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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 Neil Fuller<sup>\*1</sup>, Alex T. Ford<sup>1</sup>, Adélaïde Lerebours<sup>1</sup>, Dmitri I. Gudkov<sup>2</sup>, Liubov L.  
5 Nagorskaya<sup>3</sup>, & Jim T. Smith<sup>4</sup>

6 <sup>1</sup> Institute of Marine Sciences, School of Biological Sciences, University of Portsmouth, Ferry  
7 Road, Portsmouth, Hampshire PO4 9LY, UK.

8 <sup>2</sup> Department of Freshwater Radioecology, Institute of Hydrobiology, Geroyev Stalingrada  
9 Ave. 12, UA-04210 Kiev, Ukraine.

10 <sup>3</sup> Applied Science Center for Bioresources of the National Academy of Sciences of Belarus,  
11 27 Akademicheskaya Str., 220072 Minsk, Belarus.

12 <sup>4</sup> 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  $\mu\text{Gy/hr}$ . 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

76 waste from the US Department of Energy's Oak Ridge facility. The authors recorded  
77 increased genetic diversity in *G. affinis* individuals inhabiting two contaminated ponds (dose  
78 rates of 50 and 1313  $\mu\text{Gy/hr}$ ) as opposed to individuals from two reference populations.  
79 However, one of the aforementioned sites is also heavily contaminated with a suite of other  
80 genotoxicants (e.g. heavy metals and polycyclic aromatic hydrocarbons, Theodorakis &  
81 Shugart, 1997), meaning any observed effects cannot be attributed solely to radiation. The  
82 Chernobyl area is heterogeneously contaminated by a range of radionuclides, including  $^{90}\text{Sr}$ ,  
83  $^{137}\text{Cs}$  and transuranium isotopes ( e.g.  $^{238}\text{Pu}$ ,  $^{239,240}\text{Pu}$ , and  $^{241}\text{Am}$ , Beresford et al., 2018).  
84 Doses to aquatic macroinvertebrates are dominated by external  $\gamma$  and  $\beta$  radiation from  $^{137}\text{Cs}$   
85 and  $^{90}\text{Sr}$  (Murphy et al., 2011).

86 To the authors' knowledge, only a single study has considered radiation effects on genetic  
87 diversity in aquatic biota at Chernobyl, despite the large number of closed lake systems in  
88 the area where biota are chronically exposed to low-dose radiation. Fetisov et al., (1992)  
89 studied genetic and morphological differences in seven populations of the zebra mussel,  
90 *Dreissena polymorpha*, in the Chernobyl area using an allozyme method. Based on data for  
91 five loci, no significant influence of radiation on *D. polymorpha* genetic structure was  
92 recorded, though thermal regime was found to influence genetic diversity. However the use  
93 of allozymes in genetic diversity studies has been questioned, particularly at small spatial  
94 scales and using a small number of loci (e.g. Turlure et al., 2014). Furthermore, the influence  
95 of radiation was not the direct focus of the study and no dose rates were provided,  
96 necessitating further robust study into the impacts of chronic radiation exposure on the  
97 genetic diversity of aquatic invertebrates. The 2011 accident at the Fukushima Dai-Ichi  
98 nuclear power plant led to further contamination of marine and freshwater environments that  
99 will persist for decades (IAEA, 2015). This further emphasises the need for robust studies of  
100 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 detritivorous isopod crustacean  
121 commonly found in freshwater systems across Europe. Crustaceans are one of the  
122 International Commission 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  $F_{st}$ ) 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 Instruments 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 HindIII (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.

### 231 *3.2 Genetic Differentiation and Isolation-by-Distance*

232 Genetic differentiation values ( $F_{st}$ ) 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.  $F_{st}$  values greater than 0.15 are generally considered to indicate  
235 significant differentiation (Frankham et al., 2002). The smallest  $F_{st}$  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  $\mu\text{Gy/hr}$  respectively) along with a site of low-level contamination (Dvorische, dose rate  
248 of 0.691  $\mu\text{Gy/hr}$ ) 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 Pripjat 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 ( $\pi$ ), 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,  $H_o$  = Observed heterozygosity,  $F$  = Inbreeding coefficient and  $\pi$  =  
281 Nucleotide diversity. Values are shown as  $\pm$  SD for genetic diversity measures calculated at  
282 the individual level (e.g.  $H_e$ ,  $H_o$  and  $F$ ).

