Supporting Information. Pages S1 to S19. Figures S1 to S3. Tables S1 to S6.

Impact of environmental radiation on the health and reproductive status of fish from Chernobyl

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Figure S1. Mean activity concentration of ^{241}Am in liver (A) and muscle (B), ^{239,240}Pu in liver (C) and muscle (D) and ^{238}Pu in liver (E) and muscle (F) of perch and roach (*n* = 3 to 5, mean ± Sd, Bq/kg, w.w.) collected in the CEZ. The activity concentrations in liver of fish were higher than in muscle and the highest levels of transuranium isotopes were found in perch from Glubokoye lake.

Figure S2. Figure showing the relative abundance of fish (%) species in each lake. The number of fish were recorded during the June 2015 session performed in Belarus and Ukraine.

Figure S3. Distribution of the GSI values of perch (A) and roach (B) collected from the 7 different lakes in September 2014 and 2015. Interquartile ranges are represented by the box. The boxes are delimited by the first (25% of samples) and the third (75% of samples) quartile. The line in each box is the median of the distribution.

Table S1. Number of perch and roach collected in September 2014 and 2015, and used for health and reproductive status assessment.

Table S2. Hydrological parameters and initial Cs deposition (A). Nutrient concentrations (NO_3^-, NO_2^- and PO_4^{3-} in µg/L, mean ± SD, *n* = 3 per lake) (B) and physicochemical parameters (Dissolved Oxygen, DO, in %, pH, temperature, conductivity in µS/cm) (C) measured in the surface water samples of the 7 lakes in September 2014 and 2015.

Table S3. Major alkali and alkali-earth element water concentrations (Na, Mg, S, K and Ca in mg/L, mean ± SD, *n* = 3 per lake) (A) and trace elements concentrations (As, Sr, Cd, Cs, Pb and U in µg/L, mean ± SD, *n* = 3 per lake) (B) determined by ICP-MS in the surface water samples of the 7 lakes in September 2014 and 2015.

Table S4. Mean activity concentration of ^{241}Am in liver (A) and muscle (B), ^{239,240}Pu in liver (C) and muscle (D) and ^{238}Pu in liver (E) and muscle (F) of perch and roach (*n* = 3 to 5, mean ± Sd, Bq/kg, w.w.) collected in the CEZ.

Table S5. Total length (cm), body weight (g), Fulton condition index, hepatosomatic index, gonad weight (g) (mean ± SD) measured in female perch and roach collected at Glubokoye (H) (*n* = 23 and 15 respectively), Yanovsky (H) (*n* = 11 and 21 respectively), Cooling Pond (H) (*n* = 16 and 3 respectively), Gorova (L) (*n* = 17 and 18 respectively), Svatoye (M) (*n* = 16 and 9 respectively), Stoyacheye (L) (*n* = 13) and Dvoriche (L) (*n* = 7 and 16 respectively). The age (yr) (mean ± SD) was measured in female perch and roach collected at Glubokoye, Yanovsky, Cooling Pond and Gorova.

Table S6. Number of micronucleus counted per 1000 erythrocytes (mean ± SD, *n* = 5).

Methods

Fish collection

The fulton condition, hepatosomatic and gonadosomatic index were determined as follow: K = body weight/ (total length (cm)^3) x 100; HSI = (liver weight/body weight) x 100 and GSI = (gonad weight/body weight) x 100, respectively.

Water chemistry

After filtration on a 0.2 µm mesh size filter and acidification (2% v/v of HNO_3 65% trace metal grade, Sigma), major alkali and alkali-earth element water concentrations (Na, Mg, S, K and Ca in mg/L, mean ± SD, n = 3 per lake) and trace elements concentrations (As, Sr, Cd, Cs, Pb and U in µg/L, mean ± SD, n = 3 per lake) were measured in the 7 lakes (Table S3) with quadrupole-based ICP-MS system (Model iCAPQ; Thermo Scientific) equipped with CCTED, ‘collision cell mode’ (7% Hydrogen in Helium) with kinetic energy discrimination (KED) to eliminate polyatomic interferences. Samples entered the ICP-QMS through an auto-sampler (Cetac ASX-520) via a nebuliser (Thermo-Fisher Scientific; 1mL min^{-1}) and spray chamber. Internal standards were introduced to the sample stream via a separate line including Ge (50 µg/L), Rh (20 µg/L) and Ir (10 µg/L) in 2% HNO_3. Two sets of external mulit-element calibration standards for major elements (0-30 mg/L) and trace elements (0-100 µg/L) were prepared from Certiprep(TM) multi-element stock solutions.

