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Does Chernobyl-derived radiation impact the developmental stability of *Asellus aquaticus* 30 years on?

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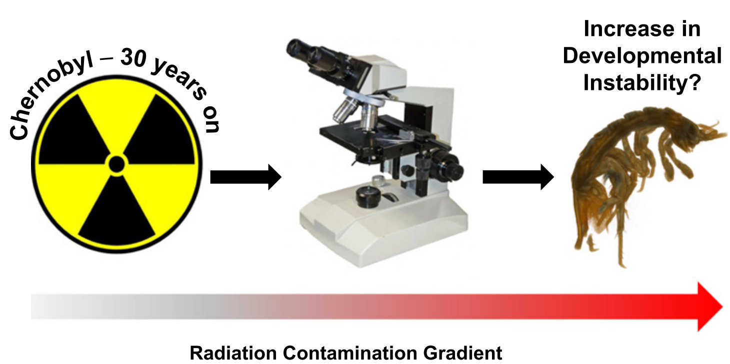
HIGHLIGHTS

- 30 year impacts of Chernobyl on development of *Asellus aquaticus* assessed
- Fluctuating asymmetry (FA) used as measure of developmental stability
- No increase in developmental stability along gradient of radioactive contamination
- Findings suggest resilience of aquatic invertebrate populations to radionuclides.
- Helps to understand the impacts of chronic exposures to radiation on ecosystems

GRAPHICAL ABSTRACT



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ABSTRACT

Effects of long-term, environmentally relevant doses of radiation on biota remain unclear due to a lack of studies following chronic exposure in contaminated environments. The 1986 Chernobyl accident dispersed vast amounts of radioactivity into the environment which persists to date. Despite three decades of research, impacts of the incident on non-human organisms continues to be contested within the scientific literature. The present study assessed the impact of chronic radiation exposure from Chernobyl on the developmental stability of the model aquatic isopod, *Asellus aquaticus* using fluctuating asymmetry (FA) as an indicator. Fluctuating asymmetry, defined as random deviations from the expected perfect bilateral symmetry of an organism, has gained prominence as an indicator of developmental stability in ecotoxicology. Organisms were collected from six lakes along a gradient of radionuclide contamination in Belarus and the Ukraine. Calculated total dose rates ranged from 0.06–27.1 μGy/h. Fluctuating asymmetry was assessed in four meristic and one metrical trait. Significant differences in levels of pooled asymmetry were recorded between sample sites independent of sex and specific trait measured. However, there was no correlation of asymmetry with radiation doses, suggesting that differences in asymmetry were not attributed to radionuclide contamination and were driven by elevated asymmetry at a single site. No correlation between FA and measured environmental parameters suggested a biotic factor driving observed FA differences. This study appears to be the first to record no evident increase in developmental stability of biota from the Chernobyl region. These findings will aid in understanding the response of organisms to chronic pollutant exposure and the long term effects of large scale nuclear incidents such as Chernobyl and Fukushima.

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1. Introduction

The developmental stability of an organism is demonstrated by its capacity to produce an optimal phenotypic form under a specific set of environmental conditions (Palmer, 1994). Bilateral symmetry offers a method of determining levels of developmental stability founded on an a priori understanding of the ideal form; perfect bilateral symmetry. Fluctuating asymmetry (FA) refers to subtle, random deviations from the expected bilateral symmetry displaying a normal distribution with a mean of zero (Palmer and Strobeck, 1986). FA analyses have gained prominence as both an environmental monitoring tool and in evolutionary biology studies owing to the apparent ease with which such studies can be conducted and analysed compared with other phenotypic fitness indicators (Van Dongen, 2006). An increase in FA has been linked to a range of extrinsic and intrinsic stressors including organic pollutants (Jenssen et al., 2010), temperature (Vishalakshi and Singh, 2008a) and genetic stressors such as inbreeding (Özener, 2010). Studies have demonstrated relationships between levels of FA and traditional measures of fitness (Bakker et al., 2006; Silva et al., 2016), although the reliability of FA as a fitness indicator has been criticised (see Kruuk et al., 2003; Vishalakshi and Singh, 2008b).

The 1986 Chernobyl accident dispersed an estimated 5300 PBq of radioactivity into the environment (UNSCEAR, 2000) contaminating large areas of Europe. However, 30 years after the accident controversy still exists regarding the biological consequences of the incident and the dose levels at which these occur (Beresford and Copplestone, 2011; Beresford et al., 2016). For example, an order of magnitude decline in above ground invertebrates inhabiting Chernobyl (bumble bees, spiders, grasshoppers, butterflies and dragonflies) was recorded over a dose range of 0.1–10 μGy/h by Møller and Mousseau (2009) 20 years after the accident. Such dose rates fall within the range of exposure to terrestrial wildlife as a consequence of naturally occurring radionuclides (i.e. ^{40}K, ^{238}U and ^{232}Th) in the United Kingdom (Beresford et al., 2008). Conversely, no impact of radiation dose rates on the abundance and diversity of aquatic macroinvertebrates was found by Murphy et al. (2011) in Chernobyl contaminated lakes. The Tōhoku earthquake-tsunami at the Fukushima Dai-ichi nuclear power plant (NPP) in 2011 led to further contamination of the environment with radionuclides, with release estimates ranging from 7 to 50 and 1–5.5 PBq of ^{137}Cs for atmospheric and direct marine source term releases respectively (IAEA, 2015). At present, the radiological consequences of the Fukushima incident on non-human biota are unclear. These incidents underpin the necessity of robust evaluation of the impacts of radiation on wildlife.

