Chronic Radiation Exposure at Chernobyl Shows No Effect on Genetic Diversity in the Freshwater Crustacean, Asellus aquaticus Thirty Years On

Neil Fuller, Alex T Ford, Adélaïde Lerebours, Dmitri I Gudkov, Liubov Nagorskaya, Jim Smith

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1 **Chronic Radiation Exposure at Chernobyl Shows No Effect on Genetic**

2 **Diversity in the Freshwater Crustacean, *Asellus aquaticus* Thirty Years On**

3 Running Head: Genetic Diversity in Chernobyl Crustaceans

4 <u>Neil Fuller</u>*[1], Alex T. Ford[1], Adélaïde Lerebours[1], Dmitri I. Gudkov[2], Liubov L.

5 Nagorskaya[3], & Jim T. Smith[4]

6 [1] Institute of Marine Sciences, School of Biological Sciences, University of Portsmouth, Ferry

7 Road, Portsmouth, Hampshire PO4 9LY, UK.

8 [2] Department of Freshwater Radioecology, Institute of Hydrobiology, Geroyev Stalingrada

9 Ave. 12, UA-04210 Kiev, Ukraine.

10 [3] Applied Science Center for Bioresources of the National Academy of Sciences of Belarus,

11 27 Academicheskaya Str., 220072 Minsk, Belarus.

12 [4] School of Earth & Environmental Sciences, University of Portsmouth, Burnaby Building,

13 Burnaby Road, Portsmouth, Hampshire PO1 3QL, UK

14 *Corresponding author: Neil Fuller (neil.fuller@port.ac.uk)

15 **Abstract**

16 Analysis of genetic diversity represents a fundamental component of ecological risk

17 assessments in contaminated environments. Many studies have assessed the genetic

18 implications of chronic radiation exposure at Chernobyl, generally recording an elevated

19 genetic diversity and mutation rate in rodents, plants and birds inhabiting contaminated

20 areas. Only limited studies have considered genetic diversity in aquatic biota at Chernobyl,

21 despite the large number of freshwater systems where elevated dose rates will persist for

22 many years. Consequently the present study aimed to assess the effects of chronic radiation

23 exposure on genetic diversity in the freshwater crustacean, *Asellus aquaticus*, using a

24 genome-wide SNP approach (Genotyping-by-sequencing). It was hypothesised that genetic

25   diversity in *A. aquaticus* would be positively correlated with dose rate. *A. aquaticus* was

26   collected from six lakes in Belarus and the Ukraine ranging in dose rate from 0.064 to 27.1

27   µGy/hr. Genotyping-by-sequencing analysis was performed on 74 individuals. A significant

28   relationship between geographical distance and genetic differentiation confirmed the

29   Isolation-by-Distance model. Conversely, no significant relationship between dose rate and

30   genetic differentiation suggested no effect of the contamination gradient on genetic

31   differentiation between populations. No significant relationship between five measures of

32   genetic diversity and dose rate were recorded, suggesting that radiation exposure has not

33   significantly influenced genetic diversity in *A. aquaticus* at Chernobyl. This is the first study to

34   adopt a genome-wide SNP approach to assess the impacts of environmental radiation

35   exposure on biota. These findings are fundamental to understanding the long-term success

36   of aquatic populations in contaminated environments at Chernobyl and Fukushima.

37   Keywords: Chernobyl, Genetic Diversity, Crustacean, Genotyping-by-sequencing

38 **Introduction**

39   Studies of the impacts of contaminants on genetic diversity have increased in recent years

40   owing to the advent of affordable DNA sequencing services and the recognised importance

41   of maintaining genetic diversity in the conservation of wildlife populations (Frankham et al.,

42   2014; Giska et al., 2015; Rumisha et al. 2017). Alterations to genetic diversity may lead to

43   reduced susceptibility to environmental change (e.g. Ehlers et al., 2008) and increased

44   extinction potential. Furthermore, changes to genetic diversity may reflect long-term pollutant

45   impacts more adequately than many commonly used biomarkers which reflect transient

46   stress on populations (Anderson et al., 1994; Bickham et al., 2000). Ionising radiation is a

47   known mutagen with the capacity to cause a range of alterations to the genome, including

48   chromosomal aberrations, micronuclei formation and gene mutations (Morgan, 2003). Whilst

49   the genetic consequences of acute, high doses of radiation are well understood (Little et al.,

50   1997; Tucker et al., 2005), the impacts of chronic sub-lethal doses over multiple generations

51   are comparatively less clear (Baker et al., 2017).

52   Following the 1986 Chernobyl accident, the worst nuclear accident in history, a number of

53   studies assessed genetic effects of radiation exposure on both humans and wildlife (Dubrova

54   et al., 1996, 1997; Ellegren et al., 1997; Matson et al., 2000). Elevated mutation rates have

55   been recorded in the offspring of liquidators involved in the clean-up operation (e.g.

