

**This is a self-archived version of an original article. This version may differ from the original in pagination and typographic details.**

**Author(s):** Mappes, Tapio; Boratynski, Zbigniew; Kivisaari, Kati; Lavrinienko, Anton; Milinevsky, Gennadi; Mousseau, Timothy A.; Møller, Anders P.; Tukalenko, Eugene; Watts, Phillip

**Title:** Ecological mechanisms can modify radiation effects in a key forest mammal of Chernobyl

**Year:** 2019

**Version:** Accepted version (Final draft)

**Copyright:** © 2019 The Authors.

**Rights:** CC BY 3.0

**Rights url:** <https://creativecommons.org/licenses/by/3.0/>

**Please cite the original version:**

Mappes, T., Boratynski, Z., Kivisaari, K., Lavrinienko, A., Milinevsky, G., Mousseau, T. A., Møller, A. P., Tukalenko, E., & Watts, P. (2019). Ecological mechanisms can modify radiation effects in a key forest mammal of Chernobyl. *Ecosphere*, 10(4), Article e02667.  
<https://doi.org/10.1002/ecs2.2667>

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

TAPIO MAPPES,<sup>1,†</sup> ZBYSZEK BORATYŃSKI,<sup>1,2</sup> KATI KIVISAARI,<sup>1</sup> ANTON LAVRINIENKO,<sup>3</sup> GENNADI MILINEVSKY,<sup>4</sup>  
TIMOTHY A. MOUSSEAU,<sup>5</sup> ANDERS P. MØLLER,<sup>6</sup> EUGENE TUKALENKO,<sup>1,3,7</sup> AND PHILLIP C. WATTS<sup>1,3</sup>

<sup>1</sup>Department of Biological and Environmental Science, University of Jyväskylä, P.O. Box 35, Jyväskylä FI-40014 Finland

<sup>2</sup>CIBIO/InBIO, Research Center in Biodiversity and Genetic Resources, University of Porto, Vairão PT-4485-661 Portugal

<sup>3</sup>Ecology and Genetics, University of Oulu, Oulu FI-90014 Finland

<sup>4</sup>Physics Faculty, Taras Shevchenko National University of Kyiv, 64/13 Volodymyrska Street, Kyiv UA-01601 Ukraine

<sup>5</sup>Department of Biological Sciences, University of South Carolina, Columbia, South Carolina 29208 USA

<sup>6</sup>Ecologie Systématique Evolution, Université Paris-Sud, CNRS, AgroParisTech, Université Paris-Saclay, Orsay Cedex F-91405 France

<sup>7</sup>National Research Center for Radiation Medicine of the National Academy of Medical Science, Kyiv 04050 Ukraine

**Citation:** Mappes, T., Z. Boratyński, K. Kivisaari, A. Lavrinienko, G. Milinevsky, T. A. Mousseau, A. P. Møller, E. Tukalenko, and P. C. Watts. 2019. Ecological mechanisms can modify radiation effects in a key forest mammal of Chernobyl. *Ecosphere* 10(4):e02667. 10.1002/ecs2.2667

**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.

**Received** 19 September 2018; revised 19 February 2019; accepted 25 February 2019. Corresponding Editor: Robert R. Parmenter.

