Reproduction in the freshwater crustacean Asellus aquaticus along a gradient of radionuclide contamination at Chernobyl

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Reproduction in the freshwater crustacean *Asellus aquaticus* along a gradient of radionuclide contamination at Chernobyl

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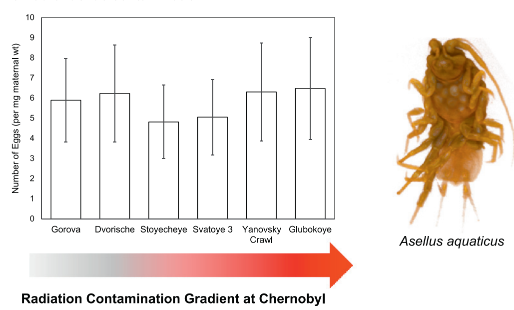
HIGHLIGHTS

- We assessed effects of Chernobyl radiation on crustacean reproduction.
- Fecundity of *Asellus aquaticus* assessed at dose rates from 0.06–27.1 μGy/h.
- No association of radiation with reproductive endpoints in *A. aquaticus*.
- Findings support proposed benchmarks for the protection of aquatic populations.
- Data can assist in management of radioactively contaminated environments.

GRAPHICAL ABSTRACT

- **No effects observed on reproduction of *Asellus aquaticus* along a gradient of radionuclide contamination**



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ABSTRACT

Nuclear accidents such as Chernobyl and Fukushima have led to contamination of the environment that will persist for many years. The consequences of chronic low-dose radiation exposure for non-human organisms inhabiting contaminated environments remain unclear. In radioecology, crustaceans are important model organisms for the development of environmental radioprotection. Previous laboratory studies have demonstrated deleterious effects of radiation exposure on crustacean reproduction. However, no studies have documented the effects of chronic radiation exposure on the reproduction of natural crustacean populations. Based on data from laboratory exposures, we hypothesised that populations of the freshwater isopod *Asellus aquaticus* exposed to radiation for thirty years at Chernobyl would display reduced reproductive output and altered timing of reproduction. To test this hypothesis, *A. aquaticus* was collected from six lakes at Chernobyl over two years with total dose rates ranging from 0.06–27.1 μGy/h. No significant differences in the fecundity, mass of broods or proportion of reproducing female *A. aquaticus* were recorded. Significant differences in the body mass of gravid females were recorded suggesting different timings of reproduction, however this was not related to radiation contamination. No significant effect of a range of environmental parameters on *A. aquaticus* reproduction was recorded. Our data suggests current dose rates at Chernobyl are not causing discernible effects on the reproductive output of *A. aquaticus*. This study is the first to assess the effects of chronic low-dose radiation exposure on the reproductive output of an aquatic invertebrate at Chernobyl. These findings are consistent with proposed radiological protection benchmarks for the maintenance of wildlife populations and will assist in management of environments impacted by radiation.

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1. Introduction

The accident on 26th April 1986 at the Chernobyl nuclear power plant (CNPP) led to the release of an estimated 5300 PBq (5.3×10^{17} Bq) of radioactivity into the environment (UNSCEAR, 2000). Vast areas of the former Soviet Union (fSU) and Western Europe were contaminated with radioactive substances, leading to radiation exposure of human and non-human organisms. A total of 220,000 people across Belarus, Russia and the Ukraine were permanently evacuated and a 30 km exclusion zone was established around the CNPP (UNSCEAR, 2000). Radioactivity in the aquatic environment was of concern owing to the proximity of the CNPP to the Pripyat river system and potential for contamination of the Kiev reservoir. Whilst dose rates decreased significantly in subsequent years owing to decay of short lived radionuclides and settling of radioactive substances to sediments, a number of 'closed' lake systems around Chernobyl retained relatively high levels of radioactivity. Such systems are typified by a lack of significant inflow or outflows of water, and are widespread in the Pripyat flood plain area and fSU countries affected by the Chernobyl accident (Smith and Beresford, 2005). The 2011 accident at the Fukushima Daiichi Nuclear Power Plant (FDNPP) caused by the Great East Japan Earthquake further contaminated a range of aquatic environments with radionuclides (IAEA, 2015), including a range of freshwater irrigation ponds and closed lakes. Understanding the effects of chronic radiation exposure on the aquatic environment is therefore a highly pertinent issue.

Immediately after the Chernobyl accident, wildlife inhabiting the exclusion zone were subjected to high doses of radiation with significant effects recorded for a range of organisms (See Smith and Beresford, 2005; Hinton et al., 2007). The chronic effects of Chernobyl-derived radiation on the environment are much less clear and remain highly controversial within the scientific community. No influence of radioactive contamination was observed on the abundance and diversity of macroinvertebrate species in Chernobyl affected lakes 18 years post-accident (Murphy et al., 2011). Recent studies of mammal communities in Chernobyl-affected areas (e.g Deryabina et al., 2015; Webster et al., 2016) have reported abundant mammal populations irrespective of the potential for radiation effects at the individual level. Conversely, population-level declines in the abundance of birds (Galván et al., 2011; Møller and Mousseau, 2007) and mammals (Møller and Mousseau, 2013) have been recorded in areas around Chernobyl. In Fukushima impacted coastal areas, a decline in the abundance and density of sessile intertidal biota was recorded (Horiguchi et al., 2016). However, difficulties in assessing the combined impact of the tsunami and elevated radiation dose rates prevent a definitive understanding of the cause of the decline. The lack of a scientific consensus regarding the long-term environmental and human health impacts of nuclear accidents have caused heightened concern amongst the wider public regarding radiation safety (Drottz-Sjöberg and Sjoberg, 1990; Orita et al., 2015). It is imperative that radioecology studies reporting no effect are regarded in the same manner as those finding detrimental radiation effects. This will enable a balanced judgement of the risk posed by anthropogenic radiation in the environment.