283 **Figure 4.** – Relationship (Spearman's  $\rho = -.110$ ,  $p > 0.05$ ) 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,  $\pi$ , in populations of *A. aquaticus* across central and Eastern  
297 Europe of  $0.00189 \pm 3.79E-06$  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 Pripjat 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 ( $F_{st}$  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  $\mu\text{Gy/hr}$ ) 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  $\text{km}^2$  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  $\mu\text{Gy/hr}$  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  $\text{mGy/d}$  for the duration of  
335 their lifecycle (Chesser et al., 2000, 2001). Similarly, many of the studies demonstrating

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

339 Maximum external dose rates in aquatic systems immediately following the accident were  
340 100 – 200 mGy/d from bottom sediments (Kyshev et al., 2005), showing a relatively rapid  
341 decline to 20 – 50 mGy/d within two months owing to the decay of short lived radionuclides  
342 (Kyshev et al., 2005). Dose rates further declined through 1986, with mean dose estimates  
343 for benthic fish in 1986 being  $22 \pm 9.0$  mGy/d, significantly lower than the previously  
344 mentioned red forest area (Kyshev & Sazykina, 2012). Though these dose rates were  
345 calculated for the Chernobyl cooling pond, doses at the most contaminated site in the  
346 present study, Glubokoye, were likely similar. Whilst these dose rates would be expected to  
347 cause significant sub-lethal effects, it is unlikely that direct mortality and a resultant  
348 bottleneck was induced in crustaceans based on existing laboratory sensitivity data (see  
349 Fuller et al., 2015 for review). In crabs for example, chronic exposure to  $^{60}\text{Co}$  dose rates of  
350 6960 mGy/d were necessary to elicit mortality in *Callinectes sapidus*, over an order of  
351 magnitude greater than doses in the immediate aftermath of the accident (Engel 1967).

352 Taking this into account, it is unlikely that the Chernobyl accident would have caused a  
353 significant bottleneck in crustacean populations, accounting for the lack of a reduced genetic  
354 diversity observed in the present study. This was further reinforced by Murphy et al., (2011),  
355 who recorded no significant reduction in the abundance of macroinvertebrates in eight  
356 Chernobyl-affected lakes in 2003 and 2004. However, it is important to consider that the lack  
357 of observed genetic effects in the present study does not indicate that discernible effects  
358 have not occurred in *A. aquaticus* as a consequence of the Chernobyl accident. As proposed  
359 by DeWoody, (1999) population sampling may have taken place at time wherein radiation-  
360 induced mutation and elevated genetic diversity may have facilitated a more rapid recovery  
361 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.

## 399 **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.

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415 optimisation of restriction enzymes, sequencing and bioinformatics analysis was performed  
416 by the Cornell University Genomic Diversity Facility.

417

418

## 419 **7. Data Accessibility Statement**

420 Filtered SNP, genetic distance (FST) and heterozygosity data are available through the Open  
421 Science Framework (doi:10.17605/OSF.IO/7WH5A). Other genetic diversity data are  
422 available through the Natural Environment Research Council (NERC) Environmental  
423 Information Data Centre (EIDC, doi:10.5285/47f036c4-e319-4825-9cb8-f27977eb20dd).

## 424 **8. References**

425 Anderson, S., Sadinski, W., Shugart, L., Brussard, P., Depledge, M., Ford, T., ... & Wogan,  
426 G. (1994). Genetic and molecular ecotoxicology: a research framework. *Environmental*  
427 *Health Perspectives*, 102(Suppl 12), 3.

428 Andrews, K. R., Good, J. M., Miller, M. R., Luikart, G., & Hohenlohe, P. A. (2016).  
429 Harnessing the power of RADseq for ecological and evolutionary genomics. *Nature Reviews*  
430 *Genetics*.

