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Evaluation of cataract formation in fish exposed to environmental radiation at Chernobyl and Fukushima

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Keywords: Radiation; Cataract; Fish; Chernobyl; Fukushima; Radiocaesium; Radiostrontium; Dose; SAXs

Abbreviations: CMOS, Complementary metal-oxide-semiconductor; DMSO, dimethyl sulphoxide; GLM, generalised linear model; HEPES; N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid; h, hours; ICRP, International Commission on Radiological Protection; IR, ionising radiation; mGy milli-Gray; PFA, paraformaldehyde; PBS, phosphate buffered saline; SAXS, small angle X-ray scattering.

Highlights

Most detailed analysis of cataract formation in free-living wild animals exposed to radiation

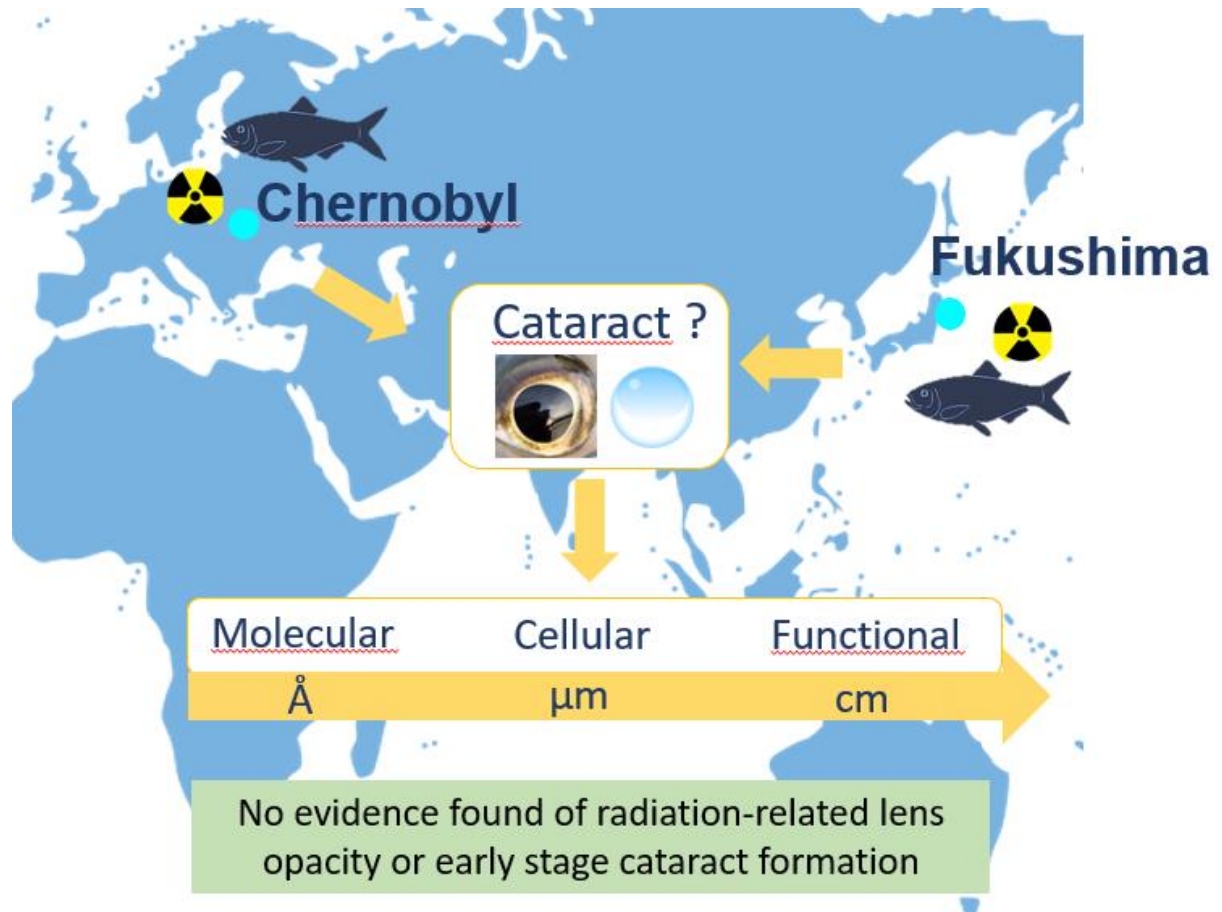
Protein aggregation, epithelial cell density and focal length studied

Epithelial cell density study helps to inform on radiation impacts over time as the lens develops

No evidence found of radiation-related lens opacities

No evidence found of early stage cataract formation

Graphical Abstract



Abstract

Recent studies apparently finding deleterious effects of radiation exposure on cataract formation in birds and voles living near Chernobyl represent a major challenge to current radiation protection regulations. This study conducted an integrated assessment of radiation exposure on cataractogenesis using the most advanced technologies available to assess the cataract status of lenses extracted from fish caught at both Chernobyl in Ukraine and Fukushima in Japan. It was hypothesised that these novel data would reveal positive correlations between radiation dose and early indicators of cataract formation.

The structure, function and optical properties of lenses were analysed from atomic to millimetre length scales. We measured the short-range order of the lens crystallin proteins using Small Angle X-Ray Scattering (SAXS) at both the SPring-8 and DIAMOND synchrotrons, the profile of the graded refractive index generated by these proteins, the epithelial cell density and organisation and finally the focal length of each lens.

The results showed no evidence of a difference between the focal length, the epithelial cell densities, the refractive indices, the interference functions and the short-range order of crystallin proteins (X-ray diffraction patterns) in lens from fish exposed to different radiation doses. It could be argued that animals in the natural environment which developed cataract would be more likely, for example, to suffer predation leading to survivor bias. But the cross-length scale study presented here, by evaluating small scale molecular and cellular changes in the lens (pre-cataract formation) significantly mitigates against this issue.

1 **Introduction**

2

3 The formation of cataract in humans due to occupational or accidental exposure to acute
4 ionising radiation is well documented (ICRP, 2012). Data on cataract incidence with long
5 follow up periods of atomic bomb survivors, astronauts, residents of the Chernobyl nuclear
6 exclusion zone and radiation workers have shown that radiation associated lens opacities
7 occurred at much lower doses than previously thought (Bouffler et al., 2012). Consequently,
8 the International Commission on Radiological Protection (ICRP) decreased the risk threshold
9 of the absorbed dose from 2 to 0.5 Gy (ICRP, 2012), which is within the range of possible
10 cumulative lifetime doses to which animals at Chernobyl and Fukushima could be exposed.

11

12 Little is known about the effect of long-term chronic exposure through the life span of
13 organisms exposed to ionising radiation in the natural environment. In the laboratory,
14 amphibians, rabbits, rodents and fish have all been used as models for the effects of radiation
15 upon the eye and the lens (Worgul et al, 1976; Barnard et al, 2019; von Sallmann 1951; Geiger
16 et al., 2006). Lens development is very highly conserved from teleost fish to man (Wu et al.,
17 2015; Greiling et al., 2010; Cardozo et al., 2023; Morishita et al., 2021; Kamei and Duan, 2021;
18 Wang et al., 2021). Studies in the natural environment have proved contradictory. While a
19 significant increase in cataract incidence was claimed in birds and voles at Chernobyl
20 (Mousseau and Møller, 2013; Lehmann et al., 2016) (the latter at cumulative doses lower than
21 1 mGy). A study at Fukushima at cumulative dose rates up to 1600 mGy did not, however, find
22 any significant effects on cataract formation in wild boar (Pederson et al., 2020).