Reproductive endpoints are commonly used in environmental and toxicological studies owing to the potential for long term population level effects and ecological relevance (Lewis and Watson, 2012). A number of studies have documented reproductive effects on biota at Chernobyl. Møller et al. (2005) studied reproduction of barn swallows, *Hirunda rustica*, at Chernobyl and found reduced clutch sizes, lower hatching success and a smaller brood size of hatchlings in Chernobyl population as compared to controls. In murine rodents, a number of studies reported perturbations to reproduction and elevated embryonic mortality (Krylova et al., 1991; Testov and Taskaev, 1990), though effects appeared to be limited to the initial phase after the accident. In aquatic systems, gonadal abnormalities including asymmetry and oocyte resorption were positively correlated with contamination levels in a range of fish species including roach, *Rutilus rutilus*, perch, *Perca fluviatilis* and the goldfish *Carassius auratus* over a period from 1992 to 2005 (Belova et al., 2007). Tsytsugina and Polikarpov (2003) studied modes of reproduction and cytogenetic effects on populations of three species of Oligochaeta in contaminated areas in 1995–1996. The authors described a shift from asexual to sexual modes of reproduction at contaminated sites in two species; reproductive output was not directly quantified. This study, however, sampled only a single contaminated and control site precluding a robust understanding of the drivers of observed reproductive effects. To the authors' knowledge, no study has directly studied the impact of chronic radiation exposure on the reproduction of aquatic invertebrates at Chernobyl.

Members of the subphylum Crustacea are abundant in aquatic ecosystems globally and are gaining prominence as model organisms owing to increasing knowledge of crustacean genomics and biological systems. The International Commission on Radiological Protection (ICRP) developed the concept of reference animals and plants (RAPs) to use model organisms as a systematic basis for developing environmental radioprotection measures (ICRP, 2008). Due to their ubiquity in aquatic environments and well characterized biology, a marine crustacean of the family Cancridae has been selected as one of eight RAPs, highlighting the importance of understanding radiation effects on crustaceans for both members of the scientific and regulatory communities. At present, the effects of environmentally relevant doses of radiation on crustaceans are poorly understood (See Fuller et al., 2015 for review) owing to a lack of long-term studies in contaminated environments. In a previous study, no evidence of developmental effects (as measured using fluctuating asymmetry) of radiation exposure on the isopod crustacean, *Asellus aquaticus* at Chernobyl (Fuller et al., 2017) were found. The present study aimed to assess the effects of chronic radiation exposure on the reproduction of *A. aquaticus*. *A. aquaticus* is a benthic detritivore widespread in freshwater systems across Europe, playing a fundamental role in leaf litter degradation and nutrient cycling (Graça et al., 1993). *A. aquaticus* are widely considered to be semelparous (Chambers, 1977; Murphy and Learner, 1982; Steel, 1961 but see Maltby, 1991), with both the duration of both the breeding period and duration of embryo development driven by temperature (Andersson, 1969; Murphy and Learner, 1982; Økland, 1978). *A. aquaticus* has been used as a model organism in ecotoxicology in response to a range of toxicants including polycyclic aromatic hydrocarbons (De Lange et al., 2005), pesticides (Lukančič et al., 2010) and heavy metals (Van Ginneken et al., 2015).

Previous studies of *A. aquaticus* inhabiting polluted environments have recorded effects on reproduction. Populations below a coal mine effluent displayed a lower reproductive effort (defined as mg/offspring per mg/female) and modification of life history toward fewer larger offspring, which appeared to have a genetic basis (Maltby, 1991). Similarly, Tolba and Holdich (1981) recorded a lower fecundity in *A. aquaticus* individuals collected from sites with higher degrees of pollution. Controlled laboratory exposures have demonstrated a range of deleterious effects of chronic alpha and gamma radiation on crustaceans including delayed reproduction, reduced fecundity and survival of offspring (Alonzo et al., 2006, 2008; Gilbin et al., 2008 and Parisot et al., 2015). To the author's knowledge, no study has empirically tested the effects of radiation on crustacean reproduction following chronic low-dose radiation exposure in the field. Based on laboratory studies, we hypothesised that *A. aquaticus* individuals exposed to radiation at Chernobyl for thirty generations would display reduced reproductive output and altered timing of reproduction. To test this hypothesis, *A. aquaticus* individuals were collected from six lakes along a gradient of radionuclide contamination in Belarus and the Ukraine and fecundity, brood mass and maternal weight were assessed. Maternal weight was used as a proxy of reproductive timing (Donker et al., 1993).

2. Materials & methods

2.1. Sampling sites & collection of Asellus aquaticus

A. aquaticus was collected from six lakes along a gradient of radionuclide contamination in Belarus and the Ukraine in May – June of 2015 and 2016 (See Supplementary Information, Fig. S1). Lakes were selected based on long-term exposure to a gradient of radiation doses and historical measurements of radioactivity. Sites were visited once per sampling year and samples collected by kick netting in littoral zones from three different sub-sites at each lake using a 1 mm mesh size net (EFE, UK). The number of gravid (individuals bearing eggs) and non-gravid females collected is displayed in Table S1. The same sub-sites were visited the following year. Individuals were sorted lakeside and immediately preserved in 96% ethanol in individual Eppendorf tubes. A full description of the sampling sites is available in Fuller et al. (2017).

2.2. Hydrochemical parameters

A range of different hydrochemical variables including conductivity, oxygen saturation, pH and temperature were performed in situ using a multiparameter probe (HANNA Instruments 9828; see Table 1) at three sub-sites of each lake.

2.3. Calculation of radiation dose rates

A full description of the methods used to determine external and internal dose rates to *A. aquaticus* at sampling sites are provided in Fuller et al. (2017). Briefly, calculations were made using decay corrected deposition values of radiocaesium and strontium and dose conversion coefficients (DCCs) derived from geometry data of *A. aquaticus* using the ERICA tool (v 1.2, See Supplementary Information Table S2, Fig. S2). Internal dose rates were calculated based on average, decay corrected measurements of ^{137}Cs and ^{90}Sr in lakes at various depths taken during previous sampling in 2003. Total dose rates (individual absorbed dose rate) are provided in Table 1.