After filtration on a 0.45 µm mesh size filter and preservation (1% v/v of $ZnCl_2$ 50% w/v), concentrations of inorganic macronutrients (NO_3^-, NO_2^- and PO_4^{3-} in µg/L, mean ± SD, n = 3 per lake) were determined using a QuAAtro segmented flow nutrient analyser with autosampler (SEAL Analytical, UK). Analysis was done according to standard procedures.

Micronucleus test

A total of 1000 cells were scored blindly for each fish. Micronuclei were identified as small, dark blue stained bodies of chromatin within the cytoplasm, outside the nucleus. Only structures of similar staining intensity as the nucleus were scored as micronuclei.

Histological analyses

Liver and Gonad sections were fixed for 24 h in 10% neutral buffered formalin before transfer to 70% ethanol for subsequent histological assessment. Livers and gonads were processed in a vacuum infiltration processor using standard histological protocols (18) and embedded in paraffin wax. Using a rotary microtome, sections of 5-7 µm were taken and subsequently stained with haematoxylin and eosin (H&E).

Statistical analyses

Generalised linear models were used to assess the potential differences across the sites of the radionuclide specific activities, biometric parameters, oocyte distribution and size and chromosomal damage for both species. The effect of age on the oocyte distribution has been also assessed. The significance of parameters at the different sites was assessed using the contrast method (Hastie, 1992 *Statistical Models* in Wadsworth & Brooks/Cole Publishing Inc. Belmont) with the esticon function in R (DoBy package, (Hojsgaard, 2004, *Scandinavian Journal of Statistics*, 31:143–158)). Pearson correlation were used to assess the potential correlation between GSI and % of immature oocytes, and between DO, pH, T°C, conductivity, Na, Mg, Ca, K, stable Sr water concentration, ^{90}Sr and ^{137}Cs activity concentrations in perch and roach using the Hmisc package in R.

Results

Water chemistry

Correlation analyses revealed that the conductivity is positively correlated with the water concentration levels of stable Sr^{2+} (cor: 0.8, p = 0.03), Na^{+} (cor: 0.76, p = 0.048), Mg^{2+} (cor: 0.82, p = 0.02) and Ca^{2+} (cor: 0.75, p = 0.05) to a lesser extent (limit of significancy) across the lakes. No correlations were found between the conductivity and the ^{137}Cs specific activity in perch (cor: -0.4, p = 0.38) and roach (cor: -0.01, p = 0.99) across the contaminated lakes. The conductivity is negatively correlated to the ^{90}Sr specific activity in perch (cor: -1, p = 0.047) and in roach (cor: -0.99, $p = 0.09$) to a lesser extent ($p > 0.05$) across the lakes located in the 10 km exclusion zone. No correlations were found between the water concentration levels of K^{+} and the ^{137}Cs specific activity in perch (cor: -0.47, $p = 0.29$) and roach (cor: -0.58, $p = 0.23$) across the contaminated lakes. No correlations were found between the water concentration levels of Ca^{2+} and the ^{90}Sr specific activity in perch (cor: -0.66, $p = 0.54$) and roach (cor: -0.6, $p = 0.59$) across the contaminated lakes. Stronger negative correlation coefficients (although non significant) were found between the water concentration levels of stable Sr^{2+} and the specific activity of ^{90}Sr in perch (cor: -0.8, $p = 0.41$) and roach (cor: -0.76, $p = 0.45$) across the lakes located in the 10 km exclusion zone.

Significant contamination of fish from the CEZ with ^{137}Cs, ^{90}Sr, ^{241}Am, $^{239,240}Pu$ and ^{238}Pu

Fish from the CEZ are significantly contaminated with ^{241}Am (Figure S1 A and B, Table S3). Concentration levels of ^{241}Am in liver and muscle of perch from Glubokoye (H) reached 44 and 4 Bq/kg w.w. respectively and were higher than in perch from Cooling Pond (H) reaching 23 and 3 Bq/kg w.w. in liver ($p = 0.001$) and muscle ($p = 0.01$) respectively; and from Yanovsky (H) lake reaching 22 and 3 Bq/kg w.w. in liver ($p = 0.002$) and muscle ($p = 0.02$) respectively (Figure S1 A for liver and B for muscle, Table S4). Concentration levels of ^{241}Am in liver and muscle of roach did not vary significantly across the lakes ($p = 0.06$) (Figure S1 A and B respectively).

