurements (Studies I - IV)

Radiation was measured from ground level next to each trap with hand-held radiation detector (Gamma-Scout w/ALERT Radiation detector/Geiger Counter, Gamma-Scout GmbH & Co. KG, Germany, with shielding set to detect only gamma radiation). These resulted 20 readings per site in 2011 and 16



readings per site in years following years and these were averaged to get radiation level ( $\mu\text{Sv/h}$ ) in the environment for each trapping site. Chernobyl area presents a mosaic of contaminated and clean (background level radiation) areas and sometimes these are only few hundred meters apart (Beresford *et al.* 2019). The doses bank voles are exposed to, are expected to correlate significantly with the radiation level due to their small home ranges (Kozakiewicz *et al.* 2007).

In trappings from year 2015 onwards, some of the bank vole individuals were subjected to gamma spectrometry to estimate the whole-body radionuclide ( $\text{Cs}^{137}$ ) burden and thus internal radiation exposure. The  $\text{Cs}^{137}$  activity for each individual was measured using the SAM 940 radionuclide identifier system (Berkeley Nucleonics Corporation, San Rafael, CA, USA) equipped with a 3"x3" NaI detector. The detector was enclosed in 10 cm thick lead shielding to reduce the noise from background radioactivity. The system was calibrated with reference standard sources. After correcting for the laboratory background, the  $\text{Cs}^{137}$  activity was assessed from the obtained spectra in the energies range 619-707 keV (with cesium photo peak at 662 keV), with the use of the phantom with known activity and similar to bank vole geometry. Animals were weighted prior to measurements and individual body mass was used to standardize radioactivity across individuals. Since these measurements were obtained from only the individuals that could be measured on the day of capture, these were used to estimate how well external and internal exposure correlate. These two measurements ended up being highly correlated and external measurement were estimated to suffice ( $R^2=0.757$ , see Supplementary Figure 2 from chapter IV). Importantly, due to the use of gamma spectrometry, the estimates of the absorbed internal radiation dose consider only the exposure from  $\text{Cs}^{137}$ . Recent study shows that radiation dose of Chernobyl bank voles comes mainly from external sources of  $\text{Cs}^{137}$  and  $\text{Sr}^{90}$  (Beresford *et al.* 2018). The total radiation dose for bank voles inhabiting contaminated areas derived from approximately 63% external and 37% internal radiation exposure (Beresford *et al.* 2018). The actual dose of each individual can vary significantly, even within sites, depending on ingested food sources, but are strongly correlated with measurements from trapping sites (Chesser *et al.* 2000, Garnier-Laplace *et al.* 2013).

### 2.3 Food manipulation experiment (I)

Population sizes can be affected by the abundance of resources (Koskela *et al.* 1998). Therefore, additional food sources were placed to experimental areas and abundances of the bank vole in the CEZ were recorded. In addition, the summer and winter abundances as well as the breeding probability were estimated.

Abundance index of bank voles were estimated in early summer 2011 and in winter 2016, from the area within 50 km of the CNPP. At each location, 20

traps were placed in line for one night, with each trap separated by about 10 m and the minimum distance between trapping locations was 500 m. Animals were captured with live traps during summer, and with snap traps in winter. The breeding probability of females was estimated by taking all captured females to the laboratory where they were followed every day until their litter was born. Number of offspring (litter size) was counted when pregnant females gave birth. Head width of a mother (as a proxy of body size) was measured to the nearest 0.1 mm with a digital caliper, and animals were weighed to the nearest 0.1 g using a digital balance.

The experimental populations for the feeding experiment were chosen at the beginning of the 2014 breeding season. 18 feeding locations from contaminated areas (radiation range 1.16– 30.54  $\mu\text{Sv/h}$ , mean 7.45  $\mu\text{Sv/h}$ ) and 18 locations from clean areas (background radiation range 0.10–0.22  $\mu\text{Sv/h}$ , mean 0.15  $\mu\text{Sv/h}$ ) were selected, and both contaminated and clean locations were divided randomly into three experimental groups with six populations in each group. Groups were: control (no food manipulation), rodent food (RM1, Special Diet Services), and rodent food containing indole-3-carbinol, a chemical that can potentially protect against oxidative stress (Fan *et al.* 2009). Food was provided at each feeding station and the minimum distance between the feeding stations was 1 km. An abundance index of bank voles was estimated prior to the beginning of the experiment and after the breeding season using a 3 x 9 trapping grid (an inter-trap distance of 20 m) with feeding station in the middle of the trapping grid. Each feeding area was trapped for five days. As the experiment was conducted in open populations, the bank vole abundances represent a combination of reproduction, mortality, immigration and emigration, thus these different ecological mechanisms could not be separated in the present study.

## 2.4 Sperm analysis methods (II)

### 2.4.1 Sperm morphometry

Sperm competition and viable sperm can have an important role in species survival. Male reproductive success is determined largely on sperm numbers (Tourmente *et al.* 2011). However sperm morphometry is also shown to have a connection with sperm swimming speed (Mossman *et al.* 2009). To identify possible changes in bank vole sperm in radioactive areas, multiple sperm traits were studied. Relative testis size (RTS) was calculated to control for male reproductive status, since this has been shown to correlate well with mating rate and ejaculate mass as well as within-litter multiple paternity (Parker and Ball 2005, Ramm *et al.* 2005). RTS was calculated as in Kenagy and Trombulak (1986): testes mass/0.031 x body mass x 0.77 (Kenagy and Trombulak 1986).

Specimens examined for sperm morphometry (captured in 2011 and 2015) were sacrificed by cervical dislocation after capture and their body mass, body



length and head width were recorded. After this, frozen bodies were transported to Jyväskylä, Finland for morphometric measurements. Morphometric sperm measurements from individuals captured in 2011 were collected two years later (2013) from frozen samples, so to imitate this, samples from the individuals captured 2015 were also done two years later (2017). From thawed bodies, testes and epididymides were dissected from and testis weight was measured with an analytic balance (Mettler Toledo, XS105 DualRange, precision: 0.01g). In the epididymis, the cauda has the highest concentration of mature sperm, thus the samples were collected from this part. Incisions were made as shown in lines in Fig. 8 from near vas deferens. Mature spermatozoa were collected in phosphate buffered saline (PBS, pH 7.4) and this sperm suspension was used to assess the sperm numbers and dimensions.

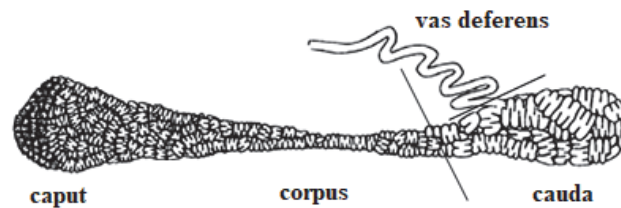


FIGURE 8 To collect sperm samples, cauda was separated from the rest of the epididymis. Incisions were made as shown in lines in the figure near the point where vas deferens connects to epididymis.

Sperm sample was loaded on a hemocytometer and sperm numbers were counted using Leitz Diaplan microscope (Leitz, Wetzlar, Germany) with 500x magnification as described by (Wang 2002). Hemocytometer grid squares for sperm counts were chosen randomly and only the spermatozoa lying completely inside the secondary square or lying on or touching the upper and left lines of the secondary square were counted. Two secondary squares were used to estimate mean number of sperm (mean count) in each sample. The total number of sperm in cauda was calculated by multiplying this mean count with the dilution factor (PBS volume divided with hemocytometer secondary square volume) as shown in the following equation from (Wang 2002).

$$\text{total sperm} = \text{mean count} \times \frac{\text{total volume of PBS (2ml)}}{\text{secondary square vol. (4} \times 10^{-6} \text{ ml)}} \quad (1)$$

Sperm morphometry was assessed from sperm smears at a 1000x magnification under bright field (LEICA DM-RBE Fluorescence Microscope). From each smear, 30 images of separate sperm cells were captured using a microscope camera (2000R Fast camera). Sperm morphometric measures were obtained for each sperm cell using ImageJ v.1.50i Software (National Institutes of Health, Bethesda, MD, USA) and included head length (HL), width (HW), mid-piece length (MPL), width (MPW), length of the principal piece (PPL) and total length of the spermatozoon (SPL) as shown in Fig. 9. Two measurements were calculated: sperm head area (HA: HL x HW,  $\mu\text{m}^2$ ) and mid-piece volume (MPV: calculated as cylinder:  $\pi \times \text{MPL} \times \text{MPW}$ ,  $\mu\text{m}^3$ ).

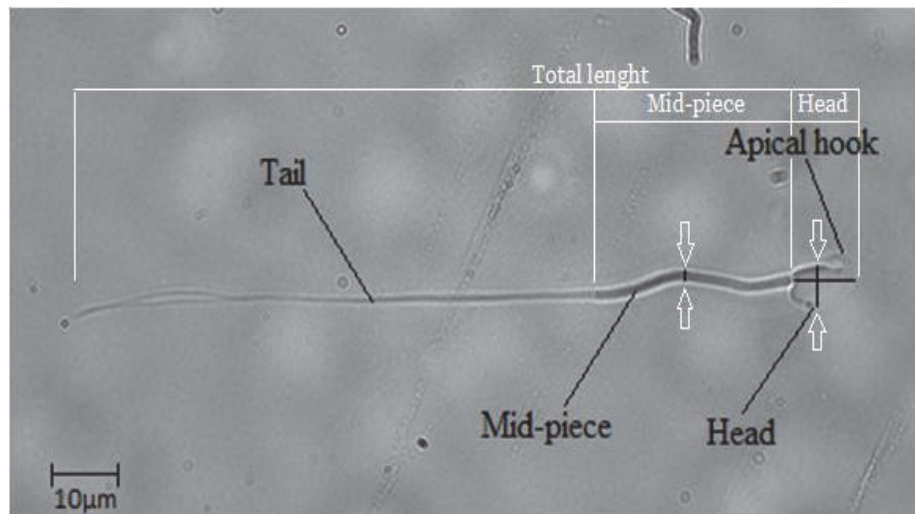


FIGURE 9 Bank vole sperm morphometrics. The measuring points for head width, head length and mid-piece length are shown with lines. Mid-piece width and head width measurements are shown with arrows. Total length and mid-piece length were measured by following the shape of the spermatozoon. Pictures were taken with Retica 2000R Fast camera connected to LEICA DM-RBE Fluorescence Microscope with 1000x magnification. Length and width measurements were done with ImageJ v.1.50i Software (National Institutes of Health, Bethesda, MD, USA).