Previous studies have demonstrated an increase in FA in populations of biota inhabiting areas impacted by elevated levels of radionuclides (Gileva and Nokhrin, 2001; Møller, 2002). For example, Oleksyk et al. (2004) demonstrated a 3.6 fold increase in mean skull FA in populations of the yellow necked mouse, *Apodemus flavicollis*, inhabiting areas closer to the failed Chernobyl reactor compared with reference populations over a range of dose rates from 0.107 to 4.146 μGy/h. Further, a high degree of FA was recorded in the freshwater mollusc, *Dreissena polymorpha* and floating pondweed *Potamogeton natans* from aquatic systems impacted by the Chernobyl incident (Yavnyuk et al., 2009). Williams et al. (2001) recorded an increase in morphological abnormalities in larval chironomids inhabiting Belarusian lakes impacted by the Chernobyl incident at ambient dose rates of 8–20 μSv/h, although FA was not directly quantified. FA therefore appears to be an appropriate indicator of radiation-induced developmental stress in organisms from the Chernobyl region.

This study aimed to assess the impacts of chronic radiation exposure along an established gradient of radionuclide contamination on the development of the water louse, *Asellus aquaticus* using FA as an indicator. FA has previously been induced in laboratory populations of *Asellus aquaticus* subjected to elevated temperatures (Savage and Hogarth, 1999) and is an effective indicator of developmental stability. *A. aquaticus* is a detritivorous isopod common in temperate freshwater ecosystems across Europe (Williams, 1962) that has gained prominence as an indicator species in ecotoxicity testing of sediment-borne contaminants (De Lange et al., 2005; McCahon and Pascoe, 1988).

2. Materials & methods

2.1. Sampling sites & collection of A. aquaticus

Six lakes were chosen ranging from 3 to 225 km in distance from the Chernobyl NPP. These sites exhibited varying degrees of contamination as a consequence of the Chernobyl incident (see Fig. 1). Where possible, localities with historic environmental data sets were selected. Samples were collected in littoral zones and amongst vegetation in June 2015 at three different sub sites of each lake by kick netting using a 1 mm mesh size net (EFE, UK). Following sieving, *A. aquaticus* were sorted and immediately preserved in 96% ethanol. Prior to analysis, samples were placed in randomly coded boxes to prevent measurement bias, a pervading problem in FA studies (Palmer, 1994).

2.2. Environmental parameters

Hydrochemical variable measurements, including conductivity oxygen saturation, pH and temperature were performed in situ using a multiparameter probe (HANNA Instruments 9828) at three stations of each lake. All lakes had similar fish communities comprised mainly of perch, roach and rudd (Smith et al., 2005; Murphy et al., 2011). Table 1 displays the measured environmental variables and available bathymetric data from Smith et al. (2005).

2.3. Estimation of external dose rates at sample sites

In the present study, external dose rates were calculated using deposition values of radiocaesium and strontium at sampling sites and dose conversion coefficients (DCC's) based on user inputted data for the geometry of *A. aquaticus* (Height = 2.2 mm, width = 1.7 mm, length = 4.7 mm and mass = 4.1 mg) using the ERICA tool (V1.2). DCC's for external dose rates were calculated to be 3.85×10^{-4} and 4.91×10^{-4} μGy/h per Bq/kg for ^{137}Cs and ^{90}Sr respectively.

Decay corrected activity concentrations of ^{137}Cs and ^{90}Sr in sediments were first calculated (Bq kg^{-1} fresh weight) as:

$$C_{sediment} = \frac{(D_{total}) \text{ x } (e^{-\lambda_r t})}{P_{sediment} \text{ x } d_{sediment}}$$

Where $C_{sediment}$ = Fresh weight activity concentration of sediment Bq kg D_{total} = Total deposition of radionuclide in Bq m^{-2}λ_r = Decay constant for radionuclide in d^{-1}t = time in days $P_{sedimentl}$ = wet sediment bulk density in kg m^{-3}$D_{sediment}$ = Depth of sediment in m within which the radionuclide has become mixed.

The dose estimates were based on a wet sediment bulk density of 1300 kg m^{-3} and assuming mixing to a depth of 0.15 m (Smith et al., 2005). The organism was assumed to occupy the sediment-water interface. Strontium – 90 (^{90}Sr) and radiocaesium (^{137}Cs) are the two major contributors to environmental radiation doses received by biota over chronic time scales (IAEA, 1995). Sr-90 is associated with fuel particles and shows a rapid decline with distance from Chernobyl relative to radiocaesium (Mück et al., 2002). Consequently, total doses from Strontium were only considered at Yanovsky Crawl and Glubokoye lake (3 and 10 km from the Chernobyl NPP respectively) as concentrations at other water bodies are known to be insignificant (Murphy et al., 2011) in comparison with radiocaesium. A generic value of 0.06 μGy/h was added to estimate cumulative external radiation doses in study lakes to account for natural background radiation (Murphy et al., 2011).