23

24 An in-vitro laboratory study (Kocemba and Waker, 2021) on rainbow trout lenses found no
25 change in focal length variability at doses up to 2200 mGy (acute). Studies finding significant
26 effects of radiation on cataract in wildlife at Chernobyl (Mousseau and Møller 2013, Lehmann
27 et al., 2016) have generated significant media coverage and, if true, would represent a major
28 challenge to current radiation protection regulation. The Lehman et al. (2016) study has,
29 however, been criticised due to its subjective nature, poor sample preservation and the lack of
30 evidence to support its conclusions (Smith 2020). Similarly, a study of cataracts in birds
31 (Mousseau and Møller 2013) has been criticised for lack of ophthalmologic expertise in
32 identifying cataract incidence (Pederson et al. 2020).

33

34 An opaque lens is highly disadvantageous for the survival of wild organisms as clear vision is
35 required for hunting, feeding and to evade predation. Therefore, studies finding no effect of
36 radiation on cataract incidence could select for individuals lacking cataracts or with
37 compromised vision. Pre-cataract phenotypes can be identified by *in vivo* evaluation methods
38 that measure the loss of lens transparency because of protein aggregation and epithelial cell
39 density changes (Markiewicz et al., 2015; Barnard et al 2018; Vigneux et al 2022). Our study
40 uses two different techniques to measure such changes which can indicate early-stage cataract
41 formation and are therefore more sensitive and less subjective than previous evaluations which
42 apparently observed radiation induced cataract in free-living organisms (Mousseau and Møller
43 2013; Lehmann et al., 2016). The first method we use measures cell densities using confocal
44 light microscopy and image analysis software to create three-dimensional maps for the
45 epithelium (Kalligeraki et al., 2020). The second method measures potential ultrastructural
46 changes to the constituent crystallin proteins within the lens fibre cells using the low angle X-
47 ray diffraction method. Changes in the structural conformation of lens crystallin proteins
48 manifest themselves as protein aggregates leading then to an opacity. The degree of this opacity
49 can be accurately and sensitively measured using small angle X-ray scatter (SAXS) (Regini et
50 al., 2004; Regini and Meek, 2009).

51

52 Fish species are highly relevant to study ionising radiation effects since they reflect the health
53 of the environment and can be used as surrogates in the assessment of the effects of stressors
54 on human health (Sipes et al., 2011; Horzmann & Freeman, 2018; Pinna et al., 2023).
55 Moreover, they are considered as the most radiosensitive aquatic species (Sazykina and
56 Kryshev, 2003) and have been highly exposed in freshwater systems at Chernobyl and both
57 freshwater and marine systems in Fukushima. At Chernobyl, the total dose absorbed in a year
58 for perch and roach can reach 142 and 129 mGy respectively and a cumulative dose at the time
59 of catch is, respectively, about 645 and 710 mGy over a 5-year life span. These levels are more
60 than 100 times higher than the levels hypothesised to induce cataract in voles from Chernobyl
61 (Lehmann et al., 2016) and higher than the ICRP recommended thresholds for human cataract
62 risk. It should be noted, however, that (as discussed below) dose estimation to the lens of free-
63 living organisms is highly uncertain.

64

65 For the first time, the methods applied here provide an objective and quantitative analysis of
66 potential radiation influence of cataract formation in free-living organisms. Uniquely, fish lens

67 sample from both Chernobyl and Fukushima are analysed, allowing comparison of fish of
68 different exposure and life histories. It was hypothesised that these novel data would reveal
69 positive correlations between radiation dose and cataract formation.

70

71 **Methods**

72

73 *Optimisation of lens cryopreservation*

74 An experiment was performed to optimise the cryopreservation buffer composition suitable to
75 preserve the structure of the fresh lens from collection in the field to the laboratory analyses.
76 To our knowledge this is the first cryopreservation buffer composition optimised for lens
77 preservation. Rainbow trout lenses were used to test the efficiency of 21 different
78 cryopreservation buffers by using a teleost buffer (composed of 111 mM NaCl; 5.4 mM KCl;
79 1 mM CaCl₂; 0.6 mM MgSO₄ and 5 mM HEPES) and varying the concentration of sucrose
80 (100 to 200 mM), glycerol (0 to 100%) and DMSO (0 to 100%) for optimal preservation of the
81 lens. A total of 31 fish and their 62 lenses were used. Three replicates were used for each
82 condition except for one in which two replicates were used. Samples were kept for a period of
83 8 days at -196 °C in the dry shipper used for field samples collection. They were defrosted at
84 4°C for 24h.

85 The most intact lenses were assessed by observing transparency and any signs of opacity or
86 structural changes using an optical microscope (Zeiss) together with statistical analyses (glm,
87 binomial).

88

89 *Field study*

90 Field studies were carried out in seven lakes in Ukraine and Belarus and two ponds and a river
91 in Japan (Figure 1). Studies on the genetic and physiological health of fish in seven lakes in
92 Belarus and Ukraine with a long-term exposure history to a gradient of radiation dose have
93 brought significant accompanying data to enable calculation of dose rate and quantification of
94 potential confounding factors such as parasite loads, water chemistry (major cations, nitrates)
95 environmental parameters (pH, T°C, dissolved oxygen) and other radioisotopes specific
96 activity (Lerebours et al., 2018; 2020). Several studies in Japan in fish from water ponds and
97 rivers from Fukushima prefecture characterised the Cs activity and distribution (Wakiyama et
98 al., 2017; Wada et al., 2019) also enabling a robust dose rate calculation.

99 One lens of each of 103 perch (*Perca fluviatilis*) collected in spring 2016 at Chernobyl in seven
100 lakes representing a gradient of radiation dose were analysed for refractive index, length radius
101 and focal length measurements (Table S1). Perch from a control lake (Dvorische), outside the
102 exclusion zone, and from a highly contaminated lake (Glubokoye), inside the exclusion zone,
103 were analysed for refractive index and Bragg Spacing using the Spring 8 synchrotron and using
104 the Diamond synchrotron (Table S2). An old (>12 years) carp (*Cyprinus carpio*) with visible
105 cataract from the cooling pond was collected and analysed as a positive control. Carp
106 (*Carassius auratus*) were also collected in ponds inside the exclusion zone in the Fukushima
107 prefecture (Suzuuchia and Funazawa Ponds; Wakiyama et al., 2017) and outside the exclusion
108 zone, in Abukuma river (Shinobu Dam; Mitamura et al., 2022) for SAXS analyses (Table S2).

109

110 The age was determined by counting the number of annuli on the scales (Chernobyl lakes) and
111 otoliths (ponds from Fukushima prefecture). Age is given in SI Table S1, being in the range 4-
112 5 years for Chernobyl fish and 6-7 years for fish at Fukushima.

113

114 *Dose estimation*

115 Internal and external dose was estimated from measurements of radioactivity in whole fish,
116 fish lenses and sediments.

117

118 Lens samples were analysed at GAU-Radioanalytical were as follows: lenses were transferred
119 to counting vials for analysis as received by gamma spectrometry. High-resolution gamma
120 spectrometric analysis was performed using HPGe detectors. Detectors were calibrated against
121 a mixed radionuclide standard solution. The standard was used to prepare a source of identical
122 geometry to that of the samples. Gamma spectra were analysed and individual radionuclides
123 quantified using Fitzpeaks spectral deconvolution software (JF Computing Services).

124

125 For radiochemical analysis, lens samples were spiked with ^{85}Sr , ^{232}U , ^{242}Pu and ^{243}Am tracers
126 for chemical recovery monitoring.

127

128 Measurements of radioactivity in sediments at Fukushima were taken from Konoplev et al.
129 (2018) and those in fish followed the radioanalytical method described in Wada et al. (2019)
130 Measurements of radioactivity in sediments and fish at Chernobyl were taken from Lerebours
131 et al. (2018). Internal and external doses were calculated using the ERICA tool (Brown et al.
132 2008).