**Copyright:** © 2019 The Authors. This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

† **E-mail:** tapio.mappes@jyu.fi

### 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

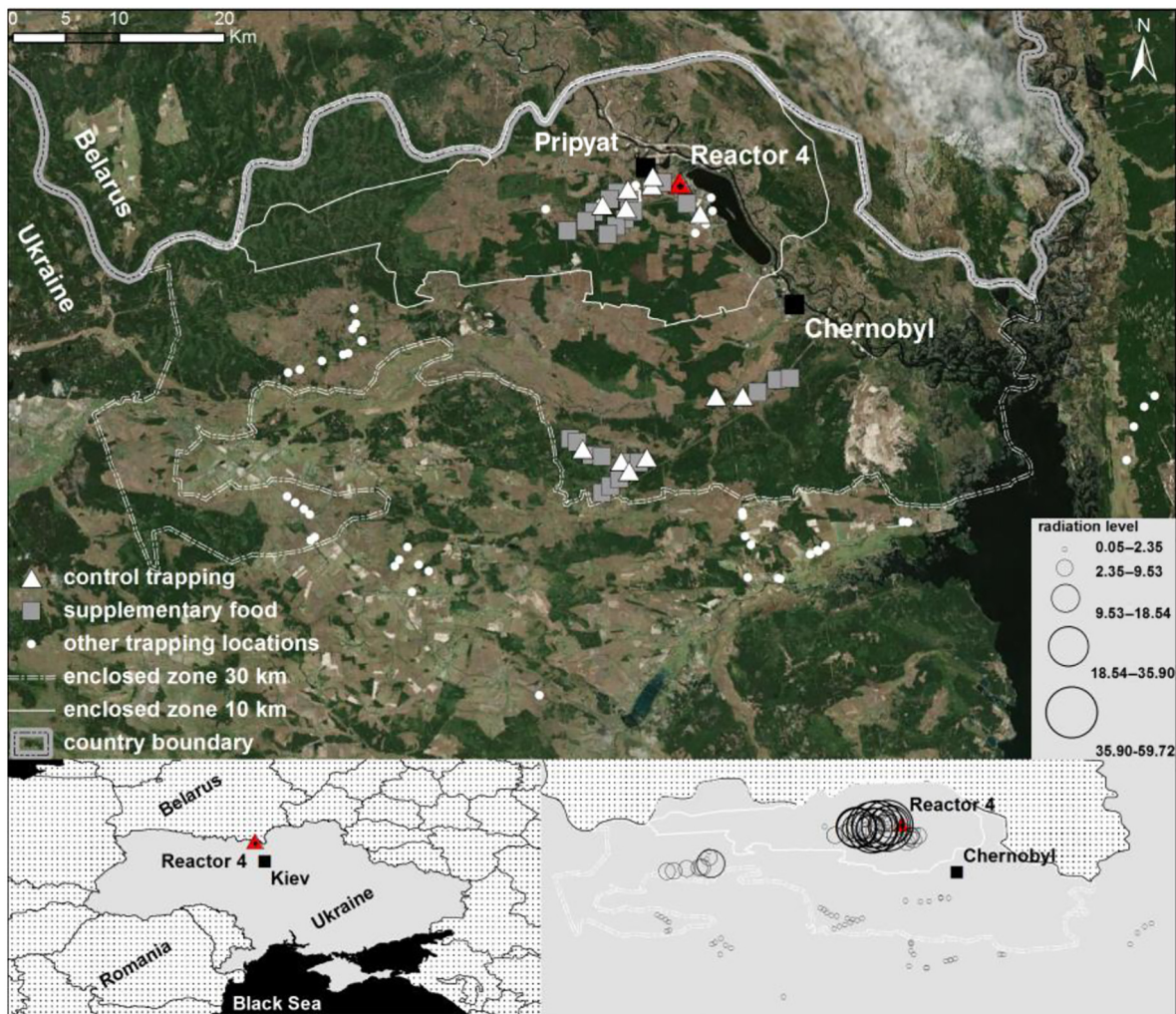


Fig. 1. Map of the Chernobyl Exclusion Zone (Ukraine) with locations where bank voles were trapped. The figure is created using Esri ArcGIS 10.0. Satellite imagery CNES/Airbus DS, Earthstar Geographics Source: Esri, DigitalGlobe, GeoEye, i-cubed, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AEX, 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