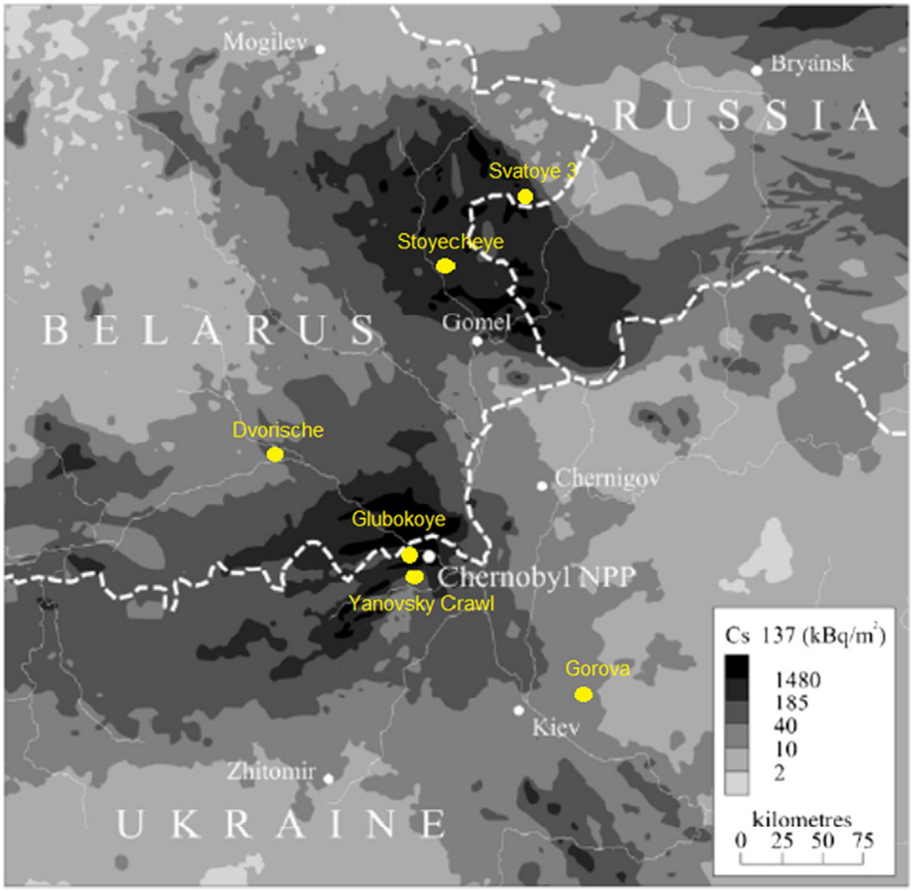

Fig. 1. Map indicating the six sample sites for *A. aquaticus*. Radiocaesium deposition is indicated. Reproduced from Smith and Beresford (2005) with permission.

2.4. Estimation of internal dose rates at sample sites

Internal dose rates were calculated based on average measurements of ^{137}Cs and ^{90}Sr in lakes at various depths (see Table 3). Dose estimates were made using the ERICA tool (V 1.2) and DCC's for internal beta-gamma radiation based on user-defined geometry (see Section 2.3). DCC values of 1.9×10^{-4} and 6.3×10^{-4} μGy/h per Bq/kg were calculated for ^{137}Cs and ^{90}Sr respectively. Since no data exists for activity concentrations of ^{137}Cs and ^{90}Sr in the least contaminated lake, Gorova, a value of 0.063 μGy/h was added to account for background exposure following Kryshev and Sazykina (1995).

2.5. Calculation of fluctuating asymmetry

Four traits were selected for analysis of FA in *A. aquaticus*; first antennae length, propodos and merus width of the first paraeopod and carpus width of the second paraeopod. Selection of these traits was based on previous studies using similar characters to demonstrate a relationship between environmental stress and FA in *Asellus aquaticus* (e.g. Savage and Hogarth, 1999). A preliminary study (Data not shown) further demonstrated that the selected traits were robust, easily quantifiable and damage during handling would not bias measurements. As an additional measure, the number of segments of the first antennae was quantified following three replicate counts. Asymmetry in segment number has been previously demonstrated in *A. aquaticus* and appears to be linked to antennal length asymmetry (Savage and Hogarth, 1999). A total of 3988 measurements were conducted on 394 organisms across the six lakes. Organisms were sexed by analysis of the pleopods and measurements of body length conducted following Bertin et al. (2002). Characters were dissected, mounted on slides and photographed using a Leica DFC310 camera following flattening under a 22×40 mm coverslip. Two independent blind measurements were taken on each trait using ImageJ (v 1.48). All measurements were conducted by one researcher (NF) over a two week period to minimise inter-observer variability (Lee, 1990). FA was calculated using the FA1 index as: FA = Mean |R-L|, where R and L represent the right and left side trait measurements in μm respectively. For comparisons of mean pooled FA values across

Table 1
Physical and chemical characteristics of the six study lakes. ND = No Data.