133

134 *Focal length and effective refractive index measurements*

135 The effective focal length and (uniform) refractive index measurements were made using a
136 previously published approach (Young et al., 2018) with a modified set up, as shown in
137 Supplementary Information Figure S1. A custom 3D printed holder was used to suspend the
138 sample lens in a diluted solution of fluorescein such that its optical axis was aligned vertically.
139 A 488 nm fibre-coupled laser source (Stradus Versalase, Vortran) was mounted directly above
140 the sample lens above a collimator. The incident illumination was focused by the sample lens
141 into the fluorescein solution causing localised fluorescence excitation. The fluorescence
142 emission was imaged through a glass viewing window and a spectral filter (MDF-GFP,
143 Thorlabs) by a CCD (QI Click Mono, 01-QICLICK-R-F- M-12, QImaging) that was aligned
144 horizontally on the bench. A brightfield image was also captured by removing the fluorescence
145 filter. The pixel scale of the camera was calibrated by imaging a diffraction grating with two
146 line pairs per mm and the line separation determined to be 10.4 pixels.

147

148 The optimum value for the refractive index was recorded and the effective focal length of the
149 lens was calculated using the ball lens equation:

150

$$151 \quad f = \frac{nR}{2(n - 1)}$$

152

153 where n is ratio of the refractive index of the lens, n_{lens} , to the refractive index of the
154 surrounding medium (assumed to be water, $n_{\text{water}} = 1.333$) and R is the radius of the lens. The
155 method and calibration were verified using a BK7 glass ball lens (04VQ06, Comar), which was
156 found to have a refractive index of 1.526 ± 0.008 . This was in good agreement with the
157 refractive index of BK7 Schott glass at 488 nm, which is 1.522.

158

159 *Hoechst Staining, cell density measurements of the lens epithelia*

160 The measurement of the epithelial cell densities in lenses taken from fish caught in control and
161 exposed lakes is as described (Kalligeraki et al., 2020). The globes had been fixed in 4% (w/v)
162 PFA in phosphate buffered saline (PBS) and the lenses were removed from the globe prior to
163 staining with 10 μ M Hoechst 33324 (Merck Life Sciences, UK) after permeabilising with 0.5%
164 (w/v) Triton-X-100 in PBS for up to an hour at room temperature. The lenses were washed in
165 PBS, then mounted on the acrylamide support and positioned prior to imaging using a Leica
166 SP5 II confocal microscope equipped with an HXC APO \times 10/0.40 NA oil (n = 1.518)
167 immersion objective (Supplementary Information Figure S2). Data collection and analysis are
168 as described (Kalligeraki et al., 2020), collecting data from the meridional rows of the lens
169 epithelium where radiation damage has been reported in mouse models exposed to low dose
170 ionising radiation (IR) (Markiewicz et al., 2015).

171

172 *SPring-8 X-ray interferometric analyses*

173 Fresh piscine lenses from four perch *P. fluviatilis*, two inside (Lake Glubokoye) and two
174 outside (Lake Dvorische) the exclusion zone of Chernobyl and an old carp, *C. carpio*, from the
175 Cooling Pond used as a positive control, were transported from UK to Japan for measurement
176 of refractive index at the SPring-8 synchrotron. One lens was analysed from each of the fish
177 from Dvorische and two lenses from each fish from Glubokoye. Samples were set in 2%
178 agarose gel within a special cell for measurement on beamline BL20B2 using X-ray phase
179 tomography based on X-ray Talbot interferometry (Hoshino et al., 2010; Hoshino et al., 2011;
180 Momose, 2005). More detail on the Spring-8 methods can be found in Supplementary
181 Information.

182 *DIAMOND synchrotron analyses*

183 Small angle X-ray scattering (SAXS) studies on the fish lenses collected from Chernobyl and
184 Fukushima were also conducted at the Diamond Synchrotron (Didcot, Oxfordshire UK) on
185 small angle beam line I22 (Smith et al., 2021). The lenses were placed in an airtight Perspex
186 sample holder with Mylar windows which are X-ray transparent. Each sample holder was
187 secured on to a motorised stage which could be moved horizontally and vertically in respect to
188 the X-ray beam. The X-ray diffraction patterns were collected separately from each lens. The
189 X-ray camera was 6.25 metres in length. The data were collected using an X-ray beam size
190 80 μ m x 250 μ m, with a wavelength of 1 \AA , directed perpendicularly to the whole lens for a 2D

191 grid scan of each lens. The exposure time for each individual X-ray diffraction pattern was 0.1
192 sec at 0.5 mm intervals along the lens horizontal and vertical meridians. Individual X-ray
193 diffraction patterns were recorded using a Pilatus P3-2M(SAXS) detector. Each 2D grid scan
194 varied in size length in both the x and y directions depending on the size of each individual
195 lens. After the data were processed and the background subtracted from each individual X-ray
196 pattern, a montage of all X-ray patterns for each lens was created separately using Diamond's
197 in house DAWN 2.11.0 software package (Basham et al., 2015; Filik et al., 2017).

198

199 *Statistical analyses*

200 All statistical tests were performed using R Studio (v4.0.1). Any potential difference in the
201 refractive index, lens radius and focal length was assessed by performing a t-test. The
202 efficiency of the different buffers on the structural appearance of the lenses after a freezing
203 period of 8 days was assessed by using a generalised linear model (glm) and using a binomial
204 family.

205

206 **Results**

207

208 *Cryopreservation optimisation*

209 The cryopreservation buffer showing the best result ($p < 0.05$) was composed of the teleost
210 buffer and 200 mM of sucrose, 60% (v/v) of glycerol and 40% (v/v) DMSO and was thus used
211 to preserve field samples.

212 *Dose estimation*

213 Fish lens samples were analysed for ^{60}Co , ^{90}Sr , ^{137}Cs , ^{241}Am and isotopes of Pu as well as a
214 range of natural radionuclides. All artificial radionuclides were below l.o.d. except for ^{137}Cs
215 which ranged from 0.08 Bq/g (f.w.) in lake Suuzuchi to 0.32 Bq/g (f.w.) in lake Glubokoye.
216 All natural radionuclides were below l.o.d. except for ^{40}K which was 0.56 Bq/g (f.w.) in
217 Funazawa and 0.64 Bq/g (f.w.) in the Chernobyl Cooling Reservoir, all others being below
218 l.o.d.

219

220 As expected, uptake of artificial radionuclides to the lens was very low, so dose to the lens
221 from radioactivity within the lens is negligible compared to that from the fish body and bed
222 sediment. It was therefore appropriate to estimate dose to the lens from the combination of
223 internal dose from $^{134,137}\text{Cs}$ in the fish body and external dose from sediment. The ERICA

224 software was used to estimate internal and external dose based on measured fish and sediment
225 activity concentration. Sediment activity concentration from the lakes in Japan were obtained
226 from Konoplev et al., (2018). It was assumed that each fish spent 50% of its time near to the
227 sediment and 50% in open water.

228

229 Estimated doses to the lens are given in Table 1. External dose rates are estimated to be
230 significantly greater than internal in the Japanese lakes, but are of the same order as internal in
231 the three most contaminated Ukrainian lakes due to the significance of ^{90}Sr beta dose within
232 the CEZ. Uncertainty in external dose estimates is driven primarily by the assumed occupancy
233 factor (the amount of time the fish is close to bed sediments). Given this and other uncertainties,
234 estimated dose rates should be treated as relative rather than absolute. Natural background
235 radiation doses to aquatic organisms have been estimated to be in the range $0.022\text{--}0.18\ \mu\text{Gy h}^{-1}$
236 (Garnier-Laplace et al., 2006) though could be significantly higher in high natural background
237 radiation areas. In the Chernobyl Cooling Pond, Kryshev and Sazykina (1995) estimated
238 natural radiation dose rates to be approximately $0.04\text{--}0.08\ \mu\text{Gy h}^{-1}$.