56   Weinberg et al., 2001) and amongst children born in heavily contaminated areas (Dubrova et

57   al., 1996, 1997). Similarly, studies have demonstrated elevated genetic diversity and

58   mutation rates in a range of wildlife inhabiting contaminated areas (e.g. Baker et al., 2017;

59   Ellegren et al., 1997). However, these findings are not ubiquitous, with a number of studies

60   finding   no significant effects of Chernobyl-derived radiation on genetic variation (e.g.

61   DeWoody, 1999; Furitsu et al., 2005; Livshits et al., 2001).

62   Though the majority of studies suggest an increase in genetic diversity and mutation rates in

63   contaminated areas of Chernobyl, a reduction in genetic diversity could also have occurred

64   owing to population bottlenecks induced by high dose rates immediately following the

65   accident. Bottleneck events occur when the size of a population is reduced due to

66   contaminant exposure, leading to a small subset of genotypes available for recovery and

67   expansion (van Straalen & Timmermans, 2002). This would lead to reduced genetic diversity

68   due to the direct removal of genotypes, known as 'genetic erosion'. To the author's

69   knowledge, no study has demonstrated a reduced genetic diversity in wildlife at Chernobyl.

70   Despite the large number of studies assessing genetic effects of environmental radiation on

71   natural populations of rodents (e.g. Baker et al., 2017; Matson et al., 2000; Wickliffe et al.,

72   2003) and plants (Geras'kin & Volkova, 2014; Kovalchuk et al., 2000; Tsyusko et al., 2006;

73   Volkova et al., 2018), only two studies have considered effects on aquatic biota. Theodorakis

74   & Shugart (1998) studied genetic variation using random amplified polymorphic DNA (RAPD)

75   methods in mosquito fish (*Gambusia affinis*) inhabiting ponds contaminated with radioactive

waste from the US Department of Energy's Oak Ridge facility. The authors recorded increased genetic diversity in *G. affinis* individuals inhabiting two contaminated ponds (dose rates of 50 and 1313 µGy/hr) as opposed to individuals from two reference populations. However, one of the aforementioned sites is also heavily contaminated with a suite of other genotoxicants (e.g. heavy metals and polycyclic aromatic hydrocarbons, Theodorakis & Shugart, 1997), meaning any observed effects cannot be attributed solely to radiation. The Chernobyl area is heterogeneously contaminated by a range of radionuclides, including ^{90}Sr, ^{137}Cs and transuranium isotopes (e.g. ^{238}Pu, ^{239,240}Pu, and ^{241}Am, Beresford et al., 2018). Doses to aquatic macroinvertebrates are dominated by external γ and β radiation from ^{137}Cs and ^{90}Sr (Murphy et al., 2011).

To the authors' knowledge, only a single study has considered radiation effects on genetic diversity in aquatic biota at Chernobyl, despite the large number of closed lake systems in the area where biota are chronically exposed to low-dose radiation. Fetisov et al., (1992) studied genetic and morphological differences in seven populations of the zebra mussel, *Dreissena polymorpha,* in the Chernobyl area using an allozyme method. Based on data for five loci, no significant influence of radiation on *D. polymorpha* genetic structure was recorded, though thermal regime was found to influence genetic diversity. However the use of allozymes in genetic diversity studies has been questioned, particularly at small spatial scales and using a small number of loci (e.g. Turlure et al., 2014). Furthermore, the influence of radiation was not the direct focus of the study and no dose rates were provided, necessitating further robust study into the impacts of chronic radiation exposure on the genetic diversity of aquatic invertebrates. The 2011 accident at the Fukushima Dai-Ichi nuclear power plant led to further contamination of marine and freshwater environments that will persist for decades (IAEA, 2015). This further emphasises the need for robust studies of the long-term genetic consequences of environmental radiation on aquatic biota.

101 Many of the studies of radiation-induced genetic changes highlighted above used techniques

102 such as restriction fragment length polymorphisms (RFLPs, e.g. Theodorakis & Shugart,

103 1998), amplified fragment length polymorphisms (AFLPs, e.g. Volkova et al., 2018) or

104 microsatellites (e.g. Ellegren et al., 1997). Recent studies have shown that genome-wide

105 analysis of single nucleotide polymorphisms (SNPs) provide less biased measures of genetic

106 diversity as compared to these more traditional techniques (Fischer et al., 2017). SNPs are

107 more widely abundant across the genome as compared to microsatellites and RFLP-based

108 techniques, and are generally more reproducible (Davey et al., 2011; Schlötterer, 2004). The

109 advent of affordable sequencing coupled with continual advancements in technologies have

110 led to the application of genome-wide SNP approaches to a wide range of questions,

111 including evolutionary history (Pollinger et al., 2010), conservation (Larson et al., 2014) and

112 ecotoxicology (Giska et al., 2015).