#### 2.4.2 Sperm velocity parameters

In 2015, sperm mobility from freshly captured bank vole males was measured. Males were sacrificed by cervical dislocation and weighted. Testes were removed and weighted. The left epididymis was frozen whole for later assessment of sperm numbers and morphometry as specified above. For sperm mobility analysis, fresh mature spermatozoa were collected from the right cauda epididymis swiftly after male was sacrificed. Spermatozoa were allowed to swim out into prewarmed (37°C) medium (Nutrient mixture F-10 Ham [Sigma Aldrich N6635] and 1% bovine serum albumin [BSA, Sigma Aldrich B4287, pH 7]), incubated for ten minutes and after this a sample (30  $\mu$ l) from this sperm suspension was used for assessment of sperm mobility parameters. I used a computer assisted semen analysis (CASA) system (ISAS v1, PROiSER R+D, Spain) with a phase contrast microscope (UB 203i Trinocular microscope) and microscope camera (PROiSER 782 monochrome). The sperm suspension was placed into one well in a microscope slide (Erie Scientific Company: ER-308B-CE24, 10 wells, 6.7mm) and examined. Four 200-image videos were recorded for each male's sperm sample. From these, the program gives an output with the following sperm velocity parameters: proportion of progressive (movement  $>80 \mu\text{m/s}$ ), motile (20-80  $\mu\text{m/s}$ ) and static (non-motile) sperm. Measurement was repeated two times for each sample. First measurement was taken after a 10-minute incubation (initial mobility: progressive<sub>1</sub>, motile<sub>1</sub> and static<sub>1</sub>) and second after a 1-hour incubation (swimming endurance: progressive<sub>2</sub>, motile<sub>2</sub> and static<sub>2</sub>). Previous research showed that sperm of

muroid rodents stays viable up to three hours (Tourmente *et al.* 2015) and our own preliminary study on Finnish bank voles showed similar results. Due to this, assumption was that if some form of radiation-induced damage were present, differences in velocity parameters would emerge very fast. In addition to velocity and endurance the CASA system also records sperm kinetic parameters as described by (Mortimer 2000): VCL (curvilinear speed  $\mu\text{m/s}$ ), VSL (rectilinear speed  $\mu\text{m/s}$ ), VAP (average path velocity  $\mu\text{m/s}$ ), LIN (linearity index %), ALH (average amplitude of the head lateral moving  $\mu\text{m}$ ) and BCF (beat frequency Hz).

## 2.5 Reciprocal transplant experimental setting (III)

Local adaptation manifests as an improved fitness of particular group in its own habitat when compared to groups from other habitats (Kawecki and Ebert 2004). Common approaches for estimating fitness in local adaptation studies are: assessing the competition between genotypes, measuring population growth rate (population level) and measuring individual level fitness in traits such as juvenile survival or fecundity (Kawecki and Ebert 2004). Reciprocal transplant method compares individuals from two (or more) type of origins, and in the experiment both are reared in both their home type environment and also in the “other” type of environment, allowing both populations to be reared in the both environments (Kawecki and Ebert 2004). If any local adaptation is present the local population will outperform the immigrant population (Kawecki and Ebert 2004).

To test the possibility of local adaptation to radioactive environment we performed a reciprocal transplant experiment in the CEZ in two consecutive years 2015 and 2016. The basic design of the study was a capture-mark-recapture experiment. Trapping locations were established in the CEZ within 50 kilometres of the power plant. These sites were divided into two categories based on the level of radiation measured from each location: “clean” (less than one micro grey ( $\mu\text{Gy}$ ) per hour) and “contaminated” (more than 10  $\mu\text{Gy}/\text{hour}$ ) areas. Each trapping site was around 1 hectare in size, with 16 box traps for live trapping, spaced in a grid with inter-trap distance of 20m (Fig. 10).

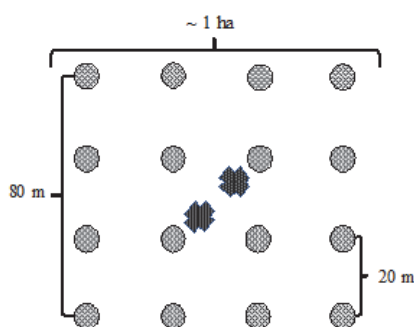


FIGURE 10 Bank vole trapping scheme. Circles represent placement of live traps in each trapping location. Vole families were released in the centre of each site to avoid dispersal out of that location, as marked by the X's in the diagram above.

The trapping and release of the vole families was done according to the reciprocal transplant experimental design (Fig. 11). This arrangement produced four treatments based on translocation: "clean-clean", "clean-contaminated", "contaminated-clean", and "contaminated-contaminated".

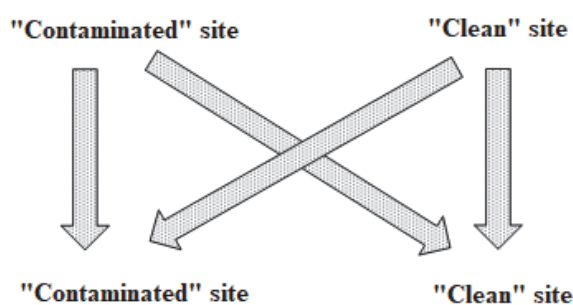


FIGURE 11 Bank voles were transplanted to foreign locations to control for the stress of establishing new home territories while caring for new-borns. Voles caught in "contaminated" areas were either released into different "contaminated" areas or into "clean" areas, and voles trapped in "clean" areas were either released into different "clean" or "contaminated" areas.

Traps were set and monitored daily for a total of three days. Caught voles were brought into the laboratory, where pregnant females were microchipped for identification, weighed (Binsol Analytics scale: Precisa Serie 165 BJ 1000C, precision: 0.01g), and their head width and body length (nose tip to anus) were measured with a digital caliper. Females were anaesthetised with isoflurane (IsoFlo®, USP) before microchipping and body measurements. The females were then held in individual cages with ad libitum food (rodent food: RM1, Special Diet Services) and water until their litter was born, when they were weighed again for their postpartum weight.

The new-born pups were counted, weighed, had their hind feet lengths and head width measured, sex determined under a light microscope, and each pup was marked with toe code for identification. Two to three days after birth, two mothers and their respective litters were released together into a new location

(Fig. 12). Trapping was conducted in experimental areas before release, allowing the released females to set up new home territories without as much competition from non-experimental animals as possible. Females and their litters were released in pairs to simulate intraspecific competition, as this species is territorial when rearing pups. The recapture of vole mothers and their litters started 20 days after their release, for a total of three days; and an additional trapping day was added, if any voles were trapped on the third day. Recaptured voles were identified and new body measurements (head width, body length, and mass) were recorded. The newly pregnant mothers were monitored for the birth of their second litters. The mothers were weighed again, and these second-litter pups were also counted, weighed, had their head width and hind feet lengths measured, and marked with toe codes. All recaptured mothers and weanlings were sacrificed in the end of the experiment by cervical dislocation. Second litters were sacrificed by overdose of anesthesia and frozen for further analysis.



FIGURE 12 On the left *M. glareolus* newborn pups. On the right female bank vole with her one week old litter. Female on the right is not related to the experiment. ©Kati Kivisaari

## 2.6 Organ sampling methods (IV)

Ionizing radiation can induce direct (e.g. cellular) and indirect (e.g. oxidative stress) organ damage and lead to changes in the size of physiologically active organs (Navarrete *et al.* 2011, Azzam *et al.* 2012). Organs that make up a high proportion of the basal metabolic rate (thus are energetically costly) such as the heart, kidney and liver, have been shown to respond to high doses of IR in radiotherapy setting (Konarzewski and Diamond 1995, Stone *et al.* 2003). This is believed to be related to increased amount of reactive oxygen species created by both high metabolism and radiation (Azzam *et al.* 2012). Kidneys filtrate blood and are exposed to water soluble products, including Cs137, and thus are expected to suffer from elevated internal radioactive exposure (Nelson *et al.* 1961). Heart has limited proliferative capacity (Bhattacharya and Asaithamby 2016) and relatively high radiotolerance (Darby *et al.* 2010), thus changes in heart mass in response to contamination were not expected. High acute doses of

radiation (>10 Gy) have been shown to damage liver in humans (Dawson *et al.* 2002). Similar laboratory studies have been conducted with mice; however, I am unaware of any studies done with wild rodents from the CEZ. Analysis of telomere lengths and expression of telomerase from these organs resulted in shorter telomeres in liver and increase in telomerase expression in brain and liver in the CEZ (Kesäniemi *et al.* 2019). Prior research has also demonstrated the negative effects of IR to skin, gastrointestinal system and testes (Paris *et al.* 2001, Chute 2012, Yamashiro *et al.* 2013).

Throughout all the years and experiments, the organ sampling methods stayed relatively similar. Dissected organs were selected prioritizing the importance in ongoing experiment and collecting as much as possible within limited time. Safety protocol in the CEZ was a limiting factor for the field seasons and for this reason, some organs had to be dissected from thawed individuals in Finland after the field experiments were finished. Individuals were sacrificed through cervical dislocation, sexed and their body mass, body length and head width were recorded. Internal organs (brain, heart, liver, kidneys, lungs and spleen, or some combination of these) were dissected, cleaned of fat and connective tissue, blotted dry with tissue paper and weighted with an analytic balance (Mettler Toledo, XS105 DualRange, precision: 0.01g). Weighted organs were stored in Eppendorf tubes and frozen for transport. For chapter IV, we studied how visceral organs (brain, heart, spleen, kidneys and lungs) differ in their response to ionizing radiation in the environment. Experimental animals that went through the experimental setup (e.g. food addition experiment) before dissection and were not used for this analysis (see manuscripts for further info). Different stages of pregnancy in captured females were accounted by subtracting the embryo mass from whole body mass. Relative organ masses were calculated from raw organ mass by taking the residuals of the raw organ mass and body mass regression.