239

240 *Cell density and optical properties*

241 Optical properties of the lenses taken from fish from seven different lakes across a gradient of
242 contamination at Chernobyl are summarised in Supplementary Information Table S3. No
243 significant differences were seen between the optical properties of the lenses taken from fish
244 caught from different lakes at Chernobyl (Figure 2).

245

246 Nucleus density measured in 20 lenses from one low contamination lake (Dvorische) and one
247 high contamination lake (Glubokoye) showed no significant differences between lakes (Figure
248 3 see also SI Figures S3; S4 for data from other lakes). Variance increased in areas of higher
249 density, whereas areas of lower density showed higher order in both lakes. To demonstrate
250 variance within samples, we have included standard deviation on error bars in Figure 3.

251

252 *Spring-8 Refractive Index analysis*

253 High resolution refractive index analysis at Spring-8 showed no evidence of a difference in
254 refractive index between the relatively uncontaminated Lake Dvorische and the most
255 contaminated lake in this study, Lake Glubokoye (Figure 4).

256

257 A montage from a Spring-8 2D grid scan from a Crucian Carp lens collected from the Shinobu
258 dam of the Abukuma river, Fukushima, is shown in Supplementary Information Figure S5.
259 Each individual X-ray pattern from the lens in the montage is dominated by a single X-ray
260 reflection which is a broad diffuse ring. The putative interpretation of this reflection is that it
261 is an interference function, and originates from the average nearest neighbour spacing between
262 the crystallin proteins. This measurement is known as the Bragg spacing (d). The width or
263 thickness of this reflection gives an indication of crystallin proteins ordering within the lens
264 fibre cells, with a narrower interference function indicating a high degree of ordering (Regini
265 et al, 2004, Regini and Meek, 2009).

266

267 The inference function spacings from a lens taken from Suzuuchi Pond (approx. dose rate 5.5
268 $\mu\text{Gy h}^{-1}$) were calculated. From each montage, a column of individual X-ray diffraction
269 patterns was chosen next to the vertical meridian of the lens. A circular integration was
270 performed on each pattern and the resulting X-ray intensity (I) profiles plotted as a function of
271 inverse space (Q). Figure 5 shows a typical intensity profile from a Suzuuchi Crucian Carp lens
272 with the peak of the interference clearly visible.

273

274 Figure 6 shows a plot of the Bragg spacings from the central meridians of the 2D grid scans of
275 Crucian Carp lenses from Glubokoye lake and the relatively uncontaminated Lake Dvorische
276 as a function of distance across the lens. As can be seen in both lenses, the Bragg spacing
277 decreases from one periphery of the lens towards to centre, and then increases again to the
278 opposite periphery. This is expected due to increase in crystallin protein concentration, and
279 hence a decrease in the average nearest neighbour spacing between the crystallin proteins. The
280 change in the protein concentration is directly responsible for the refractive index gradient
281 found in many different types of animal lenses (Pierscionek and Regini, 2012). There is an
282 asymmetry in the spacing trends in both lenses towards the edge of each lens. This may be due
283 to the individual differences in refractive index gradients of the lenses at the edges (see Figure
284 6), which in turn would lead to a localised variation in the Bragg spacings. Overall, the spacings
285 between the two lenses is remarkably similar and only vary by a maximum of 10 Å. If there
286 had been a large amount of insoluble large amorphous aggregates leading to cataract formation
287 in one of the lenses, we would expect to see a much larger change in the spacings between the
288 two accompanied by a dramatic widening of the profile of the interference function as observed

289 by Suárez et al. (1993). Therefore, the data indicate that there are no appreciable structural
290 differences at this length scale between the Crucian Carp lenses from the contaminated
291 (Glubokoye) compared to uncontaminated (Dvorische) lake.

292

293 As a positive control, we performed a DIAMOND 2D grid scan on a Chernobyl Carp lens with
294 a dense nuclear cataract which was clearly visible. The density and location of this opacity
295 suggests that it is age-related and not caused by radiation. The oldest cells in the lens are found
296 in its centre (lens nucleus) and these are more prone to age-related cataract formation (Quinlan
297 and Clark, 2022). It was found that in the vast majority of the individual X-ray diffraction
298 patterns from the lens that the interference function was not present. We attribute this to the
299 formation of many large insoluble amorphous aggregates. Some extremely diffuse interference
300 functions were observed in some individual X-ray diffraction patterns at the edges of the lens,
301 away from the nucleus. Figure 5 (b) shows the Log X-ray intensity profile of a such a pattern.
302 As can be seen, the interference function has become so broad that it has almost disappeared,
303 it is also much closer to the centre of the pattern when compared to that in Figure 5 (a) i.e. a
304 larger Bragg spacing.

305

306 **Discussion**

307

308 This study spans the length scales from mm to sub-nm resolution by measuring the optical
309 properties, refractive index profiles, epithelial cell densities and X-ray synchrotron analysis of
310 molecular aggregation and cataract formation. We found no evidence of a significant impact
311 of radiation on lens development, protein and cell organisation and optical function in fish
312 sampled from waterbodies at Chernobyl and Fukushima. The total dose absorbed in a year for
313 perch and roach in Chernobyl lakes reached 142 and 129 mGy respectively with a cumulative
314 dose at the time of catch of 645 and 710 mGy respectively over a 5-year life span. These levels
315 are more than 100 times higher than the doses previously hypothesised to induce cataract in
316 voles from Chernobyl (Lehmann et al., 2016). Our study agrees with more recent findings
317 reported for animals at Fukushima where wild boar were exposed to cumulative doses up to
318 1600 mGy without any significant signs of cataract formation (Pederson et al., 2020).

319

320 Exposure to high acute dose or chronic low dose IR can result in measurable changes in lens
321 structure and function (see Uwineza et al., 2019 for a recent review). For example,

322 accumulative doses of 0.5, 1 and 2 Gy for different dose rates of 0.063 Gy/min and 0.3 Gy/min
323 increased epithelial cell division (Barnard et al., 2022; Markiewicz et.al., 2015) altered
324 epithelial cell density and disorganisation of the epithelial cells in the transitional zone and
325 meridional rows at the most distal edge of the epithelium (Markiewicz et. al., 2015). Long-term
326 consequences of such exposures can be altered lens shape (Markiewicz et. al., 2015) and lens
327 opacification (Barnard et. al., 2022) depending on the radiosensitivity of the mouse strain used.
328 When the loss of visual acuity was monitored by Optical Coherence Tomography (OCT), then
329 posterior subcapsular cataracts were the most prevalent cataract type observed (Pawliczek
330 et.al., 2022), even after a single acute dose of 0.5 Gy (Kunze et. al., 2021).

331

332 For humans, the Lifespan Study of atomic bomb survivors and other studies showed that the
333 eye lens is a particularly radiosensitive tissue with a long latency observed between exposure
334 and the appearance of vision-impairing cataract (Hamada et al., 2020). This has called into the
335 question the concept of a defined threshold dose (Hamada et.al., 2020). The United States
336 National Council on Radiation Protection and Measurements (NCRP) has not assigned a
337 specific threshold dose because of the limitations associated with available epidemiological
338 studies (NCRP 2016), demonstrating the importance of gathering data from all sources to
339 determine whether cataract is either a tissue reaction or a stochastic effect or a mixture of both
340 by the lens (Hamada et. al., 2020).

