# Impact of environmental radiation on the health and reproductive status of fish from Chernobyl

*[1,2]\*Adélaïde Lerebours, [3]Dmitri Gudkov, [4]Liubov Nagorskaya, [3]Alexander Kaglyan, [4]Viktor Rizewski, [4]Andrey Leshchenko, [5]Elizabeth H Bailey, [1]Adil Bakir, [6]Svetlana Ovsyanikova, [7]Gennady Laptev, and [1]Jim T. Smith*

[1]School of Earth and Environmental Sciences, University of Portsmouth, Portsmouth, PO1 3QL, United Kingdom

[2]School of Biological Sciences, University of Portsmouth, Portsmouth, PO1 2DY, United Kingdom

[3]Institute of Hydrobiology of the National Academy of Sciences of Ukraine, UA-04210 Kiev, Ukraine

[4]Applied Science Center for Bioresources of the National Academy of Sciences of Belarus, 220072 Minsk, Belarus

[5]School of Biosciences, University of Nottingham, Loughborough, LE12 5RD, United Kingdom

[6]Belarussian State University, Faculty of Chemistry, Research Laboratory of Radiochemistry, 220030 Minsk, Belarus

[7]Ukrainian HydroMeteorological Institute, 03028 Kiev, Ukraine

Keywords: radiation, fish, reproduction, Chernobyl.

Corresponding Author: * Adélaïde Lerebours, phone number: +353 (0)9174 2516, e-mail: ade.lerebours@hotmail.fr.

1    TOC Art

2



*Chernobyl*

*Specific activity*

$^{137}$Cs $^{90}$Sr

$^{241}$Am $^{238}$Pu $^{239, 240}$Pu
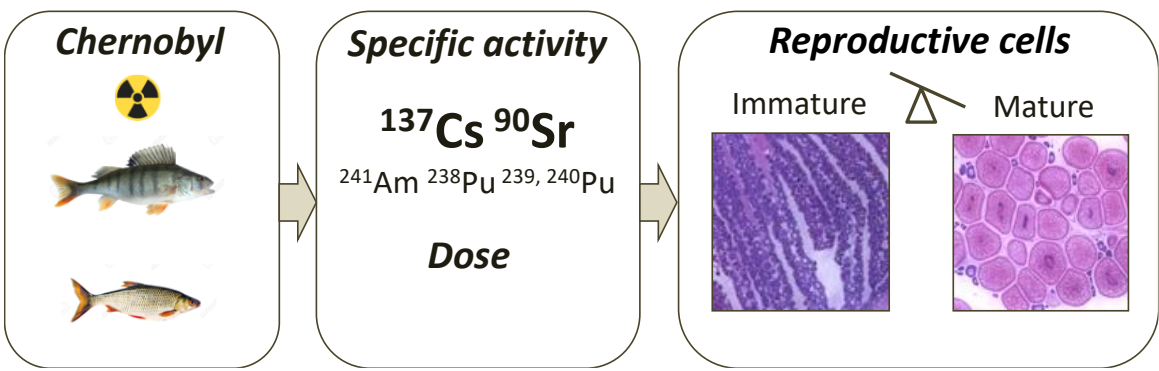
*Dose*

*Reproductive cells*

Immature     Mature

3

4

5

6    **Abstract**

7    Aquatic organisms at Chernobyl have now been chronically exposed to environmental radiation

8    for three decades. The biological effects of acute exposure to radiation are relatively well

9    documented, but much less is known about the long-term effects of chronic exposure of

10   organisms in their natural environment. Highly exposed fish in freshwater systems at Chernobyl

11   showed morphological changes in their reproductive system in the years after the accident.

12   However, the relatively limited scope of past studies did not allow robust conclusions to be

13   drawn. Moreover, the level of the radiation dose at which significant effects on wildlife occur is

14   still under debate. In the most comprehensive evaluation of the effects of chronic radiation on

15   wild fish populations to date, the present study measures specific activities of $^{137}$Cs, $^{90}$Sr and

16   transuranium elements ($^{238}$Pu, $^{239,240}$Pu and $^{241}$Am), index conditions, distribution and size of

17   oocytes, as well as environmental and biological confounding factors in two fish species perch

18   (*Perca fluviatilis*) and roach (*Rutilus rutilus*) from seven lakes. In addition, relative species

19   abundance was examined. The results showed that both fish species are, perhaps surprisingly, in

20   good general physiological and reproductive health. Perch, however, appeared to be more

21   sensitive to radiation than roach: in the most contaminated lakes, a delay of the maturation of the

22   gonads and the presence of several undeveloped phenotypes were evident only for perch and not

23   for roach.

24

25

26

3

**Introduction**

Wildlife has been chronically exposed to environmental radiation from the Chernobyl accident for the past 30 years. The biological effects of acute exposure to radiation in laboratory settings have been relatively well studied (Frederica radiation database: www.frederica-online.org[1]), but relatively little is known about the effect of long-term chronic exposure of organisms in the natural environment. The fate of wildlife remaining in the Chernobyl Exclusion Zone (CEZ) is under debate and controversy continues on the dose rate at which significant environmental impacts occur. Previous studies found no evidence of effects of radiation on aquatic macroinvertebrate or mammal populations[2,3] whereas others found reduced abundance of insect, spider, bird and mammal populations[456] at Chernobyl and Fukushima. Environmental studies on the long-term effects of radionuclide contamination at Chernobyl are of crucial importance for refining the environmental protection regulations, underpinning the public and political debate on risks of exposure to ionizing radiation and predicting the long-term impact on the environment of the more recent nuclear accident at Fukushima.

Fish are considered to be the most radiosensitive aquatic organisms[7] and have been highly exposed in freshwater systems at Chernobyl since the accident on the 26th of April 1986. At Fukushima, both freshwater and marine fish have been exposed since the March 2011 accident. At Chernobyl, the highest dose rate to fish after the accident was estimated to be 400 μGy/h[8]. Doses rates rapidly declined after the accident due to decay of short-lived isotopes, decreased bioavailability of $^{137}$Cs and its accumulation to bottom sediment[9]. In the first month after the accident, the $^{137}$Cs activity concentration were the highest in prey fish whereas a few years later, the highest concentrations were recorded in predatory fish such as perch and pike[10].

49   Bioaccumulation of $^{137}$Cs in fish muscles increases with size in silver carp, catfish and pike-

50   perch from Cooling Pond[11] and the trophic level in the foodweb[12]. $^{90}$Sr (a β emitter) and $^{137}$Cs (a

51   β and γ emitter) are the main radionuclides of concern due to their long radioactive half-life (28

52   and 30 years respectively), though transuranium elements $^{238}$Pu (α emitter), $^{239,240}$Pu (α emitters)

53   and $^{241}$Am (α and low energy γ emitter) of radioactive half-life 88, 24000, 6500 and 432 years

54   also need to be considered.

55   In fish from lakes contaminated by the Chernobyl accident, morphological changes were most

56   frequently recorded in the reproductive system. The occurrence of anomalies apparently

57   remained high after several generations post-accident in different fish species[13,14] despite the

58   continuing decrease of $^{137}$Cs specific activity[15]. Only one study in the literature relates the

59   biological effects of radiation on fish after the Fukushima Dai-ichi NPP accident[16]. The author

60   found a higher number of melano-macrophages centers in different tissues and a lower number

61   of leucocytes in exposed carps from Fukushima ponds but the causative link with radiation has

62   yet to be confirmed due to the low number of lakes studied[16]. Ionising radiation induces DNA

63   damage but only a few studies have investigated DNA damage in relation to long term exposure

64   to radionuclides in the environment. A study led on catfish from Cooling Pond did not find any

65   positive correlation between radiation exposure level and chromosomal damage[17]. However,

66   these two studies were restricted to a small number of lakes, therefore, no robust quantification

67   of the observed effects could be achieved.

68   The present study is, to our knowledge, the largest study of radiation effects on fish in the

69   natural environment. It assesses whether three decades of direct and multi-generational exposure

70   to radiation from the Chernobyl accident significantly affect the physiology of freshwater fishes.

71  For this purpose, perch (*Perca fluviatilis*) and roach (*Rutilus rutilus*) were collected from 7 lakes

72  exposed a gradient of contamination and located inside and outside the CEZ.

73     It was hypothesised that 3 decades of exposure to radiation was sufficient to negatively affect

74  the general health and reproductive status of natural populations of perch and roach.