Lake	Conductivity (μS/cm)	Oxygen saturation (%)	pH	Temperature (°C)	Max depth (m)[a]	Surface area (km²)[a]	Distance from Chernobyl (km)
Svyatoye-3	122.1	81.6	7.8	23.00	2.9	0.250	225
Stoyacheye	230	89.4	8.7	24.05	17.0	0.460	157
Dvorsiche	200	68.9	7.82	23.73	3.5	0.128	95
Glubokoye	184.8	66.3	7.92	24.92	7.3	0.100	10
Yanovsky Crawl	275	108.5	9.4	23.29	ND	ND	3
Gorova	178.5	185	9.69	22.17	ND	ND	125

[a] Data from Smith et al. (2005).

Table 2
Estimated activity concentrations of sediment and concomitant external doses received by *Asellus aquaticus* based on radiocaesium and strontium deposition at six localities in Belarus and Ukraine. Data from Smith et al. (2005) unless otherwise stated.

Lake	^{137}Cs deposition at site (kBq m^{-2})	^{137}Cs activity of sediment (Bq kg^{-1})	External dose rate ^{137}Cs (μGy/h)	^{90}Sr deposition at site (kBq m^{-2})	^{90}Sr activity of sediment (Bq kg^{-1})	External dose rate ^{90}Sr (μGy/h)	Total external dose rate (μGy/h)
Glubokoye	15500[a]	40,706	7.84	12,000	30,526	7.51	15.35
Yanovsky Crawl	14800[b]	38,867	7.5	16,300	41465[a]	10.2	17.7
Svyatoye 3	1748	4591	0.886	ND	N/A	N/A	0.886
Stoyacheye	288	756	0.145	ND	N/A	N/A	0.145
Dvorische	100[c]	262[d]	0.0513	ND	N/A	N/A	0.0513
Gorova	10	26	1.93E−03	ND	N/A	N/A	1.93E−03

[a] Data from Sansone and Voitsekhovitch (1996).
[b] Data from Nazarov and Gudkov (2009).
[c] De Cort et al. (1998).
[d] Data from Ukrainian Hydrometerological Institute, Kiev.

sites, the size-corrected FA2 index was employed: FA2 = mean [|R − L| / (R + L) / 2)] to account for differences in trait size as suggested in Palmer (1994).

2.6. Statistical analyses

A two-way mixed model ANOVA was performed on asymmetry data for each trait x lake combination, with individual as a random factor and orientation as a fixed factor. This procedure tests for the significance of all between-sides variation relative to measurement error (ME) (Palmer, 1994). This method also tests significance of directional asymmetry (DA), a pattern of variation wherein one side is consistently larger than the other, relative to FA simultaneously. Additional one-sample t-tests were performed against a mean of zero to further test for the presence of DA (Baker and Hoelzel, 2013). The aforementioned procedures cannot deduce whether the observed pattern of variation is due to true FA or asymmetry. Asymmetry refers to a pattern of R-L variation characterised by a bimodal distribution either side of zero (Palmer and Strobeck, 1986). Tests for asymmetry were performed using conventional kurtosis statistics and one-sample Kolmogorov-Smirnov tests. Finally the presence of size dependence, the association between organism size and magnitude of FA, must be accounted for as it can confound observed differences in FA between sites if different size ranges are present. Non-parametric tests of association are preferred for such analysis since they do no assume homogeneity of variance (Palmer and Strobeck, 2003). Spearman's rank-order correlation coefficients were therefore used to test the degree of size dependence of each trait with *A. aquaticus* body length.

Differences in levels of FA between sites were tested using multiple approaches. Firstly, a one-way ANOVA with Bonferroni post hoc tests was performed on size corrected FA data to analyse FA differences amongst lake populations. A general linear model (GLM) was then applied where site and sex were fixed factors and trait a random factor to ascertain the influence of sex on observed FA values. Mean asymmetry values were calculated by averaging replicate measurements to minimise the impact of measurement error (Arnqvist and Martensson, 1998). Owing to the non-normal distribution of the metrical segment asymmetry data, nonparametric analysis of variance (Kruskal-Wallis H Test) was used to analyse asymmetry differences between sites. A Spearman's rank-order correlation was used to assess the relationship between percentage of individuals exhibiting segment asymmetry and total dose rate.

The relationship between asymmetry and measured environmental parameters was analysed using regression analysis on log-transformed data. Bathymetric features such as lake area and maximum lake depth were considered to be time invariant and thus were not included in the analysis.

3. Results

3.1. Estimated radiation doses received by biota

Tables 2 and 3 display the estimated external and internal radiation doses at each of the sampling sites based on ^{137}Cs and ^{90}Sr activities in sediment and water. Maximum and minimum total doses were calculated at Glubokoye lake (27.1 μGy/h) and Gorova respectively (0.06 μGy/h, see Table 3).