2.4. Reproductive output in Asellus aquaticus at Chernobyl

Adult (>3 mm in length, Hasu et al., 2007) *A. aquaticus* individuals were first sexed following Bertin et al. (2002) by analysis of the pleopods. Individuals were measured and weighed using the Leica Application Suite (v 4.5) and a Kern ABT 120-5DM (DE) analytical fine balance with a precision of ±0.02 mg respectively. Embryos were removed from the marsupium using a glass Pasteur pipette and photographed individually using a Leica DFC310 camera. Individuals were then reweighed to provide an estimate of the total weight of the brood. Embryos were staged following the method of Holdich (1968). A total of 354 gravid individuals were analysed over the two years of sampling (See Supplementary Information, Table S1).

2.5. Statistical analyses

Differences in brood sizes and weights between sites were tested using linear mixed effects models via the nlme package (Pinheiro et al., 2015) in R Studio Version 1.01 (R Studio Team, 2016). Sampling site, year and developmental stage of the brood were used as fixed effects and maternal weight as a random effect. In crustaceans, larger females typically produce greater numbers of eggs (e.g. Oh and Hartnoll, 1999). Owing to heterogeneity in the relationship between maternal weight and egg parameters between lakes in the present study, a random slopes and intercepts model was used. Models were fit using maximum likelihood methods and validated by analysis of residuals at each level of the random effect. Post-hoc multiple comparisons were used with Tukey contrasts via the multcomp package (Hothorn et al., 2008).

Differences in the percentage of females with broods between sites of varying contamination and sampling years was tested using binary logistic regression with the glm function. Female reproductive status (gravid = 1, non-gravid = 0) was used as the binary dependent variable where site and sampling year were predictors. Differences in the body mass of gravid *A. aquaticus* between sample sites were tested using a Kruskal-Wallis and post-hoc Dunn's test with Benjamini-Hochberg correction via the dunn.test package (Dinno, 2017).

Where significant differences between sites of varying contamination were present, the relationship between measured environmental characters and reproductive parameters were tested using linear regression. This was conducted on pooled environmental data for all sub-sites. Where assumptions of regression were violated, spearman's rho was used. All statistics were conducted in R Studio (R Core Development Team, 2016).

3. Results

3.1. Proportion of breeding females

The proportion of females with broods was not related to radiation dose rate (Fig. 1, linear regression F = 3.262, df = 1, 10, $r^2 = 0.246$ $p = 0.101$). The number of adult *A. aquaticus* with broods was significantly different between sites (logistic regression, $\chi = 8.65$, df = 5, $p < 0.001$), but not between sampling years ($\chi = 1.85$, df = 1, $p = 0.065$). The most contaminated site, Glubokoye, had the greatest proportion of females with broods over two years of sampling (Mean ± SEM 56.4 ± 13.9%), significantly greater than all sites excluding Svatoye 3 (49.1 ± 4.7%, Post-hoc Tukey's contrast, $p > 0.05$). The lowest proportion of females with broods was recorded at Stoyecheye (10.3 ± 7.3%). The proportion of females with broods was not correlated with any of the measured environmental parameters; dissolved oxygen (F = 0.3447, df = 1, 10, $r^2 = 0.033$, $p = 0.570$), water temperature (F = 0.357, df = 1, 10, $r^2 = 0.034$, $p = 0.564$), conductivity (F = 0.925, df = 1, 10, $r^2 = 0.085$, $p = 0.359$) and pH (F = 0.107, df = 1, 10, $r^2 = 0.011$, $p = 0.750$).

Table 1
Radiation dose rates (individual absorbed dose rate) and environmental parameters at six lakes along a gradient of radionuclide contamination at Chernobyl. Values for environmental parameters are presented as mean ± SD for values taken at three sub-sites of each lake.

Site	Sampling date	Dose rate (μGy/h)	Temperature (°C)	Oxygen saturation (%)	pH	Conductivity (μS/cm)
Gorova	11/6/2016	0.064	22.4 ± 0.05	113 ± 16.2	8.60 ± 0.02	256 ± 0.41
	23/6/2015	0.064	22.2 ± 0.05	185 ± 26.5	9.69 ± 0.02	179 ± 0.30
Dvorische	29/05/2016	0.691	23.2 ± 0.06	80 ± 1.17	7.60 ± 0.17	197 ± 0.15
	11/06/2015	0.786	23.7 ± 0.06	68.9 ± 1.00	7.82 ± 0.17	200 ± 0.00
Stoyecheye	27/05/2016	0.774	22 ± 0.05	102 ± 2.00	8.30 ± 0.02	241 ± 1.48
	08/06/2015	0.872	24.1 ± 0.06	89.4 ± 1.76	8.70 ± 0.02	230 ± 1.41
Svatoye 3	24/05/2016	2.03	20.1 ± 0.23	92 ± 1.80	8.00 ± 0.15	114 ± 0.70
	05/06/2015	2.2	23 ± 0.70	81.6 ± 1.60	7.8 ± 0.14	122 ± 0.75
Yanovsky Crawl	05/06/2016	20.42	20.2 ± 0.11	140 ± 2.90	9.00 ± 0.04	265 ± 0.97
	19/06/2015	20.6	23.3 ± 0.11	109 ± 2.25	9.40 ± 0.04	275 ± 1.00
Glubokoye	03/06/2016	26.4	23.6 ± 0.06	112 ± 14.10	7.60 ± 0.18	199 ± 1.22
	16/06/2015	27.1	24.9 ± 0.06	66.3 ± 8.32	7.92 ± 0.19	185 ± 1.14

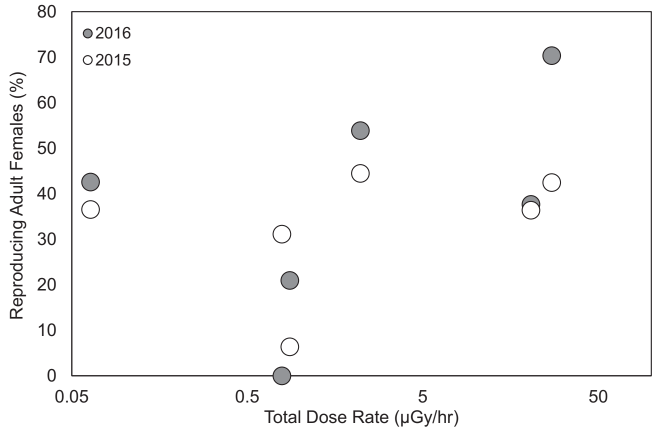

Fig. 1. Scatterplot of the relationship between the proportion of reproducing female *Asellus aquaticus* and total dose rate at six sites of varying contamination at Chernobyl.