75  **Methods**

76  *Fish collection*

77     For the health and reproductive status assessment 124 perch and 82 roach (Table S1) of similar

78  weight (Wb) and total length (Lt)were collected in September 2014 (Perch: Wb = 84 $\pm$ 25 g, Lt

79  = 19$\pm$ 2 cm; Roach: Wb = 92 $\pm$ 20 g, Lt = 20 $\pm$ 1 cm) (from 7 lakes in Belarus and Ukraine) and

80  in September 2015 (Perch: Wb = 77 $\pm$ 27 g, Lt = 18 $\pm$ 2 cm; Roach: Wb = 91 $\pm$ 27 g, Lt = 20 $\pm$ 2

81  cm) from the 4 lakes in Ukraine (Figure 1) using 3 gill nets of 20 m length and 21 mm mesh size

82  to ensure the capture of homogenous groups of mature fish. 38 perch and 60 roach were also

83  collected in March 2015 just before spawning. The relative abundance of fish species in each

84  lake was evaluated by recording the number of fish caught for each species during an additional

85  sampling session in June 2015. Perch and roach were carefully removed from the nets and kept

86  alive into tanks containing aerated water. Fish fell unconscious by a blow to the head and were

87  then killed by performing a concussion of the brain to limit as much as possible the suffering as

88  recommended by the UK Home Office procedure (Animals Scientific Procedures Act, 1986[18]).

89  Scales were sampled for age determination. The body weight, total length and presence of

90  external signs of disease and macroscopic tumours were noted for each fish using methodology

91  specified by ICES[19]. The presence of liver parasites was recorded. The Fulton condition index,

92 K, the hepatosomatic index, HSI, and the gonadosomatic index, GSI, were determined as

93 described in SI.

94 *Sampling sites*

95 Lake description. Seven lakes situated in Belarus and Ukraine (Figure 1) were selected

96 according to their hydrological properties (Table S2) and the long-term exposure to a gradient of

97 radiation doses (Table S2). The lakes are situated at distances from 1.5 to 225 km of the

98 Chernobyl NPP. Glubokoye, Yanovsky lakes and Cooling Pond are the high (H) contaminated

99 lakes, Svyatoye lake is a medium (M) contaminated lake, and Stoyacheye, Dvoriche and Gorova

100 lakes are the low (L) contaminated lakes.

101 *Water chemistry*

102 Multiple chemical parameters were measured to assess the presence of potential confounding

103 abiotic factors in each lake during all sampling sessions.

104 The pH, temperature, dissolved oxygen (DO) and conductivity ($\mu$S/cm) were measured (Table

105 S2, B). Water samples were collected at three different locations within the surface waters of

106 each lake for elemental (Na, Mg, S, K, Ca, As, Sr, Cd, Cs, Pb and U) and nutrient ($NO_3^-$, $NO_2^-$

107 and $PO_4^{3-}$) analysis in September 2014 and 2015. The methods used are described in SI.

108 *Activity measurements of $^{137}Cs$, $^{90}Sr$, $^{238}Pu$, $^{239,240}Pu$ and $^{241}Am$*

109 The activity concentration of $^{137}Cs$ was measured on the whole body of 5 additional fish

110 collected in September 2014 from each of the seven lakes using a $\gamma$ spectrometer with lithium-

111 drifted germanium detector (DGDK-100, Russia, detection limit: 0.6 Bq). The activity

7

112    concentration of $^{90}$Sr was measured on the whole body of 5 fish from Glubokoye (H), Cooling

113    Pond (H) and Yanovsky (H) lakes using a radiochemical oxalate procedure with measurement of

114    the $^{90}$Y radiochemistry, as daughter product, using the α, β radiometer (UMF-2000, Russia,

115    detection limit on the β channel: 0.01 Bq).

116        The activity concentration of $^{238}$Pu, $^{239,240}$Pu and $^{241}$Am were measured in liver and muscle

117    (and skin) of 3 to 5 additional fish from Glubokoye (H), Cooling Pond (H) and Yanovsky (H)

118    lakes as these radionuclides were mainly deposited in the vicinity of the nuclear power plant

119    after the accident. The measurements were performed using radiochemical extraction

120    chromatography separation on Sr-Resin and TRU-Resin (Eichrom, USA) followed by alpha-

121    spectrometry on α8 instrument (BSI, Latvia, detection limit on the α channel: 0.001 Bq). The

122    activity concentration of $^{241}$Am linked to γ emissions was measured using a γ spectrometer with

123    high purity germanium detectors GMX-40 (AMETEC, Ortec, USA).

124        Uncertainties of $^{137}$Cs, $^{90}$Sr and transuranium activity measurements didn't exceed 20%, 15%

125    and 25% respectively at a confidence interval of 0.95.

126

127    *Dose calculation*

128        External doses were calculated from $^{137}$Cs γ radiation, as this is the dominant contributor to

129    external dose while external exposure to $^{90}$Sr and $^{137}$Cs β radiation are minor as the water

130    provides effective shielding for external β particles. The $^{137}$Cs external doses were estimated

131    using the calculated radioactivity concentration in sediment and external dose coefficient using

132   the ERICA tool[20]. The average activity concentration in surface sediments was estimated from

133   the decay-corrected deposition of $^{137}$Cs (Bq/m$^2$) to each of the lakes and assuming that the

134   majority of the $^{137}$Cs is within the 15 cm surface sediment[9] and that the sediment density is 1300

135   kg/m$^3$. External dose rates were calculated using the dose conversion factor: $1.45 \times 10^{-4}$ μGy/h per

136   Bq/kg ww [20] considering an occupation factor of 0.5 at the sediment surface. Internal doses were

137   calculated for $^{137}$Cs in perch and roach from all the lakes while internal doses from $^{90}$Sr, $^{241}$Am,

138   $^{238}$Pu and $^{239,240}$Pu, were calculated in fish collected in the vicinity of the NPP (Glubkoye (H),

139   Yanovsky (H) and Cooling Pond (H)); $^{90}$Sr, $^{241}$Am, $^{238}$Pu and $^{239,240}$Pu activity concentrations are

140   not significant in the lakes outside the near zone[9]. For the calculation of $^{137}$Cs internal dose, the

141   dose conversion coefficient factor: $4.32 \times 10^{-6}$ mGy/d per Bq/kg [20]  and the $^{137}$Cs specific activity

142   were used. For the calculation of $^{90}$Sr internal dose, the dose conversion coefficient factor:

143   $1.51 \times 10^{-5}$ mGy/d per Bq/kg [20] and the $^{90}$Sr specific activity were used. For the calculation of

144   $^{241}$Am, $^{238}$Pu and $^{239,240}$Pu internal dose, the dose conversion coefficient factor: $7.61 \times 10^{-5}$, 7.61

145   $\times 10^{-5}$, $7.2 \times 10^{-5}$ mGy/d per Bq/kg [20] and the specific activities were used for $^{241}$Am, $^{238}$Pu and

146   $^{239,240}$Pu respectively.

147   *Micronucleus test*

148   The loss of genetic material from the nucleus of blood cells (erythrocytes) was investigated

149   applying the micronucleus test to 5 fish that were also used for histological analyses using a

150   standard procedure as described in SI.

151   *Histological analyses*

152    A standardised cross section of liver and gonad were fixed and processed according to standard

153    protocols described in SI. The liver sections were examined for microscopic pre-tumour and

154    tumour lesions according to BEQUALM and ICES criteria[18], and lesions associated to nuclear

155    and cellular polymorphism, cell death, inflammation and regeneration. For the female gonad

156    sections, the distribution of immature or mature oocytes was determined by counting the number

157    of perinuclear and cortical alveolar oocytes in a defined surface area at magnification 10 using a

158    microscope (Zeiss axiozoom), and the relative frequency of a germ cell stage was calculated as

159    follows: (number of oocytes at a given stage/total number of oocytes) x 100. Oocyte surface was

160    measured using Zen Pro software.

161    *Statistical analyses*

162    Statistical analyses were performed using R version 3.1.2. After satisfying the assumptions of

163    the normal distribution of the residuals, generalised linear models were used. If the normality of

164    the residuals was not respected, a Kruskal-Wallis rank test was applied. When significant, a

165    Wilcoxon rank test and a Bonferroni correction of the α error were performed. Pearson

166    correlation tests were performed. Further details are provided in SI.

167

168    **Results**

169    *Water chemistry*

170    Electrical conductivity varied from 120 to 318 µS/cm and the pH from 6.3 to 8.6 at the water

171    surface of the lakes. Dissolved oxygen varied from 48 to 125% and the temperature from 15.6 to

172　20.1°C at the water surface of the lakes (Table S2). Surface water concentrations of $NO_3^-$ and

173　$NO_2^-$ varied from 49 to 259 µg/L and from 1.5 to 11.9 µg/L respectively across the lakes (Table

174　S2). Surface water concentrations of $PO_4^{3-}$ varied from 1.4 to 15 µg/L across the lakes (Table

175　S2). Concentrations of toxic trace elements (As, Cd, Pb and U) were low (< 1 µg/L) in surface

176　waters of all lakes (Tables S3).