Dose rate estimates based on deposition of radionuclides at study sites are subject to a significant degree of uncertainty, owing to the necessary simplifications and assumptions made to calculate these values (see Murphy et al., 2011). However, direct measurements of external radiation doses at Svyatoye-3 by Pungkun (2012) using a thermoluminescent dosimeter (TLD) array provided good agreement with dose estimates in the present study (0.8–1.8 μGy/h for measured average sediment concentrations), suggesting estimates in the present study provide an adequate assessment of radiation doses received by biota.

3.2. Departures from normality and measurement error

Conventional kurtosis statistics of each trait × lake combination are shown displayed below in Table 4. The majority of lake × trait combinations demonstrated slight leptokurtosis which is not attributed to antisymmetry. Where platykurtic distributions existed, Kolmogorov-Smirnov tests and visual inspections of FA frequency distributions

Table 3
Estimated internal and total doses received by *Asellus aquaticus* at six lakes along a contamination gradient in Belarus and the Ukraine based on activity concentrations of ^{137}Cs and ^{90}Sr in water and sediment. ^{137}Cs and ^{90}Sr in water based on average measurements at different depths in 2003.

Lake	^{137}Cs in water (Bq l^{-1})	Internal dose ^{137}Cs (μGy/h)	^{90}Sr in water (Bq l^{-1})	Internal dose ^{90}Sr (μGy/h)	Total internal dose (μGy/h)	Total dose rate (μGy/h)
Glubokoye	7.57	1.16	77.7	10.5	11.66	27.1
Yanovsky Crawl	2.2	0.336	18.7[a]	2.53	2.866	20.6
Svatoye #3	7.8	1.19	N/A	N/A	1.19	2.2
Stoyacheye	4.24	0.647	N/A	N/A	0.647	0.872
Dvorische	4.29	0.655	N/A	N/A	0.655	0.786
Gorova	ND	0.063	N/A	N/A	0.063	0.064

[a] Data based on average measurements from 1997 to 2008 from Nazarov and Gudkov (2009).

Table 4
Kurtosis statistics of each lake × trait combination.

	Gorova	Dvorische	Stoyacheye	Svyatoye-3	Yanovsky Crawl	Glubokoye
First antennae	−0.113	1.348	0.963	2.743	3.794	2.456
Propodos width	1.974	0.702	0.278	1.851	1.173	3.151
Merus width	2.923	0.035	−0.242	−0.642	0.824	0.879
Carpus width	0.128	0.507	0.087	0.641	0.807	0.763

revealed no significant differences from normality ($p > 0.05$). Analysis of the metrical dataset (number of antennal segments) revealed a leptokurtic distribution (high, narrow peak) which was significantly different from the normal distribution (Kolmogorov–Smirnov test, $p < 0.05$). This was attributed to the low range of observed R-L values in the data and is frequently observed in metrical traits (i.e. pectoral fin rays in fishes [Johnson et al., 2004; Young et al., 2009]). Since antisymmetry is characterised by a platykurtic distribution (Palmer and Strobeck, 1986), and segment asymmetry did not significantly differ from 0, antisymmetry was not considered.

3.3. Size dependence

No correlation between body length of asellids and magnitude of asymmetry (Spearman's rank order correlation coefficient, $p > 0.05$) was recorded indicating that size dependence was not evident in the present study.

3.3.1. Fluctuating asymmetry

Significant differences in FA were recorded amongst sample sites ($p < 0.05$, see Table 5) indicating differences in developmental stability between localities (see Table 5).

A post-hoc Bonferroni test revealed significant differences in FA values between Svyatoye-3 and all sites excluding Dvorische and Glubokoye lake (see Fig. 2) when information from multiple traits was considered ($p \leq 0.05$). At Svyatoye-3, mean pooled asymmetry of four traits was 0.063 ($n = 228$), significantly greater than Yanovsky Crawl (0.043, $n = 274$), Stoyacheye (0.032, $n = 271$) and Gorova (0.045, $n = 277$). First antennae length exhibited the greatest degree of asymmetry overall (0.052, $n = 398$), however differences between trait were not significant ($p > 0.05$). Lowest mean asymmetry values were recorded for the carpus of the second paraeopod (0.044, $n = 394$). The general linear model revealed no significant differences in asymmetry between sexes, independent of locality or specific trait measured ($p > 0.05$, see Table 5).

3.3.2. Fluctuating asymmetry in segment number

No significant differences in the magnitude of segment asymmetry (calculated as |R-L|) were recorded between sampling sites (Kruskal-Wallis H, $p > 0.05$). Maximum right-left differences of 4 segments were displayed at lakes Glubokoye and Svyatoye-3. The highest % of individuals exhibiting segment asymmetry was Svyatoye-3, wherein 35.6% of asellids exhibited asymmetry in segment number. The lowest number of individuals displaying segment asymmetry (21.7%) was recorded at Gorova. Antennal segment asymmetry and total dose rate approached a positive relationship ($r^2 = 0.51$), however this relationship was non-significant (Spearman's rank-order correlation, $p > 0.05$). The raw data showed that in 72% of cases, asymmetry of segment number and antennal length was in the same direction, suggesting that antennal asymmetry in length and number of segments is linked as originally proposed in Savage and Hogarth (1999).