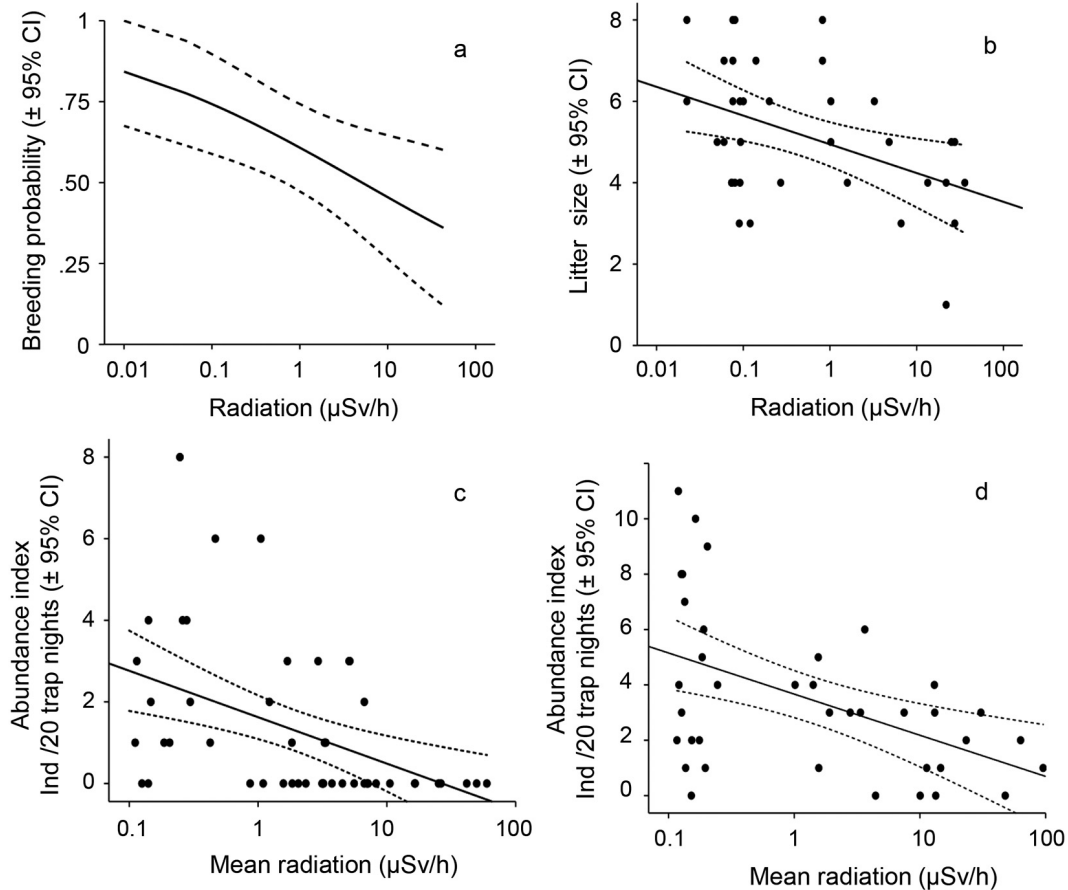


Fig. 2. (a) Background radiation level predicts the breeding probability of bank vole females in Chernobyl where fifty-nine adult females were caught (2011:  $n = 25$ ; 2013:  $n = 34$ ), of which 36 (61%) were pregnant. Predictive curve ( $\pm 95\%$  CI) is estimated by binary logistic regression (coefficient  $-0.591$ ,  $t = -2.073$ ,  $df = 57$ ,  $P = 0.043$ ). (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

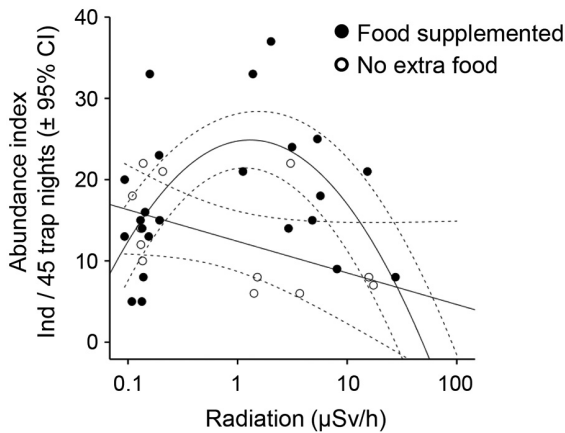


Fig. 3. The effects of feeding experiments on bank vole populations in 18 control areas (range 0.10–0.22  $\mu\text{Sv/h}$ , mean 0.15  $\mu\text{Sv/h}$ ) and 18 contaminated areas (range 1.16–30.54  $\mu\text{Sv/h}$ , mean 7.45  $\mu\text{Sv/h}$ ). In the areas where additional food was provided ( $n = 24$ ), 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 (Lavrinenko 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.

## ACKNOWLEDGMENTS

We gratefully acknowledge logistic support and help in Ukraine by Igor Chizhevsky and the Chernobyl EcoCenter. This study was financially supported by Academy of Finland grants to TM (268670) and PCW (287153), Emil Aaltonen Foundation and Oskar Oflund Foundation to KK, the postdoctoral grantee from the Portuguese Foundation for Science and Technology

(RH/BPD/84822/2012) to ZB, and the Graduate School of the University of Oulu to AL. Additional support was provided by the CNRS (France), the Samuel Freeman Charitable Trust, the Fulbright Program, the American Council of Learned Societies, and the College of Arts and Sciences at the University of South Carolina. Authors after the first author are listed in alphabetical order. TM, ZB, KK, AL, GM, TAM, APM, ET, and PCW designed the study and contributed to acquisition of field data and experiments; TM carried out the statistical analyses and drafted the manuscript; TM, ZB, KK, AL, GM, TAM, APM, ET, and PCW contributed to writing the manuscript and gave final approval for publication. The authors declare no competing financial interests.