113 Taking into account these knowledge gaps, the present study aimed to assess the impact of

114 chronic radiation exposure on the freshwater crustacean, *Asellus aquaticus*, along a gradient

115 of contamination at Chernobyl using a genome-wide SNP approach. Genotyping-by-

116 sequencing (GBS) is a simple, cost-effective reduced representation method for assessing a

117 vast number of SNPs across the genome (Narum et al., 2013). GBS has been applied to a

118 wide range of both model and non-model organisms and has been demonstrated to be a

119 useful technique for population genomic analyses even where reference genomes are not

120 available (e.g. White et al., 2013). *Asellus aquaticus* is a detrivorous isopod crustacean

121 commonly found in freshwater systems across Europe. Crustaceans are one of the

122 International Comission on Radiological Protection's (ICRP) eight reference animals and

123 plants (RAPs), meaning these organisms will be used to support the evolving system for

124 environmental radioprotection (ICRP, 2007). *A. aquaticus* is commonly used in ecotoxicology

125 studies of sediment-borne contaminants (De Lange et al., 2005; McCahon & Pascoe, 1988)

126 and has been used as an indicator of water quality (Whitehurst, 1991). Previous studies have

127 demonstrated no effects of chronic radiation exposure at Chernobyl on the development and

128 reproduction of *A. aquaticus* (Fuller et al., 2017, 2018). However, to the authors knowledge

129 no studies have been conducted on the lethal tolerance of *A. aquaticus* to ionising radiation.

130 Based on the number of studies documenting an increase in genetic diversity in biota at

131 Chernobyl (e.g. Baker et al., 2017; Matson et al., 2000; Volkova et al., 2018), we

132 hypothesised that populations of *A. aquaticus* would display elevated genetic diversity along

133 a gradient of radionuclide contamination at Chernobyl. In addition, the influence of the

134 gradient in dose rate on genetic differentiation (measured as Fst) was assessed following

135 Giska et al., (2015) and Rumisha et al., (2017).

136 **2. Materials & Methods**

137 *2.1 Field Sampling & Collection of A. aquaticus*

138 Field sampling was conducted in May-June of 2016 at six lakes in Belarus and Ukraine (see

139 Figure 1) as described in Fuller et al., (2017, 2018). Sampling sites were selected based on

140 historical measurements of radioactivity and exposure to a gradient of radionuclide

141 contamination. *A. aquaticus* were collected by kick netting in littoral zones using a 1 mm

142 mesh size net (EFE, UK), sorted lakeside and immediately preserved in 96% Ethanol. A total

143 of 455 *A. aquaticus* individuals were collected across all sites during the sampling period.

144 Numbers of *A. aquaticus* individuals sequenced are shown in Section 2.4.

145 **Figure 1. –** Map of the six study sites sampled for *Asellus aquaticus* in relation to distance
146 from the Chernobyl Nuclear Power Plant (CNPP). Map generated using qGIS (v2.18) with
147 data from DIVA-GIS (Hijmans et al., 2001).

148 *2.2 Environmental Conditions*

149 A range of different environmental parameters were measured at three sub-sites of each lake

150 using a multiparameter probe (HANNA Insturments 9828). Sampling dates and

151 environmental conditions are shown in Table 1.

152 *2.3 Calculation of Dose Rates*

153 A full description of the methods used to calculate doses to *A. aquaticus* is presented in

154 Fuller et al., (2017). Briefly, external and internal dose rates to *A. aquaticus* were calculated

155 based on decay-corrected deposition values for radiocaesium and strontium (in 1986) and

156 average activity concentrations in water at various depths measured in 2003. Accurate dose

157 conversion coefficients (DCCs) for *A. aquaticus* were generated within the ERICA tool

158 (Version 1.2) by user-inputted geometry (Height = 2.2mm, width = 1.7mm, length = 4.7mm

159 and mass = 4.1mg) and used for subsequent dose assessments using ERICA. Total dose

160 rates for *A. aquaticus* at each of the six lakes are shown in Table 1.

161 **Table 1. –** Environmental conditions, location, sampling date and total dose rate at six sites
162 of varying contamination in Belarus and the Ukraine. Error bars are standard deviations
163 based on measurements at three different sub-sites of each lake.

164 *2.4 Genomic DNA Extractions & Quality Control*

165 DNA was extracted from a total of 74 *A. aquaticus* individuals using a QIAGEN DNeasy

166 Blood & Tissue Kit (QIAGEN, Germany) following some amendments to the manufacturer

167 protocol to maximise DNA yield. Individuals were first homogenised in 180 µl of Buffer ATL.

