Quantitative Analysis of Effects of a Single ^{60}Co Gamma Ray Point Exposure on Time-Dependent Change in Locomotor Activity in Rats

Keiko Otani[1*], Megu Ohtaki[1¶], Nariaki Fujimoto[2¶], Aisulu Saimova[3&], Ynkar Kairkhanova[4&], Darkhan Uzbekov[5&], Nailya Chaizhunusova[6&], Nulran Aukenov[7&], Tolebay Rakhypbekov[8&], Hitoshi Sato[9&], Noriyuki Kawano[1&], Masaharu Hoshi[1¶]

[1] The Center for Peace, Hiroshima University, Hiroshima, Japan

[2]Research Institute for Radiation Biology and Medicine, Hiroshima University, Hiroshima, Japan

[3]Maxillo-facial and facial plastic surgery, Semey Medical University, Semey, Kazakhstan

[4]Microbiology, Semey Medical University, Semey, Kazakhstan

[5]Pathological anatomy and forensic medicine, Semey Medical University, Semey, Kazakhstan

[6]Public health, Medical University, Semey, Kazakhstan

[7]Science and human resources, The Ministry of Healthcare, Semey, Kazakhstan

[8]Board of Directors, Astana Medical University, Astana, Kazakhstan

[9]Radiological Sciences, Ibaraki Prefectural University of Health Sciences, Ibaraki, Japan.

*Corresponding author

Email: ohtani@hiroshima-u.ac.jp (KO)

¶These authors contributed equally to this work.

[&]These authors also contributed equally to this work.

Abstract

Fatigue is one of the earliest nonspecific symptoms of radiation exposure in humans, but its etiology, mechanism, and dose dependency remain unexplained. Investigating initial behavioral changes caused by irradiation of animals might provide important information to aid understanding of early health effects of radiation exposure and clinical features of radiation injury. Although previous studies in rodents suggested that radiation exposure leads to reduced activity, detailed properties of the effects were unrevealed due to a lack of proper statistical analysis, which is needed to better elucidate details of changes in locomotor activity. Ten-week-old male Wistar rats were subjected to single point external whole-body irradiation with 60Co gamma rays at 0, 2.0, 3.5, and 5.0 Gy (4 rats per group).

Infrared sensors were used to continuously record locomotor activity of each rat. Cumulative number of movements during the night was defined as “activity” for each day. A non-linear mixed effects model accounting for individual differences and daily fluctuation of activity was applied to analyze the rats’ longitudinal locomotor data. Despite a small number of animals per group, our statistical method successfully revealed characteristics of the changes in locomotor activity after radiation exposure, showing that 1) reduction in activity occurred immediately—and in a dose-dependent manner—after irradiation and 2) recovery to pre-irradiation levels required almost one week, with the same recovery rate in each dose group. In addition to improving our understanding of radiation effects on locomotor activity, this statistical framework should be useful to analyze other data with similar structure.

1. Introduction

In humans, one of the earliest effects of radiation exposure to the whole body or to a large portion of the whole body is a prodromal period of nonspecific signs and symptoms such as nausea, emesis, fatigue, fever, and anorexia [1−2]. The prodromal syndrome is generally mild or absent at total body doses of 1 Gy or less and occurs from minutes to days following exposure [3−5]. However, it is unclear to what extent these symptoms are psychogenic versus radiation-induced. Therefore, the relationship between initial symptoms and radiation dose is not well understood.

52 Early effects of irradiation have been studied in regard to radiation therapy. In a detailed
53 study of the incidence and severity of side effects during the course of radiation therapy, fatigue was
54 the most prevalent and the most severe symptom reported by patients [6]. With fractionated doses of
55 radiation for cancer treatment, radiation-induced fatigue sets in within a few days after start of
56 treatment and decreases after treatment completion [7]. Although the underlying mechanisms of
57 fatigue have been studied under several disease conditions, an understanding of the etiology,
58 mechanisms, and risk factors of radiation-induced fatigue remains elusive, and this symptom
59 remains poorly managed [8-10]. Investigating initial radiation-related behavioral changes by using
60 animals might provide important information to aid understanding of the health effects of radiation
61 exposure and clinical features of radiation injury.

62 In animals, there have been many studies of radiation-induced behavioral effects, and
63 performance decrement after irradiation has been noted in several reports. A sub-lethal dose of
64 gamma radiation suppressed aggressive behavior in male mice [11], a lethal dose of gamma
65 radiation suppressed locomotor activity in mice [12], and a sub-lethal dose of X-irradiation
66 suppressed volitional activity in rats [13]. Landauer (2002) provided a review of expected
67 performance decrement after radiation exposure [14]. These reports showed that ionizing radiation
68 temporarily suppresses animals' behavior, but that the effect does not continue for a long period.
69 York et al. reported that, 6 h after gamma irradiation with 50 or 200 cGy, spontaneous locomotor

activity in mice was 35% or 36% lower, respectively, than in sham irradiated controls, and that their activity recovered to sham irradiated level 12 h after irradiation [15].

Although many animal behavioral experiments have a time-dependent data structure with variation among individuals, analyses have typically been performed only at individual time points with no parameterization of the trend in activity over time. Therefore, quantitative analyses have not been made directly on the chronological features. To obtain more detailed and accurate information from data obtained in animal behavior experiments with time-dependent structure and individual variability, application of statistical theory would suggest that analysis based on a mixed effects model [16−17] is both appropriate and effective.

The purpose of the present study was therefore to examine in detail the changes over time in locomotor activity of rats immediately after external irradiation with ^{60}Co gamma rays by using such statistical models. Specifically, we aimed to assess the time when reduction of locomotor activity begins, the time when locomotor activity recovers to pre-irradiation level, the dose dependency of the degree of reduction in locomotor activity, and the dose dependency of the rate of recovery. There are individual differences in animal behavior that cannot be ignored, even if the animal type, gender, and weight are uniform. In addition, when animals are observed over a long period of time, it is expected that common changes in behavior will occur due to indoor conditions such as temperature, humidity, and noise, which can change daily, and it is necessary to adjust for these sources of variation.

2. Materials and Methods

2.1. Experimental Design and Data Collection

2.1.1. Animals. The experiment was approved by the Animal Experiment Committee of Semey Medical University, Republic of Kazakhstan, and was conducted in accordance with the Institutional Guide for Animal Care and Use. Ten one-week-old male Wistar rats were purchased from the Kazakh Scientific Center of Quarantine and Zoonotic Diseases, Almaty, Kazakhstan and allowed free access to a basal diet and tap water. Animal rooms were maintained at 19-22 °C with relative humidity 30–70% and a 12 h light cycle. Body weights were measured twice a week during the experiment. At 11 weeks of age, the rats were randomly divided into four groups: control (4 rats) and three irradiated groups (4 rats/group). Each irradiated group received 2, 3.5, or 5.0 Gy of whole body gamma irradiation. Controls were handled with all conditions the same as with the other groups, except that they were not irradiated (dose 0 Gy). The $LD_{50(30)}$ for this strain of Wistar rats is 7 Gy with cobalt-60 radiation [18].

2.1.2. Irradiation with ^{60}Co gamma-rays Irradiation was performed with a Teragam K-2 unit (UJP Praha, Praha-Zbraslav, Czech Republic) at the Regional Oncology Dispensary of Semey. Rats were irradiated at 1 m distance from the ^{60}Co source at a dose rate of 2.6 Gy/min. Half of the radiation dose was administered from the top and the other half was administered from the bottom. A

105 radiophotoluminescence glass dosimeter, GD-302M [Chiyoda Technol Co., Tokyo, Japan], was used
106 for measuring the doses.