## 3 RESULTS AND DISCUSSION

### 3.1 Field survey

#### 3.1.1 Bank vole densities in the CEZ and effects of food addition (I)

Breeding success decreased linearly with increasing radiation levels without any visible threshold level. In addition, the probability of bank voles being pregnant decreased significantly with increasing radiation level. Litter sizes varied between 1 and 8 and there was a significant decrease in litter size with increasing radiation level. Abundance index of bank voles was between 0 to 11 individuals per trapping location. Both summer and winter abundances decreased significantly with increasing radiation.

Food supplementation increased abundances curvilinearly: in locations with low radioactive contamination ( $< 1 \mu\text{Sv/h}$ ), the food supplementation increased abundance index significantly, however when radiation level increased past this point, there was a strong decrease in abundances. In control populations where no food was added, abundances decreased when moving to higher radiation level.

In food supplemented group, population sizes increased with radiation up to  $1 \mu\text{Sv/h}$ , however in areas with higher radiation despite the food addition population sizes decreased. Food stations could possibly spread parasites or infectious diseases in the bank vole population resulting the strong drop in population sizes (Sibly and Hone 2002). It is also possible that the number of predators could be lower in around  $1 \mu\text{Sv/h}$  areas thus reducing predation risk to a level that is unable to limit population increases when there is no food stress (Møller and Mousseau 2013). However, the interactions between radiation and these ecological processes remains unknown.



### 3.1.2 Environment qualities (I)

In all trappings among years 2011-2017, more effort was needed to capture enough animals from the most contaminated locations. Radiation could also affect bank voles indirectly through their diet. Thus, vegetation data from three different layers: cover of forest litter (< 50 cm), bushes (0.5-2 m), and tree canopy were collected. Vegetation cover of bushes and tree canopy decreased with increasing radiation in the environment; however, the cover of forest litter did not correlate with radiation. More importantly, vegetation did not seem to influence bank vole abundances. Neither forest litter, bush level nor tree canopy had a significant effect on the number of animals captured. In addition, there was no interaction between radiation level and vegetation. Thus, the effect of vegetation in the bank vole environment was considered less important. It is possible that there are some differences in the numbers of plant species that are potential food sources of the bank vole, but this cannot be analyzed from the available data.

## 3.2 Sperm morphology and swimming – The effects of radiation on male reproduction (II)

Spermatozoa varies greatly in their form and dimension (Malo *et al.* 2006). Male reproductive success is strongly dependent on sperm morphometry and swimming speed (Malo *et al.* 2006). In promiscuous species, such as bank vole, sperm competition increases the importance of high quality sperm (Ramm *et al.* 2005). Since there is very strong association between promiscuity in males and relative testis size (Kenagy and Trombulak 1986, Hosken and Ward 2001, Ramm *et al.* 2005), thus relative testis mass (RTS) was used in the analyses instead of raw data (see manuscript II for more information). There was a large variation in sperm numbers in cauda, but this this was not related to radiation level. However, RTS had a strong positive correlation with sperm numbers. RTS correlated positively also with preputial gland mass and prostate gland mass, and these results were consistent with previous studies (Ramm *et al.* 2005).

Six sperm morphometric traits were measured: total sperm length, principal piece length, head length and width, and mid-piece length and width. In addition, two traits were calculated: sperm head area and mid-piece volume. As expected, nearly all sperm components correlated positively with each other. However, radiation did not seem to influence other sperm traits excluding the smaller mid-piece volume. Sperm mid-piece is believed to be responsible for generating the energy needed for sperm motility (Turner 2003). However, without measuring the sperm swimming velocity, this link is dubious.

Therefore, in 2015, also sperm velocity from fresh samples was measured before recording sperm morphometric properties. Sperm velocity was analyzed in three groups: proportion progressive (most likely to fertilize), motile (rest of



the living sperm) and static (non-motile sperm). The measurement was taken two times: first after 10-minute incubation to assess the initial velocity and after 1-hour incubation to record sperm swimming endurance. RTS was not significantly related to sperm velocity (after 10-minute incubation) nor with swimming endurance (after 1-hour incubation). In addition, following kinetic parameters were recorded: VCL ( $\mu\text{m/s}$ ), VSL ( $\mu\text{m/s}$ ), VAP ( $\mu\text{m/s}$ ), LIN (%), ALH ( $\mu\text{m}$ ) and BCF (Hz), but found no significant relationship between radiation level and these.

The results showed a significant positive relationship between radiation level and the proportion of static sperm (after 10-minute incubation). This correlation was even stronger after the 1-hour incubation period, indicating that sperm swimming endurance is affected. The proportion of progressive sperm was not significantly related to radiation after 10-minute incubation. However, there was a significant negative relationship between proportion of progressive sperm and radiation after 1-hour incubation. No significant relationship was observed between radiation and the proportion of motile sperm after first or second time point.

The number of quality sperm is a key elements of male reproductive success (Tourmente *et al.* 2011). The volume of sperm mid-piece correlates positively with sperm swimming velocity and endurance in rodents (Firman and Simmons 2010, Montoto *et al.* 2011), implying that the found reduction in mid-piece volume could reduce fertility in bank voles that engage in sperm competition. It is also generally accepted that a large proportion of immobile sperm can be indicative of low male fertility (Turner 2006). There is a time lag between ejaculation and fertilization, which varies greatly between species (Orr and Brennan 2015). In addition, female reproductive tract filters out the sperm with inferior quality (Orr and Brennan 2015). Radiation is an environmental stressor that can cause trade-offs between investment in survival versus reproduction, resulting in low quality sperm (Congdon *et al.* 2001). These results show one possible mechanism underlying the lower population sizes in high radiation locations of the CEZ.

### **3.3 Reciprocal transplant experiment - Radiation effects on female reproduction (III)**

Life history trait theory assumes is that there are trade-offs between growth, reproduction and longevity (Cole 1954, Congdon *et al.* 2001). Several factors such as mating systems, resources, predation and competition can affect life history traits (Stearns 1989, Montiglio *et al.* 2018). Contaminant effects have the potential to alter the reproductive rates and/or the mortality rates of a population and may consequently determine population densities and survival (Congdon *et al.* 2001). To test the possible hormetic effects of radiation to bank vole litter sizes and survival, a reciprocal transplant experiment was conducted.

Litter sizes have been shown to decrease with increasing radiation (chapter I) and I expected a similar result also here. However, in my dataset, females captured from contaminated sites only tended to have smaller first litters when compared to their counterparts captured from clean sites, but statistical significance for this was not acquired. A total of 105 females were released with their litters and average recapture rate for mothers was 42%. The interaction among treatments was nearly significant, showing that females transferred to similar area where they originated from tended to be recaptured more likely than those that moved to different radiation environment. Size of the first litter on the other hand seemed to have significant negative effect on female survival. This supports the trade-off between survival and reproduction. Bank vole females have been shown to have lower survival if their litter size is increased (Koivula *et al.* 2003). For recaptured weanlings, only important factor seemed to be the origin of the mother. Weanlings survived significantly better when female was captured from clean sites. There is a possibility that radiation effects are stronger in utero and some of the offspring might be aborted before birth. Lower litter sizes in chapter (I) could be explained by this. Unweaned mice are relatively resistant towards radiation (Spalding and Trujillo 1962), so the bank vole pups with clean mother could be more resistant to the environment while being dependant on mothers milk. Size of the litter did not have any significant effect on survival or the pups nor did the size of the female. Recaptured females from 2016 were allowed to give birth to second litters after treatment. The interaction between the capture location type and experimental location type was not statistically significant, however when moving female and their litter from contaminated locations to similar type of location, females tended to give birth to larger number of pups in their second litter. However, with the sample size (23 litters) no statistically significant increase in the reproductive effort was found.

Recapture rates were not very high in this experiment, likely because if the litter did not survive, the female left the area. Survival of the litter is also dependent of the female survival, thus if female disappears very early (either leaves the area or is captured by a predator) the litter will not survive. The age of an animal can have a large impact on radioresistance. In laboratory mice the largest changes happen between 1-3 months of age, fetuses are very sensitive, where neonatal mice are somewhat resistant, weanlings (20-30 days old) are again sensitive, young adults (3-4 months) are the most resistant and in adults resistance declines throughout age (Spalding and Trujillo 1962). From released pups, only 15% (29 weaned litters, where at least one pup survived) were recaptured as weanlings. Low number of recaptured weanlings could result from the fact that young individuals cannot handle the combined stress effects from the translocation and radiation. Translocation stress however should be equal in all groups, and this does not explain why females from clean locations weaned offspring more successfully.

### 3.4 Radiation effects on bank vole organs (IV)

Laboratory studies have shown that relative organ masses (accounted for variation in size of an individual) are reliable indicators of toxicity (Sellers *et al.* 2007, Piao *et al.* 2013). However, much less is known about the effects of the IR in natural populations. In this study, possible differences in organ (brain, heart, spleen, kidneys and lungs) masses in areas with varying ionizing radiation level in the environment were investigated. Organs such as the brain and liver were expected to show strong negative responses to radioactive contamination, since these organs are energetically costly (Aiello and Wheeler 1995, Navarrete *et al.* 2011).