Fish from the CEZ are significantly contaminated with $^{239,240}Pu$ (Figure S1 C and D, Table S4). Concentration levels of $^{239,240}Pu$ in liver and muscle of perch from Glubokoye (H) reached 23 and 1 Bq/kg w.w. respectively and were higher than in perch from Cooling Pond (H) reaching 9 and 0.1 Bq/kg w.w. in liver ($p = 0.0005$) and muscle ($p = 0.01$) respectively, and Yanovsky (H) lake reaching 8 and 0.1 Bq/kg w.w. in liver ($p = 0.0005$) and muscle ($p = 0.02$) respectively (Figure S1 C for liver and D for muscle, Table S4). Concentration levels of ^{239,240}Pu in liver and muscle of roach did not vary significantly across the lakes ($p = 0.32$) (Figure S1 C and D respectively).

Fish from the CEZ are significantly contaminated with ^{238}Pu (Figure S1 E and F, Table S3). Concentration levels of ^{238}Pu in liver and muscle of perch from Glubokoye (H) reached 8 and 1 Bq/kg w.w. respectively and were higher than in perch from Cooling Pond (H) reaching 3 and 0.04 Bq/kg w.w. in liver ($p = 0.002$) and muscle ($p = 0.01$) respectively; and from Yanovsky (H) lake reaching 3 and 0.02 Bq/kg w.w. in liver ($p = 0.002$) and muscle ($p = 0.02$) respectively (Figure S1 E for liver and F for muscle, Table S4). Concentration levels of ^{238}Pu in liver and muscle of roach did not vary significantly across the lakes ($p = 0.46$) (Figure S1 E and F respectively, Table S4).

Discussion

^{137}Cs and ^{90}Sr specific activities

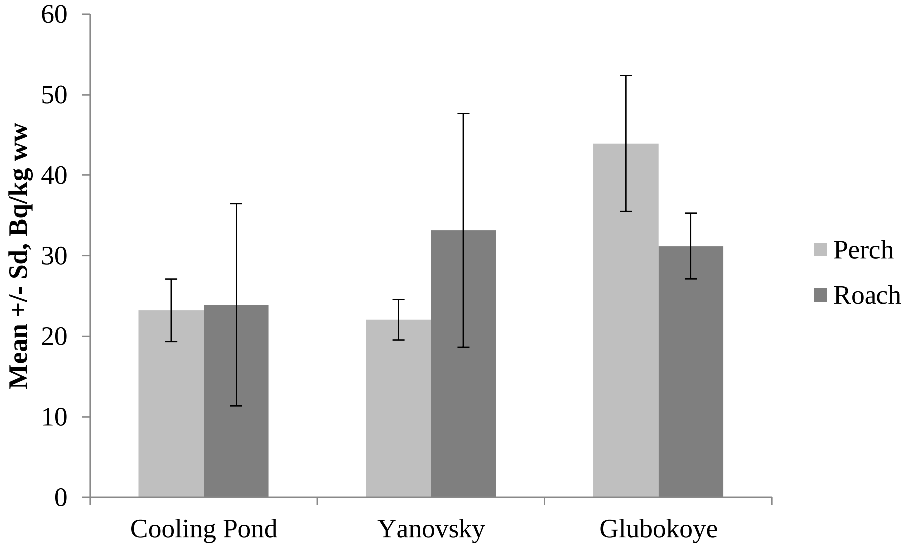
The ^{137}Cs activity concentration measured in the perch (mean: 7844 Bq/kg) and in the roach (mean: 2905 Bq/kg) from Glubokoye lake exceeded the EU maximum permitted level in imported foodstuffs (1250 Bq/kg). Activity concentrations of ^{137}Cs in roach from the other CEZ lakes (Yanovsky mean: 781 Bq/kg; Cooling Pond mean: 1231 Bq/kg) and Svyatoye lake (mean: 587 Bq/kg) were higher than Ukrainian and Japanese limits for human consumption (150 and 100 Bq/kg respectively). Activity concentrations of ^{90}Sr in both species exceeded the EU maximum permitted level (750 Bq/kg) in Yanovsky (mean: 3603 Bq/kg in perch; 2572 Bq/kg in roach) and Glubokoye (mean: 13636 Bq/kg in perch; 12556 Bq/kg in roach) and the Ukrainian permitted level (35 Bq/kg) in the Cooling Pond (mean: 79 Bq/kg in perch; 157 Bq/kg in roach).