107 **2.1.3. Measurements of daily locomotor activity** Locomotor activities (hereafter
108 abbreviated as “activities”) of the rats were measured with infra-red sensors (Model NS-AS01;
109 Neuroscience, Inc., Tokyo, Japan) placed 16 cm above the open-top cages (26.5 x 43 x 14.5 cm).
110 Numbers of movements were counted on the basis of change in the strength of infra-red rays emitted
111 from the animals. The rats were placed in separate cages, each outfitted with a sensor, and
112 movements were continuously counted by a computerized analysis system (16 channel Multi-digital
113 Counter System [MDC] and DAS System software, Neuroscience, Inc. Tokyo, Japan).
114 Measurements were started 3 days before irradiation and continued for 20 days after irradiation.

115 **2.1.4. Ethical approval** All applicable international, national, and/or institutional guidelines for
116 the care and use of animals were followed. The animal experiment was approved by the Animal
117 Experiment Committee of Semey Medical University, Republic of Kazakhstan (Protocol No 5 dated
118 16.04.2014), and conducted in accordance with the Institutional Guide for Animal Care and Use.

119 ## 2.2. Statistical analyses

120 **2.2.1. Definition of daily activity** Because rats are nocturnal animals [19], cumulative
121 number of movements was recorded during the period between 18:00 and 06:00; the number of

122 movements so recorded was defined as activity of a rat in one day. As shown in Fig 1, rates of
123 increase in cumulative movements (slopes) were steeper during nighttime (18:00–05:59) than
124 during daytime (06:00–17:59); i.e., the rats were more active at night, as expected.
125

126 **Fig 1. Cumulative number of movements of each of the 16 rats over a 36-hour period.**
127

128 This suggests that the activity defined in this study represents the nocturnal characteristic of rats and
129 it shows that the measure has relevance as an indicator of a rat's activity.

130 **2.2.2. Data modeling** Logarithmic values of daily activity of each rat as a function of elapsed
131 time relative to day of irradiation are shown for each group in Fig 2.
132

133 **Fig 2. Daily activity of each of four rats belonging to four groups.** The vertical axis shows
134 logarithm of daily activity (number of nocturnal movements) and the horizontal axis shows elapsed
135 time in days relative to the day of irradiation (indicated by arrows): (a) the control group, (b) 2.0 Gy
136 group, (c) 3.5 Gy group, and (d) 5.0 Gy group.
137

138 An acute decrease in activity after irradiation followed by quick recovery to the pre-irradiation level can
139 be seen in every exposed group, whereas no such change or trend was observed in the control

group. There also was large inter-animal variation with daily fluctuation in activity. Therefore we assumed a non-linear mixed effects model [16−17] that takes into account the dose dependency of the decrease in activity, the dose dependency of the recovery rate, individual differences among animals, and daily fluctuations within individual animals. For comparison, we fit a simple non-linear regression model in which individual differences and daily fluctuations were not taken into account.

2.2.3. Non-linear mixed effects model (NLMM) Let y_{it} be the log transformed observed activity of rat i at time t in days since irradiation with dose D_i $(t=-3,\ldots,20;\ i=1,\ldots,16)$, where "$t=0$" indicates day of irradiation. We assume the model

$$y_{it} = f(t \mid D_i, \theta) + \delta_i + \eta_t + \varepsilon_{it},$$

$$f(t \mid D_i, \theta) = \xi_0 + \xi_1 t + \xi_2 t^2 - \left(\beta_1 D_i + \beta_2 D_i^{\,2}\right) \cdot \exp\left[\left\{-\omega_1 \cdot \mathrm{e}^{-\omega_2 (D_i - D_0)}\right\} t\right] \cdot h(t),$$

$$\delta_i \sim N(0,\ \psi^2),\ \eta_t \sim N(0,\ \varphi^2),\ \varepsilon_{it} \sim N(0,\ \sigma^2),\quad t=-3,-2,\ldots,20,\ i=1,\ldots,16, \qquad (1),$$

where $\theta = (\xi_0,\ \xi_1\ ,\ \xi_2,\ \beta_1,\ \beta_2,\ \omega_1, \omega_2)$ denotes unknown parameters for fixed effects to be estimated. The term $\xi_0 + \xi_1 t + \xi_2 t^2$ expresses the time dependency of activities without radiation exposure. The term $\beta_1 D_i + \beta_2 D_i^{\,2}$ expresses whether the dose effect in the initial decrease is linear ($\beta_2 = 0$) or quadratic ($\beta_2 \neq 0$), and the term $-\omega_1 \cdot \mathrm{e}^{-\omega_2 (D_i - D_0)}$ denotes whether the recovery rate depends on dose ($\omega_2 \neq 0$) or not ($\omega_2 = 0$). D_0 denotes a fixed pre-assigned dose value for covariate centering (in this study 2.75 Gy is adopted), $\Delta = (\psi^2,\ \varphi^2, \sigma^2)$ are unknown dispersion parameters to be estimated, and the terms δ_i, η_t and ε_{it} represent independent random effects

due to individual variability, daily fluctuation, and measurement error, respectively. The function $h(t): h(t)=0\ (t<0),\ h(t)=1\ (t\geq 0)$ denotes the Heaviside function of t to indicate pre- and post-irradiation dichotomy.

Let $\mathbf{y}=(\mathbf{y}_1',\ldots,\mathbf{y}_{16}')'$, $\mathbf{y}_i=(y_{i,-3},\ldots,y_{i,20})'$, $i=1,\ldots,16$. It follows from Model (1) that $\mathbf{y}$ has a multivariate normal distribution with mean $\boldsymbol{\mu}(\boldsymbol{\theta})=(\boldsymbol{\mu}_1(\boldsymbol{\theta})',\ldots,\boldsymbol{\mu}_{16}(\boldsymbol{\theta})')'$, $\boldsymbol{\mu}_i(\boldsymbol{\theta})=f(\mathbf{t}\,|\,D_i,\theta)$, $\mathbf{t}=(-3,-2,\ldots,20)'$, $i=1,\ldots,16$, and variance-covariance matrix $\Omega(\boldsymbol{\Delta})=I_{16}\otimes\left(\rho^2 J_{41}+\sigma^2 I_{41}\right)+J_{16}\otimes\psi^2 I_{41}$, where I_m denotes an m-dimensional unit matrix, and $J_m=\mathbf{1}_m\otimes\mathbf{1}_m'$. Then the likelihood function of $(\boldsymbol{\theta},\boldsymbol{\Delta})$ can be expressed as $L(\boldsymbol{\theta},\boldsymbol{\Delta})=\frac{1}{(2\pi)^8\sqrt{|\Omega(\boldsymbol{\Delta})|}}\exp\left(-\frac{1}{2}\{\mathbf{y}-\boldsymbol{\mu}(\boldsymbol{\theta})\}'\,\Omega(\boldsymbol{\Delta})^{-1}\{\mathbf{y}-\boldsymbol{\mu}(\boldsymbol{\theta})\}\right)$. Therefore, the maximum likelihood estimates of $(\boldsymbol{\theta},\boldsymbol{\Delta})$, denoted by $(\hat{\boldsymbol{\theta}},\hat{\boldsymbol{\Delta}})$, are obtained by minimizing the quantity $Q(\theta,\boldsymbol{\Delta})=\log\left(|\Omega(\boldsymbol{\Delta})|\right)+\{\mathbf{y}-\boldsymbol{\mu}(\boldsymbol{\theta})\}'\,\Omega(\boldsymbol{\Delta})^{-1}\{\mathbf{y}-\boldsymbol{\mu}(\boldsymbol{\theta})\}+16\times\log(2\pi)$. When $\psi^2=\varphi^2=0$, Model (1) reduces to an ordinary non-linear regression model (NLRM).

2.2.4. Algorithm and software for implementation of data analyses The unknown parameters were estimated by using an algorithm for optimization with the limited-memory version of the Broyden–Fletcher–Goldfarb–Shanno method [20] to maximize the likelihood derived from the model (1), and the AIC (Akaike Information Criterion) [21] and BIC

(Bayesian information criterion) [22−23] were calculated. The function 'optim' in the R software ver. 3.5.1 was used for carrying out numerical analyses.