168 Following addition of 40 µL Proteinase K, samples were incubated at 56 °C for 16 hours to

169 allow for lysis to occur. After lysis, samples were centrifuged at 20,000 g for 5 minutes and

170 the supernatant transferred to a new microcentrifuge tube. This step was performed to

171 remove the non-lysed, largely chitinous tissue as this may impact the quality of DNA for

172 downstream applications in *A. aquaticus* (Verovnik et al., 2003). Samples were then

173 processed according to the animal tissue spin-column protocol outlined in the QIAGEN Blood

174 and Tissue Kit. Quality of extracted genomic DNA was assessed using a NanoDrop

175 spectrophotometer, and restriction enzyme digests with Hind*III* (Thermo Fisher, UK) were

176 performed on 10% of DNA samples to assess suitability for genomic library construction.

177 Digests were performed according to manufacturer protocol and visualised on a 1% agarose

178 gel.

179 *2.5 Genotyping-by-Sequencing*

180 A full description of GBS methods is provided in Elshire et al., (2011). Briefly, extracted DNA

181 was arrayed on to a 96-well sealed fully skirted PCR plate (Applied Biosystems, US) and

182 shipped to the Cornell University Genomic Diversity Facility for GBS analysis. Following

183 optimisation, PstI (CTGCAG) was selected as the most appropriate restriction enzyme owing

184 to the greater number of fragment sizes generated. Each DNA sample was digested with PstI

185 and ligated with a barcoded and a common adaptor. Following ligation of the adaptor,

186 samples were pooled within a single Eppendorf tube and purified using a Qiagen QIAquick

187 PCR Purification Kit (Qiagen, USA). The library was then subjected to the polymerase chain

188 reaction (PCR) using primers that corresponded to the ligated adaptors. After appropriate

189 dilution of samples, sequencing was performed using the Illumina Hi-Seq 2000 at the Cornell

190 University Core Laboratories Centre.

191 *2.6 Bioinformatics & SNP Calling*

192 Bioinformatics analysis was conducted by collaborators at the Genomic Diversity Facility

193 using the UNEAK (Universal Network-Enabled Analysis Kit, Lu et al., 2013) pipeline and the

194 TASSEL software version v 3.0 (Trait Analysis by aSSociation, Evolution and Linkage,

195 Bradbury et al., 2007). The UNEAK pipeline was developed to overcome the issues

196 associated with SNP discovery in absence of a reference genome, and has been shown to

197 provide accurate (> 92% accuracy) *de novo* genotype calling (Lu et al., 2013; Torkamaneh et

198 al., 2016). The UNEAK pipeline works by first trimming all sequence reads to 64 bp. Reads

199 of 64bp that are identical are then identified as tags, and pairwise alignment identifies pairs

200 of tags with a single base pair mismatch. Such tags are aligned to form a network and these

201 networks are pruned to remove putative sequencing errors (low frequency alleles). Pruning

202 was conducted according to an error rate threshold parameter of 0.01%. Only reciprocal tag

203 pairs are used for subsequent SNP calling, meaning only biallelic loci were studied. VCF

204 tools (v0.1.12a) was then used to calculate depth and missingness statistics for generated

205 SNP files. Heterozygosity and minor allele frequencies were also calculated using VCF tools.

206 *2.7 Statistical Analyses*

207 The R package SNPrelate (Zheng et al., 2012) was used to calculate additional diversity

208 statistics and to create a principal component analysis (PCA) plot of genotypes from filtered

209 SNP data. A matrix of Fst values were calculated according to the method of Weir &

210 Cockerham (1984). Other measures of genetic diversity such as Tajima's D, nucleotide

211 diversity and expected heterozygosity were calculated using the R Package PopGenome

212 (Pfeifer et al., 2014). In order to test for the presence of Isolation-by-distance (IBD) a Mantel

213 test was used with geographical distance (in km) and Fst values via the R package vegan

214 (Oksanen et al., 2013). To test for the influence of the gradient in dose rate on genetic

215 differentiation, a partial mantel test was used based on Pearson's product-moment

216 correlation with 1000 permutations. This test measures the association between two matrices

217 (e.g. genetic distance and Euclidean distance in dose rate) whilst accounting for a third

218 potentially confounding matrix (geographical distance). The association between calculated

219 genetic diversity measures and dose rates was calculated using Spearmans rank order

220 correlation coefficient. Power analysis was employed to determine the probability of seeing

221 different effect sizes based on sample size using the R package "pwr" (Champely &

222 Champely, 2007).

223 **3. Results**

224 *3.1 Data Quality & Coverage*

225 Illumina sequencing of 74 individuals on one lane yielded a total of 492 402 538 reads. Of

226 these, 372 029 165 were classed as 'good' reads containing a unique barcode. Depth of

227 coverage was on average 25.74. A depth of coverage > 20 times is preferred in SNP studies

228 to reduce the uncertainty associated with calling SNPs following low-coverage sequencing

229 (e.g. 5 x depth of coverage, Nielsen et al., 2011). The UNEAK pipeline identified a total of

230 32,321 SNPs which was trimmed to 14,463 following the data filtration steps.