From the results, noticeable was that bank vole organs did not have equal response to radiation. There was a significant negative relationship between body mass and radiation. Residual organ masses (linear regression: organ mass  $\sim$  body mass) were calculated since contaminated areas hosted smaller individuals and organ masses were positively correlated with body mass. In principal component analysis (PCA) using these residual organ masses, the organs were grouped into three components that accounted for over 80% of variation. The first component (PC1) had loadings in heart and spleen, PC2 had loadings in brain and PC3 in liver. Subsequent analysis revealed significant effect of radiation in two of three models: a positive relationship between PC 1 (heart and spleen) and radiation, negative relationship between PC2 (brain) and radiation, and no significant relationship between PC3 (liver) and radiation. There was also a significant difference between two sexes in PC2 (brain mass in females was significantly smaller). When testing all organs (brain, heart, liver, spleen) separately and including additional data for kidneys and lungs, analyses showed very similar results. Radiation had a significant negative effect on brain mass and kidney mass. Heart mass was positively correlated with radiation and there was a nearly significant positive correlation between spleen mass and radiation. The masses of brain, kidney and spleen, were also significantly smaller in female than male voles. Lung and liver masses did not show significant radiation effects, nor differences between sexes.

Both results showed the negative effect of the radiation on brain mass. Brain is very sensitive against oxidative stress damage (Cobley *et al.* 2018) and this could be behind a similar results described in bird species (Møller *et al.* 2011). This brain mass difference between clean and contaminated areas is also visible in newborn bank voles (Mappes *et al. unpublished*). Thus, the effect of reduced brain mass could persist into adulthood already from doses gained during pregnancy. It is also still unclear whether the smaller brain could cause any differences in learning or survival. However, the fallout from Chernobyl has been shown to negatively affect the verbal IQ in Norwegian children who were exposed to fallout in utero (Heiervang *et al.* 2010).

Principal component analysis revealed a positive correlation between radiation and heart and spleen mass and separate organ analysis also showed

positive correlation with radiation and heart mass. This increase could result from increased heart rate on the contaminated site. Contaminated sites have been shown to have lower abundance of insects (Møller and Mousseau 2009). In addition, bank vole abundances go down with increasing radiation (chapter I). It is thus possible that bank voles need to use more effort to find insects as well as mating partners and this would increase their heart rate. This could potentially explain the increase in heart mass (Ford 1976). Spleen mass increase could result from two possibilities: increased need to destroy damaged white blood cells or increased chance of infections. The spleen is known to increase size with ongoing infections (Mebius and Kraal 2005). So there could be an interaction between radioactive pollution and infectious diseases (Morley 2012).

For the liver, no significant effect of radiation. In one laboratory study, liver was found to be apoptosis resistant with minimal effects after being exposed to X-ray radiation (10 Gy, 0.25 Gy/min), whereas spleen tissue was found to be more radiosensitive (An and Seong 2006). The doses used in the mentioned study are much higher than in Chernobyl where annual doses can get close to 2 Gy only in parts of the Red forest. These data therefore support the notion of the liver as a very radioresistant organ.

Despite protective measures, increased use of medical radiation (x-rays, nuclear medicine) in modern medical science increases the exposure of professionals to low-dose IR (Picano *et al.* 2012) highlighting the importance of studies involving chronic low doses. There are multiple confounding factors (e.g. seasonality, nutritional state, age of individuals, presence of predators, diseases) when dealing with animals in their natural environment, however using adult individuals from the same area can control for some of these.

## 4 CONCLUSIONS

The effects of radiation in the CEZ have been under debate since the accident. There seems to be very strong opinions regarding whether the radiation level in Chernobyl can cause any adverse effects. End result seems to change based on whether samples are collected within the Ukrainian or Belarussian side and where the control areas are located (see eg. Møller and Mousseau 2013, Deryabina *et al.* 2015, Webster *et al.* 2016). Excluding humans from the system also seems to have a positive impact on some species (Geras'kin *et al.* 2008); however, but this does exclude the possible negative effects of radiation. Comparing sites with significant human influence on areas with nearly no human influence is questionable. For this reason, I did my studies within the exclusion zone and in areas where largest variation should be in the level of radiation. However, there might be some interactions among species in the ecosystem that cannot be seen in this type of studies, and more studies should be conducted to study these possible interactions. In addition, the effects from birds to are not expected to translate straight to rodents or to humans, since the diets vary widely, and all species have their specific ecological environment. Radiosensitivity in the wild remains as a poorly studied field of science. Sensitivity of the laboratory animals does not translate straight to wildlife and in the wild reduced abundance of any species can alter the whole ecosystem. The effects of the radiation can result also from historic dose (Beresford *et al.* 2019). In long living species accumulation of large doses could be possible, however the within the lifetime of a bank vole, the effects from historical doses seem unlikely. However, prenatal stages could be more sensitive to lower doses and possible changes could persist into adulthood (Hande *et al.* 1990, Verreet *et al.* 2016)

The present study confirmed that the abundances of bank voles are indeed decreasing with increasing radiation. However, it is still questionable whether this is caused by direct or indirect effects of radiation. In chapter I, the lowered abundances of bank voles in the contaminated sites of the CEZ were presented. In addition, the food addition did not seem to increase population sizes after the radiation level in the environment exceeded 1  $\mu\text{Sv/h}$ . It is unclear whether

these biological effects would follow the direct exposure to gamma radiation from the surrounding environment or by exposure to radioactive particles accumulated from food (Sazykina and Kryshev 2006). Mushrooms, for example can be an enormous source of radionuclides (Kalač 2001). Food resources of voles such as plants, small invertebrates and fungi (Calandra *et al.* 2015) are likely altered in contaminated areas (Tikhomirov and Shcheglov 1994) and also some effects of radiation on tree and bush cover were found. Our recent finding is that bank vole diet seems to contain fungi and insects when available (Lavrinienko *et al.* *unpublished*). Predation rate, could also be lower in contaminated areas, since mammalian predators (Møller and Mousseau 2013) of rodents have decreased abundances in contaminated areas. This could explain the differences between our results and earlier studies where populations of small mammals were manipulated only by exposure to external radiation (Mihok *et al.* 1985, Mihok 2004).

Chapter II revealed problems with male reproduction in voles from areas with high level of radiation. Large sperm mid-pieces are associated with energy reserves used for sperm swimming (Anderson and Dixson 2002). Decrease in sperm mid-piece volume with increasing radiation was found, and in addition, an increased proportion of static sperm in increased radiation levels. Both results support the hypothesis that radiation damage depletes the energy reserves of the sperm. This would not stop males from reproducing; however, it could affect the number of offspring that they are able to sire and thus result the smaller populations in the contaminated site of the CEZ.

Chapter III tested the effects of radiation on female reproduction and survival. Females from contaminated sites tended to have smaller litter sizes than females from clean sites, but unlike in chapter I this was not statistically significant. However, the smaller population sizes in the contaminated areas of the CEZ seems to be an ongoing trend through the years. In the translocation experiment, only the larger size of the first litter seemed to negatively affect the survival. Females giving birth to smaller litters could be younger and not putting as much effort to their first litter and thus survive better. In case of the weanling survival, only the origin of the mother seemed to affect the number of weaned pups. After the experiment, recaptured females gave birth to second litter, however only around 50% of the females were pregnant after recapture. Even though the largest litters resulted from the females that came from contaminated origin and were put to contaminated experimental area, the interaction between capture location and treatment location was not significant. It has been questioned whether the Chernobyl area could be multi stressor environment with other environmental pollutants such as lead (Pb) (Beresford *et al.* 2019). However, this has not been substantiated by the few available studies conducted in the area (Jago *et al.* 1998, Beresford *et al.* 2018). It is possible that females going through a stressful situation (contaminated environment combined with laboratory handling) have decided to put a lot of effort to their final litter. Larger first litters also seemed to lower the survival of the females in the experiment, which supports this idea.

Results from chapter IV showed the negative effects of radiation on brain and kidney mass and positive effect on heart and spleen mass in the contaminated sites of the CEZ. There are results showing increased antioxidant levels and suggesting that bank voles are coping with oxidative stress in Chernobyl (Mustonen *et al.* 2018, Kesäniemi *et al.* 2019). Smaller brain volume has been also reported in the birds species of Chernobyl (Møller *et al.* 2011) and our recent results from Fukushima mice show similar trend with the brain mass (Mappes *et al.* *unpublished*). Increased heart and spleen masses in the contaminated sites could also result from indirect radiation effects. Smaller abundances as seen in chapter I could mean that more effort is needed for finding the mating partners. Similarly, the smaller abundance of insects (Møller and Mousseau 2009) could mean more work for finding some of the food sources. Both effects increase movement in the environment and increase the heart rate that has been shown to increase the heart size (Ford 1976). High level of radionuclides is also associated with an altered gut microbiota in bank voles (Lavrinenko *et al.* 2018), potentially indicating that radiation exposure is associated with small change in diet.

The effects of ionizing radiation on bank vole population of Chernobyl seem to be very complicated. This thesis aimed to take an experimental approach to find out some of the effects altering the population sizes. Given the risks of future radiation related accidents (Wheatley *et al.* 2016), studies investigating the potential environmental and health consequences of chronic exposure to chronic low doses (less than 2 Gy/year) of radionuclides especially in natural conditions seem warranted. Our group and our collaborators have strived to experimentally study the bank vole population of the CEZ, but we have barely scratched the surface on the radioecological studies in Chernobyl.

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## YHTEENVETO (RÉSUMÉ IN FINNISH)

### Ionisoivan säteilyn vaikutus metsämyyriin Tšernobylässä

Ukrainan Tšernobylässä huhtikuussa 1986 sattunut ydinkatastrofi tarjoaa harvinaislaatuisten mahdollisuuden tutkia alhaisen kroonisen säteilyn vaikutuksia kasveihin ja eläimiin. Alueella on kuitenkin tehty yllättävän vähän tutkimusta. Luonnonvaraisilla nisäkkäillä tehty kokeellinen tutkimus on edustaa edelleen murto-osaa Tšernobylässä tehdystä tutkimuksesta. Tutkijat ovat yhä vakuuttuneempia siitä, että säteilyn haittavaikutusten arviointia pitäisi tehdä myös ekosysteemitasolla. Keskittyminen ravintoverkon avainlajeihin voisi antaa luotettavaa kuvaa säteilyn yleisistä vaikutuksista. Harvat kokeelliset nisäkästutkimukset viittaavat siihen, että alhaisellakin kroonisella säteilyllä voi olla merkittäviä vaikutuksia eliöiden menestykseen. Morfologiset muutokset, lisääntyneet mutaatiot, eliniän lyhentyminen, poikkeamat neurologisessa kehityksessä, populaatiokoon pientyminen, lisääntymiskyvyn heikkeneminen ja paikalliset lajien sukupuutot ovat kaikki korkeilla säteilyalueilla havaittuja muutoksia.