Doses span the lowest protection level for an ecosystem

There are uncertainties in the estimate of the external dose to organisms since habitat occupancy is not precisely known and varies during different stages of the life cycle. The embryonic stage of the fish may be the most externally exposed as the embryos grow close to the bottom sediments, the main source of external radiation. The estimation of the external dose was calculated by considering a homogeneous distribution of the ^{137}Cs in the 15 cm upper layer of the sediment whereas in reality this is likely to be a heterogeneous distribution. Despite these inevitable uncertainties, internal activity concentration correlates strongly with surface contamination density hence relative dose rates are robust despite potential errors in absolute dose rate estimation.

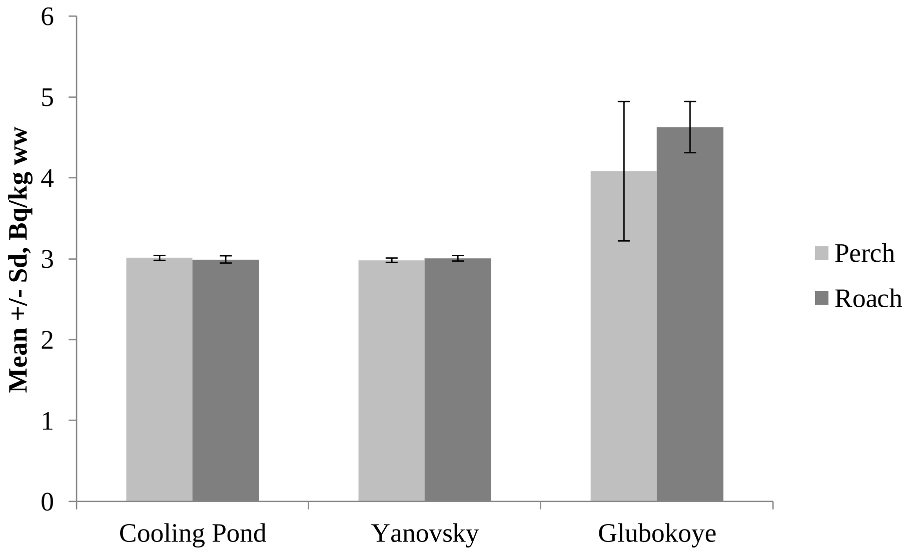
Figure S1.

A)



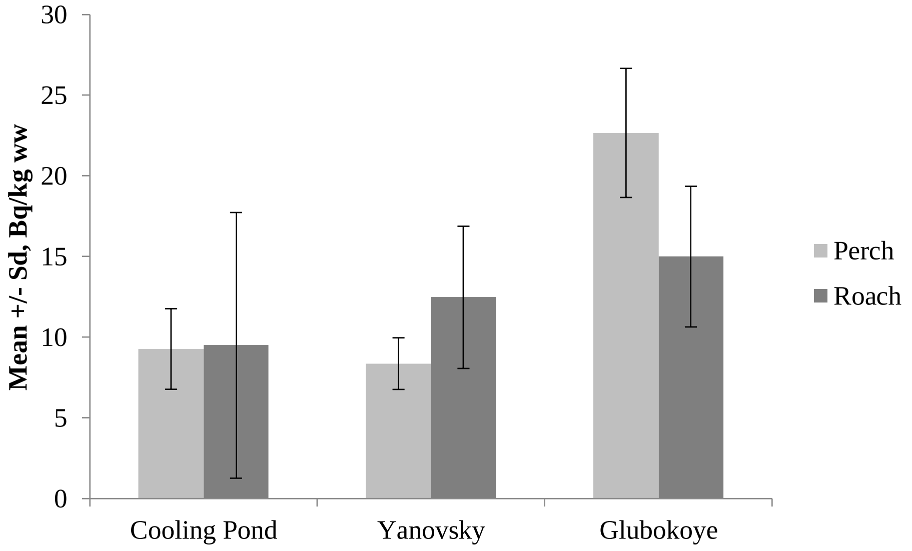
60
50
40
30
20
10
0
Mean +/- Sd, Bq/kg ww
Cooling Pond
Yanovsky
Glubokoye
Perch
Roach