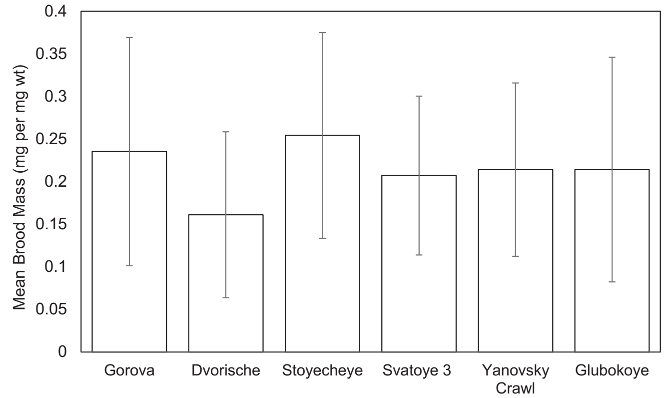

Fig. 3. Mean brood mass normalised to maternal weight of *A. aquaticus* collected from six sites along a gradient of radionuclide contamination. Sites are plotted in order of increasing contamination from left to right. Error bars represent standard deviations.

3.2. Fecundity

The number of eggs (brood size) produced by female *Asellus aquaticus* did not vary between sampling year ($F_{1,80} = 7.5$, $p = 0.740$, See Supplementary Information Tables S3, S4) or with the developmental stage of the brood ($F_{1,\,80} = 0.602$, $p = 0.502$). The greatest number of eggs was produced at Glubokoye Lake over the two sampling years (Mean ± SD, 6.477 ± 2.259 eggs per mg maternal weight, See Fig. 2) with the fewest eggs being produced at Stoyecheye Lake (4.811 ± 1.824 eggs per mg maternal weight), however no significant effect of sampling site on brood sizes was recorded ($F_{1,\,80} = 2.402$, $p = 0.494$). This was further emphasised by the lack of relationship between total dose rate and brood sizes (Spearman's rank-order correlation, rho = 0.008, $p = 0.877$).

3.3. Brood mass

Brood mass did not vary significantly between sampling years ($F_{1,\,70} = 3.653$, $p = 0.441$) or between sampling sites of varying radionuclide contamination ($F_{1,\,70} = 0.562$, $p = 0.456$, See Fig. 3). Developmental stage was found to have a significant effect on the mass of the brood however ($F_{1,\,70} = 25.060$, $p = 0.0018$). This was owing to a significantly (Post-hoc Tukey contrasts, $p = 0.0018$) greater mass of broods in the final stage of development (Mean ± SE, 2.847 ± 0.590 mg) compared to the first stage of development (1.045 ± 0.073 mg). This effect was independent of sampling site as indicated by the non-significant interaction between sampling site and developmental stage ($F_{1,\,70} = 0.642$, $p = 0.426$).

3.4. Maternal body mass

No significant differences in the body mass of gravid *Asellus aquaticus* were recorded between sampling years (Kruskal-Wallis test, $\chi^2 = 0.347$, df = 1, $p = 0.558$). Significant differences in maternal body mass were recorded between sampling sites (Kruskal-Wallis test, $\chi^2 = 109.4$, df = 5, $p = 0.000$), owing to a significantly greater mass of *A. aquaticus* at Stoyecheye (18.938 ± 6.904) and Svatoye 3 (11.730 ± 4.735) compared to all other sites (See Fig. 4, Dunn's test, p = 0.000). Differences in body mass were not related to total radiation dose rate (Spearman's rank-order correlation, rho = −0.081, p = 0.129) or any of the other measured environmental variables; conductivity (rho = −0.062, $p = 0.245$), temperature (rho = −0.077, p = 0.149), pH (rho = 0.108, $p = 0.449$) and dissolved oxygen (rho = 0.878, $p = 0.878$).

4. Discussion

The research hypothesis for this work was that populations of the isopod crustacean *Asellus aquaticus* chronically exposed to ionising radiation at Chernobyl would display reduced reproductive output and altered timing of reproduction. In order to test this hypothesis, gravid females were collected from six sites along a gradient of radionuclide contamination in areas impacted by Chernobyl in 2015 and 2016. Reproductive output was assessed and weight of gravid females was

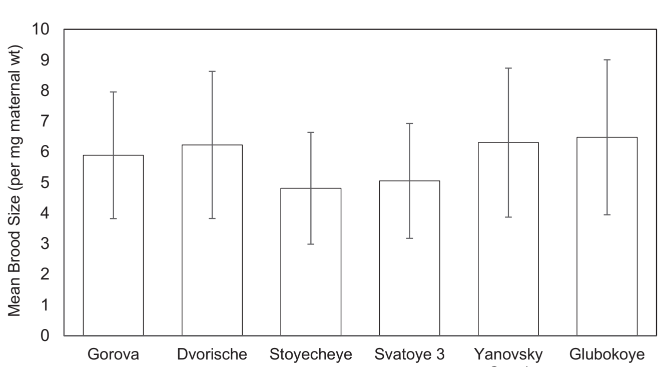

Fig. 2. Mean brood sizes normalised to maternal weight in *Asellus aquaticus* from six sites along a gradient of radionuclide contamination in Belarus and the Ukraine. Sites are plotted in order of increasing contamination from left to right. Error bars represent standard deviation.