3.2 Genetic Differentiation and Isolation-by-Distance

232 Genetic differentiation values (Fst) are shown in Table 2. Greatest values were observed

233 between Svatoye and Glubokoye lakes (0.272), suggesting these populations were the most

234 genetically isolated. Fst values greater than 0.15 are generally considered to indicate

235 significant differentiation (Frankham et al., 2002). The smallest Fst values were recorded

236 between Glubokoye and Yanovsky (0.085), the two closest sites geographically. A highly

237 significant relationship between geographical distance in kilometres and genetic

238 differentiation was recorded (see Figure 2A, Mantel Test, r = 0.786, p < 0.01), confirming the

239 Isolation-by-distance hypothesis.

240 Conversely, no significant effect of the gradient in dose rate (Euclidean distance) on genetic

241 differentiation was recorded when accounting for geographical distance (see Figure 2B,

242 Partial Mantel Test, r = 0.251, p > 0.05). This indicates that dose rate did not significantly

243 influence genetic differentiation amongst populations at Chernobyl. However, geographical

244 distance was found to be a driver of genetic differentiation. A principal component analysis

245 (PCA) plot of genotypes based on genome-wide filtered SNP data is shown in Figure 3. Two

246 of the highly contaminated sites, Glubokoye and Yanovsky Crawl (dose rates of 26.4 and

247 20.4 µGy/hr respectively) along with a site of low-level contamination (Dvorische, dose rate

248 of 0.691 µGy/hr) were differentiated from other sites in terms of genetic similarity. These sites

249 were located in closest proximity geographically, reinforcing the strong influence of the

250 geographical gradient on determining genetic similarity. Furthermore, all of the previously

251 mentioned lakes (Dvorische, Yanovsky Crawl and Glubokoye) are floodplain lakes that

252 historically would have been linked to the Pripyat river, reinforcing the potential for gene flow

253 and therefore genetic similarity of these populations.

254 **Figure 2** –Relationship between A) geographical distance and genetic differentiation (Fst)
255 and B) gradient in dose rate and genetic differentiation in *Asellus aquaticus* from six lakes of
256 varying contamination in the Chernobyl area.

257 **Figure 3 -** Principal component analysis of genotypes based on genome-wide SNP data of
258 *A. aquaticus* collected from six lakes along a gradient of radionuclide contamination at
259 Chernobyl.

260 *3.3 Genetic Diversity*

261 Calculated genetic diversity statistics for each population are shown in Table 3. Expected

262 heterozygosity (H_e), the most commonly used measure of gene diversity (Nei, 1973), ranged

263 from 0.234 at Glubokoye to 0.241 at Yanovsky Crawl. No significant relationship between H_e

264 and total dose rate was recorded (see Figure 4, n = 74, Spearman's rho = -.110, p > 0.05).

265 Nucleotide diversity (π), a measure of polymorphism within the population, ranged from

266 0.0016 at Svatoye to 0.0026 at Gorova. No significant relationship between nucleotide

267 diversity and dose rate was recorded (Spearman's rho = -0.333, p > 0.05).

268 Similarly, no significant relationship between dose rate and observed heterozygosity (H_o) or

269 the within population inbreeding coefficient, F, was recorded (Spearman's rho = -0.018, p >

270 0.05 and rho = -0.031, p > 0.05 respectively). Coupled with the lack of a relationship between

271 genetic differentiation and dose rate, this provides evidence that genetic patterns in *A.*

272 *aquaticus* at Chernobyl were not influenced by radiation dose rate. Tajima's D statistic was <

273 0 for all populations (see Table 3), with the lowest value recorded at Glubokoye (-1.099) and

274 the greatest value recorded at Svatoye (-0.612). A moderate negative relationship between

275 Tajima's D values and dose rate was recorded, though this was non-significant (Spearman's

276 rho = -0.5, p > 0.05).

277
278 **Table 3 –** Calculated genetic diversity measures based on genome-wide single nucleotide
279 polymorphism data in *Asellus aquaticus* from six lakes in the Chernobyl region. H_e =
280 Expected heterozygosity, H_0 = Observed heterozygosity, F = Inbreeding coefficient and π =
281 Nucleotide diversity. Values are shown as ± SD for genetic diversity measures calculated at
282 the individual level (e.g. H_e, H_0 and F).

283 **Figure 4. –** Relationship (Spearmans rho = -.110, p > 0.05) between H_e, expected
284 heterozygosity, in *Asellus aquaticus* and dose rate at six sites of varying radionuclide
285 contamination in Belarus and the Ukraine.