Tšernobylin alueella säteilytasot ovat paljon alhaisempia kuin mitä laboratorio-oloissa on testattu ja säteilytaso vaihtelee suuresti jopa muutaman metrin välillä. Tutkimuksissani käytettyjen pyyntialueiden taustasäteily mitattiin jokaiselta pyyntialueelta ja jokaisen pyydyksen vierestä, ja lisäksi mittaukset toistettiin joka vuosi. Näin saatiin tarkempi kuva taustasäteilyn tasosta eri pyyntialueilla ja saatiin kuvaa säteilytason vuosittaisesta vaihtelusta. Yksilöön vaikuttaa kuitenkin taustasäteilyn lisäksi ravinnon mukana elimistöön päässeet radioaktiiviset partikkelit, jotka vaurioittavat hajotessaan soluja. Tšernobylin alueella radioaktiivisista aineista vahingollisimpia ovat beetasäteilevät partikkelit (esim. cesium ja strontium), jotka ovat kehoon päästessään toksisia.

Keskityn väitöskirjassani pieniin nisäkkäisiin, jotka ovat avainasemassa metsäekosysteemeissä ja toimivat tärkeänä indikaattorina, kun pyritään ennustamaan säteilyn vaikutusta koko ekosysteemiin. Tutkimuslajina oli metsämyyrä (*Myodes glareolus*), joka on merkittävä laji Tšernobylin metsäekosysteemeissä, ja lisäksi käyttökelpoinen laboratorio-oloissa. Lajin runsaslukuisuus Tšernobylin alueella takasi lisäksi luotettavan otoskoon vuosittaisissa pyynneissä. Tšernobylin alueella taustasäteilyn määrä ennustaa selkeästi populaatiokokoa ja lajien leviämiskykyä useissa eliöryhmissä, erityisesti linnuilla ja hyönteisillä. Myös metsämyyrän yksilömäärät ja lisääntymistodennäköisyys putosivat merkittävästi säteilytason kasvaessa. Ensimmäisessä osakokeessa tutkin lisäksi voiko ravinnon määrän lisääminen auttaa selviämään säteilystressistä. Ruuan lisääminen alueelle kasvatti populaatiokokoa, kun säteilytaso oli noin 1  $\mu\text{Sv/h}$ . Tätä korkeamman säteilyn alueella populaatiokokoa kuitenkin pieneni lisäravinnosta huolimatta.

Selitystä pientyneisiin populaatiokokoihin radioaktiivisilla alueilla hain sekä koiraiden että naaraiden lisääntymisominaisuuksista. Toisessa osakokeessa keskityin metsämyyräkoiraiden siittiöiden morfologiaan ja uintikykyyn. Havait-

sin säteilyn aiheuttavan siittiövaurioita ja lisäksi lisäävän liikkumattomien siittiöiden määrää spermanäytteissä. Tämä voisi mahdollisesti heikentää metsämyyräyksilön menestystä spermakilpailussa. Naarilla lisääntymismenestyksestä kertoo erityisesti poikuekoko. Ensimmäisessä osakokeessa havaittiin lisääntymistodennäköisyyden pienentyvän säteilyn kasvaessa. Kolmannessa osakokeessa siirsin metsämyyräemoja poikueineen alkuperäistä pyyntipaikkaa radioaktiivisempaan ympäristöön tai puhtaalle kontrollialueelle. Tavoitteena oli selvittää sopeutumista säteilevään ympäristöön. Suuria eroja ryhmien välille ei syntynyt, joten on mahdollista, että pitkäaikainen altistus ja poikasten kohdussa saama annos vaikuttavat voimakkaammin yksilöissä havaittuihin vaurioihin ja pienentyneisiin populaatiokokoihin.

Korkean säteilyn tiedetään aiheuttavan solutason vaurioita ja solukuolemia. Lisäksi vaurioituneet solut voivat signaloida myös viereiset solut tuhoutumaan (bystander effect). Jälkimmäistä on havaittu myös mata-lammilla säteilyannoksilla ja tästä syystä tutkin metsämyyrien sisäelimiä ja selvitin, olisiko jonkinlaista elinten massan vähenemistä havaittavissa näissä yksilöissä. Selkeimpänä tuloksena havaitsin metsämyyrien aivojen massan korreloivan negatiivisesti ympäristön säteilyn kanssa. Sydämen ja pernan massa taas päinvastoin kasvoi lisääntyvän säteilyn myötä. Ensimmäisessä osakokeessa havaittu yksilömäärän väheneminen ja muissa kokeissa havaittu hyönteisten vähentynyt määrä alueilla voisi selittää sydämen koon kasvua, sillä ravinnon tai parittelukumppanien aktiivinen etsintä lisää sydämen syketiheyttä ja voisi täten myös kasvattaa sen kokoa. Pernal kokoon vaikuttaa paljon infektioriski, sillä perna tuhoaa vaurioituneita valkosoluja ja suurentuneen pernan taustalla on hyvin usein jokin infektio-tauti. On siten mahdollista, että Tšernobylin radioaktiivisen alueen metsämyyrillä esiintyy säteilyvaurioiden lisäksi myös infektio-tauteja.

Tämän tutkimuksen myötä olen löytänyt useita vaikutusreittejä, jolla säteily voisi vaikuttaa metsämyyrän populaatiokokoon. Säteilyn vaikutukset alhaisella säteilytasolla eivät ole yksiselitteisiä ja stressivaikutuksia on hyvin vaikea erotella luonnonpopulaatioissa. Kroonisen säteilyaltistuksen tutkiminen voi kuitenkin auttaa varautumaan tuleviin ydinonnettomuuksiin ja niiden seurauksiin. Lisäksi tutkimus säteilyn vaikutuksista pikkunisäkkäisiin antaa mahdollisuuden tehdä oletuksia säteilyn suorista ja epäsuorista vaikutuksista myös koko ekosysteemiin.

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## ORIGINAL PAPERS

### I

#### **ECOLOGICAL MECHANISMS CAN MODIFY RADIATION EFFECTS IN A KEY FOREST MAMMAL OF CHERNOBYL**

by

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## Ecological mechanisms can modify radiation effects in a key forest mammal of Chernobyl

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**Abstract.** Nuclear accidents underpin the need to quantify the ecological mechanisms which determine injury to ecosystems from chronic low-dose radiation. Here, we tested the hypothesis that ecological mechanisms interact with ionizing radiation to affect natural populations in unexpected ways. We used large-scale replicated experiments and food manipulations in wild populations of the rodent, *Myodes glareolus*, inhabiting the region near the site of the Chernobyl disaster of 1986. We show linear decreases in breeding success with increasing ambient radiation levels with no evidence of any threshold below which effects are not seen. Food supplementation of experimental populations resulted in increased abundances but only in locations where radioactive contamination was low (i.e., below  $\approx 1 \mu\text{Sv/h}$ ). In areas with higher contamination, food supplementation showed no detectable effects. These findings suggest that chronic low-dose-rate irradiation can decrease the stability of populations of key forest species, and these effects could potentially scale to broader community changes with concomitant consequences for the ecosystem functioning of forests impacted by nuclear accidents.

**Key words:** Chernobyl; chronic radiation; food supplementation; forest ecosystem; ionizing radiation; key species; *Myodes vole*; nuclear accident; population increase; population sensitivity; reproductive success.

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### INTRODUCTION

We have entered the Anthropocene, a period when human actions dominate the well-being and functioning of the Earth's environment. Detrimental effects of human actions on biota are well-documented ranging from global impacts, such as climate change and ocean acidification, to more local events, such as loss of habitat, and pollution by metals or nutrients. One particularly

controversial source of human impact is related to the accidental release of radionuclides. The Chernobyl nuclear accident (1986) is a model for studies of the impact of chronic exposure to low-dose radioactive contaminants on wildlife (Anspaugh et al. 1988). The explosion at the former Chernobyl Nuclear Power Plant (NPP) released a wide array of fission products, including cesium-137 and strontium-90, and unspent nuclear fuel (plutonium-239), that were

dispersed widely (about 200,000 km<sup>2</sup>) over large parts of Northern Ukraine, southeastern Belarus, and western Russia, with less but still detectable contaminants distributed over much of Fennoscandia and Central Europe (Evangelidou et al. 2013). Contamination levels derived from the former Chernobyl NPP are highly variable with background radiation levels sometimes varying by two orders of magnitude between places separated by a few hundred meters (Fig. 1). This variation in contamination levels, where high and low levels of radiation rate occur

in relatively close proximity, allows for sensitive and replicated analyses of biological effects of exposure to radioactive contamination (Mousseau and Møller 2011).