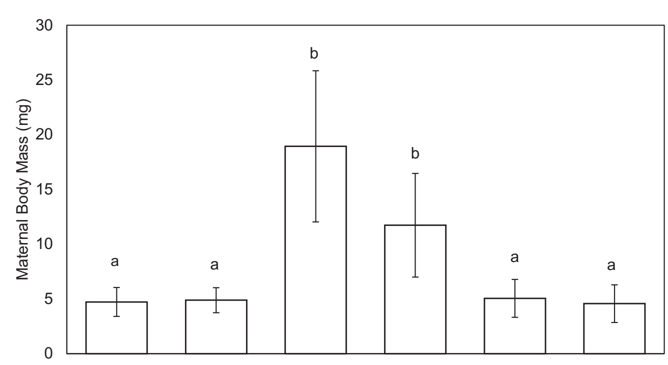

Fig. 4. Body mass of gravid *A. aquaticus* from six sites along a gradient of radionuclide contamination in Belarus and the Ukraine. Matching letters represent no significant difference, different letters represent significant differences (Dunn's test, $p < 0.05$). Error bars are ±SD.

used as a proxy of timing of reproduction. No significant differences in the numbers or mass of offspring produced at sites of varying radionuclide contamination was found in the present study. Significant differences in the body mass of ovigerous females was found, suggesting different timing of reproduction between localities. However, this was not related to radiation dose rates or any of the measured potential confounding factors. No support for these hypotheses were found within the data.

The present study finds no evidence that current dose rates in aquatic systems at Chernobyl (maximum total dose rate of 27.1 μGy/h, See Table 1) are impacting the reproduction of *A. aquaticus*. However, reproduction of *A. aquaticus* populations may have been affected immediately following the accident. Dose rates from sediment in aquatic systems in the immediate aftermath of the accident were estimated to be in the range of 100–200 mGy/d. Based on laboratory studies of crustaceans exposed to radiation (e.g. Parisot et al., 2015), such dose rates would be anticipated to cause deleterious effects on reproduction, though drastic differences in sensitivities between crustacean species have been recorded (See Fuller et al., 2015 for review).

A range of different organisations have proposed dose thresholds and benchmarks below which no detrimental impacts on populations of aquatic organisms are expected. For example, UNSCEAR concluded that maximum dose rates of ≤400 μGy/h to an individual within an aquatic population would not have detrimental effects at the population level, owing to a lack of evidence suggesting significant effects on reproduction at ≤200 μGy/h (Copplestone et al., 2008; UNSCEAR, 2008). For the ICRP's reference crustacean (ICRP, 2008), a derived consideration reference level (DCRL) of 400–4000 μGy/h has been proposed, within which there is some chance of deleterious effects to individuals. The lack of observed effects in the present study at dose rates of up to 27.1 μGy/h is consistent with these benchmark values. Lower dose benchmarks of 10 μGy/h have been proposed as generic screening values for environmental radioprotection in the absence of detailed organism-specific data (ERICA & PROTECT projects, Larsson, 2008; Howard et al., 2010). In this study of reproductive output in a single crustacean species, no negative effects at dose rates higher than those generic values were observed. Though the possibility of adaptation of populations at Chernobyl cannot be ruled out, this suggests that these lower benchmarks may be overly conservative for protection at the population level for some groups of organisms. Emphasis should therefore be placed on the development of species specific benchmarks for protection of the environment.

Recent studies have documented reproductive effects in crustaceans chronically exposed to radiation doses below those in the present study. Parisot et al. (2015) demonstrated reduced fecundity and delayed reproduction in *Daphnia magna* following multigenerational laboratory exposure to ^{137}Cs at 7 μGy/h. Two of the six sampling sites in the present study exceeded this dose rate (Yanovsky Crawl and Glubokoye Lake, see Table 1) with no detectable reproductive effects. Disparities between controlled laboratory studies and those conducted in the field have been reviewed by Garnier-Laplace et al. (2013). In contrast to the present study, the authors found greater sensitivity to radiation in organisms studied in the field compared to those exposed under controlled conditions. One possible explanation for the differences between the two studies may be adaptation of *A. aquaticus* to chronic radiation exposure. *A. aquaticus* populations are univoltine, meaning approximately thirty generations have occurred since the nuclear accident (Brattey, 1986). Crustaceans have been shown to gain tolerance to pollutants in as few as seven generations (Sun et al., 2014), further suggesting adaptation as a mechanism for the lack of effect in the present study. This highlights the necessity of field studies to validate and contextualise the results of laboratory experiments. Further comparative research into the effects of low-dose radiation on crustacean reproduction in the lab and field will enable a greater understanding of the importance of adaptation in species response to radiation.

In the present study, no significant relationship between measures of female reproduction and a range of environmental parameters was recorded. In *A. aquaticus,* duration of both the breeding period and embryonic development is related to temperature and the number of degree days above a minimum temperature (Andersson, 1969; Murphy and Learner, 1982; Økland, 1978). Studies have further suggested a role of photoperiod and food availability in governing reproductive patterns in *A. aquaticus* (Tadini and Valentino, 1969). In the present study, sampling was conducted once per year at each locality over two sampling years. Analysis of additional environmental conditions throughout the year such as food availability and the timing of spring would allow for greater understanding of the factors driving *A. aquaticus* reproduction within these lakes, and may explain the lack of a relationship between environmental parameters and *Asellus* reproduction. However, sampling throughout the year was not possible owing to logistical and permitting restrictions. In the majority of reported cases *A. aquaticus* individuals reproduce only once during their lifespan and are considered semelparous (Chambers, 1977; Murphy and Learner, 1982; Steel, 1961) though multiple broods have been recorded (e.g. Maltby, 1991). Therefore, sampling once per year is adequate to gain an understanding of typical reproductive output in *A. aquaticus.*