431 Baker, R. J., Dickins, B., Wickliffe, J. K., Khan, F. A., Gaschak, S., Makova, K. D., & Phillips,  
432 C. D. (2017). Elevated mitochondrial genome variation after 50 generations of radiation  
433 exposure in a wild rodent. *Evolutionary applications*, 10(8), 784-791.

434 Bazin, E., Glémin, S., & Galtier, N. (2006). Population size does not influence mitochondrial  
435 genetic diversity in animals. *Science*, 312(5773), 570-572.

436 Beresford, N. A., Barnett, C. L., Gashchak, S., Maksimenko, A., Guliachenko, E., Wood, M.  
437 D., & Izquierdo, M. (2018). Radionuclide transfer to wildlife at a 'Reference Site' in the  
438 Chernobyl Exclusion Zone and resultant radiation exposures. *Journal of environmental*  
439 *radioactivity*.

440 Bickham, J. W., Sandhu, S., Hebert, P. D., Chikhi, L., & Athwal, R. (2000). Effects of  
441 chemical contaminants on genetic diversity in natural populations: implications for  
442 biomonitoring and ecotoxicology. *Mutation research/Reviews in Mutation research*, 463(1),  
443 33-51.

444 Bradbury, I. R., Hamilton, L. C., Dempson, B., Robertson, M. J., Bourret, V., Bernatchez, L.,  
445 & Verspoor, E. (2015). Transatlantic secondary contact in Atlantic Salmon, comparing

446 microsatellites, a single nucleotide polymorphism array and restriction-site associated DNA  
447 sequencing for the resolution of complex spatial structure. *Molecular Ecology*, 24(20), 5130–  
448 5144

449 Bradbury, P. J., Zhang, Z., Kroon, D. E., Casstevens, T. M., Ramdoss, Y., & Buckler, E. S.  
450 (2007). TASSEL: Software for association mapping of complex traits in diverse samples.  
451 *Bioinformatics*, 23(19), 2633–2635.

452 Champely, S., & Champely, M. S. (2007). The pwr package. *UCB Lyon*, 1.

453 Chesser, R. K., Sugg, D. W., Lomakin, M. D., van den Bussche, R. A., DeWoody, J. A.,  
454 Jagoe, C. H., ... Baker, R. J. (2000). Concentrations and dose rate estimates of <sup>134</sup>Cesium and <sup>137</sup>  
455 cesium and <sup>90</sup>strontium in small mammals at chornobyl, Ukraine. *Environmental Toxicology*  
456 *and Chemistry*, 19(2), 305–312.

457 Chesser, R. K., Rodgers, B. E., Wickliffe, J. K., Gaschak, S., Chizhevsky, I., Phillips, C. J., &  
458 Baker, R. J. (2001). Accumulation of <sup>137</sup>Cesium and <sup>90</sup>Strontium from abiotic and biotic  
459 sources in rodents at Chernobyl, Ukraine. *Environmental Toxicology and Chemistry*, 20(9),  
460 1927–1935.

461 Cohen, J. (1977). *Statistical Power Analysis for the Behavioral Sciences* (1<sup>st</sup> Edition). New  
462 York: Academic Press.

463 Davey, J., Hohenlohe, P., Etter, P., Boone, J., Catchen, J., & Blaxter, M. (2011). Genome-  
464 wide genetic marker discovery and genotyping using next-generation sequencing. *Nature*  
465 *Reviews Genetics*, 12(7), 499–510.

466 De Lange, H. J., De Haas, E. M., Maas, H., & Peeters, E. T. H. M. (2005). Contaminated  
467 sediments and bioassay responses of three macroinvertebrates, the midge larva *Chironomus*  
468 *riparius*, the water louse *Asellus aquaticus* and the mayfly nymph *Ephoron virgo*.  
469 *Chemosphere*, 61(11), 1700–1709.