## LITERATURE CITED

- Anspaugh, L. R., R. J. Catlin, and M. Goldman. 1988. The global impact of the Chernobyl reactor accident. *Science* 242:1513–1519.
- Bonner, W. M. 2003. Low-dose radiation: thresholds, bystander effects, and adaptive responses. *Proceedings of the National Academy of Sciences of USA* 100:4973–4975.
- Boonstra, R., R. G. Manzon, S. Mihok, and J. E. Helson. 2005. Hormetic effects of gamma radiation on the stress axis of natural populations of meadow voles (*Microtus pennsylvanicus*). *Environmental Toxicology and Chemistry* 24:334–343.
- Boutin, S. 1990. Food supplementation experiments with terrestrial vertebrates—patterns, problems, and the future. *Canadian Journal of Zoology* 68:203–220.
- Brooks, A. L. 2005. Paradigm shifts in radiation biology: their impact on intervention for radiation-induced disease. *Radiation Research* 164:454–461.
- Calabrese, E. J., and M. K. O'Connor. 2014. Estimating risk of low radiation doses – A critical review of the beir vii report and its use of the linear no-threshold (LNT) hypothesis. *Radiation Research* 182:463–474.
- Calandra, I., G. Labonne, O. Mathieu, H. Henttonen, J. Leveque, M. J. Milloux, E. Renvoise, S. Montuire, and N. Navarro. 2015. Isotopic partitioning by small mammals in the subnivium. *Ecology and Evolution* 5:4132–4140.
- Chesser, R. K., B. E. Rodgers, J. K. Wickliffe, S. Gaschak, I. Chizhevsky, C. J. Phillips, and R. J. Baker. 2001. Accumulation of (137)cesium and (90)strontium from abiotic and biotic sources in rodents at Chornobyl, Ukraine. *Environmental Toxicology and Chemistry* 20:1927–1935.
- Council, N. R. 2006. *Health Risks from Exposure to Low Levels of Ionizing Radiation: BEIR VII Phase 2*. National Academies Press, Washington, D.C., USA.
- Evangelidou, N., Y. Balkanski, A. Cozic, and A. P. Møller. 2013. Simulations of the transport and deposition of Cs-137 over Europe after the Chernobyl Nuclear Power Plant accident: influence of varying emission-altitude and model horizontal and vertical resolution. *Atmospheric Chemistry and Physics* 13:7183–7198.
- Fan, S. J., Q. H. Meng, J. Y. Xu, Y. Jiao, L. Zhao, X. D. Zhang, F. H. Sarkar, M. L. Brown, A. Dritschilo, and E. M. Rosen. 2013. DIM (3,3'-diindolylmethane) confers protection against ionizing radiation by a unique mechanism. *Proceedings of the National Academy of Sciences of USA* 110:18650–18655.
- Feinendegen, L. E. 2005. Evidence for beneficial low level radiation effects and radiation hormesis. *British Journal of Radiology* 78:3–7.
- Flowerdew, J. R., and S. A. Ellwood. 2001. Impacts of woodland deer on small mammal ecology. *Forestry* 74:277–287.
- Forbes, K. M., P. Stuart, T. Mappes, K. S. Hoset, H. Henttonen and O. Huitu. 2014. Diet quality limits summer growth of field vole populations. *PLoS ONE* 9:e91113.
- Garnier-Laplace, J., K. Beaugelin-Seiller, C. Della-Vedova, J. M. Metivier, C. Ritz, T. A. Mousseau and A. P. Møller. 2015. Radiological dose reconstruction for birds reconciles outcomes of Fukushima with knowledge of dose-effect relationships. *Scientific Reports* 5:16594.
- Garnier-Laplace, J., S. Geras'kin, C. Della-Vedova, K. Beaugelin-Seiller, T. G. Hinton, A. Real and A. Oudalova. 2013. Are radiosensitivity data derived from natural field conditions consistent with data from controlled exposures? A case study of Chernobyl wildlife chronically exposed to low dose rates. *Journal of Environmental Radioactivity* 121:12–21.
- Geras'kin, S. A., S. V. Fesenko and R. M. Alexakhin. 2008. Effects of non-human species irradiation after the Chernobyl NPP accident. *Environment International* 34:880–897.
- Gralla, F., D. J. Abson, A. P. Møller, D. J. Lang, and H. von Wehrden. 2014. The impact of nuclear accidents on provisioning ecosystem services. *Ecological Indicators* 41:1–14.
- Hansson, L. 1979. Condition and diet in relation to habitat in bank voles *Clethrionomys glareolus*: population or community approach? *Oikos* 33:55–63.
- Hohmann, U., and D. Huckschlag. 2005. Investigations on the radiocaesium contamination of wild boar (*Sus scrofa*) meat in Rhineland-Palatinate: a stomach content analysis. *European Journal of Wildlife Research* 51:263–270.
- Huitu, O., M. Koivula, E. Korpimäki, T. Klemola, and K. Norrdahl. 2003. Winter food supply limits