286 **4. Discussion**

287 The present study hypothesised that genome-wide estimates of genetic diversity in the

288 freshwater crustacean *Asellus aquaticus* would be positively related to the total dose rate at

289 six lakes in the Chernobyl region. No significant relationship between five different genetic

290 diversity metrics and dose rate was recorded, leading to rejection of this hypothesis. A strong

291 significant effect of geographical distance on genetic differentiation was evidenced,

292 suggesting Isolation-by-distance. Conversely, the gradient in radionuclide contamination was

293 not found to impact genetic differentiation. Calculated genetic diversity measures for *A.*

294 *aquaticus* in the present study were within the range of other areas of Europe as recorded by

295 Verovnik et al., (2005) and Konec et al., (2015). Verovnik et al., (2005) recorded mean

296 values for nucleotide diversity, π, in populations of *A. aquaticus* across central and Eastern

297 Europe of 0.00189 ± 3.79E-06 using mitochondrial DNA methods. Values obtained within

298 the present study were broadly similar, ranging from 0.00165– 0.00267.

299 The suggestion of IBD in the present study is in disagreement with previous work conducted

300 by Verovnik et al., (2005) who studied the mitochondrial DNA and 28s rDNA sequences of *A.*

301 *aquaticus* at a continental scale and found no evidence of IBD. The present study focused

302 largely on closed lake systems with limited connectivity (though Dvorische, Yanovsky Crawl

303 and Glubokoye are floodplain lakes of the Pripyat river which would have been connected

304 historically), which is fundamental to structuring *A. aquaticus* populations given known

305 limitations on dispersal capacity (e.g. Sworobowicz et al., 2015; Verovnik et al., 2005). This

306 may explain the presence of IBD in the present study, as it is assumed limited gene flow

307 would have occurred between enclosed, isolated populations. Methodological differences in

308 the calculation of genetic differentiation and techniques used to assess genetic structure may

309 further explain the differences between studies. For example, studies have shown significant

310   variation in the ability of genetic markers e.g. microsatellite or SNP based techniques to

311   distinguish genetic differentiation of populations (e.g. Bradbury et al., 2015).

312   Gene flow between populations is often thought to mask the effects of pollutants on genetic

313   patterns, even in species with known limited dispersal capacity (e.g. Giska et al., 2015;

314   Theodorakis et al., 2001). However, the moderate to high levels of genetic differentiation

315   observed between populations in the present study (Fst values up to 0.27) and the

316   confirmation of IBD suggests that gene flow and homogenization of populations is likely not

317   responsible for the lack of a relationship between genetic variables and dose rate. This

318   suggests that current dose rates received by *A. aquaticus* at Chernobyl (maximum of 27.1

319   µGy/hr) are either insufficient to cause a high rate of mutations and subsequent elevated

320   genetic diversity, or that effects may have occurred previously but populations have

321   recovered. These findings contradict the majority of studies which record an increase in

322   genetic diversity in a range of non-human organisms at Chernobyl (Baker et al., 2017;

323   Ellegren et al., 1997).

324   One possible explanation for these differences may be that *A. aquaticus* populations have

325   not been historically exposed to dose rates as high as those for rodents and pines that have

326   been shown to have altered genetic diversity (e.g. Baker et al., 2017; Volkova et al., 2018).

327   For example, many of the studies of genetic diversity in bank voles at Chernobyl have

328   monitored populations from the Red Forest (e.g. Baker et al., 2017; Matson et al., 2000). The

329   Red Forest refers to the highly contaminated area adjacent to the CNPP where acute doses

330   led to mortality in a 4-6 km^2 zone of pines immediately following the accident (Kryshev et al.,

331   2005). Dose rates in this area remain extremely high to date, with maximum air dose rates of

332   200 µGy/hr recorded in some 'hotspots' within the forest (N.A. Beresford, pers. comm).

333   Studies on the accumulation of radionuclides in small mammals within this area in the late

334   1990's suggested that chronic dose rates could be as high as 86 mGy/d for the duration of

335   their lifecycle (Chesser et al., 2000, 2001). Similarly, many of the studies demonstrating

genetic effects in scots pine are based on trees receiving doses orders of magnitude higher than the present study (e.g. Kuchma et al., 2011; Vornam et al., 2012), though effects at lower doses have also been recorded (e.g. Geras'kin & Volkova, 2014; Volkova et al., 2017).

Maximum external dose rates in aquatic systems immediately following the accident were 100 – 200 mGy/d from bottom sediments (Kyshev et al., 2005), showing a relatively rapid decline to 20 – 50 mGy/d within two months owing to the decay of short lived radionuclides (Kryshev et al., 2005). Dose rates further declined through 1986, with mean dose estimates for benthic fish in 1986 being 22 ± 9.0 mGy/d, significantly lower than the previously mentioned red forest area (Kryshev & Sazykina, 2012). Though these dose rates were calculated for the Chernobyl cooling pond, doses at the most contaminated site in the present study, Glubokoye, were likely similar. Whilst these dose rates would be expected to cause significant sub-lethal effects, it is unlikely that direct mortality and a resultant bottleneck was induced in crustaceans based on existing laboratory sensitivity data (see Fuller et al., 2015 for review). In crabs for example, chronic exposure to ^{60}Co dose rates of 6960 mGy/d were necessary to elicit mortality in *Callinectes sapidus*, over an order of magnitude greater than doses in the immediate aftermath of the accident (Engel 1967).