470 DeWoody, J. A. (1999). Nucleotide variation in the p53 tumor-suppressor gene of voles from  
471 Chernobyl, Ukraine. *Mutation Research/Genetic Toxicology and Environmental*  
472 *Mutagenesis*, 439(1), 25-36.

473 Dubrova, Y. E., Nesterov, V. N., Krouchinsky, N. G., Ostapenko, V. A., Neumann, R., Neil, D.  
474 L., & Jeffreys, A. J. (1996). Human minisatellite mutation rate after the Chernobyl  
475 accident. *Nature*, 380(6576), 683.

476 Dubrova, Y. E., Nesterov, V. N., Krouchinsky, N. G., Ostapenko, V. A., Vergnaud, G.,  
477 Giraudeau, F., ... & Jeffreys, A. J. (1997). Further evidence for elevated human minisatellite  
478 mutation rate in Belarus eight years after the Chernobyl accident. *Mutation Research*, 381(2),  
479 267-278.

480 Ehlers, A., Worm, B., & Reusch, T. B. H. (2008). Importance of genetic diversity in eelgrass  
481 *Zostera marina* for its resilience to global warming. *Marine Ecology Progress Series*, 355, 1–  
482 7. <http://doi.org/10.3354/meps07369>

483 Ellegren, H., Lindgren, G., Primmer, C. R., & Møller, A. P. (1997). Fitness loss and germline  
484 mutations in barn swallows breeding in Chernobyl. *Nature*, 389(6651), 593.

485 Elshire, R. J., Glaubitz, J. C., Sun, Q., Poland, J. A., Kawamoto, K., Buckler, E. S., &  
486 Mitchell, S. E. (2011). A robust, simple genotyping-by-sequencing (GBS) approach for high  
487 diversity species. *PLoS ONE*, 6(5).

488 Engel, D. W. (1967). Effect of Single and Continuous Exposures of Gamma Radiation on the  
489 Survival and Growth of the Blue Crab, *Callinectes sapidus*. *Radiation research*, 32(4), 685-  
490 691.

491 Fetisov, A. N., Rubanovich, A. V., Slipchenko, T. S., & Shevchenko, V. A. (1992). The  
492 structure of *Dreissena polymorpha* populations from basins adjacent to the Chernobyl atomic  
493 power station. *Science of the total environment*, 112(1), 115-124.

494 Fischer, M. C., Rellstab, C., Leuzinger, M., Roumet, M., Gugerli, F., Shimizu, K. K., ...  
495 Widmer, A. (2017). Estimating genomic diversity and population differentiation – an empirical  
496 comparison of microsatellite and SNP variation in *Arabidopsis halleri*. *BMC Genomics*, 18(1),  
497 69.

498 Frankham, R., Briscoe, D.A. & Ballou, J.D. (2002). *Introduction to Conservation Genetics*.  
499 Cambridge: Cambridge University Press.

500 Frankham, R., Bradshaw, C. J., & Brook, B. W. (2014). Genetics in conservation  
501 management: revised recommendations for the 50/500 rules, Red List criteria and population  
502 viability analyses. *Biological Conservation*, 170, 56-63.

503 Fuller, N., Lerebours, A., Smith, J. T., & Ford, A. T. (2015). The biological effects of ionising  
504 radiation on Crustaceans: A review. *Aquatic Toxicology*, 167, 55-67.

505 Fuller, N., Smith, J. T., Nagorskaya, L. L., Gudkov, D. I., & Ford, A. T. (2017). Does  
506 Chernobyl-derived radiation impact the developmental stability of *Asellus aquaticus* 30 years  
507 on?. *Science of the Total Environment*, 576, 242-250.

508 Fuller, N., Ford, A. T., Nagorskaya, L. L., Gudkov, D. I., & Smith, J. T. (2018). Reproduction  
509 in the freshwater crustacean *Asellus aquaticus* along a gradient of radionuclide  
510 contamination at Chernobyl. *Science of the Total Environment*, 628, 11-17.