Despite an extensive literature concerning radionuclide movement in the environment and associated genetic damage (Chesser et al. 2001, Geras'kin et al. 2008, Yablokov 2009), there are no experimental tests concerning the ecological mechanisms which determine possible radiation effects in the Chernobyl ecosystem (Møller and Mousseau 2013b). For example, the only

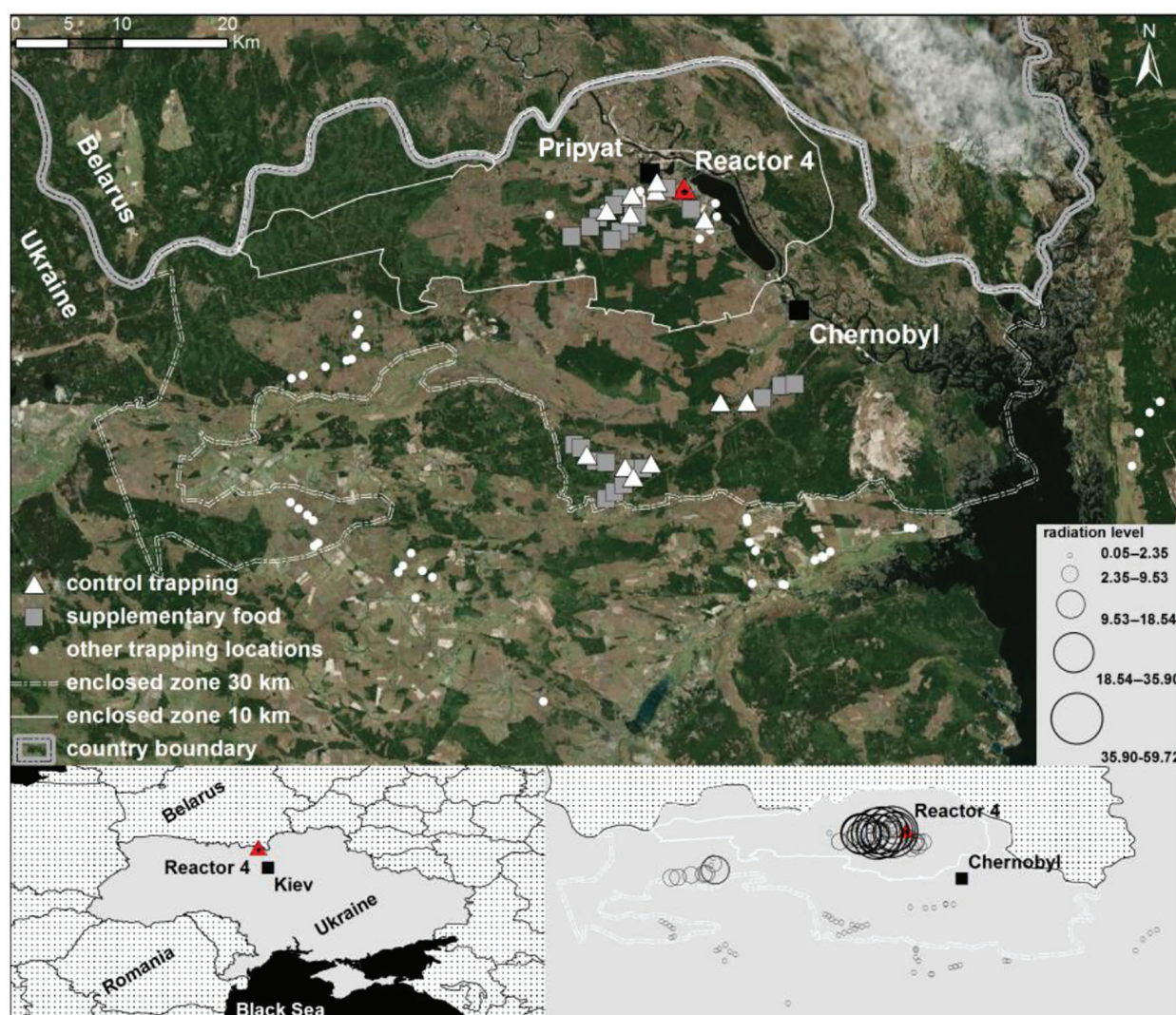


Fig. 1. Map of the Chernobyl Exclusion Zone (Ukraine) with locations where bank voles were trapped. The figure is created using Esri ArcGIS 10.0. Satellite imagery CNES/Airbus DS, Earthstar Geographics Source: Esri, DigitalGlobe, GeoEye, i-cubed, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AEX, GetMapping, AeroGRID, IGN, IGP, swisstopo, and the GIS User Community, Esri, HERE, DeLorme. Please note that several sampling sites were located at short distances as indicated by overlapping circles.



experiments quantifying the impact of exposure to radiation upon reproductive capability, and the concomitant population dynamics of small mammals, were conducted by manipulating the external radiation levels (Mihok et al. 1985, Mihok 2004). These experiments, in a North American small rodent system, suggest that animals can be very resistant to external radiation doses if other sources of radiation exposure (e.g., via ingestion of contaminated food) and ecosystem effects are excluded. Here, our aim was to experimentally test the hypothesis that ecological mechanisms (namely availability of food resources) can modify some of the putative detrimental effects of radiation in natural populations. This question is particularly relevant given recent findings that organisms living under natural conditions appear to be many times more sensitive to the deleterious effects of ionizing radiation (Garnier-Laplace et al. 2013).

The biological effects of low-dose (<100 mSv) radiation exposure are strongly debated (Bonner 2003, Calabrese and O'Connor 2014). Much controversy surrounds the validity of the Threshold model; whereby, exposure to low radiation doses is predicted to have non-significant, or even beneficial (hormesis; Boonstra et al. 2005, Feinendegen 2005) effects on individuals with detrimental consequences of radiation occurring only above a threshold dose (Tubiana et al. 2009). The alternative hypothesis of Linear No-Threshold (LNT) model has been widely tested (and supported) in laboratory animals and in epidemiological studies of humans (Land 2002, Brooks 2005, Council 2006), but much less is known concerning the shape of radiation response curves for natural ecosystems (although see Møller and Mousseau 2011, Garnier-Laplace et al. 2015). Here, we tested predictions of the LNT model that radiation has a proportional relationship with individual fitness measures and concomitant population growth rates, without any evidence for a threshold below which negative effects are not observed.

We conducted a large-scale, replicated study of the effects of radioactive contamination on the breeding characteristics and abundances of a small mammal, the bank vole *Myodes glareolus*. In addition, we experimentally determined how food limitation interacted with radiation to affect population characteristics. The bank vole is a

common and abundant terrestrial vertebrate that inhabits Eurasian forest ecosystems (Macdonald and Barrett 1993), which makes it an attractive indicator species for the health of forest ecosystems that may have been injured by anthropogenic activities.

## MATERIALS AND METHODS

### *Study species*

The bank vole is abundant (typically between 10 and 80 individuals per hectare) in most types of forest (from deciduous to coniferous) in Europe and Asia (Macdonald and Barrett 1993). Its diet is highly variable and may include various herbs, seeds, buds, roots, berries, fruits, mosses, lichens, fungi, and small invertebrates (Calandra et al. 2015). Bank voles are important prey of many owls and hawks and mammalian predators from weasels to foxes (Krebs 1996). The bank vole breeding season lasts from May to September when females produce up to four litters, each with two to ten offspring (Koivula et al. 2003, Mappes and Koskela 2004). In the wild, bank voles can live up to 1.5 yr, including one overwintering and one breeding season (Innes and Millar 1994).

### *Abundance index and breeding of females*

Abundances of bank voles were estimated in early summer (30 May–7 June 2011, 48 locations, 960 trap nights) and in winter (15–20 February 2016, 38 locations, 760 trap nights) within 50 km of the former Chernobyl NPP, Ukraine (Fig. 1). At each location, 20 traps were placed in line for one night, with each trap separated by about 10 m. Animals were caught with Ugglan Special2 live traps (Grahnb, Sweden) in summer 2011 (with sunflower seeds and potato as a bait), and with snap traps in winter 2016 (with bread and peanut oil as bait). The minimum distance between trapping locations was 500 m. To quantify habitat variation among locations, percentage vegetation cover was estimated within a 1 m radius around each trap at three layers: forest litter (vegetation of 0–50 cm high), bushes (0.5–2 m), and in the tree canopy. We selected these three measures of habitat to estimate whether contaminated and control areas are different in key components of habitat structure. Litter cover and bushes are a proxy for habitat in which bank



voles burrow and take refuge (Flowerdew and Ellwood 2001), and a cover of tree canopy can determine a general habitat selection of this forest species (Zwolak et al. 2016). Breeding characteristics (breeding or not, and litter size) were measured for all adult females caught in 2011 ( $n = 25$ , 18 locations) and in August 2013 ( $n = 34$ , an additional trapping in 24 locations). The breeding probability of females was estimated by taking all captured females to the laboratory where their possible pregnancy and breeding were followed. Number of offspring (litter size) was measured when pregnant females gave birth. Head width (a proxy of body size) was measured to the nearest 0.1 mm with a digital caliper, and animals were weighed to the nearest 0.1 g using a digital balance. Sample size (number of trapping locations) was maximized during the research periods, with time limits constrained by safety issues for humans as determined by the Chernobyl Exclusion Zone administration.

#### *Measurements of ambient radiation*

Ambient radiation levels at trapping locations were measured at 1 cm above the ground with a handheld GM dosimeter (Gamma-Scout w/ ALERT Radiation detector/Geiger Counter, Gamma-Scout GmbH and Co. KG, Germany) calibrated to measure Sieverts per hour (Sv/h). The mean ambient radiation levels varied among trapping locations from 0.01 to 95.55  $\mu\text{Sv/h}$  (Fig. 1). Given the long half-life of  $^{137}\text{Cs}$ , such measurements of radiation are highly repeatable among days and even years (Møller and Mousseau 2013a).

#### *Feeding experiment*

The experimental populations for the feeding experiment were chosen at the beginning of the 2014 breeding season. We chose 18 feeding locations from contaminated areas (range 1.16–30.54  $\mu\text{Sv/h}$ , mean 7.45  $\mu\text{Sv/h}$ ) and 18 locations from control areas (range 0.10–0.22  $\mu\text{Sv/h}$ , mean 0.15  $\mu\text{Sv/h}$ ). Both contaminated and control locations were divided randomly into three experimental groups (six populations each). The experimental groups were as follows: control (no food manipulation), rodent food (RM1, Special Diet Services), and rodent food containing the potential radio-protectant/mitigant, indole-3-carbinol (Fan et al. 2013). Since indole-3-carbinol

did not affect bank vole abundance ( $F = 0.305$ ,  $df = 2,17$ ,  $P > 0.587$ ) or interact with the radiation level ( $F = 0.001$ ,  $df = 2,17$ ,  $P > 0.996$ ), the two food supplementation groups were combined into a single food treatment in the subsequent analyses. Food was provided ad libitum at each feeding station; the minimum distance between the feeding stations was 1 km. The sample size (number of feeding places) was maximized according to the constraints caused by material (e.g., food) and time limits determined by safety regulations imposed by the Chernobyl Exclusion Zone administration on human research activity. Abundance of bank voles was estimated prior to the beginning of the experiment (early June) and after the breeding season (early October) using a  $3 \times 3$  trapping grid, with an inter-trap distance of 20 m; the feeding station was located in the middle of the trapping grid. The trapping period was five days in each feeding area. As the experiment was conducted in open populations, the bank vole abundances represent a combination of reproduction and mortality, as well as immigration and emigration: These different ecological mechanisms could not be separated in the present study. In general, bank vole females defend breeding territories of up to 0.6 ha (Mazurkiewicz 1983) (corresponding to 40–50 m radius circle), but males and non-breeding females are not territorial and they can disperse up to 1 km (Kozakiewicz et al. 2007).