177　Full results are presented in SI.

178　*Significant contamination of fish from the CEZ with $^{137}$Cs, $^{90}$Sr, $^{241}$Am, $^{239,240}$Pu and $^{238}$Pu*

179　Thirty years after the Chernobyl accident, fish from the lakes located in the CEZ and in

180　Belarus (Svyatoye (M)) are still significantly contaminated with $^{137}$Cs ($p < 0.001$) (Figure 2A).

181　Perch from Glubokoye (H) and Svyatoye (M) lakes have the highest activity concentrations of

182　$^{137}$Cs, reaching $7844 \pm 899$ and $6090 \pm 526$ Bq/kg w.w. (wet weight) respectively. Perch from

183　Yanovsky (H) and Cooling Pond (H) are contaminated to a lesser extent with $^{137}$Cs levels

184　reaching $2567 \pm 993$ and $2974 \pm 501$ Bq/kg w.w. respectively. Perch from Dvoriche (L),

185　Stoyacheye (L) and Gorova (L) lakes contained much lower $^{137}$Cs levels: $193 \pm 31$, $88 \pm 20$ and 4

186　$\pm 1$ Bq/kg w.w. respectively (Figure 2A). The activity concentration of $^{137}$Cs is higher in perch

187　than in roach ($p < 0.001$) and by a factor 2-3 for the CEZ lakes (Figure 2A).

188　Fish from the CEZ are still significantly contaminated with $^{90}$Sr (Figure 2B). Concentration

189　levels for both species significantly differ across sites ($p < 0.001$) and reached, for perch and

190　roach respectively, $13636 \pm 1618$ and $12556 \pm 1536$ Bq/kg w.w. in Glubokoye (H) lake, $3603 \pm$

191　$2364$ and $2572 \pm 694$ Bq/kg w.w. in Yanovsky (H) lake and $79 \pm 16$ and $157 \pm 23$ Bq/kg w.w. in

192　the Cooling Pond (H) (Figure 2B). These are whole fish activity concentrations, most of the $^{90}$Sr

11

193    is found in bony tissues[21]. There was no significant difference in $^{90}$Sr activity concentrations

194    between perch and roach ($p = 0.34 > 0.05$) (Figure 2B).

195       Fish from the CEZ are significantly contaminated with $^{241}$Am $^{239,240}$Pu and $^{238}$Pu (Figure S1,

196    Table S4). Concentration levels in liver were significantly higher than in muscle for both species

197    ($p < 0.001$). For instance, $^{241}$Am concentration levels in liver were 7 to 11 times higher than in

198    muscle. Concentration levels of $^{241}$Am $^{239,240}$Pu and $^{238}$Pu in liver and muscle of perch from

199    Glubokoye (H) were significantly higher than in perch from Yanovsky lake and Cooling Pond ($p$

200    $< 0.05$) (Figure S1, Table S4). Concentration levels of $^{241}$Am $^{239,240}$Pu and $^{238}$Pu in liver and

201    muscle of roach did not vary significantly across the lakes ($p > 0.05$) (Figure S1). Further details

202    are provided in SI.

203    *Dose rate to fish*

204       The $^{90}$Sr internal dose rates (whole body average) varied from 0.1 (Cooling Pond (H)) to 7.7

205    (Glubokoye (H)) μGy/h in roach and from 0 (Cooling Pond (H)) to 8.4 (Glubokoye (H)) μGy/h

206    in perch (Table 1). The $^{137}$Cs internal dose rates ranged from 0 (Gorova (L), Dvoriche (L) and

207    Stoyacheye (L)) to 0.5 and 1.4 (Glubokoye (H)) μGy/h in roach and perch respectively. The

208    highest $^{137}$Cs external dose rates to fish were calculated in lakes from the CEZ and varied from

209    5.9 μGy/h in Glubokoye (H) to 7.3 μGy/h in Cooling Pond (H) (Table 1). The external dose to

210    fish from Svyatoye (M) was 10 times lower (0.7 μGy/h). The external doses were very low for

211    the three other lakes (L). The total β and γ dose rate ranged from 7.6 μGy/h in roach from the

212    Cooling Pond (H) to 15.7 μGy/h in perch from Glubokoye (H) lake. The total α dose rate in

213    perch and roach ranged from 0.11 to 0.24 and from 0.01 to 0.02 μGy/h in the liver and muscle

214    respectively (Table 2). The $^{241}$Am internal dose rate contributes to 63% and 87% of the total α

215    dose rate in liver and muscle of fish respectively. The $^{239,240}$Pu internal dose rate contributes to

216    27% and 10% of the total α dose rate in liver and muscle of fish respectively. The $^{238}$Pu internal

217    dose rate contributes to 10% and 4% of the total α dose rate in liver and muscle of fish

218    respectively. The total α dose rate contributes to 1.2-1.9% of the total (α, β and γ) dose rate in

219    fish from the three highly contaminated lakes.

220    *Fish species abundance*

221    The relative abundance of fish species does not differ between lakes ($p = 0.59$) therefore there

222    is no evidence of negative effects of radiation exposure on fish biodiversity (Figure S2).

223    *General health condition*

224    The lengths of the fish were not significantly different across lakes ($p = 0.85$) (Table S4). The

225    body weights of perch from the different lakes did not differ, except for Svyatoye (M), where the

226    values recorded were significantly higher. The weights of the roach from Glubokoye (H) and

227    Svyatoye (M) were the highest and there was no difference between Yanovsky (H), Cooling

228    Pond (H) and Gorova (L). The Fulton condition (FC) index of roach from the different lakes did

229    not significantly vary ($p = 0.99$). The FC of the perch from Cooling pond (H), Yanovsky (H) and

230    Glubokoye (H) were smaller than for perch from Stoyacheye (L) ($p < 0.01$) but were similar to

231    the FC of the perch from Svyatoye (L), Dvoriche (L) and Gorova (L) (Table S5). The

232    hepatosomatic index (HSI) of perch did not significantly vary across sites ($p = 0.5$). The HSI of

233    roach from Glubokoye (H), Yanovsky (H) and Cooling Pond (H) were significantly higher than

234    for roach from Svyatoye (M) ($p < 0.05$) but were similar to the HSI of the roach from Dvoriche

13

235    (L) (Table S5). No disease nor gross tumours or malformations were recorded in any of the fish

236    collected. Parasites were observed in liver of the perch from Yanovsky (H), Gorova (L),

237    Stoyacheye (L) and Dvoriche (L) and the prevalence was 55%, 6%, 31% and 14% respectively.

238    The histological analyses of the liver did not reveal any pre-tumour (Foci of cellular alterations)

239    and tumour (Hepatocellular adenoma and carcinoma) lesions nor more lesions associated with

240    nuclear and cellular polymorphism, cell death, inflammation, regeneration and melano-

241    macrophage centers in exposed fish.


242    *Reproductive status*


243    The gonadosomatic index (GSI) of perch and roach were significantly lower at Yanovsky (H)

244    and Cooling Pond (H) than at Dvoriche (L) and Gorova (L) ($p < 0.05$) (Figure S3). The GSI of

245    perch from Glubokoye (H) was significantly lower than perch from Gorova (L) ($p = 0.0004$)

246    (Figure 2A). No significant difference was found between GSI of perch from Glubokoye (H),

247    Yanosvsky (H), Cooling pond (H), Svyatoye (M) and Stoyacheye (L) ($p > 0.05$) (Figure S3A).

248    No significant difference was found between GSI of roach from Glubokoye (H), Dvoriche (L)

249    and Gorova (L) ($p > 0.05$) (Figure S3B). The fish age did not significantly influence the GSI of

250    roach ($p = 0.11$) and perch ($p = 0.15$).


251    The GSI of perch was inversely correlated to the percentage of immature oocytes in Yanovsky

252    (H) (cor: -0.85, $p < 0.001$), Cooling Pond (H) (cor: -0.77, $p < 0.001$) and Glubokoye (H) (cor: -

253    0.66, $p = 0.0008$) but not in Gorova (L) (cor: -0.28, $p = 0.30$), Dvoriche (L) (cor: 0.12, $p = 0.80$),

254    Stoyacheye (L) (cor: -0.24, $p = 0.44$) and Svyatoye (M) (cor: -0.37, $p = 0.16$). The correlation

255    was significant in Cooling Pond (H) and Yanovsky (H) due to the presence of sexually immature

14

256    fish displaying lower GSI than maturing fish ($p < 0.001$). 30% and 45% of female perch had

257    gonads containing only immature oocytes in Cooling Pond (H) and Yanovsky (H) lake ($p <$

258    $0.001$) in September (Figure 3, A, B). This phenotype was still found in Yanovsky (H) (38%)

259    and Cooling Pond (H) (25%) before spawning time in March (Figure 3, C) when oocytes should

260    have been mature in all fish (Figure 3, D, F). The occurrence of the immature phenotype was

261    positively correlated with the $^{137}$Cs external dose rate (cor: 0.78; $p = 0.04$) but not to the $^{90}$Sr

262    internal (cor: -0.04; $p = 0.9$) or the total (cor: 0.43; $p = 0.3$) dose rates. All the female roach

263    collected were maturing in September and mature in March (Figure 3, E, F). The GSI of roach

264    was inversely correlated to the percentage of immature oocytes in Glubokoye (H) (cor: -0.59, $p =$

265    $0.02$), Yanovsky (H) (cor: -0.67, $p = 0.0008$), Cooling Pond (H) (cor: -0.52, *only 3 observations*),

266    Gorova (L) (cor: -0.68, $p = 0.003$) but not in Svyatoye (M) (cor: -0.32, $p = 0.4$) and Dvoriche (L)

267    (cor: -0.19, $p = 0.5$). The age did not influence the percentage of immature oocytes in perch ($p =$

268    $0.19$) and roach ($p = 0.86$).