341

342 We did not find a significant difference in density of lens epithelial cells between fish from
343 waterbodies of different levels of ionising radiation. The density and organisation of the
344 meridional rows of lens epithelial cells at the very periphery of the lens epithelium is altered
345 by aging Wu et al., 2015, by diet (Gona, 1984) and by exposure to radiation, both ionising
346 (Zintz and Beebe, 1986, Pendergrass et al., 2010, Markiewicz et al., 2015) and non-ionising
347 radiation Wei, Hao et al. 2021). Zebrafish are an established radiobiological model (Geiger,
348 Parker et al. 2006, Liu et al., 2020, Marques et al., 2020) and the developing eye and fish lens
349 is a key sensor of both toxic (Sipes et al., 2011) and radioprotective agents (Liu et al., 2020).
350 In early zebrafish development, a total IR exposure of 1.62mGy was sufficient to differentially
351 regulate genes that would be expected to have lens effects as they are transcription factors
352 expressed during and following lens development (trp53, TGFb1, cebpa, crabp2 and vegfa;
353 <https://research.bioinformatics.udel.edu/iSyTE/>). A dose of 0.4 mGy/hr continuous for 92

354 hours produced developmental deformities which included head and eye deformities (Hurem
355 et al., 2017). For non-ionising radiation, early studies had indicated the sensitivity of teleost,
356 mouse, rabbit and human to UVB (290-320nm) was similar (Cullen and Montieth-McMaster
357 1993; Cullen et al 1994).The chronic exposure of trout to levels of UVB radiation equivalent
358 to the daily accumulative dose of 500J/cm² over a 205 day period demonstrated the formation
359 of anterior opacities detected by slit-lamp analyses (Cullen et al., 1994). This study also
360 suggested that the age of the fish could influence cataract incidence (Cullen et al; 1994) and
361 that UVB exposure could accelerate the aging process (Zigman, 1983). Exposure to ionising
362 radiation is also believed to accelerate age-related cataract (Uwineza et al., 2019). In later
363 studies, the near to threshold UVB dose for rats (Michael and Brismar, 2001; Galichanin et al
364 2010; Hurem et al 2017) produced changes in the refractive index gradient (Michel and Bismar
365 2001) that were irreversible (Galichanin et al., 2008). It seems reasonable therefore to expect
366 the eyes and lenses of teleosts to be similar to other animal models.

367

368 In the perch fish taken from the lakes around Chernobyl, no statistically significant changes in
369 the distribution and density of the lens epithelial cells in the and adjacent to the meridional
370 rows in the epithelium were observed. We know that dose (Markiewicz, Barnard et al. 2015))
371 and dose rate (Barnard et al., 2019, Barnard et al., 2022) affect the mouse lens response to
372 ionising radiation. The response to low dose ionising radiation by human lenses (Della Vecchia
373 et al., 2020; Little et al., 2021; Su et al., 2021) can be associated with increased cataract
374 incidence. There is evidence the doses and dose rates experienced by voles in the Chernobyl
375 area would be sufficient to cause opacities and cataract (Kleiman et al., 2017), though methods
376 and interpretation are not presented in the Kleiman et al. (2017) summary abstract. Lens
377 opacities affect species fitness (Flink et al., 2017), responses to predators (Flink et al., 2017)
378 and diet selection (Vivas Muñoz et al., 2021), but the perch examined in the different Chernobyl
379 locations showed no statistically significant differences in their lens metrics or optical
380 properties.

381

382 A previous SAXS study of human lenses (Suárez et al., 1993) found that the inference function
383 becomes much broader and the spacing increases greatly with the age of the lenses, especially
384 past the age of 55 years in humans. These authors found that Bragg spacing (which arises from
385 the average centre to centre distance between the crystallin proteins) from a single X-ray pattern

386 from the centre of each lens increased from 142Å from a very young lens to 200Å in a lens
387 with more insoluble protein and increasing light scatter has aging events that eventually lead
388 to lens opacification (reviewed in Quinlan and Clark, 2022). The dramatic changes in the
389 interference function are explained in terms of the structural conversion of the crystallin
390 proteins with age (Regini and Meek, 2009). These proteins become denatured in response to
391 environmental stresses such as radiation, temperature, glycation and oxidation. The denatured
392 proteins are thought to combine to form water insoluble amorphous aggregates. As these
393 amorphous aggregates grow, they increase the average nearest neighbour spacing between the
394 crystallin proteins and detrimentally affect the order of proteins and water which is integral to
395 maintaining transparency. When the amorphous aggregates become comparable in size to the
396 wavelengths of the visible spectrum, they scatter light causing lens opacities. If the cataract is
397 very dense, the average nearest neighbour spacing between the crystallin proteins becomes so
398 large that the inference function disappears from the X-ray diffraction pattern (Figure 5b). This
399 isolated case is most likely to be related to the age of the fish (>12 years), given its position in
400 the central and oldest part of the lens.

401

402 We would expect that lenses which have been exposed to ionising radiation of a sufficient dose
403 would have increased levels of insoluble proteins in the form of amorphous aggregates, even
404 before opacities in the lens become visible as conceptualised in the cataractogenic load
405 hypothesis (Uwineza et.al., 2019; Quinlan and Cark, 2022). This would result in a large
406 increase in the Bragg spacing of around 60 Å. As Figure 6 illustrates, such a large increase was
407 not evident in any of the lenses (15 lenses studied) of fish from Chernobyl and Fukushima,
408 except the sample from the old (>12 years) Crucian Carp with a visible central cataract. Of
409 course, it was only feasible to study a proportion of the fish population by synchrotron methods.
410 We cannot rule out the existence of cataract or pre-cataract in fish which were not studied.
411 Nevertheless, the synchrotron method is the most sensitive available to detect altered protein-
412 protein associations and protein aggregation as indicators of any pre-cataract or cataract
413 phenotype. For 15 lenses studied by synchrotron, 40 lenses (from contaminated lakes) studied
414 for refractive index and 20 (from contaminated lakes) for epithelial cell density, this was not
415 the case, suggesting that this the likely situation for the wider fish population.

416

417 It is further noted that we cannot completely exclude radiation-induced cataractogenesis in the
418 fish populations of these lakes. Other potential indicators of cataract formation (including
419 biochemical differences such as oxidative stress) were not studied. Further, it was only possible
420 to gain a representative sample of similar age (4-5 years) fish from each of the lakes. Older fish
421 may provide better evidence of radiation induced cataract but it was not feasible to obtain a
422 large and age consistent sample of old fish due to their relatively much lower numbers in the
423 population. It should also be noted that in all studies of wild animals, particularly aquatic
424 organisms, dose rate estimation is highly uncertain owing to changes in exposure during the
425 development and lifetime of the organism and to uncertainty of movement and habitat. Thus
426 the estimated doses calculated here should be treated with caution, though relative doses
427 between groups of fish from different sites are much more accurate since development and
428 habitat occupancy factors are similar between sites. Further laboratory experiments which can
429 better control dose and dose rate (albeit with the limitation of relatively short duration) would
430 be valuable to complement studies in the natural environment.

431

432 It could also be argued that animals in the natural environment that developed cataract would
433 be more likely to be predated leading to survivor bias, but the small scale molecular and cellular
434 changes we have studied (ie pre-cataract formation) would mitigate against this.

435

436 Uncertainty in dose rate estimation could explain some differences in findings about cataract
437 formation in wild animals. However, because relative doses between waterbodies are much
438 more accurate than absolute doses, they are not sufficient to explain the difference between our
439 findings and those of Lehman et al. (2016) (voles) and Mousseau and Moller (2013) (birds)
440 studies. These latter findings of cataract formation at very low doses and dose rates are in
441 contradiction to ICRP guidance based on laboratory animal and human epidemiological data.
442 They are further not supported by the field study findings reported here for fish and previously
443 for wild boar Pederson et al. (2020)). It has been suggested that the earlier studies (Lehman et
444 al. 2016; Mousseau et al. 2013) had significant methodological and interpretation issues (Smith
445 et al. 2017, Pederson et al. 2020).