#### *Statistical analyses*

Breeding characteristics of individuals were analyzed with a generalized linear mixed model (GLMM), where either breeding probability (binomial error distribution and logit link function) or litter size (multinomial error distribution and logit link function) was the dependent variable, and ambient radiation level ( $\log_{10}$  transformed) was the predictor. As the breeding characters were studied in the two different years 2011 and 2013, we first tested whether the effects of radiation differed between years. These analyses showed that the main effect of year and its interactions with radiation were not significant (for breeding probability: the main effect,  $P = 0.375$ ; interaction,  $P = 0.521$ ; and for litter size: the main effect,  $P = 0.095$ ; interaction,  $P = 0.111$ ). Consequently, we combined the data for the two years in the same analyses. In the subsequent analyses

of breeding characteristics, year and location were included as random factors. Curve estimations (Curve Fit) were used to analyze linear or quadratic relationship between dependent variables and radiation (both log<sub>10</sub> transformed). Vegetation cover variables were arcsine transformed. The effects of food supplementation were analyzed at the population level also using a GLMM, with abundance index as the dependent variable, and food supplementation, radiation level (low or high radiation level), and their interaction as predictors (Table 1). Location was included in all models as a random factor. All statistical tests were performed using IBM SPSS v.20.0 (IBM SPSS, Chicago, Illinois, USA).

**RESULTS**

The probability of a bank vole being pregnant decreased significantly with increasing ambient radiation level (GLMM, binary logistic regression; coefficient -0.591, *t* = -2.073, *df* = 57, *P* = 0.043; linear equation: *t* = -2.407, *P* = 0.019; quadratic equation: *t* = 1.516, *P* = 0.135; Fig. 2a). Litter size of bank voles varied between 1 and 8 (mean = 5.17, SE = 0.280) and decreased significantly with increasing radiation levels (GLMM, multinomial logistic regression; coefficient -0.651, *t* = -2.206, *df* = 29, *P* = 0.048; linear equation: *t* = -2.800, *P* = 0.008; quadratic equation: *t* = -0.831, *P* = 0.412; Fig. 2b). Linear equations of both the probability of being pregnant and litter size were more significant compared to their quadratic equations, consistent with the Linear No-Threshold (LNT) model for radiation effects.

Size of females was not significantly related to radiation (*t* = -1.776, *df* = 57, *P* = 0.081), and there was no interactive effect of female size with radiation on breeding probability or litter size

(*t* = 0.231, *df* = 55, *P* = 0.818 and *t* = 0.336, *df* = 27, *P* = 0.739, respectively). Abundance of bank voles was not correlated with breeding probability or litter size (*t* = 0.622, *df* = 55, *P* = 0.537; *t* = -0.444, *df* = 27, *P* = 0.660) and did not have an interactive effect with radiation levels (*t* = 0.336, *df* = 55, *P* = 0.738; *t* = -0.860, *df* = 27, *P* = 0.397). These results suggest that radiation did not indirectly affect the breeding success of voles by changing their structural size or by modifying population densities and its possible consequences (e.g., level of intra-specific competition).

**Abundances of bank voles**

Abundance index varied from 0 to 11 individuals per trapping location. Both summer (Fig. 2c) and winter (Fig. 2d) abundances of bank voles decreased significantly with increasing ambient radiation (in summer: *r*<sup>2</sup> = 0.209, *t* = -3.490, *df* = 47, *P* = 0.001; linear equation, *t* = -3.103, *P* = 0.003; quadratic equation, *t* = -0.348, *P* = 0.730; in winter: *r*<sup>2</sup> = 0.242, *t* = -3.394, *df* = 37, *P* = 0.002; linear equation, *t* = -2.239, *P* = 0.032; quadratic equation, *t* = -0.201, *P* = 0.842). As with radiation effects on probability of pregnancy and litter size described above, the significant linear terms support the predictions of the LNT model. Ambient radiation levels also negatively covaried with abundance indexes of control populations in the feeding experiment in 2014 (see below).

Radiation effects on bank vole populations could be biased by environmental differences between contaminated and control areas. Indeed, the vegetation cover of bushes (0.5–2 m) and tree canopy decreased with increasing radiation levels (coefficient = -0.058, *t* = -0.333, *P* = 0.021; coefficient = -0.076, *t* = -0.288, *P* = 0.047, respectively). However, the most important vegetation variable for bank voles (cover of forest litter: 0–50 cm) was not significantly correlated with radiation (coefficient = -0.034, *t* = -0.182, *P* = 0.216). Moreover, any effect of vegetation on bank voles was minimal as vegetation cover, either forest litter, bushes, or tree canopy, was not correlated with abundance indexes of bank voles (coefficient = -0.955, *t* = -0.416, *P* = 0.678; coefficient = -1.138, *t* = -0.443, *P* = 0.658; coefficient = 2.457, *t* = 1.448, *P* = 0.149, respectively). And most importantly, these vegetation variables

Table 1. Results of GLMM tests of the effects of food supplementation and background radiation on abundance index of bank voles in Chernobyl.

Effects	Coefficient	SE	<i>t</i>	<i>P</i>
Abundance				
Food	-1.387	0.667	-2.080	0.038
Radiation	0.463	0.464	0.999	0.318
Food × radiation	-1.609	0.771	-2.087	0.037

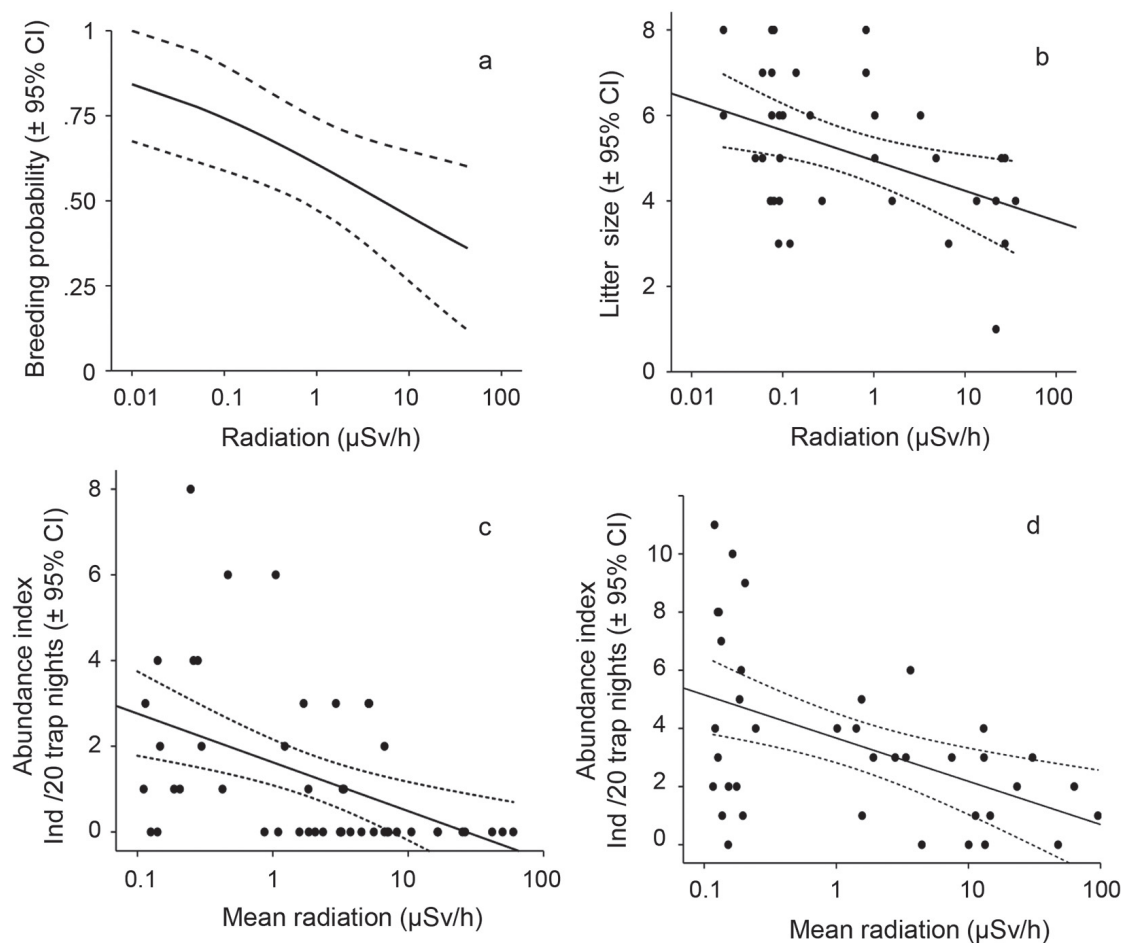


Fig. 2. (a) Background radiation level predicts the breeding probability of bank vole females in Chernobyl where fifty-nine adult females were caught (2011:  $n = 25$ ; 2013:  $n = 34$ ), of which 36 (61%) were pregnant. Predictive curve ( $\pm 95\%$  CI) is estimated by binary logistic regression (coefficient  $-0.591$ ,  $t = -2.073$ ,  $df = 57$ ,  $P = 0.043$ ). (b) Litter size of breeding females ( $n = 36$ ) decreased with an increase in ambient radiation levels ( $y = -0.71 (\pm 0.25) \times \log(x) + 4.94 (\pm 0.27)$ ) (GLMM, multinomial logistic regression; coefficient  $-0.651$ ,  $t = -2.061$ ,  $df = 29$ ,  $P = 0.048$ ). Abundances of bank voles decreased with increasing the mean ambient radiation level at the trapping area ( $n = 48$ ) in summer (c) ( $y = -1.14 (\pm 0.33) \times \log(x) + 1.63 (\pm 0.27)$ ,  $r^2 = 0.209$ ,  $t = -3.490$ ,  $df = 47$ ,  $P = 0.001$ ) and in winter (d) ( $n = 38$ ) ( $y = -1.15 (\pm 0.44) \times \log(x) + 3.67 (\pm 0.42)$ ,  $r^2 = 0.242$ ,  $t = -3.394$ ,  $df = 37$ ,  $P = 0.002$ ).

did not interact significantly with radiation level (coefficient =  $-0.718$ ,  $t = -0.237$ ,  $P = 0.813$ ; coefficient =  $-1.380$ ,  $t = -0.364$ ,  $P = 0.716$ ; coefficient =  $3.457$ ,  $t = 1.392$ ,  $P = 0.165$ , respectively).