The lack of effect on reproduction in the present study may suggest that metabolic resources are being diverted from other processes in order to meet the physiological costs of radiation exposure (Jones and Hopkin, 1996). Similarly, alterations to metabolic requirements may lead to changes in feeding behaviour. Nascimento and Bradshaw (2016) demonstrated reduced grazing activity in *Daphnia magna* exposed to acute gamma radiation from ^{137}Cs. Alonzo et al. (2006) further suggested a metabolic cost of alpha radiation exposure in *D. magna.* Given the importance of feeding in *A. aquaticus* on leaf litter decomposition and therefore nutrient cycling in freshwater ecosystems (Graça et al., 1993), future studies should focus on the metabolic impacts of radiation exposure at Chernobyl and knock-on effects on feeding in *A. aquaticus*. The advent of advanced techniques such as metabolomics in environmental toxicology (e.g. Cappello et al., 2013; Xu et al., 2015) enables greater understanding of the metabolic pathways affected by stressors in the environment. Given the previous studies suggesting a metabolic cost of radiation exposure, future studies should employ a metabolomic approach to assessing the effects of radiation on biota.

5. Conclusions

In conclusion, the present study did not observe any significant association of reproductive endpoints in crustaceans with radiation. This suggests either that there are no such effects in current *Asellus* populations, or that these effects are so subtle to be undetectable in the natural environment given other environmental influences on *Asellus* reproduction. This study is the first to monitor reproduction in crustaceans at Chernobyl. The results of this study will aid in understanding the long-term effects of radiation exposure at the population level and support the management and monitoring of radioactively contaminated environments.

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Conflict of interest statement

This project was funded by the UK Natural Environment Research Council, Radioactive Waste Management Ltd. and the Environment

Agency of England and Wales. J.T.S. has also carried out small (<£10 k) consultancy projects for a range of clients including the Japan Atomic Energy Agency and UK Radioactive Waste Management Ltd.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.01.309.

References

Alonzo, F., Gilbin, R., Bourrachot, S., Floriani, M., Morello, M., Garnier-Laplace, J., 2006. Effects of chronic internal alpha irradiation on physiology, growth and reproductive success of *Daphnia magna*. Aquat. Toxicol. 80 (3):228–236. https://doi.org/10.1016/j.aquatox.2006.09.001.

Alonzo, F., Gilbin, R., Zeman, F.A., Garnier-Laplace, J., 2008. Increased effects of internal alpha irradiation in *Daphnia magna* after chronic exposure over three successive generations. Aquat. Toxicol. 87 (3):146–156. https://doi.org/10.1016/j.aquatox.2008.01.015.

Andersson, E., 1969. Life-cycle and growth of *Aselus aquaticus* (L.) with special reference to the effects of temperature. 49. Institute of Freshwater Research, Drottningholm, pp. 5–26.

Belova, N.V., Emel'yanova, N.G., Makeeva, A.P., Ryabov, I.N., 2007. The state of the reproductive system of several fish species from water bodies polluted with radionuclides during the Chernobyl catastrophe. J. Ichthyol. 47 (5):366–384. https://doi.org/10.1134/s0032945207050050.

Bertin, A., David, B., Cezilly, F., Alibert, P., 2002. Quantification of sexual dimorphism in *Asellus aquaticus* (Crustacea: Isopoda) using outline approaches. Biol. J. Linn. Soc. 77 (4):523–533. https://doi.org/10.1046/j.1095-8312.2002.00125.

Brattey, J., 1986. Life history and population biology of larval *Acanthocephalus lucii* (Acanthocephala: Echinorhynchidae) in the isopod *Asellus aquaticus*. J. Parasitol.: 633–645 https://doi.org/10.2307/3281450.

Cappello, T., Mauceri, A., Corsaro, C., Maisano, M., Parrino, V., Paro, G.L., ... Fasulo, S., 2013. Impact of environmental pollution on caged mussels Mytilus Galloprovincialis using NMR-based metabolomics. Mar. Pollut. Bull. 77 (1):132–139. https://doi.org/10.1016/j.marpolbul.2013.10.019.

Chambers, M.R., 1977. A comparison of the population ecology of *Asellus aquaticus* (L.) and *Asellus meridianus* rac. in the reed beds of the Tjeukemeer. Hydrobiologia 53 (2):147–154. https://doi.org/10.1007/BF00029293.

Copplestone, D., Hingston, J., Real, A., 2008. The development and purpose of the FREDERICA radiation effects database. J. Environ. Radioact. 99 (9):1456–1463. https://doi.org/10.1016/j.jenvrad.2008.01.006.

De Lange, H.J., De Haas, E.M., Maas, H., Peeters, E.T.H.M., 2005. Contaminated sediments and bioassay responses of three macroinvertebrates, the midge larva *Chironomus riparius*, the water louse *Asellus aquaticus* and the mayfly nymph *Ephoron virgo*. Chemosphere 61 (11):1700–1709. https://doi.org/10.1016/j.chemosphere.2005.03.083.

Deryabina, T.G., Kuchmel, S.V., Nagorskaya, L.L., Hinton, T.G., Beasley, J.C., Lerebours, A., Smith, J.T., 2015. Long-term census data reveal abundant wildlife populations at Chernobyl. Curr. Biol. 25 (19):R824–R826. https://doi.org/10.1016/j.cub.2015.08.017.

Dinno, A., 2017. dunn.test: Dunn's Test of Multiple Comparisons Using Rank Sums. R package version 1.3.3. http://CRAN.R-project.org/package=dunn.test :p. 2017.

Donker, M.H., Zonneveld, C., Van Straalen, N.M., 1993. Early reproduction and increased reproductive allocation in metal-adapted populations of the terrestrial isopod Porcellio Scaber. Oecologia 96 (3):316–323. https://doi.org/10.1007/bf00317500.

Drottz-Sjöberg, B.M., Sjoberg, L., 1990. Risk perception and worries after the Chernobyl accident. J. Environ. Psychol. 10 (2):135–149. https://doi.org/10.1016/s0272-4944(05)80124-0.