269    *The exposed maturing perch gonads contain a higher proportion of immature oocytes.*

270    The immature fish (100% of immature oocytes) were not included in these analyses. Maturing

271    female perch gonads from Glubokoye (H) contained a higher percentage of immature oocytes

272    (68%) than fish from Cooling Pond (H) (61%, $p = 0.03$), Svyatoye (M) (58%, $p = 0.046$),

273    Stoyacheye (L) (57%, $p = 0.0006$), Dvoriche (L) (58%, $p = 0.03$) and Gorova (L) (54%, $p =$

274    $0.00008$) (Figure 4A). Female perch gonads from Yanovsky (H) (65%) contained a higher

275    percentage of immature oocytes than fish from Stoyacheye (L) ($p = 0.03$) and Gorova (L) ($p =$

276    $0.01$) (Figure 4A). Female perch gonads from Cooling Pond (H) contain a similar percentage of

277    immature oocytes than fish from the other lakes ($p > 0.05$) except for fish from Glubokoye (H)

15

278    (Figure 4A). The percentage of immature oocytes was positively correlated to the total (cor:

279    0.92, $p = 0.004$), $^{90}$Sr internal (cor: 0.78, $p = 0.04$) and $^{137}$Cs external (cor: 0.78, $p = 0.04$) dose

280    rates.

281    *The proportion of immature oocytes in roach gonads is variable across lakes*

282    Roach from Cooling Pond (H) and Yanovsky (H) had a higher proportion of immature eggs

283    (62% and 54% respectively) than roach from Dvoriche (L) lake (41%) ($p < 0.01$) but displayed a

284    similar proportion of immature eggs as roach from lake Gorova (L) (51%) and Svyatoye (M)

285    (51%) ($p > 0.05$) (Figure 4B). Roach from Glubokoye (H) (49%) lake displayed a similar

286    proportion of immature eggs as roach from Dvoriche (L) (41%), Gorova (L) (51%) and Svyatoye

287    (M) (51%) lake ($p > 0.05$) (Figure 4B).

288    The female perch and roach gonads and oocytes did not display any structural damage.

289    *No chromosomal damage was evidenced in blood cells of exposed fish*

290    The number of micronuclei did not significantly vary across the sites for both species ($p = 0.14$

291    $> 0.05$) (Table S6).

292    **Discussion**

293    Physico-chemical values correspond to good quality waters according to the European surface

294    water quality standards (OECD, Annex1) and nutrient concentrations are typical of oligotrophic

295    waters (nitrate <1-3 mg/L; phosphate $< 0.04$ mg/L, OECD, Annex1).

296    *$^{137}$Cs, $^{90}$Sr, $^{241}$Am, $^{239,240}$Pu and $^{238}$Pu specific activities*

297    Thirty years after the accident, the activity concentration of $^{137}$Cs and $^{90}$Sr are still higher than

298    the EU (1250 Bq/kg for $^{137}$Cs; 750 Bq/kg for $^{90}$Sr), Ukrainian (150 Bq/kg for $^{137}$Cs; 35 Bq/kg for

299    $^{90}$Sr) and Japanese (100 Bq/kg) maximum permissible level for human consumption in some of

300    the lakes affected by the Chernobyl nuclear accident (see SI for details).

301    Activity concentrations of $^{137}$Cs measured in perch from Glubokoye (H) and Svyatoye (M)

302    lakes were the highest and reach 7844 and 6090 Bq/kg respectively. For Svyatoye (M), which is

303    the most distant of the 7 lakes, the high values are due to the high initial amount of $^{137}$Cs

304    deposited in this area, as well as hydrology and hydrochemistry of the water body. This is a

305    closed lake with a very low water exchange rate and low natural potassium concentration, which

306    explains the slow decontamination[10,22].

307    The most important pathway of $^{137}$Cs accumulation in fish is through the diet route[23,24]. The

308    results evidenced a $^{137}$Cs biomagnification phenomenon. As a carnivorous fish, the perch

309    significantly accumulates 2-3 times more $^{137}$Cs than its omnivorous prey, the roach. This is

310    consistent with previous studies led on perch and non-predatory fish from Chernobyl lakes where

311    the $^{137}$Cs contamination levels exceeded that of non-predatory ones by 2 in smaller fish[22] or 3-10

312    times in larger[12].

313    Activity concentrations of $^{90}$Sr were the highest in perch and roach from Glubokoye lake (H)

314    reaching a mean of 13636 and 12556 Bq/kg, decreased significantly in fish from Yanovsky (H)

315    and were the lowest in fish from Cooling Pond (H). The difference of activity concentrations

316    between sites might be due to the heterogeneous deposition of burning particles just after the

317    Chernobyl NPP accident[9]. The results show that, contrary to $^{137}$Cs, $^{90}$Sr is not biomagnified

318  between roach and perch. $^{90}$Sr can be accumulated from the water via the gills[25] or through the

319  diet[26].

320    Activity concentrations of $^{241}$Am, $^{239,240}$Pu and $^{238}$Pu were higher in liver than in muscle of

321  both fish species and the liver of perch from Glubokoye (H) lake contained at least 2 times more

322  $^{241}$Am, $^{239,240}$Pu and $^{238}$Pu (44, 23 and 8 Bq/kg w.w. respectively) than perch from Yanovsky (H)

323  and Cooling Pond (H). No biomagnification of transuranium radioisotopes were evidenced

324  between roach and perch. These results corroborate previous findings from environmental

325  studies on fish from the Baltic sea[27]. The dietary route was suggested to play a major role in the

326  plutonium intake in fish. At Chernobyl, the bioavailability of plutonium isotopes varies

327  depending on the association with fuel particles.

328  *Doses span the lowest protection level for an ecosystem*

329    The estimation of the radiation dose is discussed in Supplementary material. In the present

330  study, the total dose rate to roach and perch from the CEZ lakes was estimated to range from 7.6

331  to 14.1 μGy/h and from 7.9 to 15.7 μGy/h respectively. The dose rate in fish from Glubokoye

332  (H) lake was the highest especially due to the contribution of the high $^{90}$Sr internal dose rate (7.7

333  and 8.4 μGy/h for roach and perch respectively). However, as a β emitter and mainly

334  accumulated in calcium-rich tissues, a higher dose is expected in near bone tissues than in other

335  tissues, more than a few millimetres distant. The total internal α dose rate was estimated to range

336  from 0.01 to 0.2 μGy/h in perch and roach which is approximately two orders of magnitude

337  below the total β and γ total dose rate although the relative biological effectiveness of the α dose

338  is considered an order of magnitude higher. The doses observed in the study lakes span the

339    recommended screening level for protection of an ecosystem of 10 μGy/h (ERICA project[20]).

340    Although we have observed subtle effects of radiation on reproduction of perch at dose rates

341    below relevant reference levels (40-400 μGy/h)[28], no clear evidence of population level effects

342    was evidenced. Reference levels are usually set to protect populations of species therefore, this

343    study does not contradict currently accepted levels.

344    *No effect on the relative abundance of fish species*

345    In the present study, the relative abundance of fish species did not vary across the sites,

346    suggesting no negative effect of radiation on fish biodiversity, though absolute population

347    densities were not studied. The lack of observed effect on species diversity is in agreement with

348    previous studies on aquatic macroinvertebrate and mammal populations[2,3] which showed high

349    diversity (and abundance) in the Chernobyl affected areas. Other studies, however, have found

350    reduced abundance of insect, spider and mammal populations[4,5,6]. Our results show that thirty

351    years after the accident, the fish population is in good general health. This is in accordance with

352    the lowest protection level for an ecosystem of 10 μGy/h (ERICA project[20]) and the benchmark

353    levels established for reference pelagic fish species (40-400 μGy/h)[29]. At the individual level, the

354    absence of significant variation of the Fulton condition and hepatosomatic index further supports

355    the previous finding at the population level by showing that exposure to radiation has not

356    affected the general health and energetic status of fish. Finally, no genotoxic effect was

357    evidenced as previously described in a previous study on catfish from the Cooling Pond (H)[17].