Radiation clearly affected the response of the bank vole populations to environmental changes. The population living with additional food resources increased only in low radiation areas, (up to  $1 \mu\text{Sv/h}$ ), but decreased from  $1 \mu\text{Sv/h}$  to  $30 \mu\text{Sv/h}$  (quadratic equation:  $t = -2.836$ ,  $P = 0.010$ ; Fig. 3, Table 1). Abundance indexes of the populations living without supplemental

food tended to decrease linearly with increasing ambient radiation levels ( $t = -1.909$ ,  $P = 0.085$ ; Fig. 3).

## DISCUSSION

The results presented here refute the hypothesis of there being a threshold level of radiation below which there are no effects in natural populations of animals (Tubiana et al. 2009). Support for a threshold hypothesis would be derived from a non-linear relationship between low-dose

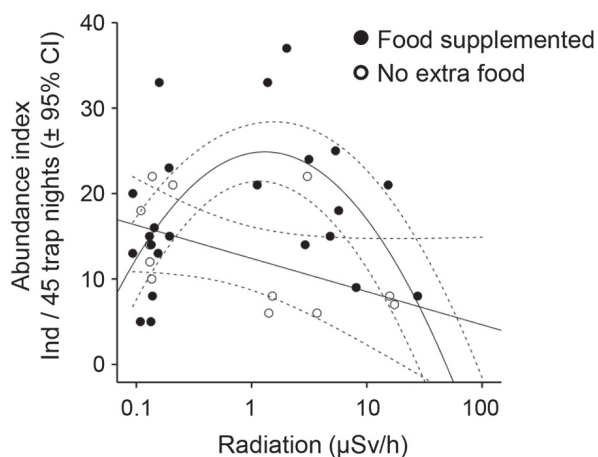


Fig. 3. The effects of feeding experiments on bank vole populations in 18 control areas (range 0.10–0.22  $\mu\text{Sv/h}$ , mean 0.15  $\mu\text{Sv/h}$ ) and 18 contaminated areas (range 1.16–30.54  $\mu\text{Sv/h}$ , mean 7.45  $\mu\text{Sv/h}$ ). In the areas where additional food was provided ( $n = 24$ ), abundances of bank voles increased with radiation level up to about 1  $\mu\text{Sv/h}$  and then decreased above this radiation level. In the areas without additional food, populations tended to decrease linearly with the increase in radiation level (see statistics in the *Results*).

radiation and its consequences, such that there are non-significant negative effects (or even beneficial effects, e.g., hormesis; Boonstra et al. 2005, Feinendegen 2005) of low radiation levels (here at 0.5–10  $\mu\text{Sv/h}$ ) and with harmful effects beginning, and increasing significantly thereafter, following a specific threshold level of radiation exposure. Contrary to the threshold hypothesis, harmful and significant radiation effects on bank vole populations can be observed even at very low levels of ambient radioactivity (1  $\mu\text{Sv/h}$  or less) and these effects increase linearly with exposure above these levels. However, our findings also show that ecological mechanisms can modify linear effects of radiation. Here, the supplemental food resources increased vole abundances up to a low level of radiation ( $\approx 1$   $\mu\text{Sv/h}$ ), but higher levels were associated with decreased abundances independently of supplemental food resources.

Chronic exposure to ionizing radiation is widely believed to have direct and indirect effects on natural populations of animals. Direct

effects of radiation exposure include an increased frequency of mutations (Møller and Mousseau 2015, but see cf. Kesäniemi et al. 2018) and/or damage to DNA that causes developmental disorders, tumors, and cancers (Møller et al. 2007). Moreover, birds inhabiting areas of high radiation have impaired sperm morphology (Møller et al. 2008), potentially providing one explanation for the lower breeding probability of bank voles. Certainly, bank voles inhabiting areas contaminated by radionuclides derived from the former Chernobyl NPP show signs of molecular stress, such as upregulation of some DNA damage response genes (Jernfors et al. 2018) and altered telomere homeostasis (Kesäniemi et al. 2019). These biological effects could be caused by direct exposure to gamma radiation from the surrounding environment or by exposure to alpha and beta particles accumulated in animals from food (Sazykina and Kryshev 2006). For example, mushrooms, an essential component of the diet of bank voles (Hansson 1979), can be an enormous source of alpha- and beta-emitting radionuclides (Mihok et al. 1989, Gralla et al. 2014). At this time, we cannot yet distinguish the direct effects of radiation from its indirect effects. Such indirect effects could be modified by quantity or quality of food resources as affected by radiation. For example, food resources of voles (mainly plants, fungi, and small invertebrates; Calandra et al. 2015) are likely altered in contaminated areas (Tikhomirov and Shcheglov 1994) and we found some impact of radiation on cover of trees and bushes. With this in mind, a high level of radionuclides is associated with an altered gut microbiota in bank voles (Lavrinienko et al. 2018), potentially indicating that radiation exposure is associated with a change in diet. Another important ecological factor, predation rate, may also be lower in contaminated areas, since, for example, both avian (Møller and Mousseau 2009) and mammalian predators (Møller and Mousseau 2013a) of rodents decrease in abundance in contaminated areas. The absence of these ecological mechanisms could be the main reasons for differences between our results and those of earlier studies where populations of small mammals were manipulated only by exposure to external radiation (Mihok et al. 1985, Mihok 2004).



A novel element of the study reported here was the use of experimental manipulations of food resources in order to test whether the effects of food stress correlate with other environmental stressors (here exposure to environmental radionuclides). The effect of food supplementation in natural populations depends upon study species and its environment. Nonetheless, food supplementation generally does not strongly increase population densities when environmental conditions are good (Boutin 1990) while densities often increase in populations experiencing harsh environments (Huitu et al. 2003, Forbes et al. 2014, Johnsen et al. 2017). Accordingly, additional food did not increase bank vole abundances in the control populations but the food treatment did have a positive effect on abundance in contaminated areas at low (<1  $\mu\text{Sv/h}$  ambient dose rates) radiation levels. However, above this level of radiation, the increasing radiation levels had a clear negative effect on abundances of bank voles despite food supplementation. Thus, the food supplementation can mitigate the detrimental effects of an environment contaminated by radionuclides up to a certain point only. We suggest that the relevant environment of bank voles can be altered at many trophic stages. For example, predation risk of avian predators could be already reduced at the elevated radiation levels (1  $\mu\text{Sv/h}$ ) (Møller and Mousseau 2013a), and thus, these predators might be unable to limit population increases when food stress of voles is artificially relaxed. Furthermore, many parasites and diseases of voles might not regulate their populations in a density-dependent manner in elevated radiation levels (Sibly and Hone 2002), although the interactions between these important ecological processes and radiation are still unknown (Morley 2012).

The interaction between ionizing radiation and other environmental stressors on natural populations is being increasingly recognized as potentially significant. For example, in a meta-analysis of the effects of Chernobyl-derived radioactive contaminants on 19 species of plants and animals living under natural conditions (Garnier-Laplace et al. 2013), it was found that organisms in the wild were more than eight times more sensitive to negative radiation effects than these same species living under laboratory or model conditions. Similarly, a study of pine trees (*Pinus sylvestris*)

living across a wide range of ambient radiations levels in Chernobyl found very strong negative effects on growth during the first three years after the disaster in 1986, with large negative effects persisting for 2.5 decades following the disaster during years of significant drought (Mousseau et al. 2013). And, a study of pollinators, fruit trees, and frugivores in Chernobyl found evidence for significant interactions among these guilds that varied across ambient radiation levels (Møller et al. 2012). These studies suggest a very large effect of ecological interactions on the susceptibility of organisms to the deleterious effects of ionizing radiation.

To conclude, in this study we used experimental manipulation of food resources to demonstrate significant effects of radiation on a key forest mammal. These findings are particularly important given the potential for ecosystem-wide consequences of the observed effects on rodents. These results suggest that rodent populations, and by implication, entire ecosystems, are likely to have been affected across perhaps 200,000  $\text{km}^2$  in Eastern, Northern, and even Central Europe where radioactive contaminants stemming from the Chernobyl disaster are still measurable in a large diversity of different species and are known to accumulate in the food chain (e.g., wild boars *Sus scrofa* in Germany, reindeer *Rangifer tarandus* in Finland and Sweden (Hohmann and Huckschlag 2005, Strebl and Tataruch 2007, Semizhon et al. 2009)). Although the consequences of exposure to low-dose radiation are very difficult to detect under most circumstances because of the complexity of biotic and abiotic factors shaping individual fitness and population processes, the experimental studies presented here provide irrefutable evidence that even very low doses can lead to significant consequences for individuals, populations, and likely even entire ecosystems.

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## II

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