Fuller, N., Lerebours, A., Smith, J.T., Ford, A.T., 2015. The biological effects of ionising radiation on crustaceans: a review. Aquat. Toxicol. 167:55–67. https://doi.org/10.1016/j.aquatox.2015.07.013.

Fuller, N., Smith, J.T., Nagorskaya, L.L., Gudkov, D.I., Ford, A.T., 2017. Does Chernobyl-derived radiation impact the developmental stability of *Asellus aquaticus* 30 years on? Sci. Total Environ. 576:242–250. https://doi.org/10.1016/j.scitotenv.2016.10.097.

Galván, I., Mousseau, T.A., Møller, A.P., 2011. Bird population declines due to radiation exposure at Chernobyl are stronger in species with pheomelanin-based coloration. Oecologia 165 (4):827–835. https://doi.org/10.1007/s00442-010-1860-5.

Garnier-Laplace, J., Geras'kin, S., Della-Vedova, C., Beaugelin-Seiller, K., Hinton, T.G., Real, A., Oudalova, A., 2013. Are radiosensitivity data derived from natural field conditions consistent with data from controlled exposures? A case study of Chernobyl wildlife chronically exposed to low dose rates. J. Environ. Radioact. 121:12–21. https://doi.org/10.1016/j.jenvrad.2012.01.013.

Gilbin, R., Alonzo, F., Garnier-Laplace, J., 2008. Effects of chronic external gamma irradiation on growth and reproductive success of *Daphnia magna*. J. Environ. Radioact. 99 (1):134–145. https://doi.org/10.1016/j.jenvrad.2007.07.004.

Graça, M.A.S., Maltby, L., Calow, P., 1993. Importance of fungi in the diet of *Gammarus pulex* and *Asellus aquaticus* I: feeding strategies. Oecologia 93 (1):139–144. https://doi.org/10.1007/BF00321203.

Hasu, T., Holmes, J.C., Valtonen, E.T., 2007. Isopod (*Asellus aquaticus*) size and acanthocephalan (*Acanthocephalan lucil*) infections. J. Parasitol. 450–457.

Hinton, T.G., Alexakhin, R., Balonov, M., Gentner, N., Hendry, J., Prister, B., ... Woodhead, D., 2007. Radiation-induced effects on plants and animals: findings of the United Nations Chernobyl forum. Health Phys. 93 (5):427–440. https://doi.org/10.1097/01.hp.0000281179.03443.2e.

Holdich, D.M., 1968. Reproduction, growth and bionomics of *Dynamene bidentata* (Crustacea: Isopoda). J. Zool. 156 (2):137–153. https://doi.org/10.1111/j.1469-7998.1968.tb05925.x.

Horiguchi, T., Yoshii, H., Mizuno, S., Shiraishi, H., 2016. Decline in intertidal biota after the 2011 great East Japan earthquake and tsunami and the Fukushima nuclear disaster: field observations. Sci. Rep. 6:20416. https://doi.org/10.1038/srep20416.

Hothorn, T., Bretz, F., Westfall, P., 2008. Simultaneous inference in general parametric models. Biom. J. 50 (3):346–363. https://doi.org/10.1002/bimj.200810425.

Howard, B.J., Beresford, N.A., Andersson, P., Brown, J.E., Copplestone, D., Beaugelin-Seiller, K., ... Whitehouse, P., 2010. Protection of the environment from ionising radiation in a regulatory context—an overview of the PROTECT coordinated action project. J. Radiol. Prot. 30 (2):195. https://doi.org/10.1088/0952-4746/30/2/s01.

IAEA, 2015. The Fukushima Daiichi Accident: Radiological Consequences. Technical Volume 4/5. International Atomic Energy Agency, Vienna.

ICRP, 2008. Environmental protection: the concept and use of reference animals and plants. ICRP publication 108. Ann. ICRP 38 (4–6).

Jones, D.T., Hopkin, S.P., 1996. Reproductive allocation in the terrestrial isopods *Porcellio scaber* and *Oniscus asellus* in a metal-polluted environment. Funct. Ecol.:741–750 https://doi.org/10.2307/2390509.

Krylova, T.V., Skurat, L.N., Dolgov, V.A., 1991 Dec. The reproductive potential of the bank vole (*Clethrionomys glareolus* Schreb) and of the tundra vole (*Microtus oeconomus* Pall.) under the conditions of the elevated radiation background of Bryansk Province. Nauchnye doklady vysshei shkoly. Biologicheskie nauki. 10, pp. 109–118.

Larsson, C.M., 2008. An overview of the ERICA integrated approach to the assessment and management of environmental risks from ionising contaminants. J. Environ. Radioact. 99 (9):1364–1370. https://doi.org/10.1016/j.jenvrad.2007.11.019.

Lewis, C., Watson, G.J., 2012. Expanding the ecotoxicological toolbox: the inclusion of polychaete reproductive endpoints. Mar. Environ. Res. 75:10–22. https://doi.org/10.1016/j.marenvres.2011.08.002.

Lukančič, S., Žibrat, U., Mezek, T., Jerebic, A., Simčič, T., Brancelj, A., 2010. Effects of exposing two non-target crustacean species, *Asellus aquaticus* L., and *Gammarus fossarum* Koch., to atrazine and imidacloprid. Bull. Environ. Contam. Toxicol. 84 (1):85. https://doi.org/10.1007/s00128-009-9854-x.

Maltby, L., 1991. Pollution as a probe of life-history adaptation in *Asellus aquaticus* (isopoda). Oikos 61:11. https://doi.org/10.2307/3545402.

Møller, A.P., Mousseau, T.A., 2007. Species richness and abundance of forest birds in relation to radiation at Chernobyl. Biol. Lett. 3 (5):483–486. https://doi.org/10.1098/rsbl.2007.0226.

Møller, A.P., Mousseau, T.A., 2013. Assessing effects of radiation on abundance of mammals and predator–prey interactions in Chernobyl using tracks in the snow. Ecol. Indic. 26:112–116. https://doi.org/10.1016/j.ecolind.2012.10.025.