- growth of northern vole populations in the absence of predation. *Ecology* 84:2108–2118.
- Innes, D. G. L., and J. S. Millar. 1994. Life-histories of *Clethrionomys* and *Microtus* (Microtinae). *Mammal Review* 24:179–207.
- Jernfors, T., J. Kesäniemi, A. Lavrinienko, T. Mappes, G. Milinevsky, A. P. Møller, T. A. Mousseau, E. Tukalenko, and P. C. Watts. 2018. Transcriptional Upregulation of DNA Damage Response Genes in Bank Voles (*Myodes glareolus*) Inhabiting the Chernobyl Exclusion Zone. *Frontiers in Environmental Science* 5:95.
- Johnsen, K., R. Boonstra, S. Boutin, O. Devineau, C. J. Krebs, and H. P. Andreassen. 2017. Surviving winter: Food, but not habitat structure, prevents crashes in cyclic vole populations. *Ecology and Evolution* 7:115–124.
- Kesäniemi, J., Z. Boratyński, J. Danforth, P. Itam, T. Jernfors, A. Lavrinienko, T. Mappes, A. P. Møller, T. A. Mousseau and P. C. Watts. 2018. Analysis of heteroplasmy in bank voles inhabiting the Chernobyl exclusion zone: a commentary on Baker et al. (2017) “Elevated mitochondrial genome variation after 50 generations of radiation exposure in a wild rodent.” *Evolutionary Applications* 11:820–826.
- Kesäniemi, J., A. Lavrinienko, E. Tukalenko, Z. Boratyński, K. Kivisaari, T. Mappes, G. Milinevsky, A. P. Møller, T. A. Mousseau, and P. C. Watts. 2019. Exposure to environmental radionuclides associates with tissue-specific impacts on telomerase expression and telomere length. *Scientific Reports* 9:850.
- Koivula, M., E. Koskela, T. Mappes, and T. A. Oksanen. 2003. Costs of reproduction in the wild: manipulation of reproductive effort in the bank vole. *Ecology* 84:398–405.
- Kozakiewicz, M., A. Chołuj, and A. Kozakiewicz. 2007. Long-distance movements of individuals in a free-living bank vole population: an important element of male breeding strategy. *Acta Theriol (Warsz)* 52:339–348.
- Krebs, C. J. 1996. Population cycles revisited. *Journal of Mammalogy* 77:8–24.
- Land, C. E. 2002. Uncertainty, low-dose extrapolation and the threshold hypothesis. *Journal of Radiological Protection* 22:A129–A135.
- Lavrinienko, A., T. Mappes, E. Tukalenko, T. A. Mousseau, A. P. Møller, R. Knight, J. T. Morton, L. R. Thompson, and P. C. Watts. 2018. Environmental radiation alters the gut microbiome of the bank vole *Myodes glareolus*. *ISME Journal* 12:2801–2806.
- Macdonald, D. W., and P. Barrett. 1993. *Mammals of Britain & Europe*. Harper Collins, London, UK.
- Mappes, T., and E. Koskela. 2004. Genetic basis of the trade-off between offspring number and quality in the bank vole. *Evolution* 58:645–650.
- Mazurkiewicz, M.. 1983. Ecological structure of the population: spatial organization of the population. *Acta Theriologica* 28:117–127.
- Mihok, S. 2004. Chronic exposure to gamma radiation of wild populations of meadow voles (*Microtus pennsylvanicus*). *Journal of Environmental Radioactivity* 75:233–266.
- Mihok, S., B. Schwartz, and S. L. Iverson. 1985. Ecology of Red-backed Voles (*Clethrionomys gapperi*) in a gradient of gamma-radiation. *Annales Zoologici Fennici* 22:257–271.
- Mihok, S., B. Schwartz, and A. M. Wiewel. 1989. Bioconcentration of fallout Cs-137 by fungi and Red-backed voles (*Clethrionomys gapperi*). *Health Physics* 57:959–966.
- Møller, A. P., F. Barnier, and T. A. Mousseau. 2012. Ecosystems effects 25 years after Chernobyl: pollinators, fruit set and recruitment. *Oecologia* 170:1155–1165.
- Møller, A. P., and T. A. Mousseau. 2009. Reduced abundance of raptors in radioactively contaminated areas near Chernobyl. *Journal of Ornithology* 150:239–246.
- Møller, A. P., and T. A. Mousseau. 2011. Efficiency of bio-indicators for low-level radiation under field conditions. *Ecological Indicators* 11:424–430.
- Møller, A. P., and T. A. Mousseau. 2013a. Assessing effects of radiation on abundance of mammals and predator-prey interactions in Chernobyl using tracks in the snow. *Ecological Indicators* 26:112–116.
- Møller, A. P., and T. A. Mousseau. 2013b. The effects of low-dose radiation: Soviet science, the nuclear industry – and independence? *Significance* 10:14–19.
- Møller, A. P., and T. A. Mousseau. 2015. Strong effects of ionizing radiation from Chernobyl on mutation rates. *Scientific Reports* 5:8363.
- Møller, A. P., T. A. Mousseau, F. de Lope, and N. Saino. 2007. Elevated frequency of abnormalities in barn swallows from Chernobyl. *Biology Letters* 3:414–417.
- Møller, A. P., T. A. Mousseau, C. Lynn, S. Ostermiller, and G. Rudolfson. 2008. Impaired swimming behaviour and morphology of sperm from barn swallows *Hirundo rustica* in Chernobyl. *Mutation Research-Genetic Toxicology and Environmental Mutagenesis* 650:210–216.
- Morley, N. J. 2012. The effects of radioactive pollution on the dynamics of infectious diseases in wildlife. *Journal of Environmental Radioactivity* 106:81–97.
- Mousseau, T. A., and A. P. Møller. 2011. Landscape portrait: a look at the impacts of radioactive contaminants on Chernobyl’s wildlife. *Bulletin of the Atomic Scientists* 67:38–46.
- Mousseau, T. A., S. M. Welch, I. Chizhevsky, O. Bondarenko, G. Milinevsky, D. J. Tedeschi, A. Bonisoli-