358    *The reproductive biology of exposed fish is potentially affected by radiation*

19

359    Previous studies on fish from the Cooling Pond (H) of the Chernobyl NPP have reported

360    negative effects of radiation on the reproductive system (sterility and anomalies of gonads) at a

361    dose rate above 83-208 μGy/h[30]. Severe anomalies of the reproductive system of fish exposed to

362    radiation have been described in carp (asymmetry, sterility, abnormal cells or absence of gonads)

363    collected several years after the Kyshtym (21 μGy/h in 1972-1975) and the Chernobyl (17 μGy/h

364    in 1989-1992) accidents. Such abnormalities were also identified in the offspring of carp from

365    the Chernobyl NPP Cooling Pond (H) born in 1989 and they were less pronounced in the

366    offspring born in 1990[7]. Gonad abnormalities have also been recorded in post-accident

367    generations ($F_2$-$F_4$) of perch (sterilisation process of male, destruction of oocytes) and roach

368    (sterilisation process of female, destruction and resorption of oocytes, degenerations, abnormal

369    morphology) from Cooling Pond and Glubokoye Lake[13] although the number of fish sampled in

370    that study was too low to draw a robust conclusion (4 and 9 specimens in Glubokoye for perch

371    and roach respectively in 2004). In our study, the gonads and oocytes did not display any of

372    those abnormalities, highlighting overall a better reproductive condition of the perch and roach

373    population, 30 years after the accident.

374    However, the results of this study provide evidence of two types of inhibitory effects on the

375    reproductive biology of exposed female perch: the suppression of gonad development and a

376    delay in the recruitment of mature oocytes. These effects can result from both the toxic action

377    mode of radionuclides and the capacity of the population to adapt to contamination pressure.

378    The suppression of gonad development in fish is the result of long-term exposure to stress.

379    Although no differences were detected in mean gonadosomatic index (GSI) of female perch

380    between the lakes, the lowest GSI values observed in perch from Yanovsky (H) and Cooling

381    Pond (H) are due to the presence of 45% and 30% sexually immature females respectively. Such

382    phenotypes were not found in roach. This phenotype, however, was not found in fish from

383    Glubokoye (H) that are absorbing the highest total and internal $^{90}$Sr dose rate. The occurrence of

384    this phenotype was only correlated to the $^{137}$Cs external dose rate ($p < 0.05$). Thus, the

385    interpretation of observed inhibition of gonad development in perch from Yanovsky lake (H) and

386    the Cooling Pond (H) might be confounded by other factors, including food availability and

387    social interactions since the phenotype was not observed at the equally contaminated Glubokoye

388    lake (H). It is possible that higher selective pressure due to the ecology of the lake (such as a

389    more highly competitive ecosystem) may have encouraged the more rapid disappearance of this

390    disadvantageous phenotype over the several generations that have passed since the accident. To

391    our knowledge, studies showing undeveloped phenotypes of fish gonads exposed to radiation are

392    scarce. One study in the literature found that 30% of female Mozambique tilapia waterborne

393    exposed during their whole life to a dose of 1.3 x $10^3$ to 1.7 x $10^3$ µGy/h of $^{90}$Sr under laboratory

394    conditions had underdeveloped ovaries[7]. This dose rate is, however, approximately 50-100 times

395    higher than current dose rates to fish at Chernobyl.

396    The maturing female perch from Glubokoye (H) (15.7 µGy/h) and Yanovsky (H) (9.8 µGy/h)

397    lake contained a higher proportion of immature oocytes (68% and 65% respectively) than perch

398    from all other lakes. This is strongly correlated to the higher dose rate ($p < 0.05$). The perch

399    containing a higher percentage of immature oocytes had a lower GSI in Yanovsky (H), Cooling

400    Pond (H) and Glubokoye (H) ($p < 0.001$). Therefore, long-term exposure to radiation was found

401    to affect the maturation of oocytes and especially the recruitment of cortical alveolar oocytes in

402    fish absorbing the highest total dose rates. In the literature, a two weeks delay in spawning has

21

403   been described in the Siberian roach exposed to a dose rate of 83-333 µGy/h, 15-20 years after

404   the acute contamination event of the Kyshtym accident (Mayak Production Association),

405   Southern Ural[7]. Moreover, UNSCEAR reported a delay in spawning in fish exposed to very high

406   dose rates of 500-1000 µGy/h[31]. In the present study, the inhibitory effect on oocyte development

407   was no longer observed in the March sampling period, just before spawning, as all oocytes were

408   mature. Therefore, the disruptions to oocyte development observed in the three most

409   contaminated lakes will not necessarily lead to population-level effects. No disruption of the

410   reproductive status of female roach gonads was observed. The mechanisms by which

411   radionuclides could exert an effect on the endocrine system and subsequently alter the gonad

412   maturation of fish are unknown. The present study shows that exposure to radionuclides affects

413   oogenesis in the wild perch population. Further research on the molecular mechanisms involved

414   is in progress.


415     The evidence presented here strongly suggests that 30 years after the Chernobyl accident, the

416   reproductive status of perch is affected by chronic, low level radiation in their natural

417   environment. A clear increase was observed in proportion of immature oocytes from 54% in fish

418   from the reference lakes to 68% in fish from the highly contaminated lake (15.7 µGy/h).

419   Evidence for a high proportion of fish with undeveloped gonads was found in two of the most

420   contaminated lakes, but not in the third similarly contaminated lake meaning that the effect could

421   not unambiguously be attributed to past or current radiation. It is unknown whether effects

422   observed here are due to three decades of multi-generational (direct) exposure to radiation and/or

423   to trans-generational effects linked to the past history of contamination. In the Cooling Pond,

424   dose rates to fish in the short term after the accident reached 400 µGy/h and external sediment

425     dose rates reached 4000 μGy/h[32]. The early high dose rates are of crucial importance because, as

426     a result of trans-generational exposure, the current dose might not be directly related to the

427     effects observed but might rather be a result of previous higher exposure history.

428       The present study uniquely assesses the effect on fish health of a wide range of environmental

429     radiation exposure levels from highly contaminated lakes within the 10 km Chernobyl exclusion

430     zone in Ukraine to lakes with dose rates close to natural background. This study of multiple

431     biological parameters, has shown that the populations of two species are generally in good

432     physiological (including reproductive) health, but that there is evidence that a proportion of the

433     female perch population has either a failure or delay in maturation of the gonads. It cannot be

434     determined whether this is an effect of ongoing chronic low dose radiation or an effect of higher

435     dose rates on previous generations. The present study does not indicate significant radiation

436     effects on fish at the population level. It is important to note that fish have high fecundity; for

437     example, each female perch can produce thousands of eggs per year[33]. Thus, although some

438     effects on perch reproduction have been observed, it seems unlikely that these effects would

439     significantly impact fish population density.

444

445   **Supporting Information**. This file contains further details of the methods, results and discussion

446   sections, figures S1 to S3 and tables S1 to S6.

447   **References**

448   (1)   Real, A.; Sundell-Bergman, S.; Knowles, J. F.; Woodhead, D. S.; Zinger, I. Effects of

449         Ionising Radiation Exposure on Plants, Fish and Mammals: Relevant Data for

450         Environmental Radiation Protection. *Journal of Radiological Protection* **2004**, *24* (4A),

451         A123–A137.

452   (2)   Murphy, J. F.; Nagorskaya, L. L.; Smith, J. T. Abundance and Diversity of Aquatic

453         Macroinvertebrate Communities in Lakes Exposed to Chernobyl-Derived Ionising

454         Radiation. *Journal of Environmental Radioactivity* **2011**, *102* (7), 688–694.

455   (3)   Deryabina, T. G.; Kuchmel, S. V.; Nagorskaya, L. L.; Hinton, T. G.; Beasley, J. C.;

456         Lerebours, A.; Smith, J. T. Long-Term Census Data Reveal Abundant Wildlife Populations

457         at Chernobyl. *Current Biology* **2015**, *25* (19), R824–R826.

458   (4)   Møller, A. P.; Mousseau, T. A. Species Richness and Abundance of Forest Birds in

459         Relation to Radiation at Chernobyl. *Biology Letters* **2007**, *3* (5), 483–486.

460   (5)   Moller, A. P.; Mousseau, T. A. Reduced Abundance of Insects and Spiders Linked to

461         Radiation at Chernobyl 20 Years after the Accident. *Biology Letters* **2009**, *5* (3), 356–359.

462   (6)   Møller, A. P.; Mousseau, T. A. Assessing Effects of Radiation on Abundance of Mammals

463         and Predator–Prey Interactions in Chernobyl Using Tracks in the Snow. *Ecological*

464         *Indicators* **2013**, *26*, 112–116.