Maximum likelihood (ML) or restricted maximum likelihood (REML) [24] estimates of the parameters in the linear mixed-effects models can be computed with the "lmer" function in the "lme4" package for R [25]. In this study, the ML method was used to compare the goodness-of-fit of models with the AIC criterion. Estimation results were almost the same with both methods.

3. Results

3.1. Result of Regression Analysis

3.1.1. Estimation of fixed effect parameters. Regression analysis was first performed with all parameters of the NLMM (full NLMM), then model selection was applied by choosing the smallest AIC to determine the optimal NLMM (optimal NLMM). The full NLRM and optimal NLRM were defined in the same way. Estimates of fixed-effect parameters and their 95% confidence intervals under the full and optimal NLMM are shown in Tables 1(a) and (b), respectively; those under the full and optimal NLRM are shown in Tables 2(a) and (b), respectively.

Table 1. Estimated fixed effects parameters in the full NLMM (a) and those in the optimal NLMM (b).

(a)

	Full NLMM					
			95% Confidence Interval			
Parameter	Estimate	SE	Lower bound	Upper bound	p-value	
β_1	0.069	0.015	0.041	0.098	0.000	**
β_2	–0.007	0.003	–0.012	–0.001	0.023	*
ω_1	10.391	4.808	0.968	19.815	0.015	*
ω_2	0.082	0.166	–0.243	0.407	0.310	
ξ_0	4.326	0.020	4.288	4.364	0.000	**
ξ_1	0.003	0.041	–0.076	0.083	0.468	
ξ_2	–0.008	0.022	-0.052	0.035	0.353	

** : $p < 0.01$, * : $0.01 \le p < 0.05$

Estimated random effect parameters: $(\psi^2, \varphi^2, \sigma^2) = (0.0018,\ 0.0019,\ 0.0015)$

Log-likelihood: 643.47, AIC: –1266.94, BIC: –1227.44

(b)

	Optimal NLMM					
			95% Confidence Interval			
Parameter	Estimate	SE	Lower bound	Upper bound	p-value	
β_1	0.066	0.016	0.033	0.098	0.000	**
β_2	–0.006	0.003	–0.012	0.001	0.036	*
ω_1	9.063	2.949	3.283	14.843	0.001	**
ξ_0	4.319	0.014	4.290	4.347	0.000	**

** : $p < 0.01$, * : $0.01 \le p < 0.05$

Estimated random effect parameters: $(\psi^2, \varphi^2, \sigma^2) = (0.0018,\ 0.0019,\ 0.0015)$

Log-likelihood: 642.90, AIC: –1271.80, BIC: –1244.15

Table 2. Estimated fixed effect parameters in the full NLRM (a) and those in the optimal NLRM (b).

(a)

Full NLRM						
			95% Confidence Interval			
Parameter	Estimate	SE	Lower bound	Upper bound	p-value	
β_1	0.075	0.023	0.030	0.120	0.001	**
β_2	–0.006	0.005	–0.016	0.004	0.104	
ω_1	3.922	1.404	1.170	6.674	0.003	**
ω_2	0.574	0.447	–0.303	1.450	0.100	
ξ_0	4.333	0.007	4.320	4.346	0.000	**
ξ_1	0.003	0.016	-0.028	0.033	0.435	
ξ_2	–0.011	0.008	-0.027	0.006	0.107	

204 ** : $p < 0.01$, * : $0.01 \le p < 0.05$

205 Estimated residual variance: $\hat{\sigma}^2 = 0.00502$

206 Log-likelihood: 744.091, AIC: –928.17, BIC: –883.56

207

208

209

210 (b)

Optimal NLRM						
			95% Confidence Interval			
Parameter	Estimate	SE	Lower bound	Upper bound	p-value	
β_1	0.049	0.005	0.039	0.059	0.000	**
ω_1	5.973	1.726	2.590	9.356	0.000	**
ξ_0	4.334	0.006	4.323	4.345	0.002	**
ξ_2	–0.010	0.003	–0.016	–0.004	0.000	**

211 ** : $p < 0.01$, * : $0.01 \le p < 0.05$

212 Estimated residual variance: $\hat{\sigma}^2 = 0.0058$

213 Log-likelihood: 742.12, AIC: –930.25, BIC: –899.02

214

215 **3.1.2. Estimation of the random effects parameters.** **In the optimal NLMM,**

216 variances of the random effects due to individual differences, daily variation, and measurement

error were 0.0018, 0.0019, and 0.0015, which account for 35%, 36%, and 29% of the total variance, respectively. Predictions of individual differences $(\hat{\delta}_1, \hat{\delta}_2, \cdots, \hat{\delta}_{16})$ and those of daily fluctuation $(\hat{\eta}_{-3}, \hat{\eta}_{-2}, \cdots, \hat{\eta}_{20})$ were obtained by calculating posterior means. The predictions $\hat{\delta}_i$ in each of the four groups (control group and three irradiated groups) and the predictions $\hat{\eta}_t$ by day are shown in panels (a) and (b) of Fig 3, respectively.

Fig 3. Predictions of random values. Predictions of random values by individual $\hat{\delta}_i$ by group are shown in panel (a) and predictions of random values by day $\hat{\eta}_t$ are shown in panel (b).

Residuals in the optimal NLMM and in the optimal NLRM are given by $y_{it} - \hat{f}(t \mid D_i, \theta) - \hat{\delta}_i - \hat{\eta}_t$ and $y_{it} - \hat{f}(t \mid D_i, \theta)$, respectively. The standard deviations of residual errors in the optimal NLMM and optimal NLRM were 0.038 and 0.071, respectively. The distributions of residuals in the NLMM and NLRM are shown in Fig 4.

Fig 4. Parallel boxplots of residual errors in the non-linear mixed model (NLMM) and ordinary non-linear regression model (NLRM).

3.2. Comparison of goodness of fit of the NLMM and the NLRM

There is a large difference between the AICs of the optimal NLMM and the optimal NLRM, which were −1271.80 and −930.25, respectively (See Table 1 (b) and Table 2 (b)). The measurement error variances of the NLMM and NLRM were 0.0015 and 0.0058 (See Table 1 (b) and Table 2 (b)). Therefore the fit of the NLMM was preferable to that of the NLRM in terms of prediction and accuracy. The estimated time dependency of activity in each group under the optimal NLMM is shown in Fig 5.

Fig 5. Estimated mean trends of daily locomotor activity in rats by dose group under the optimal NLMM..

In each of the irradiated groups, activity decreased immediately after irradiation but recovered to the pre-irradiation level within a few days with a common recovery rate irrespective of dose.

Discussion

One of the advantages of using the more complex NLMM structure, as demonstrated in this paper, is that a second-order dose dependency could be detected in the initial decrease, which was not found with the NRLM (which estimated a linear dependency). Estimated magnitudes of initial decreases at $t = 0$ by dose group and their 95% confidence intervals in the optimal NLMM and those in the optimal NLRM are shown in Fig 6.

253 **Fig 6. Fitted dose response curves from the optimal NLMM and the optimal NLRM.** The
254 estimated magnitudes of decrease at $t = 0$ by dose group and their 95% confidence intervals and
255 fitted dose-response curves with dotted line from the NLMM and the NLRM are shown in panels (a)
256 and (b), respectively. Cross marks show observed data of individual rats. The fitted dose-response
257 curve from the optimal NLMM was a downward convex quadratic curve.

258

259 The plots of predictions of individual differences $\hat{\delta}_i$ by dose group (Fig 3 (a)) show that the
260 assumption of homoscedasticity for distributions of individual difference between the four dose
261 groups seems to be satisfied. This means that the random assignment of rats to the four groups was
262 effective in terms of individual differences. The plots of predictions of time-dependent daily
263 fluctuation $\hat{\eta}_t$ (Fig 3 (b)) show that the assumption of independency of each of the random variables
264 η_t seems to be satisfied. The Durbin Watson statistic [26] for $\hat{\eta}_t$ was 2.33 (p-value 0.902),
265 indicating that no strong autocorrelation is observed in daily fluctuation.