3.3.3. Fluctuating asymmetry in relation to environmental parameters

Regression analysis found no significant relationship between pooled FA and any of the measured environmental parameters including conductivity ($R^2 = 0.063$, $p = 0.330$, $df = 1$, $F = 1.015$), temperature ($R^2 = 0.012$, $p = 0.663$, $df = 1$, $F = 0.198$), pH ($R^2 = 0.067$, $p = 0.301$, $df = 1$, $F = 1.141$) and dissolved oxygen levels ($R^2 = 0.017$, $p = 0.603$, $df = 1$, $F = 0.281$).

3.3.4. Fluctuating asymmetry in relation to total radiation dose

Mean asymmetry at the most highly contaminated lake, Glubokoye (total dose rate of 27.1 μGy/h) was 0.053, 1.2 times greater than individuals from the site of lowest contamination, Gorova (total dose rate of 0.06 μGy/h) although these differences were non-significant (Bonferroni, $p > 0.05$). Mean asymmetry at Yanovsky crawl, with total dose rates of 20.6 μGy/h, was 0.043, within the range of sites exhibiting dose rates up to two orders of magnitude lower (i.e. Gorova). The relationship between asymmetry and radiation dose rate along the contamination gradient is shown in Fig. 3. The value for the regression slope was 0.008 (see Fig. 3), demonstrating the lack of relationship between the two variables which was non-significant ($p > 0.05$).

4. Discussion

The present study aimed to determine the impact of Chernobyl-derived radionuclides on the developmental stability of the aquatic invertebrate, *Asellus aquaticus*, using fluctuating asymmetry as an indicator. Six localities were sampled encompassing the full range of radiocaesium contamination across both Belarus and Ukraine. No relationship between radiation dose rate and pooled asymmetry of four morphometric traits was found. Asymmetry in segment number of the first antennae approached a positive relationship with radiation dose rate; however this was non-significant (see Section 3.4.2).

These findings are different to those in the available literature which suggest an increase in FA in response to radiation exposure. This has been documented for a range of biota including fish (Lajus et al., 2014; Zakharov et al., 1996), small mammals (Gileva and Nokhrin, 2001; Oleksyk et al., 2004), birds (Møller, 1993), insects (Møller, 2002) and plants (Møller, 1998). Studies of aquatic invertebrates from the Chernobyl region further support an increase in FA as a result of radionuclide exposure. Yavnyuk et al. (2009) measured FA in two species, the zebra mussel *Dreisenna polymorpha* and floating pondweed, *Potamogeton natans*. The authors recorded a 25-fold increase in asymmetry of *D.*

Table 5
Results of the general linear model analysis with a mixed model ANOVA performed on size corrected asymmetry data in *A. aquaticus*. Site and sex were fixed effects, trait a random effect.

Source of variation	Mean square	F value	Df	p value	Interpretation
Site	0.014	3.238	5	0.035	FA varies between localities
Sex	0.000	0.704	1	0.803	FA does not differ between sexes
Trait	0.006	1.513	3	0.396	FA does not vary dependent on trait
Site * sex	0.001	0.309	5	0.900	FA differences between sexes not dependent on site
Site * trait	0.004	2.383	15	0.052	FA differences between sites not dependent on trait
Sex * trait	0.003	1.402	3	0.280	FA difference between traits not dependent on sex

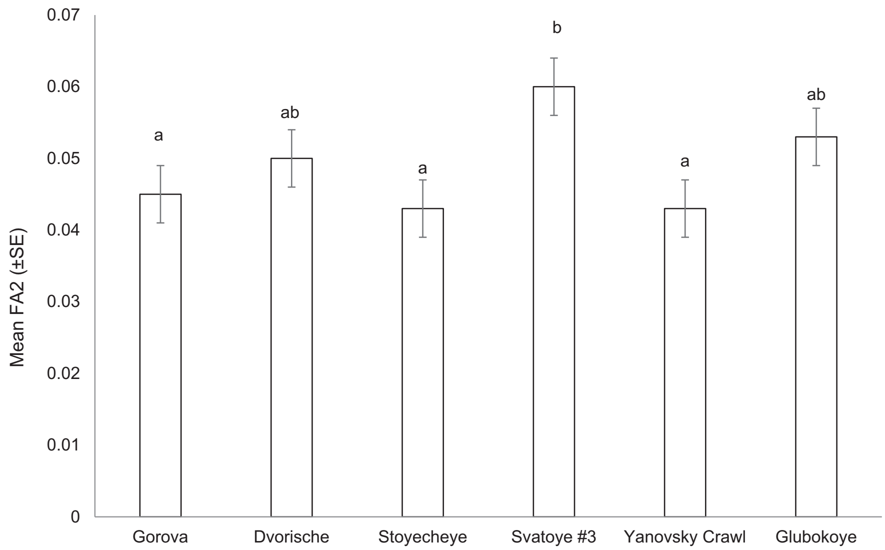

Fig. 2. Fluctuating asymmetry values (mean ± SE; FA = |R − L| / ((R + L) / 2)) pooled from four traits (first antennae length, propodos and merus width of the first paraeopod, and carpus width) in *Asellus aquaticus* collected from six lakes along a gradient of radionuclide contamination in Belarus and Ukraine. Sites are plotted in order of increasing contamination from left to right. Different letters denote significant differences ($p < 0.05$) between sites, matching letters signify no significant difference.