B)



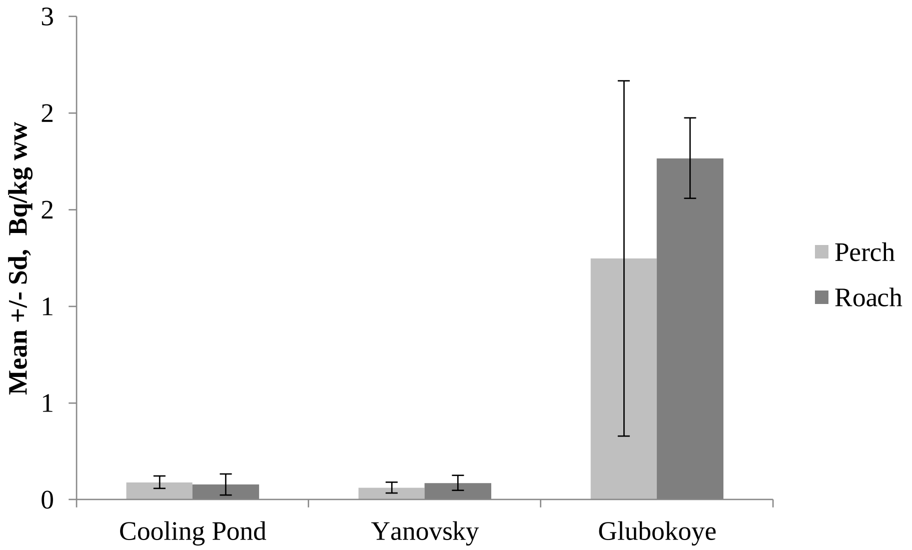
6
5
4
3
2
1
0
Mean +/- Sd, Bq/kg ww
Cooling Pond
Yanovsky
Glubokoye
Perch
Roach

C)



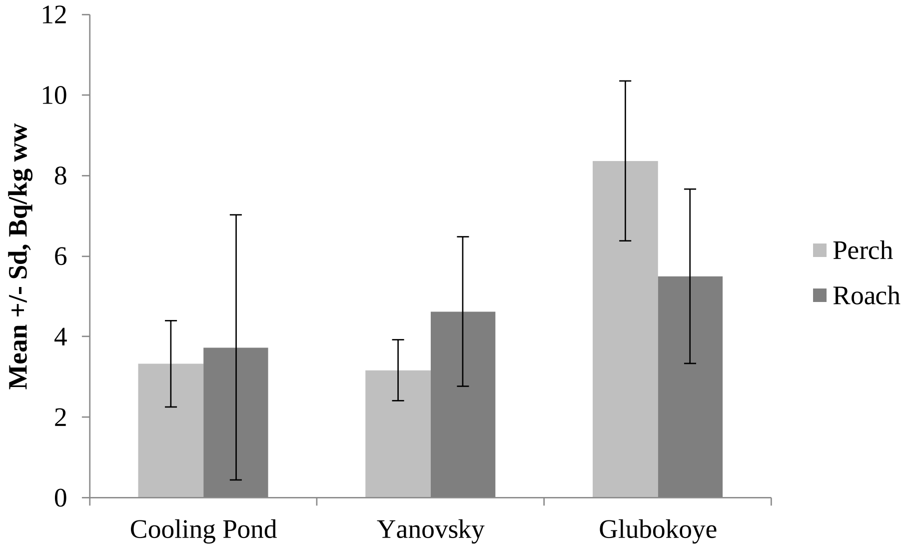
30
25
20
15
10
5
0
Mean +/- Sd, Bq/kg ww
Cooling Pond
Yanovsky
Glubokoye
Perch
Roach

D)