266 Because acute changes were the focus in this experiment, longer observation was not
267 performed, but it is necessary to investigate late effects. The irradiation was a single and sub-lethal
268 dose, so it is considered that damage was acute, disappearing in a short period of time, and
269 resilience to allow recovery from the damage was not affected by irradiation. The effects of chronic
270 low dose exposure remain as future issues to be addressed. As one important example of the need

for assessing effects of chronic exposure, a giant earthquake of magnitude M9 struck East Japan on March 11, 2011. Subsequently a 'tsunami' engulfed the Fukushima Daiichi Nuclear Power Plant (FDNPP). As a result, FDNPP reactors 1-3 suffered meltdown and significant amounts of radioactive materials have been released into the environment [27].The dose to the public is estimated to be low [28], but many Japanese people are worried about the resulting health effects of chronic low dose exposure.

In the present study, effects of irradiation on behavior of rats were investigated efficiently, despite a small number of animals with large individual differences. This was achieved by using a statistical method that accounts for inter-animal differences and daily fluctuation in activity—a non-linear mixed model fit to repeated measurements. With such an efficient approach, we were able to demonstrate a temporary, but dose-dependent, decrease in activity following irradiation and a dose-independent common recovery rate. The statistical framework for analyzing longitudinal locomotor data in this study should be generally applicable to other repeated measurement data with similar structure.

Supporting Information

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Reference

1. Christensen DM, Parrillo SJ, Glassman ES, Goans RE. Management of Ionizing Radiation Injuries and Illnesses, Part 4: Acute Radiation Syndrome. The Journal of the American Osteopathic Association. 2014: l 114 (9):702−11. doi: 10.7556/jaoa.2014.138 PMID: 25170040

2. Dörr H, Meineke V. Acute radiation syndrome caused by accidental radiation exposure − therapeutic principles. BMC Medicine. 2011; 9:126. http://www.biomedcentral.com/1741−7015/9/126

3. Dainiak N, Gent RN, Carr Z, Schneider R, Buglova E, Chao N, et al. Literature Review and Global Consensus on Management of Acute Radiation Syndrome Affecting Nonhematopoietic Organ Systems. Disaster Med Public Health Prep. 2011; 5(3): 183–201. doi:10.1001/dmp.2011.73.

4. Donnelly EH, Nemhauser JB, Smith M,Kazzi ZN, Farfán EB, Chang AS, Naeem SF. Acute Radiation Syndrome: Assessment and Management. South Med J. 2010; 103(6):541−6. doi:10.1097/SMJ.0b013e3181ddd571 PMID: 20710137

5. López M, Martín M. (2011) Medical management of the acute radiation syndrome. Reports of practical oncology and radiotherapy. 2011; 16:138–146. doi: 10.1016/j.rpor.2011.05.001 PMID: 24376971

6. Hofman M, Ryan JL, Figueroa−Moseley CD, Jean−Pierre P, Morrowa GR. Cancer−Related Fatigue: The Scale of the Problem. Oncologist. 2007; 12 (Suppl 1):4−10. doi: 10.1634/theoncologist.12−S1−4 PMID: 17573451

7. Jereczek−Fossa BA, Santoro L, Alterio D, Franchi B, Fiore MR, Fossati P, Kowalczyk A, Canino P, Ansarin M, Orecchia R. Fatigue during head−and−neck radiotherapy: prospective study on 117 consecutive patients. Int. J. Radiation Oncology Biol. Phys. 2007; 68(2): 403–415. DOI: 10.1016/j.ijrobp.2007.01.024 PMID: 17398020

8. Bower JE, Ganz PA, Tao ML, Hu W, Belin TR, Sepah S, Cole S, Aziz N. Inflammatory Biomarkers and Fatigue during Radiation Therapy for Breast and Prostate Cancer. Clin Cancer Res. 2009; 15(17): 5534–5540. doi:10.1158/1078−0432.CCR−08−2584

9. Hsiaoa CP, Dalya B, Saliganba NL. The Etiology and management of radiotherapy−induced fatigue. Expert Rev Qual Life Cancer Care. 2016 ; 1(4): 323–328. doi:10.1080/23809000.2016.1191948

10. Albuquerque K, Tell D, Lobo P, Millbrandt L, Mathews HL, Witek, Janusek LW. Impact of partial versus whole breast radiation therapy on fatigue, perceived stress, quality of life and natural killer cell activity in women with breast cancer. BMC Cancer. 2012; 12:251.http://www.biomedcentral.com/1471−2407/12/251

11. Maier DM, Landauer MR. Effects of acute sublethal gamma radiation exposure on aggressive behavior in male mice: a dose−response study. Aviat Space Environ Med. 1989; 60(8):774−8. PMID: 2775134

12. Landauer MR, Davis HD, Dominitz JA, Weiss JF. Long−term effects of radioprotector WR−2721 on locomotor activity and body weight of mice following exposure to ionizing radiation. Toxicology. 1988; 49(2−3):315−23. doi: 10.1016/0300−483x(88)90014−5 PMID: 2836973

13. Jones DC, Kimeldorf DJ, Rubadeau DO, Osborn GK, Castanera TJ. Effect of x−irradiation on performance of volitional activity by the adult male rat. Am J Physiol. 1954; 177(2):243−50. doi: 10.1152/ajplegacy.1954.177.2.243 PMID: 13158555

14. Landauer MR (2002) Radiation−Induced Performance Decrement. Military Medicine. 2002; 167(2 Suppl): 128−30. doi: 10.1093/milmed/167.suppl_1.128

15. York JM, Blevins NA, Meling DD, Peterlin MB, Gridley DS, Cengel KA, Freund GG. The biobehavioral and neuroimmune impact of low−dose ionizing radiation. Brain Behav Immun. 2012; 26(2): 218–227. doi:10.1016/j.bbi.2011.09.006.

16. Davidian M. and Giltinan DM. Nonlinear Models for Repeated Measurement Data: An Overview and Update. JABES. 2003; 8: 387–419. doi:10.1198/1085711032697

17. Pinheiro JC, Bates DM. Mixed-effects Models in S and S-PLUS. New York: Springer; 2000.

18. Tsuchihashi S, Enamoto Y, Yamada T, Nakamura W, Eto H. The Mortality and Body Weight Response of Male Wister Rats *i*n the Lethal X−ray Dose Range. J Radiat Res. 1969; 10(3)4:133−138. PMID: 5382979 doi: 10.1269/jrr.10.133

19. Sidlo J, Zaviacic M, Kvasnicka P. Night and day differences in the food−intake of laboratory rats Wistar and Koletsky strains. Bratisl Lek Listy.1995; 96(12):655−7. PMID: 8624748

20. Byrd RH, Lu P, Nocedal J, Zhu C. A limited memory algorithm for bound constrained Optimization. SIAM Journal on Scientific Computing. 1995; 16(5): 1190−1208. doi: 10.1137/0916069

21. Akaike H. Information theory and an extension of the maximum likelihood principle. Proceedings of the 2nd International Symposium on Information Theory, Petrov, B. N., and Caski, F. (eds.), Akadimiai Kiado, Budapest. 1973; 267−281

22. Schwarz GE. Estimating the dimension of a model. Annals of Statistics, 1978; 6 (2): 461–464. doi:10.1214/aos/1176344136. MR468014.