446

447 There may be differences in lens development and structure between species which could affect
448 the formation of radiation-induced cataract. Lens development across vertebrates is very well

449 conserved as discussed in the Introduction. The development and structure of fish lenses are
450 similar in terms of their protein content, type of proteins and in the protein distribution across
451 the lens which creates the refractive index (Sivak 2004; Kocemba and Waker, 2021). Hence,
452 any response to factors that cause cataract will be broadly similar. However, whilst both fish
453 and rodent lenses are abundant in γ -crystallin, the latter contain γ S-crystallin, but the former
454 are rich in γ M-crystallin (Mahler et al, 2013). The low tryptophan and high methionine content
455 in γ M-crystallin may offer a protective effect given that methionine has been shown to provide
456 radiation protection (Vuyyuri et al, 2008; Mahler et al 2013).

457

458 The present study has observed no radiation induced cataract or evidence of pre-cataract lens
459 damage in wild fish exposed to relatively high cumulative dose (but relatively low dose rate)
460 radiation. As the lens grows, each layer of lens fibres laid down by the differentiated epithelial
461 cells is a 'snap shot' or 'time capsule' of its history recorded for posterity in a similar manner
462 to tree rings. Consequently, the lens represents both an endpoint of exposure history and a
463 timeline of accumulated damage which is highly relevant under chronic exposure scenarios.

464

465

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Table 1 Estimated dose rates from Japanese, Belarussian and Ukrainian lakes. The values in the table represent dose rates from artificial radionuclides.

Site	¹³⁷ Cs sediment kBq/kg w.w.*	¹³⁴ Cs sediment kBq/kg w.w.*	Ext. dose μGy/h	Int. dose ¹³⁷ Cs w.w. kBq/kg	Int. dose ¹³⁴ Cs kBq/kg	Int. dose ⁹⁰ Sr kBq/kg	Int. dose μG/h	Total dose μGy/h
Chernobyl								
Dvorische	0.26	n.d.	Backgr.	0.19	n.d.	Not meas.	Backgr.	0.1
Gorova	0.026	n.d.	Backgr.	0.004	n.d.	Not meas.	Backgr.	Backgr.
Stoyacheye	0.75	n.d.	0.1	0.088	n.d.	Not meas.	Backgr.	0.1
Svyatoye	4.56	n.d.	0.7	6.09	n.d.	Not meas.	1.1	1.7
Glubokoye	39.7	n.d.	5.9	7.8	n.d.	13.6	9.8	15.7
Yanovsky Crawl	47.8	n.d.	7.1	2.6	n.d.	3.60	2.7	9.8
Cooling Pond	49.1	n.d.	7.3	3.0	n.d.	0.079	0.6	7.9
Fukushima								
Suzuuchi	23.2*	3.5*	4.6	3.9	0.57	Not meas.	0.86	5.5
Funasawa	32*	4.7*	6.3	3.8	0.56	Not meas.	0.83	7.1
Abukuma	35*	5.3*	7.0	0.008	0.0012	Not meas.	0.0018	7.0

* Based on sediment data from Konplev et al. (2018) and a f.w./d.w. ratio of 5.0

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Figure 1. Maps showing location of sampling sites at (a) Chernobyl and (b) Fukushima. The reference dates for the radiation levels are 1986 for Chernobyl and 2011 for Fukushima.

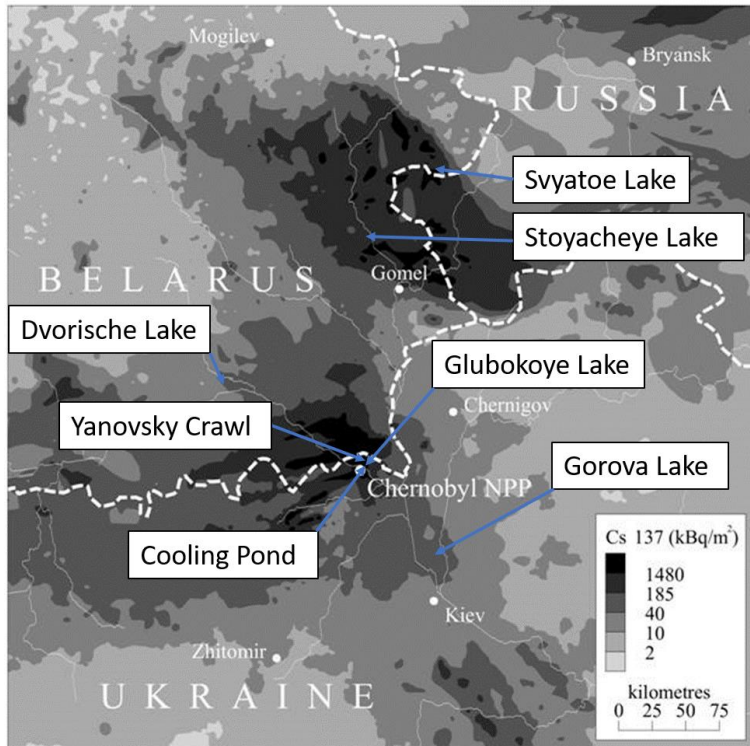
Figure 2. Lens maximum refractive index (n), focal length (mm) and radius (mm) in lakes across a gradient of contamination density at Chernobyl. Error bars show 1 Std Error (too small to be seen for refractive index).

Figure 3. Mean of cell density measurements for Lake 1; low contamination Dvorische and 2; high contamination Glubokoye; standard deviation on error bars. A t-test reveals $p_{\text{mean}} = 0.08$.

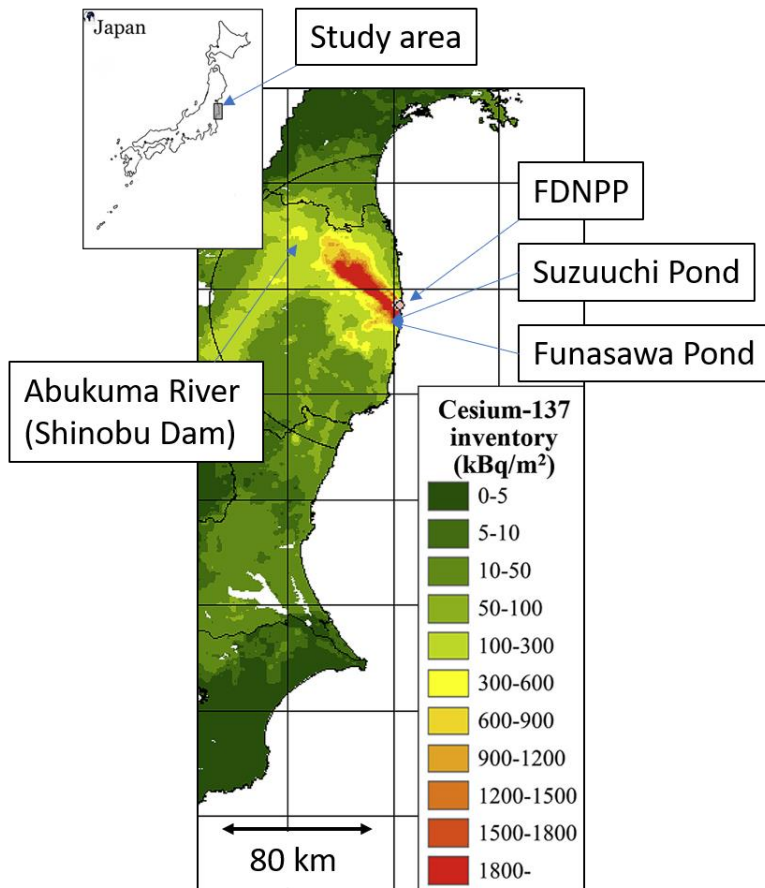
Figure 4. High resolution refractive index measurements at Spring-8 showing no difference between Lake Dvorische (relatively low radiation dose; one lens from each of two perch) and Lake Glubokoye (relatively high radiation dose; 2 lenses from each of two perch).