- Alquati, and A. P. Møller. 2013. Tree rings reveal extent of exposure to ionizing radiation in Scots pine *Pinus sylvestris*. *Trees-Structure and Function* 27:1443–1453.
- Sazykina, T., and I. I. Kryshev. 2006. Radiation effects in wild terrestrial vertebrates - the EPIC collection. *Journal of Environmental Radioactivity* 88:11–48.
- Semizhon, T., V. Putyrskaya, G. Zibold, and E. Klemt. 2009. Time-dependency of the Cs-137 contamination of wild boar from a region in Southern Germany in the years 1998 to 2008. *Journal of Environmental Radioactivity* 100:988–992.
- Sibly, R. M., and J. Hone. 2002. Population growth rate and its determinants: an overview. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* 357:1153–1170.
- Strebl, F., and F. Tataruch. 2007. Time trends (1986–2003) of radiocesium transfer to roe deer and wild boar in two Austrian forest regions. *Journal of Environmental Radioactivity* 98:137–152.
- Tikhomirov, F. A., and A. I. Shcheglov. 1994. Main investigation results on the forest radioecology in the Kyshtym and Chernobyl accident zones. *Science of the Total Environment* 157:45–57.
- Tubiana, M., L. E. Feinendegen, C. C. Yang, and J. M. Kaminski. 2009. The linear no-threshold relationship is inconsistent with radiation biologic and experimental data. *Radiology* 251:13–22.
- Yablokov, A. V. 2009. Chernobyl's Radioactive Impact on Fauna. Pages 255–280 *in* Chernobyl: consequences of the catastrophe for people and the environment. The New York Academy of Sciences, Oxford, Mississippi, USA.
- Zwolak, R., M. Bogdziewicz, and L. Rychlik. 2016. Beech masting modifies the response of rodents to forest management. *Forest Ecology and Management* 359:268–276.