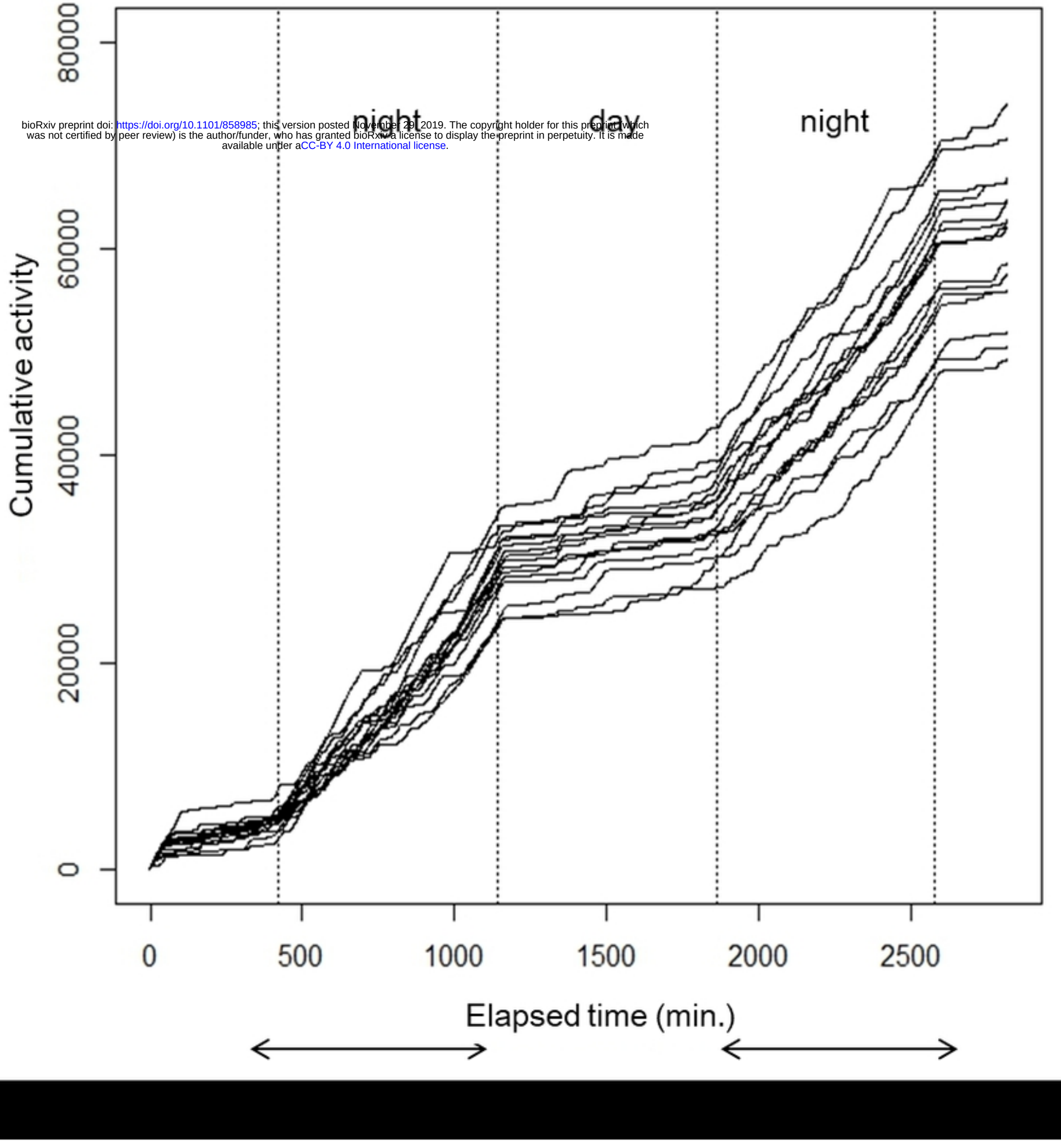
23. Konishi S, Kitagawa G. Information Criteria and Statistical Modeling. Springer; 2008.

24. Harville DA. Maximum likelihood approaches to variance component estimation and to related problems. Journal of the American Statistical Association. 1977; 72: 320–340.

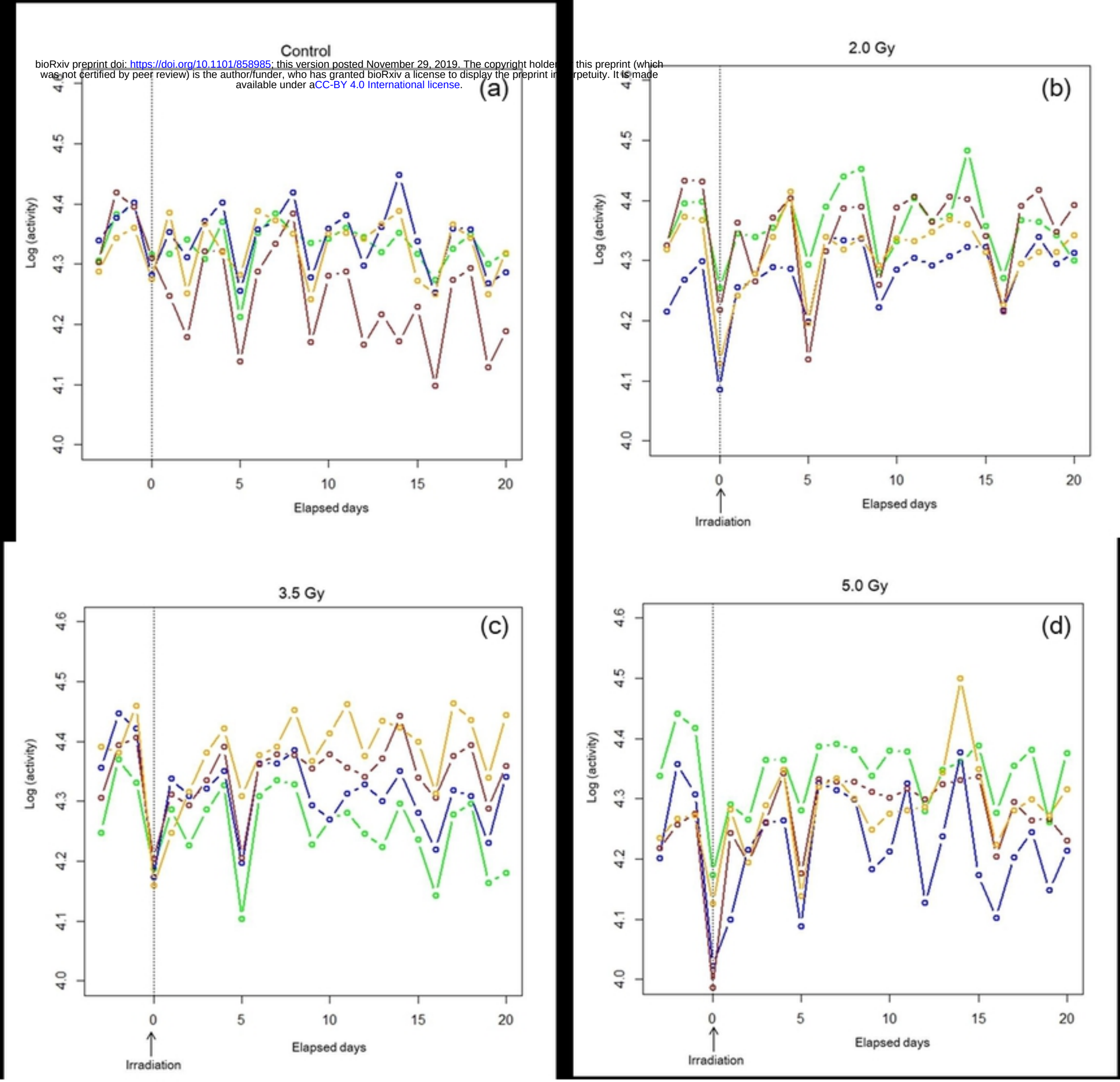
25. Bates D, Mächler M, Bolker BM, Walker SC. Fitting Linear Mixed−Effects Models Using lme4. Journal of Statistical Soft−ware. 2015; doi: 10.18637/jss.v067.i01

357 26. Durbin J, Watson GS. Testing for Serial Correlation in Least Squares Regression I. Biometrika.
358 1950; **37**, 409−−428. doi: 10.2307/2332391
359 27. Strickland E. Explainer: What went wrong in Japan's nuclear reactors. IEEE Spectrum 4. 2011:
360 https://spectrum.ieee.org/tech−talk/energy/nuclear/explainer−what−went−wrong−in−japans−nucl
361 ear−reactors
362 28. United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) (2014).
363 UNSCEAR 2013 Report to the Gene Assembly with scientific annexes, Annex B: Effects of
364 radiation exposure of children. New York: UNSCEAR.