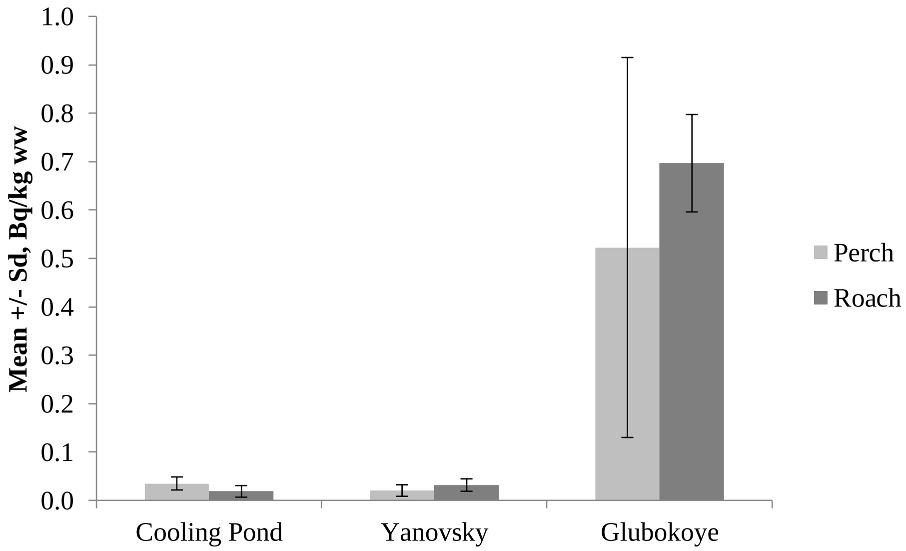
3
2
2
1
1
0
Mean +/- Sd, Bq/kg ww
Cooling Pond
Yanovsky
Glubokoye
Perch
Roach

E)



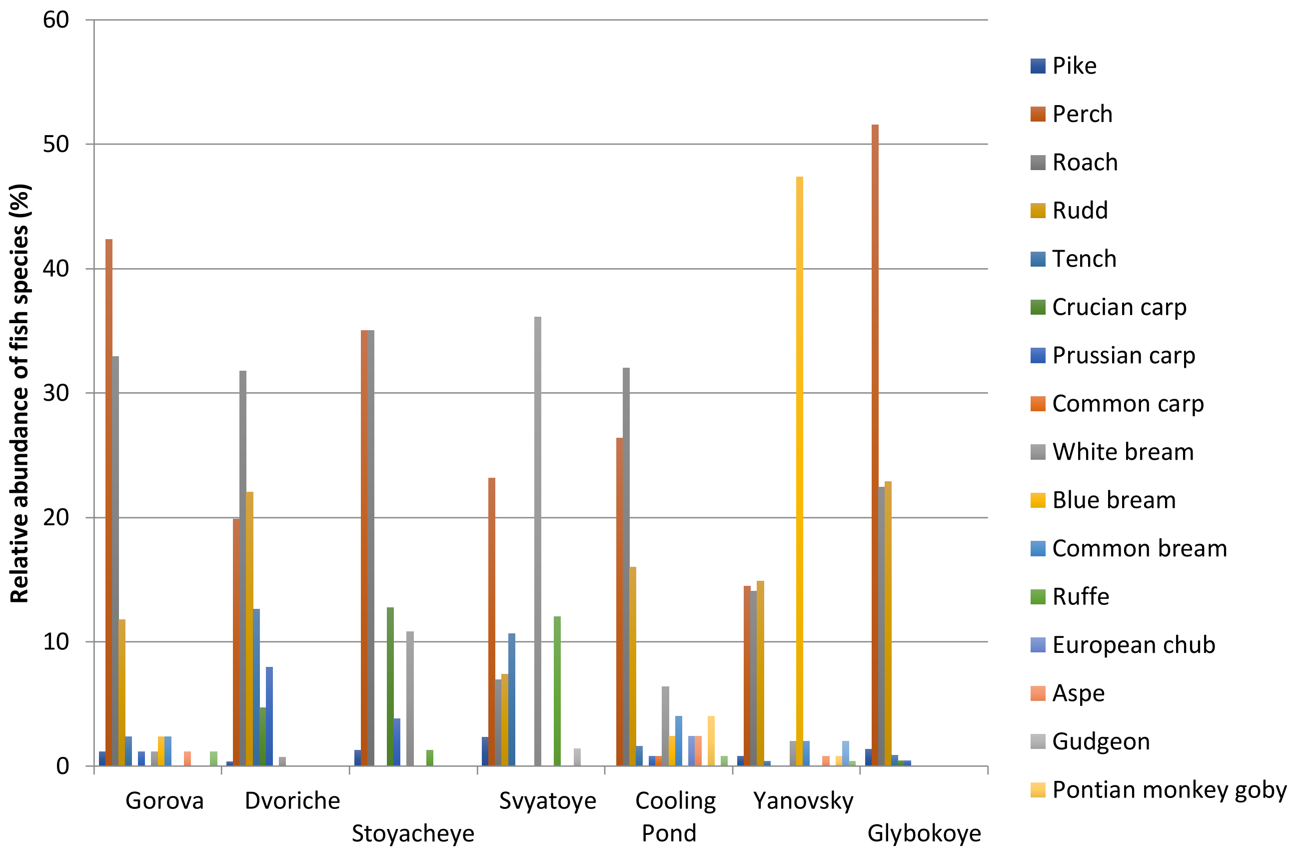
12
10
8
6
4
2
0
Mean +/- Sd, Bq/kg ww
Cooling Pond
Yanovsky
Glubokoye
Perch
Roach