511 Furitsu, K., Ryo, H., Yeliseeva, K. G., Kawabata, H., Krupnova, E. V., Trusova, V. D., ... &  
512 Nomura, T. (2005). Microsatellite mutations show no increases in the children of the  
513 Chernobyl liquidators. *Mutation Research/Genetic Toxicology and Environmental*  
514 *Mutagenesis*, 581(1), 69-82.

515 Geras' kin, S. A., & Volkova, P. Y. (2014). Genetic diversity in Scots pine populations along a  
516 radiation exposure gradient. *Science of the Total Environment*, 496, 317-327.

517 Giska, I., Babik, W., van Gestel, C. A. M., van Straalen, N. M., & Laskowski, R. (2015).  
518 Genome-wide genetic diversity of rove beetle populations along a metal pollution gradient.  
519 *Ecotoxicology and Environmental Safety*, 119, 98–105.

520 Hague, M. T. J., & Routman, E. J. (2016). Does population size affect genetic diversity? A  
521 test with sympatric lizard species. *Heredity*, 116(1), 92.

522 Hijmans, R. J., Guarino, L., Cruz, M., & Rojas, E. (2001). Computer tools for spatial analysis  
523 of plant genetic resources data: 1 . DIVA-GIS. *Plants Genetic Resources Newsletter*, 127,  
524 15–19.

525 Hinton, T. G., Alexakhin, R., Balonov, M., Gentner, N., Hendry, J., Prister, B., ... &  
526 Woodhead, D. (2007). Radiation-induced effects on plants and animals: Findings of the  
527 United Nations Chernobyl Forum. *Health physics*, 93(5), 427-440.

528 Hoffman, J. I., Simpson, F., David, P., Rijks, J. M., Kuiken, T., Thorne, M. a. S., ...  
529 Dasmahapatra, K. K. (2014). High-throughput sequencing reveals inbreeding depression in a  
530 natural population. *Proceedings of the National Academy of Sciences*, 111(10), 3775–3780.

- 531 IAEA. (2015). *The Fukushima Dai-ichi Accident: Technical Volume 4/5 Radiological*  
532 *Consequences*. Vienna: IAEA.
- 533 ICRP (2007). ICRP 103: The 2007 Recommendations of the International Commission on  
534 Radiological Protection. *Annals of the ICRP*, 37, 330.
- 535 Kimura, M. (1983). *The neutral theory of molecular evolution*. Cambridge: Cambridge  
536 University Press.
- 537 Konec, M., Prevorčnik, S., Sarbu, S. M., Verovnik, R., & Trontelj, P. (2015). Parallels  
538 between two geographically and ecologically disparate cave invasions by the same species,  
539 *Asellus aquaticus* (Isopoda, Crustacea). *Journal of evolutionary biology*, 28(4), 864-875.
- 540 Kovalchuk, O., Dubrova, Y. E., Arkhipov, A., Hohn, B., & Kovalchuk, I. (2000). Germline  
541 DNA: Wheat mutation rate after Chernobyl. *Nature*, 407(6804), 583.
- 542 Kryshev, I. I., Sazykina, T. G., & Beresford, N. A. (2005). Effects on Wildlife. In J. T. Smith &  
543 N. A. Beresford (Eds.), *Chernobyl: Catastrophe and Consequences* (pp. 267–287).  
544 Chichester: Springer-Praxis.
- 545 Kryshev, A. I., & Sazykina, T. G. (2012). Comparative analysis of doses to aquatic biota in  
546 water bodies impacted by radioactive contamination. *Journal of environmental*  
547 *radioactivity*, 108, 9-14.
- 548 Kuchma, O., Vornam, B., & Finkeldey, R. (2011). Mutation rates in Scots pine (*Pinus*  
549 *sylvestris* L.) from the Chernobyl exclusion zone evaluated with amplified fragment-length  
550 polymorphisms (AFLPs) and microsatellite markers. *Mutation Research/Genetic Toxicology*  
551 *and Environmental Mutagenesis*, 725(1), 29–35.
- 552 Larson, W. A., Seeb, L. W., Everett, M. V., Waples, R. K., Templin, W. D., & Seeb, J. E.  
553 (2014). Genotyping by sequencing resolves shallow population structure to inform  
554 conservation of Chinook salmon (*Oncorhynchus tshawytscha*). *Evolutionary*  
555 *Applications*, 7(3), 355-369.
- 556 Little, J. B., Nagasawa, H., Pfenning, T., & Vetrovs, H. (1997). Radiation-induced genomic  
557 instability: delayed mutagenic and cytogenetic effects of X rays and alpha  
558 particles. *Radiation research*, 148(4), 299-307.
- 559 Livshits, L. A., Malyarchuk, S. G., Lukyanova, E. M., Antipkin, Y. G., Arabskaya, L. P.,  
560 Kravchenko, S. A., ... & Vergnaud, G. (2001). Children of Chernobyl cleanup workers do not  
561 show elevated rates of mutations in minisatellite alleles. *Radiation research*, 155(1), 74-80.
- 562 Lu, F., Lipka, A. E., Glaubitz, J., Elshire, R., Cherney, J. H., Casler, M. D., ... Costich, D. E.  
563 (2013). Switchgrass Genomic Diversity, Ploidy, and Evolution: Novel Insights from a  
564 Network-Based SNP Discovery Protocol. *PLoS Genetics*, 9(1).
- 565 Matson, C. W., Rodgers, B. E., Chesser, R. K., & Baker, R. J. (2000). Genetic diversity of  
566 *Clethrionomys glareolus* populations from highly contaminated sites in the Chornobyl region,  
567 Ukraine. *Environmental Toxicology and Chemistry*, 19(8), 2130-2135.
- 568 McCahon, C. P., & Pascoe, D. (1988). Culture techniques for three freshwater  
569 macroinvertebrate species and their use in toxicity tests. *Chemosphere*, 17(12), 2471-2480.
- 570 Morgan, W. F. (2003). Non-targeted and delayed effects of exposure to ionizing radiation: I.  
571 Radiation-induced genomic instability and bystander effects in vitro. *Radiation*  
572 *research*, 159(5), 567-580