365



Figure



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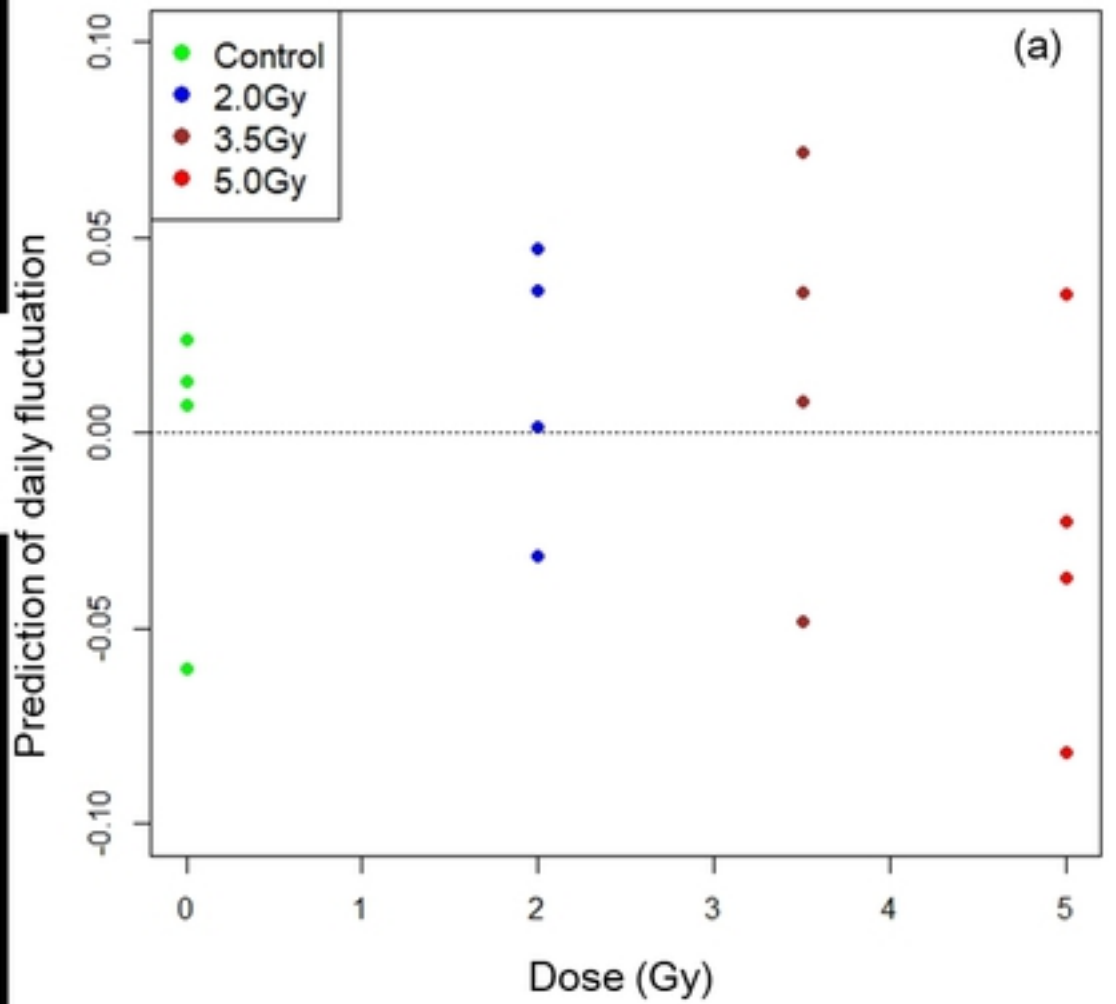
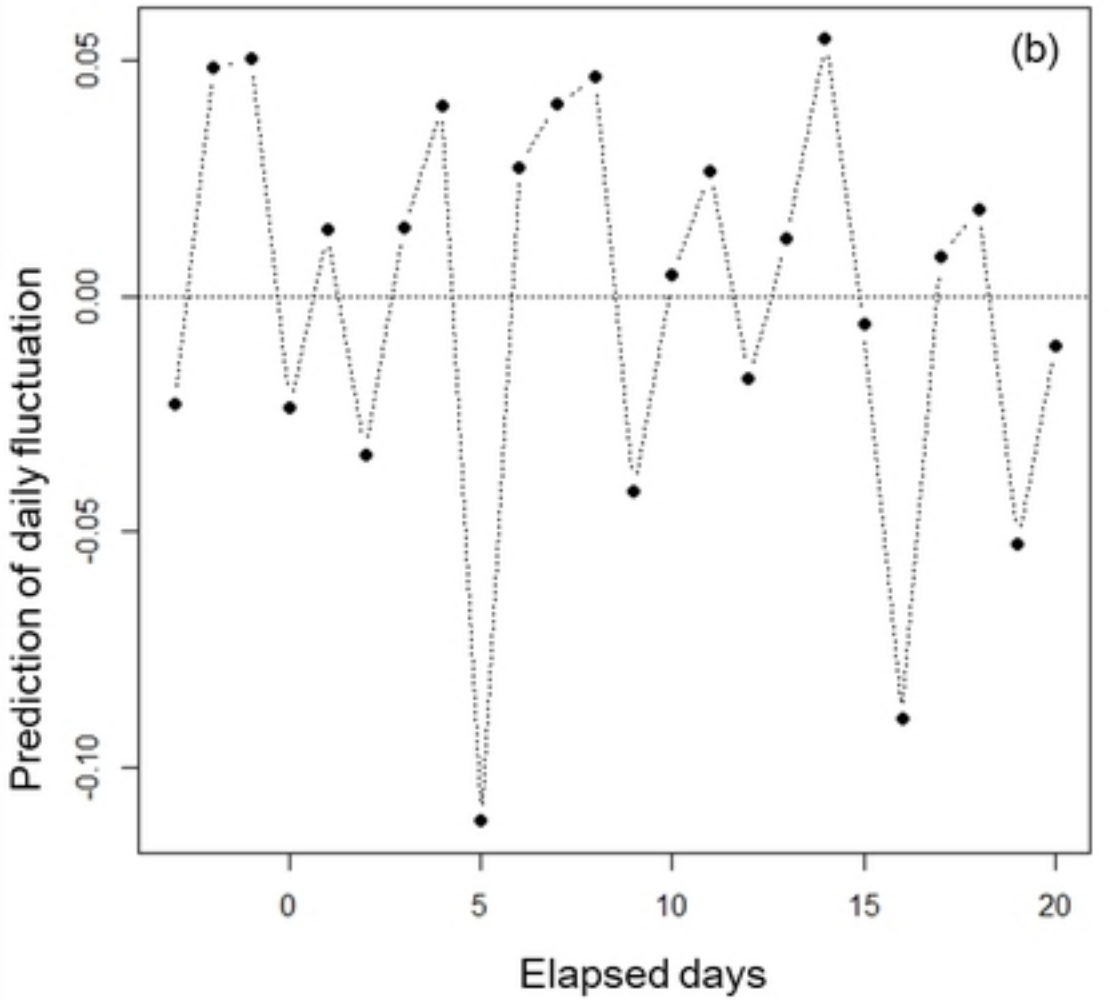
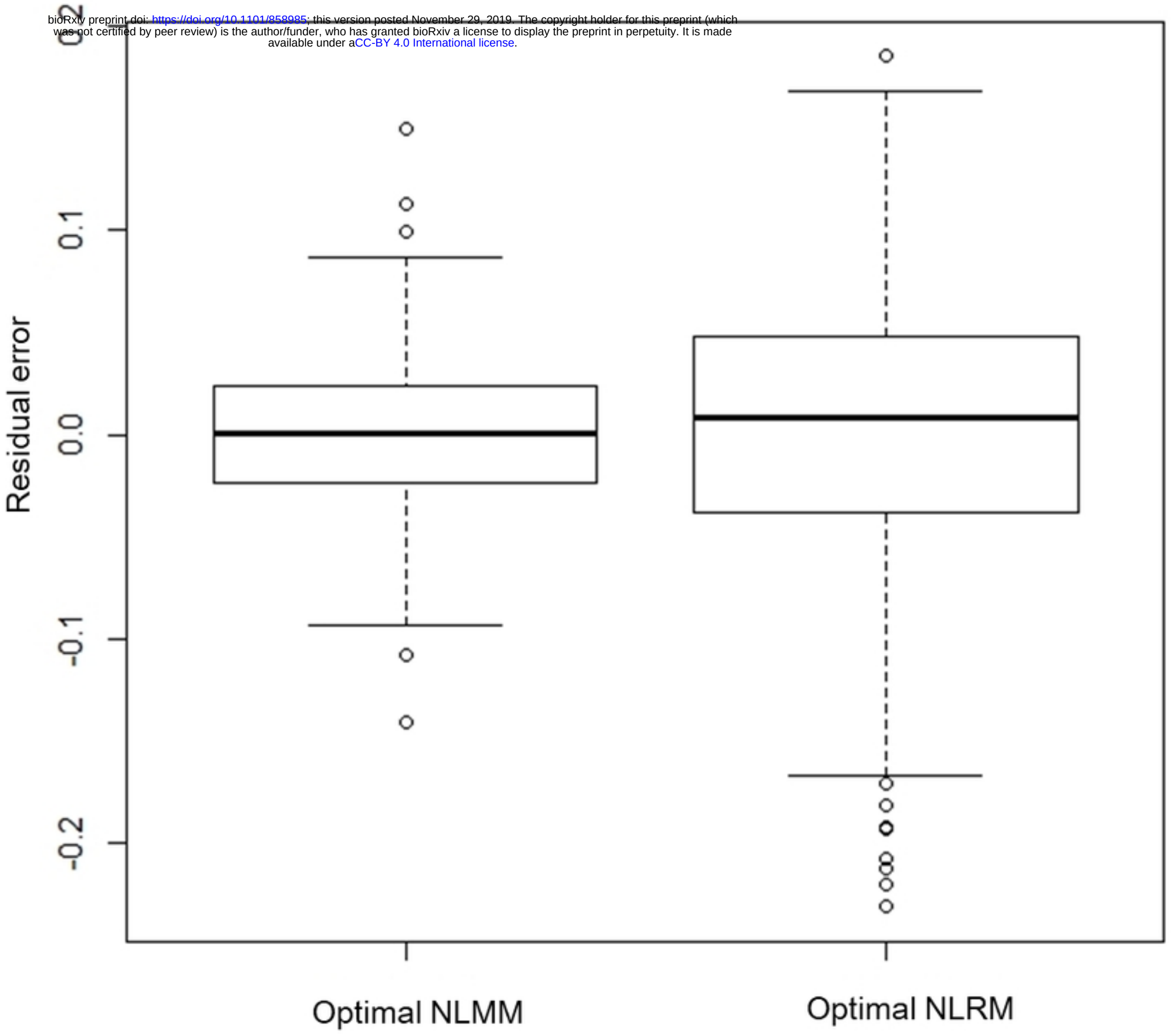