F)



1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Mean +/- Sd, Bq/kg ww
Cooling Pond
Yanovsky
Glubokoye
Perch
Roach

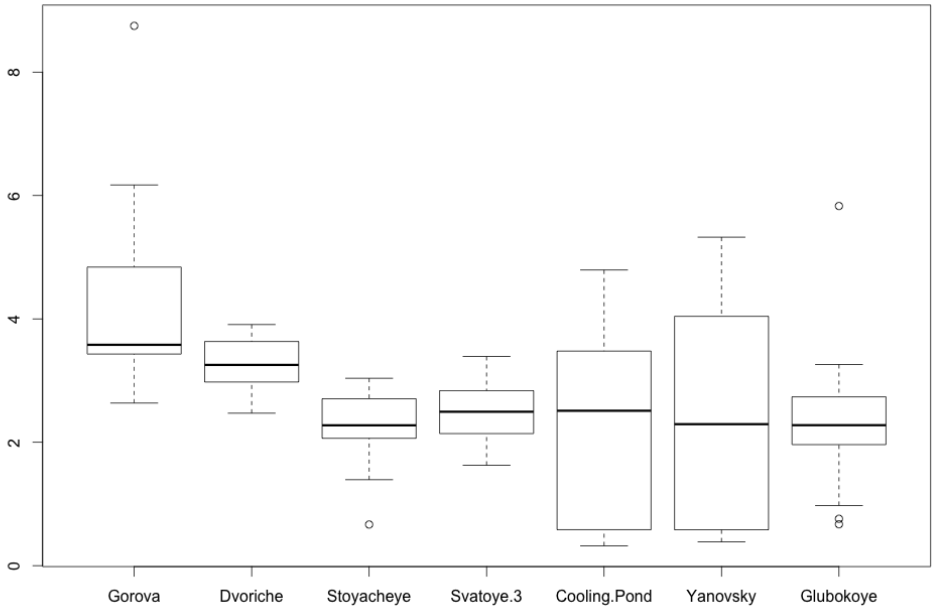
Figure S2.



60
50
40
30
20
10
0
Relative abundance of fish species (%)
Gorova
Dvoriche
Stoyacheye
Svyatoye
Cooling Pond
Yanovsky
Glybokoye
Pike
Perch
Roach
Rudd
Tench
Crucian carp
Prussian carp
Common carp
White bream
Blue bream
Common bream
Ruffe
European chub
Aspe
Gudgeon
Pontian monkey goby

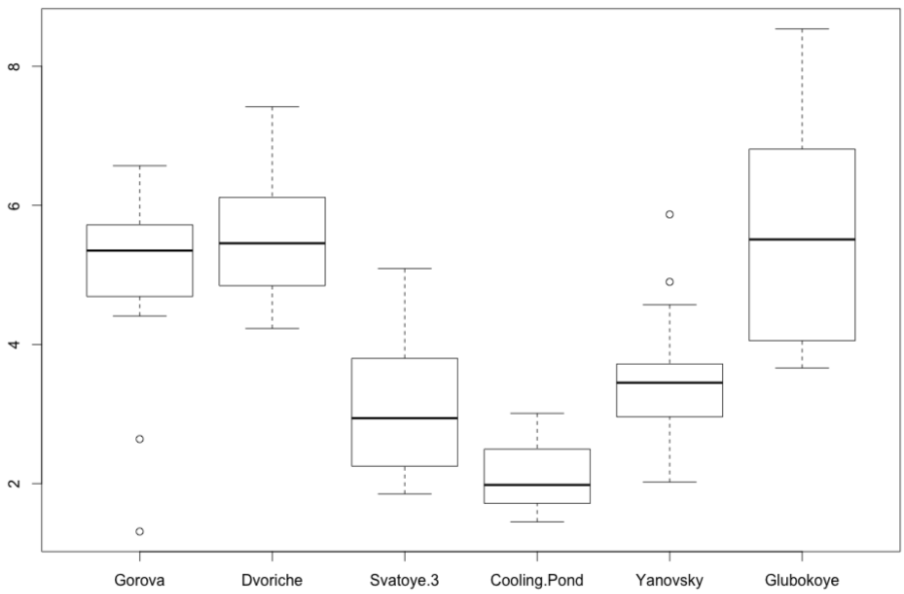
Figure S3.