Figure 5. A plot of the Log X-ray intensity scale against inverse space from an individual DIAMOND X-ray diffraction pattern from (a) a Lake Suzuuchi Crucian Carp lens showing a peak corresponding to the interference function which arises from the average centre to centre distance between the crystallin proteins; (b) a Chernobyl Carp lens with a dense nuclear age related cataract. The interference function has become swamped in the background radiation due to the disordering of the crystallin proteins in the lens with the age-related cataract.

Figure 6. A plot of the Bragg spacings from the central meridians of the 2D grid scans of Crucian Carp lenses from DIAMOND. Glubokoye (Open circles; “contaminated”) and Dvorische (Filled circles; “uncontaminated”). Neither Bragg spacing pattern shows evidence of protein aggregation and therefore early stage cataract formation.



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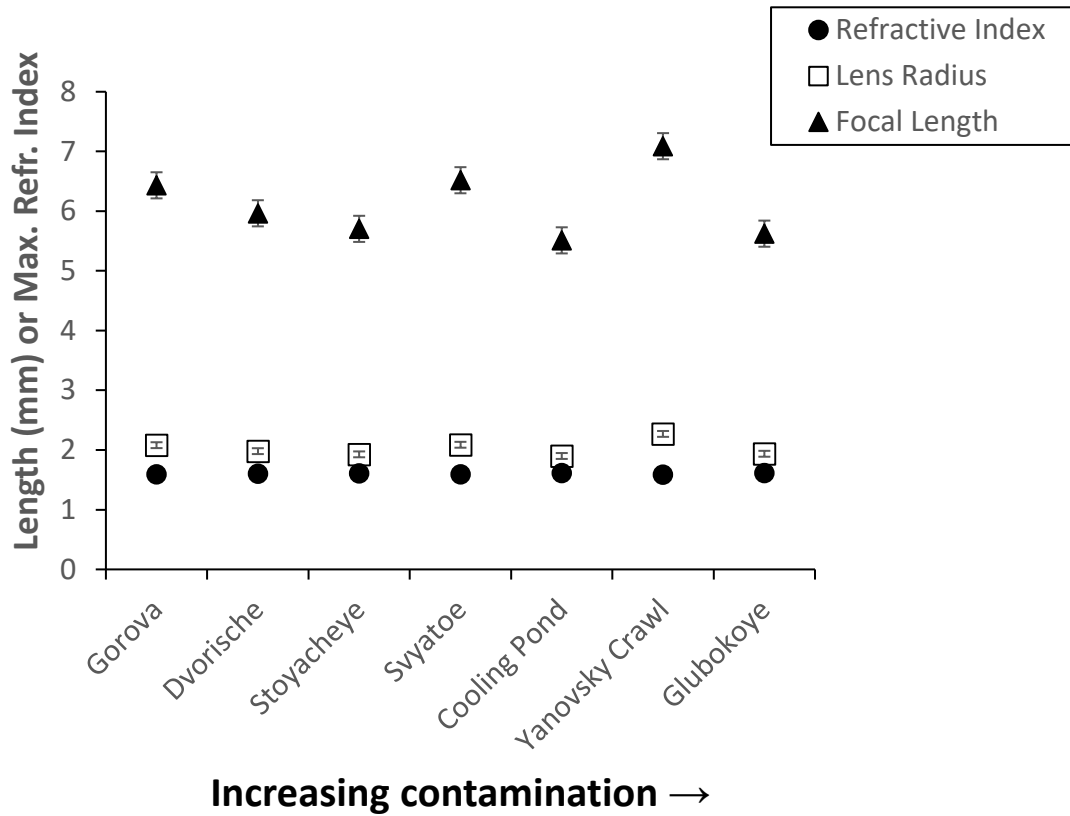
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Figure 1. Maps showing location of sampling sites at (a) Chernobyl and (b) Fukushima. The reference dates for the radiation levels are 1986 for Chernobyl and 2011 for Fukushima.

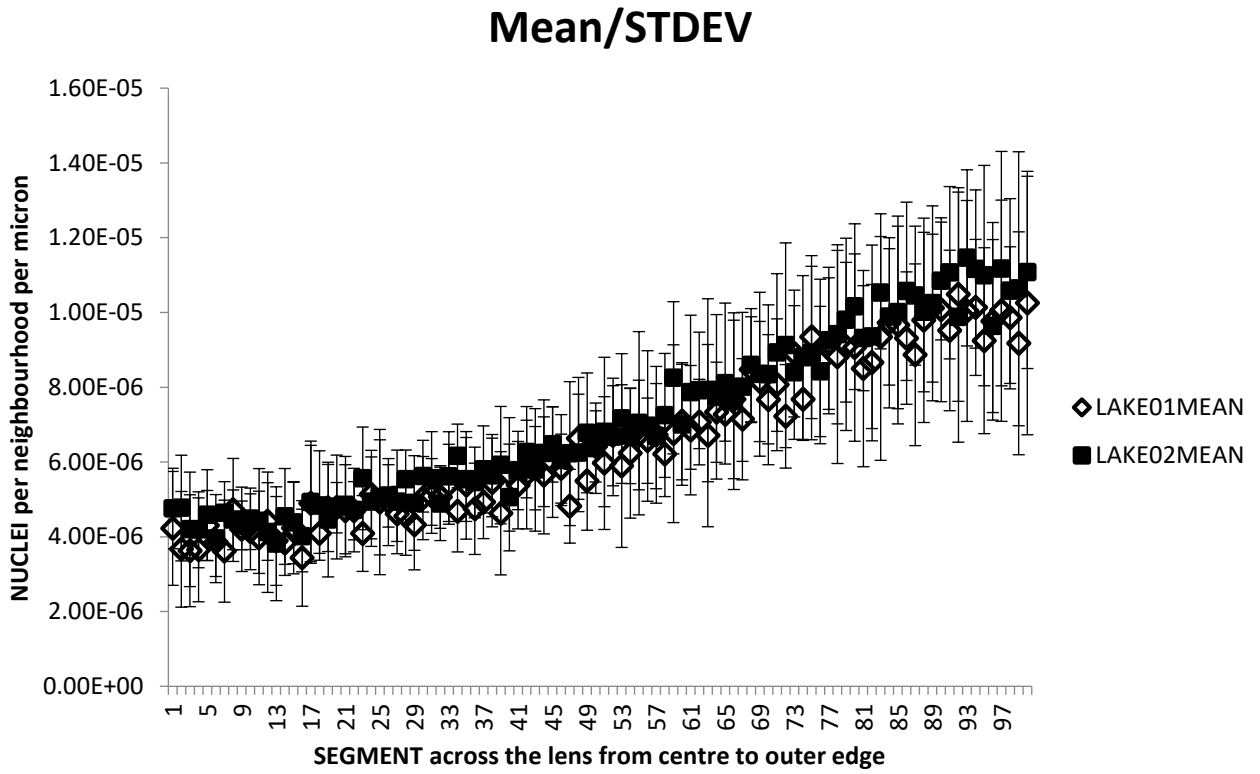
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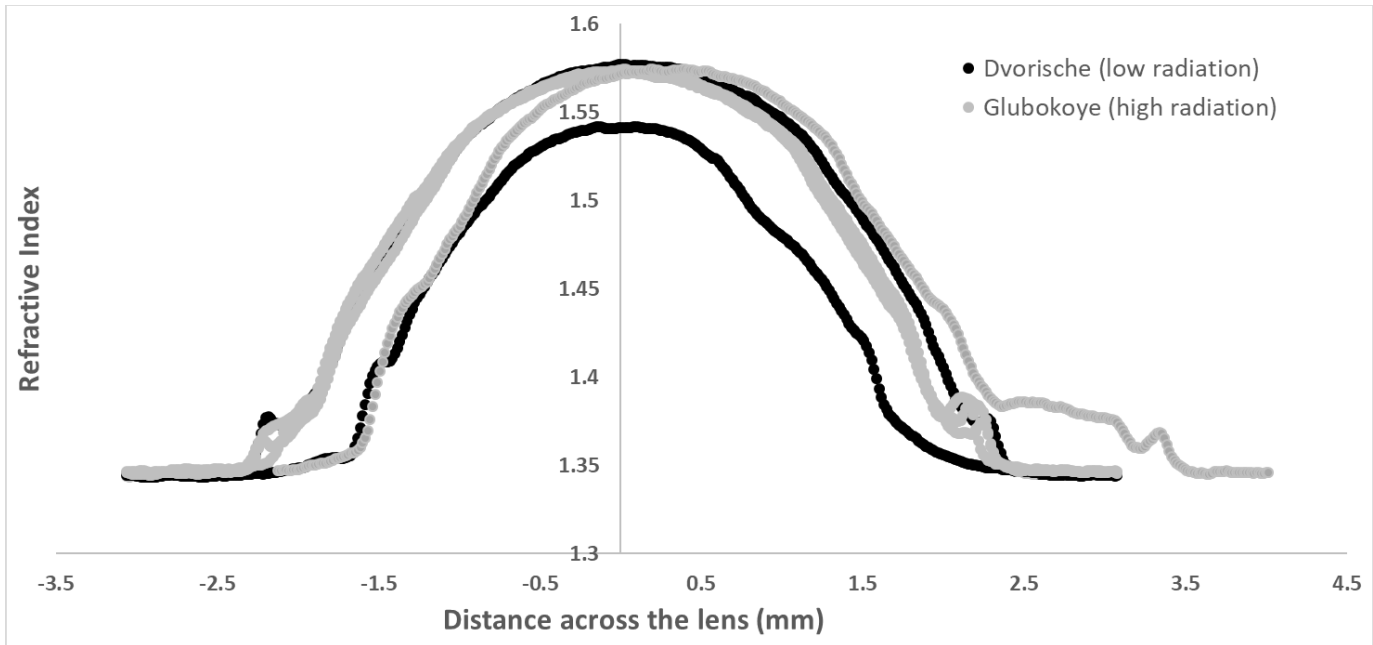
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Figure 3. Mean of cell density measurements for Lake 1; low contamination Dvorische and 2; high contamination Glubokoye; standard deviation on error bars. A t-test reveals $p_{\text{mean}} = 0.08$.