(a)
Control
2.0Gy
3.5Gy
5.0Gy
Prediction of daily fluctuation
0.10
0.05
0.00
-0.05
-0.10
0
1
2
3
4
5
Dose (Gy)


(b)
Prediction of daily fluctuation
0.05
0.00
-0.05
-0.10
0
5
10
15
20
Elapsed days

Figure



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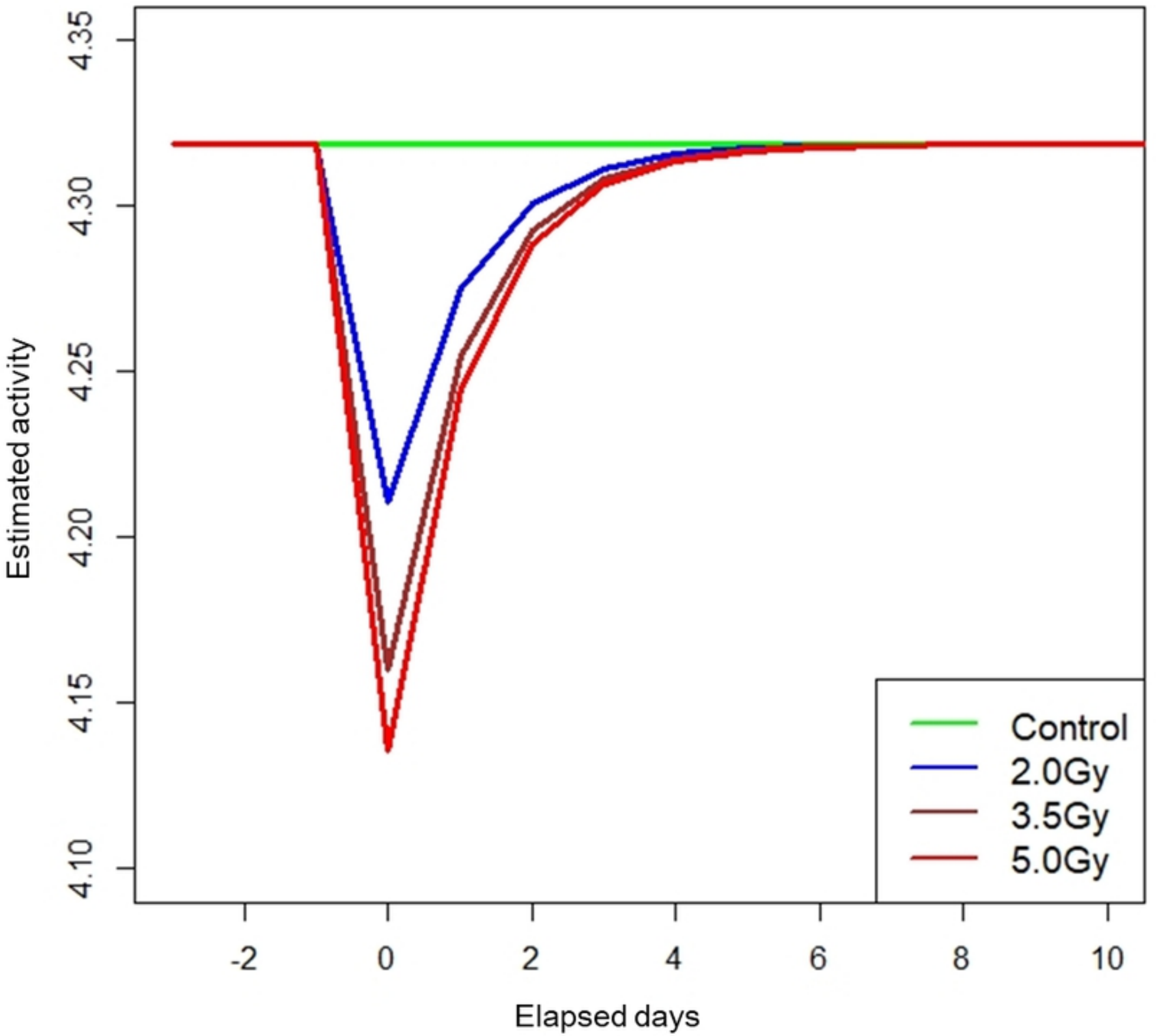