Taking this into account, it is unlikely that the Chernobyl accident would have caused a significant bottleneck in crustacean populations, accounting for the lack of a reduced genetic diversity observed in the present study. This was further reinforced by Murphy et al., (2011), who recorded no significant reduction in the abundance of macroinvertebrates in eight Chernobyl-affected lakes in 2003 and 2004. However, it is important to consider that the lack of observed genetic effects in the present study does not indicate that discernible effects have not occurred in *A. aquaticus* as a consequence of the Chernobyl accident. As proposed by DeWoody, (1999) population sampling may have taken place at time wherein radiation-induced mutation and elevated genetic diversity may have facilitated a more rapid recovery from a previous bottleneck and the resultant reduced genetic diversity.

362   One limitation of this study was the limited number of individuals sequenced per site and lack

363   of an adequate number of samples collected at various time points (see Matson et al., 2000

364   for discussion). For analysis of the relationship between total dose rate and a range of gene

365   diversity measures, 74 samples were analysed. For an 80% chance of detecting a 'medium'

366   effect size of r = 0.3 (Cohen, 1977), 84 samples would be necessary assuming a significance

367   level of 0.05. A much larger sample size of > 700 samples would be necessary for the same

368   conditions for a 'small' effect size of 0.1 (Cohen, 1977). It is therefore possible that radiation

369   may have induced a minor effect on genetic diversity in *A. aquaticus* that the present study

370   may not have had the necessary statistical power to detect. Furthermore, effective population

371   size was not estimated in this study. Under neutral theory, a given population's genetic

372   diversity is dependent on both the effective population size and the mutation rate (Kimura,

373   1983). Larger effective populations typically show greater genetic diversity than smaller

374   populations (Hague & Routman, 2016), though some comparative studies have questioned

375   this relationship (Bazin et al., 2006). The conclusions of the present study are therefore

376   based on the assumption of similar effective population sizes at these locations. In order to

377   discriminate the drivers regulating genetic variation at the population level, multiple sampling

378   years of a greater number of individuals and a robust understanding of demographic and

379   ecological processes within sampling locations is necessary (Matson et al., 2000). Ideally,

380   samples collected prior to the Chernobyl accident and those exposed to the highest dose

381   rates in the immediate aftermath would be available for population genetic analysis.

382   However, such samples were not available. Future research into the effects of chronic

383   radiation exposure on genetic variation should aim to couple sampling over long time scales

384   with a robust understanding of community dynamics.

385   To the authors' knowledge, this study is the first to apply a genome-wide SNP approach to

386   studying genetic diversity in response to radiation exposure. The majority of available studies

387   have used techniques such as AFLP, RFLP or microsatellites, though recent studies have

388 moved toward deep sequencing (e.g. Baker et al., 2017). Given the advantages of genome-

389 wide SNP discovery techniques as compared to previous approaches (Schlötterer, 2004),

390 this method would have increased analytic power to detect a relationship between radiation

391 dose rate and genetic diversity. The sequencing depth in the present study (>20x) is known

392 to provide accurate genotype calling and *de novo* SNP discovery even in non-model

393 organisms without a reference genome (Andrews et al., 2016; Torkamaneh et al., 2016).

394 Furthermore, the number of SNPs studied in the present study (14,463 following data

395 filtration) has been shown to provide reliable measures of genetic diversity in non-model

396 organisms. For example, Hoffman et al., (2014) found that inbreeding coefficients generated

397 from RAD-seq analysis of 13,000 SNPS in the oldfield mouse, *Peromyscus polionotus*

398 *subgriseus*, had strong concordance with known pedigree based values.

5. Conclusion

400 This study represents the first assessment of radiation effects on genetic variation in

401 crustaceans, internationally important model organisms in radioecology (ICRP, 2007). No

402 effect of radiation dose rate on genetic variation was recorded, which is different to the

403 majority of available literature recording increased genetic diversity and elevated mutation

404 rates in non-human organisms at Chernobyl. This was largely attributed to lower overall dose

405 rates over time as compared to more radiosensitive organisms such as pines and rodents

406 wherein clear genetic effects have been demonstrated. Coupled with previous studies

407 demonstrating no effects of radiation on reproduction and development (Fuller et al., 2017,

408 2018) in crustaceans at Chernobyl, this study will aid in understanding the long-term effects

409 of radiation exposure on populations of aquatic biota.