polymorpha collected from the Chernobyl cooling pond compared to reference areas within the Dnieper river. A smaller increase in *P. natans* FA of 1.4-fold compared to samples collected from the Kiev reservoir was documented. Molluscs have been demonstrated to accumulate and retain radionuclides at concentrations orders of magnitude higher than other aquatic biota (Frantsevich et al., 1996; Jaeschke et al., 2015) and are susceptible to elevated dose rates. For example, Gudkov et al. (2016) recorded dose rates of 350–420 μGy/h in the freshwater snail, *Lymnaea stagnalis* collected from Glubokoye lake over a study period from 1998 to 2014. Dose rate estimates to *Asellus aquaticus* in the present study for Glubokoye lake were 27.1 μGy/h, an order of magnitude lower. The lower dose rates received by *A. aquaticus* in the present study may not have been to sufficient to cause a discernible increase in developmental instability as indicated by FA.

Some studies have proposed exposure thresholds below which no discernible increase in FA above background level is expected. For example, Oleksyk et al. (2004) found significant correlations between intramuscular ^{137}Cs concentrations and right-left differences in the yellow-necked mouse, *Apodemus flavicollis* collected from around the Chernobyl exclusion zone. The authors suggested a dose range for internal exposure of 0.132–0.297 μGy/h above which FA may increase significantly above background level. Five of the six sampled sites (See Table 3) in the present study had total internal dose rates exceeding this threshold, with no discernible increase in FA. This may be owing to inter-species differences in response to elevated doses of radiation. *Asellus aquaticus* has been shown to be resistant to a range of toxicants and capable of life history adaption to favour habitation of polluted environments (Fraser, 1980; Maltby, 1991). For example, Maltby (1991) demonstrated adaptive modification of reproductive investment toward fewer, large offspring in *A. aquaticus* inhabiting chronically contaminated sites as compared to a clean site. Although radiosensitivity varies greatly within taxa (Harrison and Anderson, 1996), it is generally accepted that radiosensitivity increases with the degree of biological complexity (Copplestone et al., 2001). Organisms such as birds, trees and mammals are believed to be more radiosensitive than invertebrates (Hinton et al., 2007). Consequently, dose thresholds for induction of FA

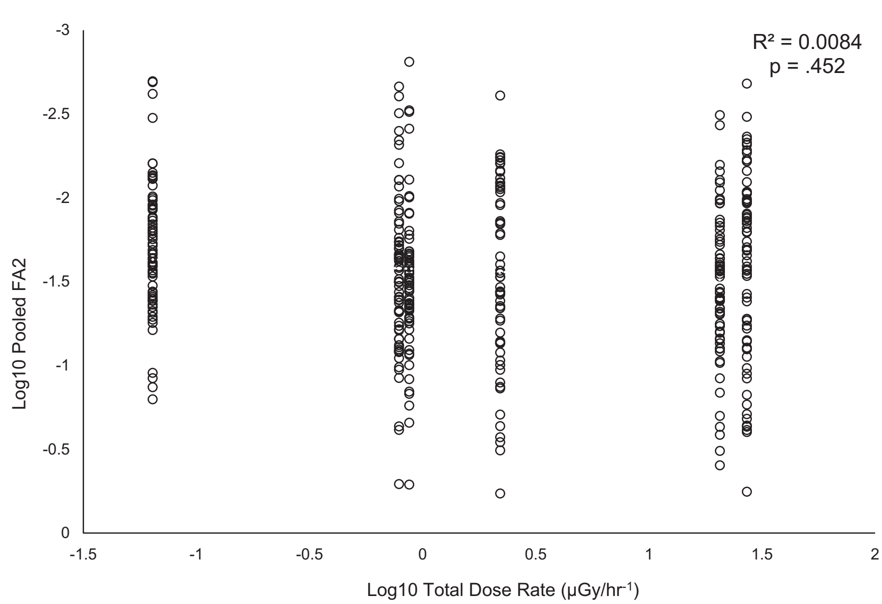

Fig. 3. Scatterplot of total dose rate in relation to pooled asymmetry of four morphometric traits (calculated as FA = |R-L|/((R + L)/2)) in *Asellus aquaticus* from the Chernobyl region.

may be significantly lower in vertebrate organisms and studies of FA may not be directly comparable.