Møller, A.P., Mousseau, T.A., Milinevsky, G., Peklo, A., Pysanets, E., Szép, T., 2005. Condition, reproduction and survival of barn swallows from Chernobyl. J. Anim. Ecol. 74 (6):1102–1111. https://doi.org/10.1111/j.1365-2656.2005.01009.

Murphy, P.M., Learner, M.A., 1982. The life history and production of *Asellus aquaticus* (Crustacea: Isopoda) in the River Ely, South Wales. Freshw. Biol. 12 (5):435–444. https://doi.org/10.1111/j.1365-2427.1982.tb00638.x.

Murphy, J.F., Nagorskaya, L.L., Smith, J.T., 2011. Abundance and diversity of aquatic macroinvertebrate communities in lakes exposed to Chernobyl-derived ionising radiation. J. Environ. Radioact. 102 (7):688–694. https://doi.org/10.1016/j.jenvrad.2011.04.007.

Nascimento, F.J., Bradshaw, C., 2016. Direct and indirect effects of ionizing radiation on grazer–phytoplankton interactions. J. Environ. Radioact. 155:63–70. https://doi.org/10.1016/j.jenvrad.2016.02.007.

Oh, C.W., Hartnoll, R.G., 1999. Size at sexual maturity, reproductive output, and seasonal reproduction of *Philocheras trispinosus* (Decapoda) in Port Erin Bay, Isle of Man. J. Crustac. Biol. 19 (2):252–259. https://doi.org/10.1163/193724099X00051.

Økland, K., 1978. Life history and growth of Asellus aquaticus in relation to environment in a eutrophic lake in Norway. Hydrobiologia 59, 243–260.

Orita, M., Hayashida, N., Nakayama, Y., Shinkawa, T., Urata, H., Fukushima, Y., ... Takamura, N., 2015. Bipolarization of risk perception about the health effects of radiation in residents after the accident at Fukushima nuclear power plant. PLoS One 10 (6), e0129227. https://doi.org/10.1371/journal.pone.0129227.

Parisot, F., Bourdineaud, J.P., Plaire, D., Adam-Guillermin, C., Alonzo, F., 2015. DNA alterations and effects on growth and reproduction in *Daphnia magna* during chronic exposure to gamma radiation over three successive generations. Aquat. Toxicol. 163: 27–36. https://doi.org/10.1016/j.aquatox.2015.03.002.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., Team, R.C., 2015. nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1–120. http://CRAN.R-project.org/package=nlme.

Smith, J.T., Beresford, N.A., 2005 Jan 1. Chernobyl: Catastrophe and Consequences. Springer, Chichester.

Steel, E.A., 1961. Some observations on the life history of *Asellus aquaticus* (L.) and *Asellus meridianus* racovitza (Crustacea:Isopoda). Proc. Zool. Soc. London 137 (1):71–87. https://doi.org/10.1111/j.1469-7998.1961.tb06162.x).

Sun, P.Y., Foley, H.B., Handschumacher, L., Suzuki, A., Karamanukyan, T., Edmands, S., 2014. Acclimation and adaptation to common marine pollutants in the copepod *Tigriopus californicus*. Chemosphere 112:465–471. https://doi.org/10.1016/j.chemosphere.2014.05.023.

Tadini, G.V., Valentino, F., 1969. Richerche sulla determinazione della stasi riproduttiva in varie razze geografiche di '*Asellus aquaticus*' (Crust. Isop.). Atti Accad. naz.Lincei Memorie. Ser. 8. Vol. 9, 3, pp. 3–50.

Team, R., 2016. RStudio: Integrated Development for R. Boston: RStudio Inc.

Team RC, 2016. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Testov, B.V., Taskaev, A.I., 1990. Dynamics of mouse-type rodent populations in the zone of the Chernobyl NPP. Biological and Radioecological Aspects of the Consequences of the Chernobyl Accident, p. 86.
Tolba, M.R., Holdich, D.M., 1981. The effect of water quality on the size and fecundity of *Asellus aquaticus* (Crustacea: Isopoda). Aquat. Toxicol. 1 (2):101–112. https://doi.org/10.1016/0166-445x(81)90033-3.
Tsytsugina, V.G., Polikarpov, G.G., 2003. Radiological effects on populations of Oligochaeta in the Chernobyl contaminated zone. J. Environ. Radioact. 66 (1):141–154. https://doi.org/10.1016/s0265-931x(02)00120-0.
United Nations Scientific Committee on the Effects of Atomic Radiation, 2000. Sources and Effects of Ionizing Radiation, ANNEX J, Exposures from Natural Radiation Sources. UNSCEAR 2000 REPORT, New York. 1 pp. 97–99.
United Nations Scientific Committee on the Effects of Atomic Radiation, 2008. Effects of Ionizing Radiation, Annex E, Exposures From Natural Radiation Sources. UNSCEAR 2008 REPORT, New York.
Van Ginneken, M., De Jonge, M., Bervoets, L., Blust, R., 2015. Uptake and toxicity of Cd, Cu and Pb mixtures in the isopod *Asellus aquaticus* from waterborne exposure. Sci. Total Environ. 537:170–179. https://doi.org/10.1016/j.scitotenv.2015.07.153.
Webster, S.C., Byrne, M.E., Lance, S.L., Love, C.N., Hinton, T.G., Shamovich, D., Beasley, J.C., 2016. Where the wild things are: influence of radiation on the distribution of four mammalian species within the Chernobyl exclusion zone. Front. Ecol. Environ. 14 (4):185–190. https://doi.org/10.1002/fee.1227.
Xu, H.D., Wang, J.S., Li, M.H., Liu, Y., Chen, T., Jia, A.Q., 2015. 1 H NMR based metabolomics approach to study the toxic effects of herbicide butachlor on goldfish (*Carassius auratus*). Aquat. Toxicol. 159:69–80. https://doi.org/10.1016/j.aquatox.2014.11.020.