573 Murphy, J. F., Nagorskaya, L. L., & Smith, J. T. (2011). Abundance and diversity of aquatic  
574 macroinvertebrate communities in lakes exposed to Chernobyl-derived ionising  
575 radiation. *Journal of environmental radioactivity*, 102(7), 688-694.

576 Narum, S. R., Buerkle, C. A., Davey, J. W., Miller, M. R., & Hohenlohe, P. A. (2013).  
577 Genotyping-by-sequencing in ecological and conservation genomics. *Molecular*  
578 *ecology*, 22(11), 2841-2847.

579 Nielsen, R., Paul, J. S., Albrechtsen, A., & Song, Y. S. (2011). Genotype and SNP calling  
580 from next-generation sequencing data. *Nature Reviews Genetics*, 12(6), 443.

581 Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., ...  
582 Wagner, H. (2013). Package "vegan." *R Package Ver. 2.0-8*, 254.

583 Pfeifer, B., Wittelsbürger, U., Ramos-Onsins, S. E., & Lercher, M. J. (2014). PopGenome: An  
584 efficient swiss army knife for population genomic analyses in R. *Molecular Biology and*  
585 *Evolution*, 31(7), 1929–1936.

586 Pollinger, J. P., Lohmueller, K. E., Han, E., Parker, H. G., Quignon, P., Degenhardt, J. D., ...  
587 & Bryc, K. (2010). Genome-wide SNP and haplotype analyses reveal a rich history  
588 underlying dog domestication. *Nature*, 464(7290), 898.

589 Rumisha, C., Leermakers, M., Elskens, M., Mdegela, R. H., Gwakisa, P., & Kochzius, M.  
590 (2017). Genetic diversity of the giant tiger prawn *Penaeus monodon* in relation to trace metal  
591 pollution at the Tanzanian coast. *Marine Pollution Bulletin*, 114(2), 759–767.

592 Schlötterer, C. (2004). The evolution of molecular markers—just a matter of fashion?. *Nature*  
593 *reviews genetics*, 5(1), 63.