465   (7)   Sazykina, T. G.; Kryshev, A. I. EPIC Database on the Effects of Chronic Radiation in Fish:

466         Russian/FSU Data. *Journal of Environmental Radioactivity* **2003**, *68* (1), 65–87.

467 (8) Kryshev, A. I. Modelling the Accidental Radioactive Contamination and Assessment of
468 Doses to Biota in the Chernobyl NPP's Cooling Pond. *Proceedings of the topical meeting*
469 *of International Union of Radioecologists* **1998**, No. mol 1, 32–38.

470 (9) *Chernobyl - Catastrophe and Consequences*; Smith, J. T., Beresford, N. A., Eds.; Springer-
471 Praxis books in environmental sciences; Springer: Berlin, 2005.

472 (10) Ryabov, I. N. Long-Term Observation of Radioactivity Contamination in Fish around
473 Chernobyl. **2002**, 11, 112-122.

474 (11) Koulikov, A. O.; Ryabov, I. N. Specific Cesium Activity in Freshwater Fish and the Size
475 Effect. *Science of The Total Environment* **1992**, *112* (1), 125–142.

476 (12) Kryshev, I. I. Radioactive Contamination of Aquatic Ecosystems Following the Chernobyl
477 Accident. **1995**, 27(3), 207-219.

478 (13) Belova, N. V.; Emel'yanova, N. G.; Makeeva, A. P.; Ryabov, I. N. The State of the
479 Reproductive System of Several Fish Species from Water Bodies Polluted with
480 Radionuclides during the Chernobyl Catastrophe. *Journal of Ichthyology* **2007**, *47* (5), 366–
481 384.

482 (14) Belova, N. V.; Emel'yanova, N. G. Status of the Reproductive System of Bony Fish from
483 the Teterev River and the Kiev Reservoir 20 Years after the Chernobyl Accident. *Journal of*
484 *Ichthyology* **2009**, *49* (9), 793–802.

485 (15) Belova, N. V. Radiobiological Analysis of Silver Carp *Hypophthalm Ichthys Molitrix* in the
486 Water Cooler of the Chernobyl Nuclear Power Plant in the Post Emergency Period. 1. The
487 State of the Reproductive System in Fish That Were Exposed to the Emergency. *Vopr.*
488 *Ikhtiol* **1993**, *33* (6), 814–828.

489    (16) Suzuki, Y. Influences of Radiation on Carp from Farm Ponds in Fukushima. *Journal of*
490        *Radiation Research* **2015**, *56* (suppl 1), i19–i23.

491    (17) Sugg, D. W.; Bickham, J. W.; Brooks, J. A.; Lomakin, M. D.; Jagoe, C. H.; Dallas, C. E.;
492        Smith, M. H.; Baker, R. J.; Chesser, R. K. DNA Damage and Radiocesium in Channel
493        Catfish from Chernobyl. *Environmental Toxicology and Chemistry* **1996**, *15* (7), 1057–
494        1063.

495    (18) The Animal (Scientific Procedure) Act 1986. *The Lancet* **1986**, *328* (8497).

496    (19) Biological Effects of Contaminants: Use of Liver Pathology of the European Flatfish Dab
497        *(Limanda Limanda L.)* and Flounder *(Platichthys Flesus L.)* for Monitoring; Feist, S. W.,
498        Ed.; ICES techniques in marine environmental sciences; International Council for the
499        Exploration of the Sea: Copenhagen, 2004.

500    (20) Brown, J. E.; Alfonso, B.; Avila, R.; Beresford, N. A.; Copplestone, D.; Hosseini, A. A
501        New Version of the ERICA Tool to Facilitate Impact Assessments of Radioactivity on Wild
502        Plants and Animals. *Journal of Environmental Radioactivity* **2016**, *153*, 141–148.

503    (21) Smith, J. T.; Sasina, N. V.; Kryshev, A. I.; Belova, N. V.; Kudelsky, A. V. A Review and
504        Test of Predictive Models for the Bioaccumulation of Radiostrontium in Fish. *Journal of*
505        *Environmental Radioactivity* **2009**, *100* (11), 950–954.

506    (22) Smith, J. T.; Kudelsky, A. V.; Ryabov, I. N.; Hadderingh, R. H. Radiocaesium
507        Concentration Factors of Chernobyl-Contaminated Fish: A Study of the Influence of
508        Potassium, and "Blind" Testing of a Previously Developed Model. *Journal of*
509        *Environmental Radioactivity* **2000**, *48*, 359–369.

510    (23) Forseth, T.; Ugedal, O.; Jonsson, B.; Langeland, A.; Njastad, O. Radiocaesium Turnover in

511          Arctic Charr (*Salvelinus Alpinus*) and Brown Trout (*Salmo Trutta*) in a Norwegian Lake.

512          *The Journal of Applied Ecology* **1991**, *28* (3), 1053.

513    (24) Elliott, J. M.; Hilton, J.; Rigg, E.; Tullett, P. A.; Swift, D. J.; Leonard, D. R. P. Sources of

514          Variation in Post-Chernobyl Radiocaesium in Fish from Two Cumbrian Lakes (North-West

515          England). *The Journal of Applied Ecology* **1992**, *29* (1), 108.

516    (25) Chowdhury, M. J.; Blust, R. A Mechanistic Model for the Uptake of Waterborne Strontium

517          in the Common Carp (*Cyprinus Carpio* L.). *Environmental Science & Technology* **2001**, *35*

518          (4), 669–675.

519    (26) Kryshev, A. I. Model Reconstruction of 90Sr Concentrations in Fish from 16 Ural Lakes

520          Contaminated by the Kyshtym Accident of 1957. *Journal of Environmental Radioactivity*

521          **2002**, *64* (1), 67–84.

522    (27) Skwarzec, B.; Struminska, D. I.; Borylo, A. Bioaccumulation and Distribution of Plutonium

523          in Fish from Gdansk Bay. *Journal of Environmental Radioactivity* **2001**, *55* (2), 167–178.

524    (28) *ICRP*; Annals of the ICRP; 108; 2008; p Volume 38 Nos. 4-6.

525    (29) Larsson, C. M. An Overview of the ERICA Integrated Approach to the Assessment and

526          Management of Environmental Risks from Ionising Contaminants. *Journal of*

527          *Environmental Radioactivity* **2008**, *99* (9), 1364–1370.

528    (30) Kryshev, A. I.; Sazykina, T. G. Comparative Analysis of Doses to Aquatic Biota in Water

529          Bodies Impacted by Radioactive Contamination. *Journal of Environmental Radioactivity*

530          **2012**, *108*, 9–14.

531    (31) *Sources and Effects of Ionizing Radiation: UNSCEAR 2008 Report to the General*

532          *Assembly, with Scientific Indexes Vol. 2*; United Nations: New York, 2011.

533    (32) Kryshev, I. I.; Sazykina, T. G. Assessment of Radiation Doses to Aquatic Organism's in the

534            Chernobyl Contaminated Area. **1995**, 13.

535    (33) Craig, J. F. Population Dynamics of Perch, Perca Fluviatilis L. in Slapton Ley, Devon.: I.

536            Trapping Behaviour, Reproduction, Migration, Population Estimates, Mortality and Food.

537            *Freshwater Biology* **1974**, *4* (5), 417–431.

538

539    **Figure and Table Legends.**

540    **Figure 1.** Map showing the sampling sites in the Mogilev (Svyatoye lake (M)) and Gomel

541    (Stoyacheye and Dvoriche lakes (L)) regions of Belarus, in the CEZ (Glubokoye lake, Yanovsky

542    lake and Cooling pond (H)) and in the eastern region of Kiev in Ukraine (Gorova lake (L)).

543    **Figure 2.** Mean activity concentration of $^{137}$Cs (A) and $^{90}$Sr (B) in perch and roach ($n = 5$, mean

544    $\pm$ Sd, Bq/kg, w.w.) collected in and outside the CEZ. Analysis found a significantly higher $^{137}$Cs

545    activity concentration in perch than in roach (A) but no difference of $^{90}$Sr activity concentration

546    between the two species (B).

547    **Figure 3.** Pictures of tissue sections showing oocytes of female perch (A, B, C and D) and roach

548    (E, F). A and C: perinuclear oocytes (immature) from perch collected in September and March.

549    B and E: corticular alveolar oocytes (maturing) from perch and roach, respectively, collected in

550    September. D and F: vitellogenic oocytes (mature) from perch and roach, respectively, collected

551    in March.

552     **Figure 4.** Distribution of immature and mature oocytes in maturing female perch (A) and roach

553     (B) gonads collected in the different lakes. The sexually immature perch (only containing

554     perinuclear oocytes) were not considered.