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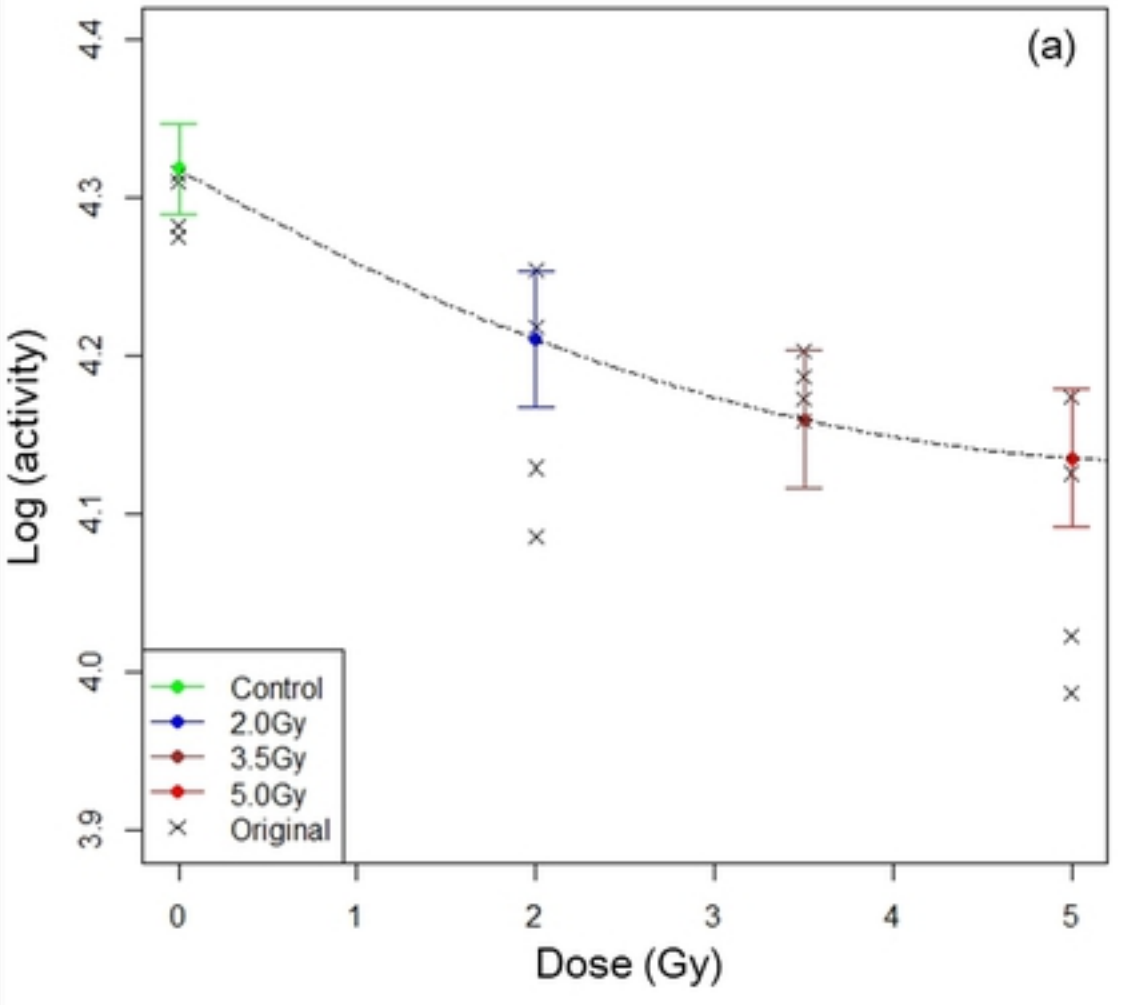
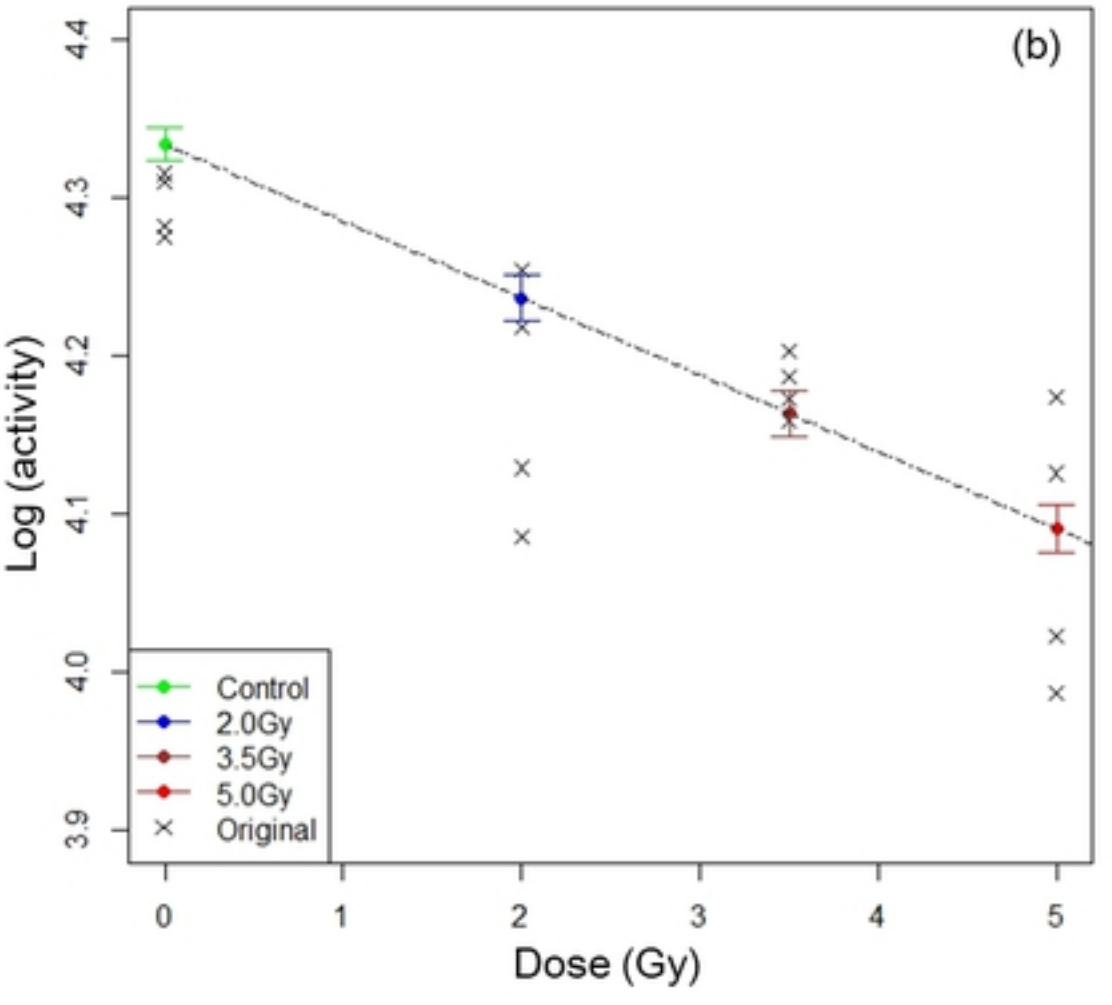

(a)
Log (activity)
Dose (Gy)
Control
2.0Gy
3.5Gy
5.0Gy
Original


(b)
Log (activity)
Dose (Gy)
Control
2.0Gy
3.5Gy
5.0Gy
Original

Figure