594 Sworobowicz, L., Grabowski, M., Mamos, T., Burzyński, A., Kilikowska, A., Sell, J., &  
595 Wysocka, A. (2015). Revisiting the phylogeography of *Asellus aquaticus* in Europe: insights  
596 into cryptic diversity and spatiotemporal diversification. *Freshwater Biology*, 60(9), 1824–  
597 1840.

598 Tajima, F. (1989). Statistical method for testing the neutral mutation hypothesis by DNA  
599 polymorphism. *Genetics*, 123(3), 585–595.

600 Theodorakis, C. W., Blaylock, B. G., & Shugart, L. R. (1997). Genetic ecotoxicology I: DNA  
601 integrity and reproduction in mosquitofish exposed in situ to  
602 radionuclides. *Ecotoxicology*, 6(4), 205-218.

603 Theodorakis, C. W., & Shugart, L. R. (1998). Genetic ecotoxicology III: the relationship  
604 between DNA strand breaks and genotype in mosquito fish exposed to  
605 radiation. *Ecotoxicology*, 7(4), 227-235.

606 Theodorakis, C. W., Bickham, J., Lamb, T., Medica, P. A., & Lyne, T. B. (2001). Integration of  
607 genotoxicity and population genetic analyses in kangaroo rats (*Dipodomys merriami*)  
608 exposed to radionuclide contamination at the Nevada Test Site, USA. *Environmental*  
609 *Toxicology & Chemistry*, 20(2), 317–326.

610 Torkamaneh, D., Laroche, J., & Belzile, F. (2016). Genome-wide SNP calling from  
611 genotyping by sequencing (GBS) data: a comparison of seven pipelines and two sequencing  
612 technologies. *PLoS One*, 11(8), e0161333.

613 Tsyusko, O. V., Smith, M. H., Oleksyk, T. K., Goryanaya, J., & Glenn, T. C. (2006). Genetics  
614 of cattails in radioactively contaminated areas around Chornobyl. *Molecular ecology*, 15(9),  
615 2611-2625.