Fluctuating asymmetry has frequently been reported to have low heritability (Kruuk et al., 2003; Leamy and Klingenberg, 2005; Woods et al., 1998) which is advantageous in its use as an environmental monitoring tool (Palmer and Strobeck, 1986) since observed patterns are expected to reflect stress within the population rather than its genetic structure. Therefore the lack of increase in FA recorded in the present study suggests that current dose rates in the Chernobyl region are insufficient to cause an increase in developmental instability in *A. aquaticus*. Assuming *A. aquaticus* to be univoltine (Brattey, 1986), populations have undergone almost thirty generations since the Chernobyl incident. It is therefore possible that organisms may have adapted to chronic radiation stress. Studies have demonstrated adaptation of aquatic invertebrate populations exposed to a range of pollutants over relatively short timeframes (Goussen et al., 2015; Hochmuth et al., 2015; Sun et al., 2014). For example, Sun et al. (2014) found evidence for adaptation to tributytltin oxide (TBTO) in the sexually reproducing marine copepod, *Tigriopus californicus* in seven generations. Elevated mutation frequencies have been reported in a range of organisms from Chernobyl (see Møller & Moussea, 2015 for meta-analysis). However, studies of potential adaption to chronic radiation stress are limited and at present inconclusive (i.e. Geras'kin et al., 2011; Klubicová et al., 2010). Future work should focus on the application of whole genome techniques to study mutation rates and potential adaptation to chronic radiation stress in Chernobyl biota.

In the present study, first antennae length exhibited the greatest degree of asymmetry (see Section 3.4). In many crustacean species, antennae are considered a secondary sexual character involved in the detection and location of receptive females (Dunn, 1998; Lefebvre et al., 2000). In *A. aquaticus*, first antennae possess aesthetascs (Wägele, 1983) which are believed to have a chemosensory function in mate detection, supporting the role of this trait as a secondary sexual character. Møller and Pomiankowski (1993) argue that patterns of FA within secondary sexual characters differ from typical morphological traits and are often significantly greater in the former. This is thought to be due to production costs in developing elaborate secondary sexual characters and the strong associated directional selection which may select against modifiers that buffer development (Manning and Chamberlain, 1994). The elevated FA in first antennae in the present study supports an increase in FA in secondary sexual characters relative to typical morphological traits.

Significantly greater mean asymmetry was observed at Svyatoye-3 relative to all sites excluding Dvorische and Glubokoye lake when data from multiple traits was considered. Differences in asymmetry were not found to be related to any of the measured environmental parameters using regression analysis (see Section 3.4.2). Elevated developmental instability at this site may be due to a biotic stressor, for example an increased parasitic burden. A number of studies have demonstrated positive correlations between parasite burden and FA (i.e. Alibert et al., 2002; Bonn et al., 1996; Cuevas-Reyes et al., 2011). *Asellus aquaticus* is an intermediate host to acanthocephalan parasites (Brattey, 1986) and has also been demonstrated to harbour *Wolbachia*, a parasitic intracellular bacteria (Bouchon et al., 1998). Acanthocephalan infection has been linked to an increase in FA in another freshwater aquatic invertebrate species, *Gammarus pulex*, which occupies an overlapping ecological niche (Alibert et al., 2002; Graça et al., 1994). Furthermore, sex ratios of *A. aquaticus* at Svyatoye-3 were skewed in the favour of females relative to other sites (0.31:0.69 males to females, Data not shown), which could be indicative of *Wolbachia* infection, since this parasite typically causes feminization of host organisms (Rigaud et al., 1999). Future research will aim to quantify the prevalence of *Wolbachia* infection in Belarusian and Ukrainian populations of *A. aquaticus* using a PCR-based approach.

5. Conclusions

No relationship between total radiation dose rates and magnitude of FA was recorded in the present study. The percentage of individuals exhibiting segment asymmetry approached a positive relationship with total dose rate; however, this was not found to be statistically significant. Significantly elevated FA was recorded at a single site, and was not attributed to radiation dose or any of the measured environmental parameters, suggesting a biotic stressor driving an increase in developmental instability. Whilst Lajus et al. (2014) recorded only a minor impact of radiation on FA in two fish species, this finding appears to be novel in the literature in finding no discernible impact of radiation on FA in biota from the Chernobyl region. The present study utilized the full range of contamination in aquatic systems from within the highly contaminated 10 km zone to 225 km in distance from the NPP (see Fig. 1), providing a comprehensive assessment of the potential effects of Chernobyl-derived radiation on FA in *A. aquaticus.* In contrast, many of the previous studies of FA in Chernobyl biota utilized only a single contaminated and reference site. For example, Møller (2002) and Lajus et al. (2014) studied FA in stag beetles and fish respectively from only a single contaminated and reference area. The greater range of dose rates in the present study strengthens any observed associations between radionuclide contamination and FA and may account for the differences between the present and previous studies of developmental instability in Chernobyl biota.

The significance of the Chernobyl incident for both members of the public and policy makers presents challenges for the scientific community, as described by Chesser and Baker (2006). It is imperative that negative results are regarded in the same manner as those reporting positive results. Recent studies (i.e. Deryabina et al., 2015; Webster et al., 2016) have recorded abundant wildlife populations in the Chernobyl exclusion zone and challenge perceptions of the detrimental impacts of radiation exposure. Clearly, the effects of radiation on non-human organisms are not ubiquitous and likely depend on dose rate, species and effect endpoint. Further robust studies of individual level effects are necessary. The lack of increase in FA in the present study challenges previous findings of the impacts of chronic radiation exposure on the development of organisms. Such findings will aid in developing risk assessments of the impact of chronic radiation exposure on biota and will help elucidate the long term impacts of large scale nuclear incidents such as Chernobyl and Fukushima.

Conflict of interest

The authors declare no conflict of interest.

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