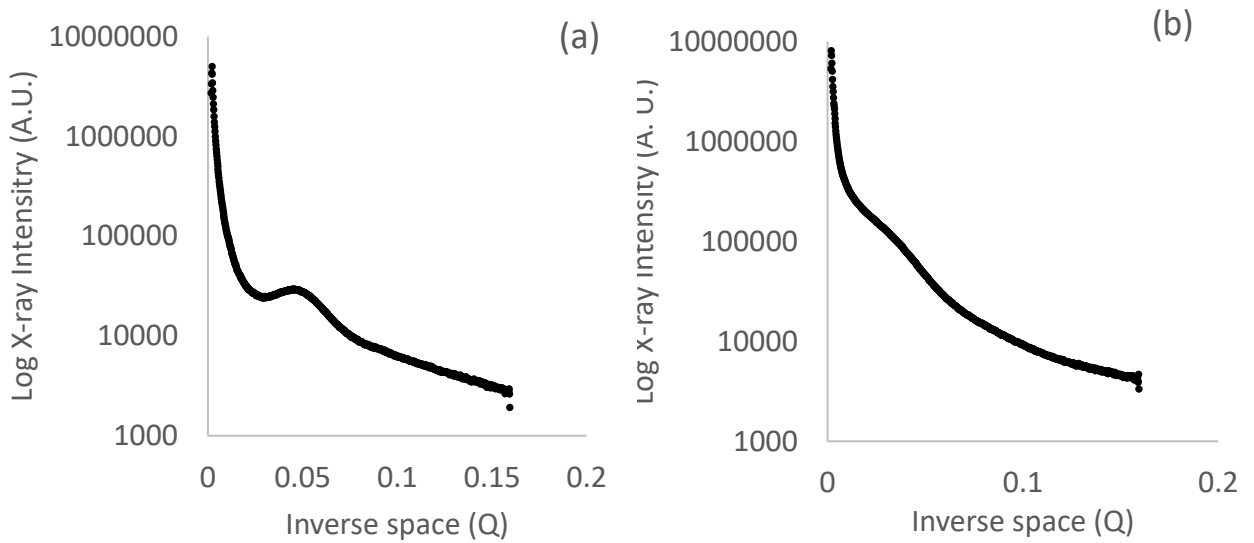
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Figure 4 High resolution refractive index measurements at Spring-8 showing no difference between Lake Dvorische (relatively low radiation dose; one lens from each of two perch) and Lake Glubokoye (relatively high radiation dose; 2 lenses from each of two perch).

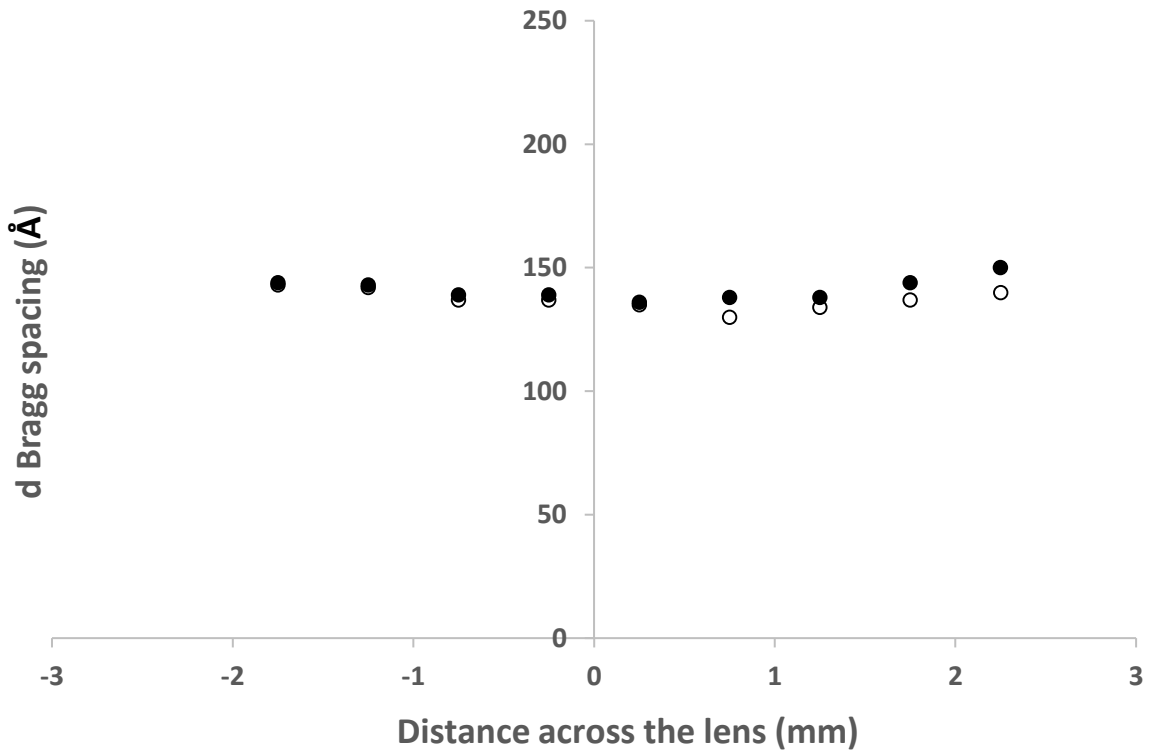
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