- 616 Tucker, J. D., Cofield, J., Matsumoto, K., Ramsey, M. J., & Freeman, D. C. (2005).  
617 Persistence of chromosome aberrations following acute radiation: I, PAINT translocations,  
618 dicentrics, rings, fragments, and insertions. *Environmental and molecular*  
619 *mutagenesis*, 45(2-3), 229-248.
- 620 Turlure, C., Vandewoestijne, S., & Baguette, M. (2014). Conservation genetics of a  
621 threatened butterfly: comparison of allozymes, RAPDs and microsatellites. *BMC*  
622 *genetics*, 15(1), 114.
- 623 van Straalen, N. M., & Timmermans, M. J. (2002). Genetic variation in toxicant-stressed  
624 populations: an evaluation of the “genetic erosion” hypothesis. *Human and Ecological Risk*  
625 *Assessment*, 8(5), 983-1002.
- 626 Verovnik, R., Sket, B., Prevorčnik, S., & Trontelj, P. (2003). Random amplified polymorphic  
627 DNA diversity among surface and subterranean populations of *Asellus aquaticus* (Crustacea:  
628 Isopoda). *Genetica*, 119(2), 155–165.
- 629 Verovnik, R., Sket, B., & Trontelj, P. (2005). The colonization of Europe by the freshwater  
630 crustacean *Asellus aquaticus* (Crustacea: Isopoda) proceeded from ancient refugia and was  
631 directed by habitat connectivity. *Molecular Ecology*, 14(14), 4355–4369.
- 632 Volkova, P. Y., Geras'kin, S. A., & Kazakova, E. A. (2017). Radiation exposure in the remote  
633 period after the Chernobyl accident caused oxidative stress and genetic effects in Scots pine  
634 populations. *Scientific Reports*, 7, 43009.
- 635 Volkova, P. Y., Geras'kin, S. A., Horemans, N., Makarenko, E. S., Saenen, E., Duarte, G. T.,  
636 ... & Kudin, M. (2018). Chronic radiation exposure as an ecological factor: Hypermethylation  
637 and genetic differentiation in irradiated Scots pine populations. *Environmental Pollution*, 232,  
638 105-112.
- 639 Vornam, B., Arkhipov, A., & Finkeldey, R. (2012). Nucleotide diversity and gene expression  
640 of Catalase and Glutathione peroxidase in irradiated Scots pine (*Pinus sylvestris* L.) from the  
641 Chernobyl exclusion zone. *Journal of Environmental Radioactivity*, 106, 20–26.
- 642 Weinberg, H. S., Korol, A. B., Kirzhner, V. M., Avivi, A., Fahima, T., Nevo, E., ... &  
643 Skvarkaja, E. (2001). Very high mutation rate in offspring of Chernobyl accident  
644 liquidators. *Proceedings of the Royal Society of London B: Biological Sciences*, 268(1471),  
645 1001-1005.
- 646 Weir, B. S., & Cockerham, C. C. (1984). Estimating F-statistics for the analysis of population  
647 structure. *evolution*, 38(6), 1358-1370.
- 648 White, T. A., Perkins, S. E., Heckel, G., & Searle, J. B. (2013). Adaptive evolution during an  
649 ongoing range expansion: The invasive bank vole (*Myodes glareolus*) in Ireland. *Molecular*  
650 *Ecology*, 22(11), 2971–2985.
- 651 Whitehurst, I. T. (1991). The *Gammarus*: *Asellus* ratio as an index of organic pollution. *Water*  
652 *Research*, 25(3), 333-339.
- 653 Wickliffe, J. K., Rodgers, B. E., Chesser, R. K., Phillips, C. J., Gaschak, S. P., & Baker, R. J.  
654 (2003). Mitochondrial DNA heteroplasmy in laboratory mice experimentally enclosed in the  
655 radioactive Chernobyl environment. *Radiation research*, 159(4), 458-464.
- 656 Zheng, X., Levine, D., Shen, J., Gogarten, S. M., Laurie, C., & Weir, B. S. (2012). A high-  
657 performance computing toolset for relatedness and principal component analysis of SNP  
658 data. *Bioinformatics*, 28(24), 3326–3328.

## 659 **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.

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

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	Dvorische	Glubokoye	Svatoye Lake	Yanovsky Crawl	Stoyecheye	Gorova	
Dvorische							681
Glubokoye	0.1235103						682
Svatoye Lake	0.255504	0.2719511					683
Yanovsky Crawl	0.1206123	0.08523209	0.254953				684
Stoyecheye	0.2278073	0.2233065	0.223041	0.21475			685
Gorova	0.2086033	0.1999745	0.263433	0.1927364	0.1918563		686
							687
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							689

**Table 2.**  
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Pairwise  
genetic  
differenti

690 ation (Fst) values in *Asellus aquaticus* collected from six lakes of varying contamination in  
691 Belarus and the Ukraine.

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N	H <sub>e</sub>	H <sub>0</sub>	F	π	Tajima's D
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701 **Figure 2** –Relationship between A) geographical distance and genetic differentiation (Fst)  
702 and B) gradient in dose rate and genetic differentiation in *Asellus aquaticus* from six lakes of  
703 varying contamination in the Chernobyl area

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

707 **Table 3** – Calculated genetic diversity measures based on genome-wide single nucleotide  
708 polymorphism data in *Asellus aquaticus* from six lakes in the Chernobyl region. H<sub>e</sub> =  
709 Expected heterozygosity, H<sub>0</sub> = Observed heterozygosity, F = Inbreeding coefficient and π =  
710 Nucleotide diversity. Values are shown as ± SD for genetic diversity measures calculated at  
711 the individual level (e.g. H<sub>e</sub>, H<sub>0</sub> 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 (Spearman's rho = -.110, p > 0.05) between H<sub>e</sub>, expected  
713 heterozygosity, in *Asellus aquaticus* and dose rate at six sites of varying radionuclide  
714 contamination in Belarus and the Ukraine.

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