555     **Table 1.** Table showing the calculated $^{90}$Sr and $^{137}$Cs internal dose rates, $^{137}$Cs external dose rates

556     and the total dose rates. All doses were corrected to 2015.


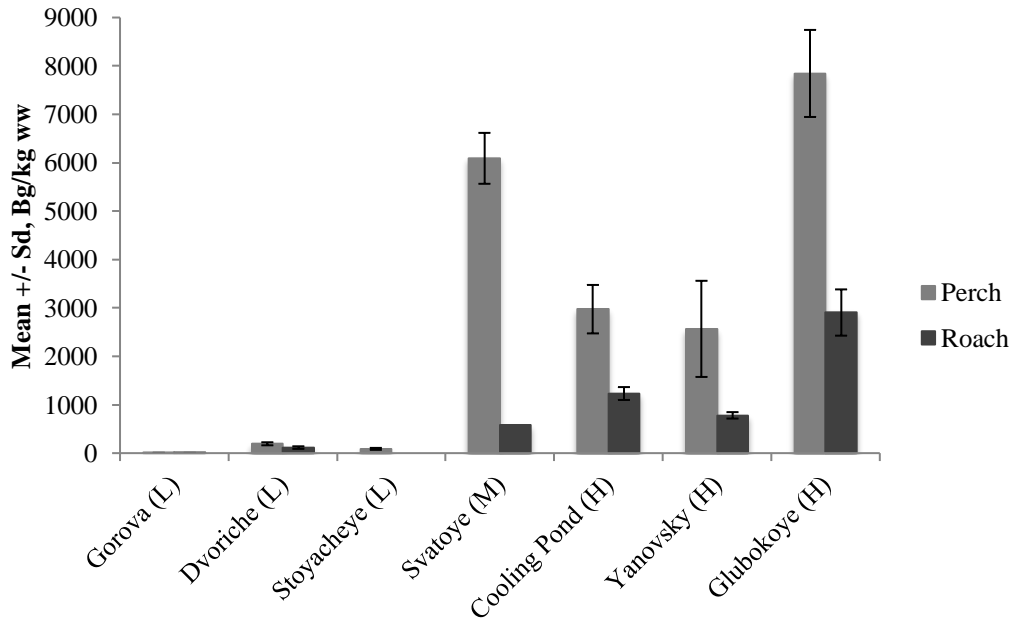557     **Table 2.** Table showing the calculated $^{241}$Am, $^{238}$Pu and $^{239,240}$Pu internal dose rates.
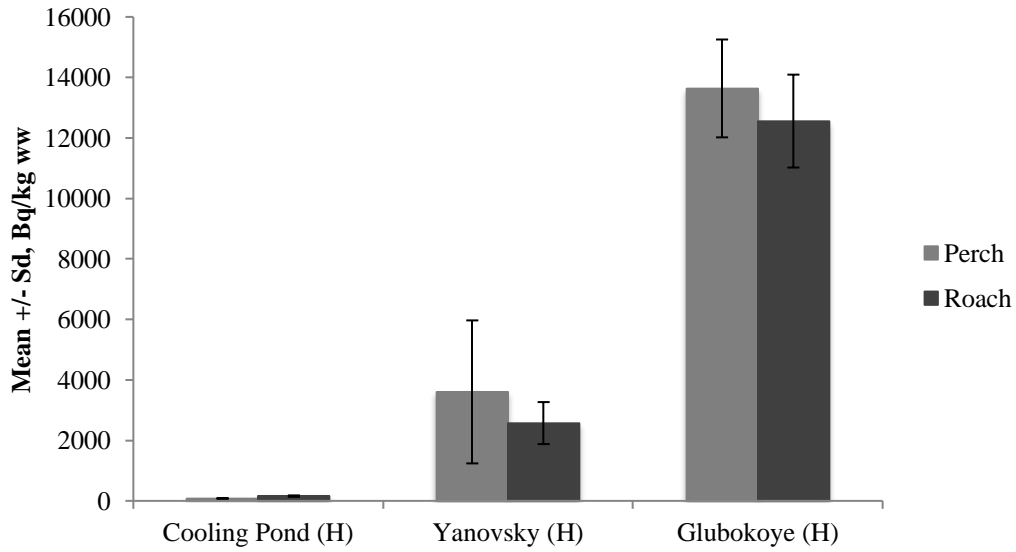
**Figure 1.**
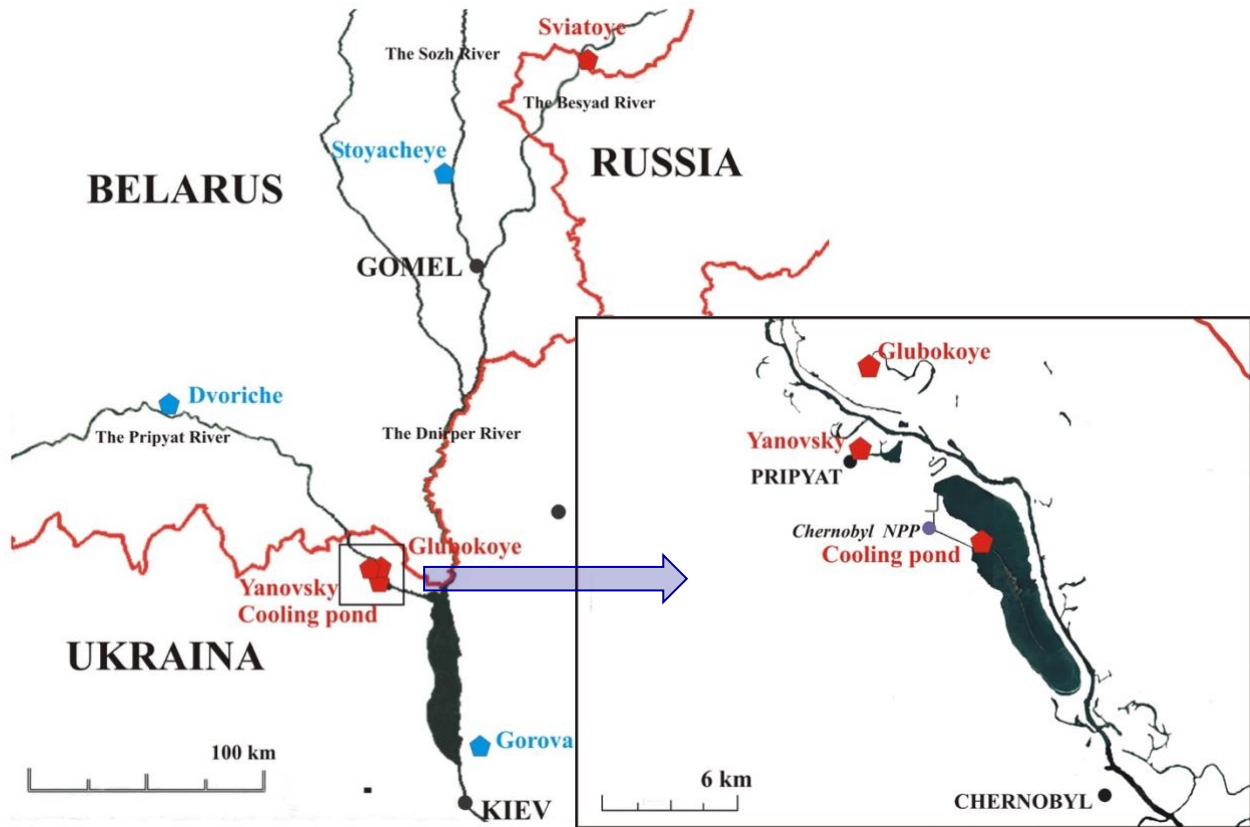
**Figure 2.**