6. Acknowledgements

7. Data Accessibility Statement

Filtered SNP, genetic distance (FST) and heterozygosity data are available through the Open

Science Framework (doi:10.17605/OSF.IO/7WH5A). Other genetic diversity data are

available through the Natural Environment Research Council (NERC) Environmental

Information Data Centre (EIDC, doi:10.5285/47f036c4-e319-4825-9cb8-f27977eb20dd).

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9. Author Contributions

660 A.T.F, J.T.S and N.F. conceived and designed the study. N.F., D.I.G., A.L. and L.L.N.

661 conducted field sampling. N.F. performed the laboratory work (DNA extractions and quality

662 control) and N.F., J.T.S and A.T.F wrote the manuscript.

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665 **10. Tables & Figures**

666 **Figure 1. –** Map of the six study sites sampled for *Asellus aquaticus* in relation to distance
667 from the Chernobyl Nuclear Power Plant (CNPP). Map generated using qGIS (v2.18) with
668 data from DIVA-GIS (Hijmans et al., 2001).

669 **Table 1. –** Environmental conditions, location, sampling date and total dose rate at six sites
670 of varying contamination in Belarus and the Ukraine. Error bars are standard deviations
671 based on measurements at three different sub-sites of each lake.

672

Site	Sampling Date	Latitude	Longitude	Dose Rate (μGy/hr)	Temperature (°C)	Oxygen Saturation (%)	pH	Conductivity (μS/cm)
Gorova	11/6/2016	50.70	30.70	0.064	22.4 ± 0.05	113 ± 16.2	8.60 ± 0.02	256 ± 0.41
Dvorische	29/05/2016	52.01	29.43	0.691	23.2 ± 0.06	80 ± 1.17	7.60 ± 0.17	197 ± 0.15
Stoyecheye	27/05/2016	52.86	30.91	0.774	22 ± 0.05	102 ± 2.00	8.30 ± 0.02	241 ± 1.48
Svatoye	24/05/2016	53.17	31.86	2.03	20.1 ± 0.23	92 ± 1.80	8.00 ± 0.15	114 ± 0.70
Yanovsky Crawl	05/06/2016	51.41	30.07	20.42	20.2 ± 0.11	140 ± 2.90	9.00 ± 0.04	265 ± 0.97
Glubokoye	03/06/2016	51.44	30.06	26.4	23.6 ± 0.06	112 ± 14.10	7.60 ± 0.18	199 ± 1.22

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	Dvorische	Glubokoye	Svatoye Lake	Yanovsky Crawl	Stoyecheye	Gorova
Dvorische						
Glubokoye	0.1235103					
Svatoye Lake	0.255504	0.2719511				
Yanovsky Crawl	0.1206123	0.08523209	0.254953			
Stoyecheye	0.2278073	0.2233065	0.223041	0.21475		
Gorova	0.2086033	0.1999745	0.263433	0.1927364	0.1918563	

Table 2. – Pairwise genetic differentiation (Fst) values in *Asellus aquaticus* collected from six lakes of varying contamination in Belarus and the Ukraine.

	N	H_e	H_0	F	π	Tajima's D

Figure 2 –Relationship between A) geographical distance and genetic differentiation (Fst) and B) gradient in dose rate and genetic differentiation in *Asellus aquaticus* from six lakes of varying contamination in the Chernobyl area

Figure 3 - Principal component analysis of genotypes based on genome-wide SNP data of *A. aquaticus* collected from six lakes along a gradient of radionuclide contamination at Chernobyl.

Table 3 – Calculated genetic diversity measures based on genome-wide single nucleotide polymorphism data in *Asellus aquaticus* from six lakes in the Chernobyl region. H_e = Expected heterozygosity, H_0 = Observed heterozygosity, F = Inbreeding coefficient and π = Nucleotide diversity. Values are shown as ± SD for genetic diversity measures calculated at the individual level (e.g. H_e, H_0 and F).

Dvorische	12	0.23426 ± 0.010	0.12343 ± 0.034	0.47537 ± 0.134	0.001779881	-1.074
Glubokoye	13	0.23373 ± 0.008	0.10739 ± 0.020	0.54170 ± 0.079	0.002438229	-1.099
Gorova	13	0.23677 ± 0.009	0.13059 ± 0.031	0.44943 ± 0.126	0.002669943	-0.659
Stoyecheye	12	0.23521 ± 0.008	0.12750 ± 0.029	0.45792 ± 0.114	0.002215685	-0.671
Svatoye	12	0.23646 ± 0.007	0.14392 ± 0.050	0.39342 ± 0.186	0.001649979	-0.612
Yanovsky Crawl	12	0.24123 ± 0.011	0.14549 ± 0.029	0.40218 ± 0.145	0.002023974	-0.984

712 **Figure 4. –** Relationship (Spearmans rho = -.110, p > 0.05) between H_e, expected
713 heterozygosity, in *Asellus aquaticus* and dose rate at six sites of varying radionuclide
714 contamination in Belarus and the Ukraine.

715