**A)**



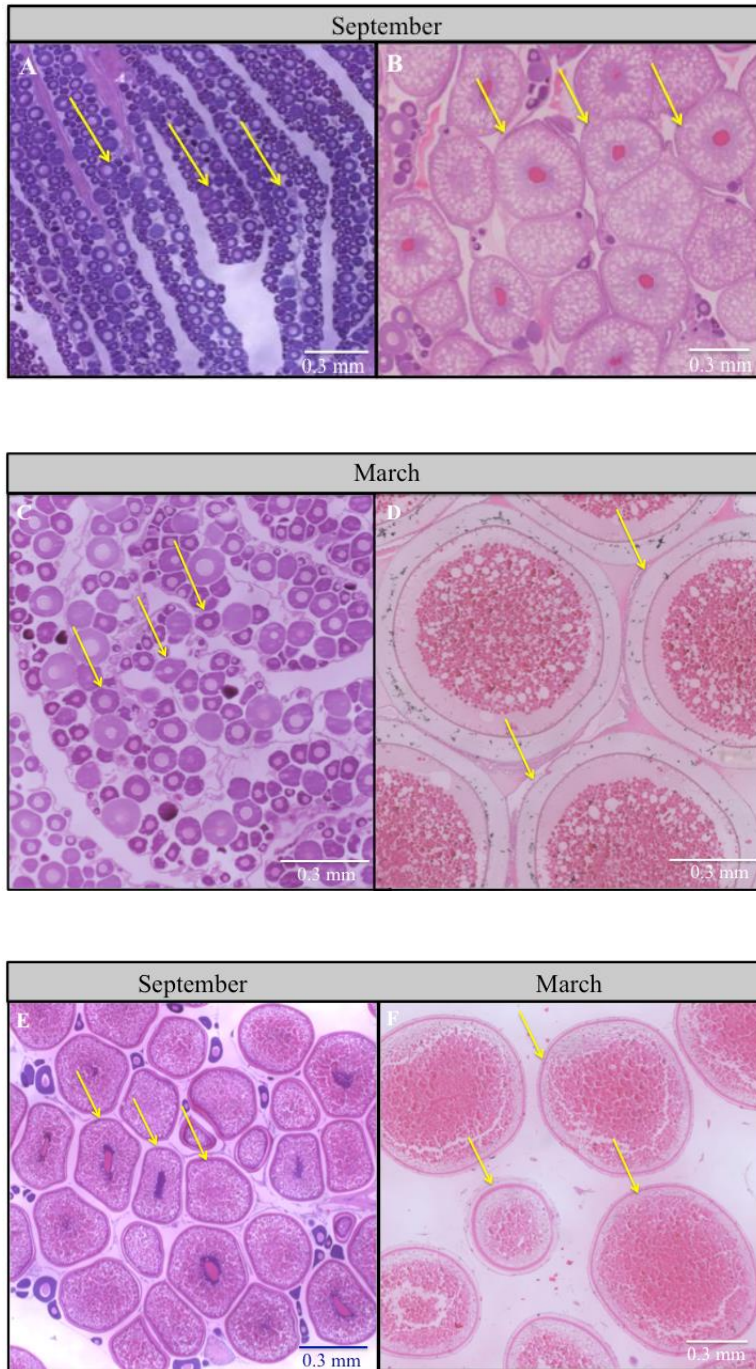**B)**

**Figure 3.**
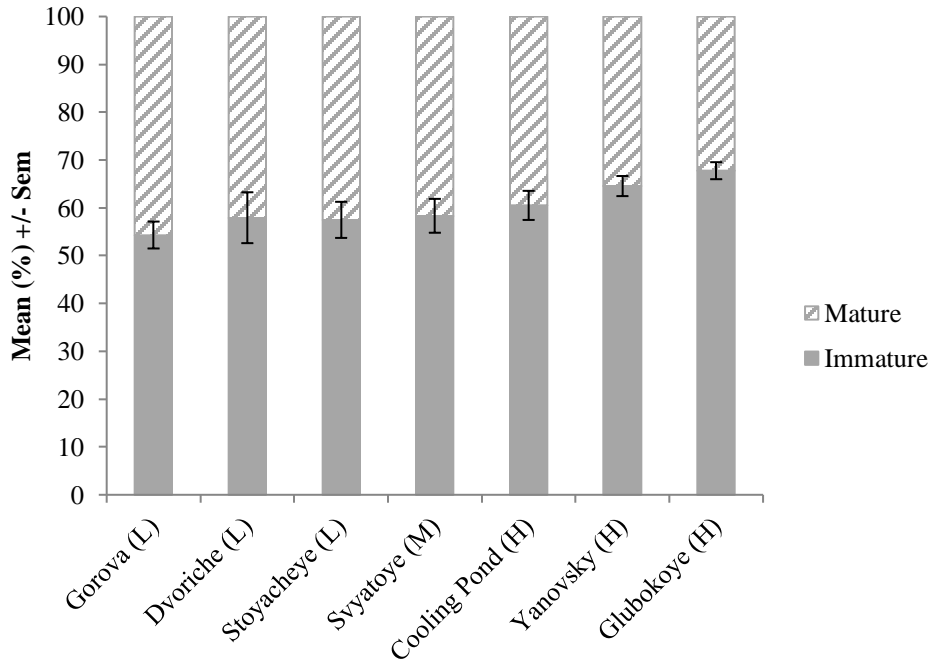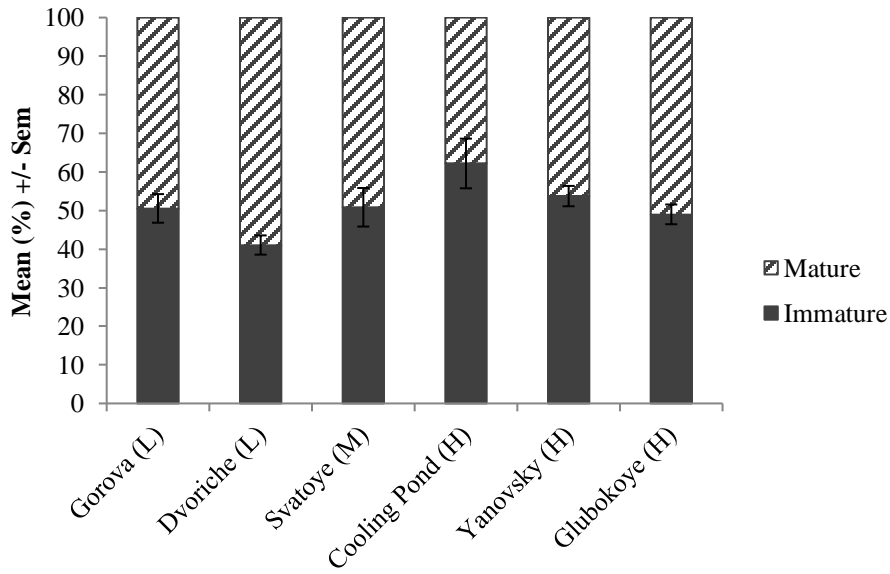
**Figure 4.**

**A)**



**B)**

**Table 1.**

| Lake | $^{137}$Cs internal dose rate (µGy/h) 2015 | | $^{90}$Sr internal dose rate (µGy/h) 2015 | | $^{137}$Cs external dose rate (µGy/h) 2015 | Total dose rate (µGy/h) 2015 | |
|---|---|---|---|---|---|---|---|
| | Roach | Perch | Roach | Perch | | Roach | Perch |
| Glubokoye (H) | 0.5 | 1.4 | 7.7 | 8.4 | 5.9 | 14.1 | 15.7 |
| Yanovsky (H) | 0.1 | 0.5 | 1.6 | 2.2 | 7.1 | 8.8 | 9.8 |
| Cooling P. (H) | 0.2 | 0.5 | 0.1 | 0.0 | 7.3 | 7.6 | 7.9 |
| Svyatoye (M) | 0.1 | 1.1 | / | / | 0.7 | 0.8 | 1.7 |
| Stoyacheye (L) | nd | 0.0 | / | / | 0.1 | 0.1 | 0.1 |
| Dvoriche (L) | 0.0 | 0.0 | / | / | 0.0 | 0.1 | 0.1 |
| Gorova (L) | 0.0 | 0.0 | / | / | 0.0 | 0.0 | 0.0 |

**Table 2.**

| Lake (H) | $^{241}$Am Internal dose rate (µGy/h) 2015 | | | | $^{239,240}$Pu Internal dose rate (µGy/h) 2015 | | | | $^{238}$Pu Internal dose rate (µGy/h) 2015 | | | | Total $\alpha$ internal dose rate (µGy/h) 2015 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Roach | | Perch | | Roach | | Perch | | Roach | | Perch | | Roach | | Perch | |
| | Liver | Muscle | Liver | Muscle | Liver | Muscle | Liver | Muscle | Liver | Muscle | Liver | Muscle | Liver | Muscle | Liver | Muscle |
| Glubokoye | 0.10 | 0.01 | 0.14 | 0.01 | 0.05 | 0.01 | 0.07 | 0.0040 | 0.02 | 0.002 | 0.03 | 0.002 | 0.16 | 0.02 | 0.24 | 0.02 |
| Yanovsky | 0.11 | 0.01 | 0.07 | 0.01 | 0.04 | 0.0003 | 0.03 | 0.0002 | 0.01 | 0.0001 | 0.01 | 0.0001 | 0.16 | 0.01 | 0.11 | 0.01 |
| Cooling P. | 0.08 | 0.01 | 0.07 | 0.01 | 0.03 | 0.0002 | 0.03 | 0.0003 | 0.01 | 0.0001 | 0.01 | 0.0001 | 0.12 | 0.01 | 0.11 | 0.01 |