A)



0
2
4
6
8
Gorova
Dvoriche
Stoyacheye
Svatoye.3
Cooling.Pond
Yanovsky
Glubokoye

B)



2
4
6
8
Gorova
Dvoriche
Svatoye.3
Cooling.Pond
Yanovsky
Glubokoye

Table S1.

	Roach		Perch	
	2014	**2015**	**2014**	**2015**
Glubokoye (H)	5	10	2	21
Yanovsky (H)	10	11	13	9
Cooling Pond (H)	1	2	9	16
Svyatoye (M)	9	/	17	/
Stoyacheye (L)	/	/	13	/
Dvoriche (L)	16	/	7	/
Gorova (L)	5	13	6	11

Table S2.

A

Lake	Maximum depth (m)	Surface (km^2)	Distance from CNPP (km)	^{137}Cs deposition (kBq/m^2) "corrected to 6/05/1986"
Cooling Pond (H)	18	22	1.5	19180
Yanovsky (H)	11	0.8	2.3	18650
Glubokoye (H)	7.3	0.1	10	15500
Svyatoye (M)	5.1	0.25	225	1778
Stoyacheye (L)	17	0.46	167	293
Dvoriche (L)	3.5	0.128	95	100
Gorova (L)	2	0.05	88	10

B

Lakes	NO_3^-	NO_2^-	PO_4^{3-}
Glubokoye (H)	49.3 ± 1.8	1.8 ± 0.5	8.1 ± 1.7
Yanovsky (H)	76.1 ± 44.7	9.8 ± 14.7	6.0 ± 1.1
Cooling Pond (H)	57.9 ± 21.9	11.9 ± 3.9	15.0 ± 15.5
Svyatoye (M)	159.3 ± 81.8	2.0 ± 1.0	1.7 ± 0.5
Stoyacheye (L)	259.2 ± 182.7	1.5 ± 0.5	1.4 ± 0.9
Dvoriche (L)	201.0 ± 39.7	2.1 ± 0.2	1.4 ± 0.4
Gorova (L)	79.6 ± 68.7	2.5 ± 0.1	5.6 ± 1.0

C

Lake	DO (%)	pH	T (°C)	Conductivity (µS/cm)
Glubokoye (H)	105	7.6	18.1	193
Yanovsky (H)	108	8	17.7	277
Cooling Pond (H)	48	8.6	15.6	318
Svyatoye (M)	110	6.3	18.9	120
Stoyacheye (L)	125	7.1	19.3	226
Dvoriche (L)	95	6.3	20.1	186
Gorova (L)	55	8.3	17.2	255

Table S3.

A

Lake	Na	Mg	S	K	Ca
Glubokoye (H)	5.6 ± 1.4	3.8 ± 0.4	1.1 ± 0.7	1.5 ± 0.2	26.2 ± 0.8
Yanovsky (H)	16.1 ± 1.3	4.9 ± 0.2	13.8 ± 1.8	4.8 ± 0.7	25.4 ± 1.4
Cooling Pond (H)	11.1 ± 1.4	7.9 ± 0.2	8.1 ± 1.1	3.2 ± 0.6	37.0 ± 1.3
Svyatoye (M)	2.02 ± 0.2	2.7 ± 0.1	0.3 ± 0.1	4.0 ± 0.2	18.0 ± 0.3
Stoyacheye (L)	2.7 ± 0.1	5.6 ± 0.3	0.8 ± 0.2	6.0 ± 0.3	33.2 ± 1.7
Dvoriche (L)	3.6 ± 0.1	5.7 ± 0.1	1.0 ± 0.1	2.4 ± 0.03	28.2 ± 0.5
Gorova (L)	6.2 ± 1.3	7.0 ± 0.3	2.4 ± 1.2	3.6 ± 0.7	37.8 ± 6.4

B

Lake	As	Sr	Cd	Cs	Pb	U
Glubokoye (H)	0.4 ± 0.05	103.6 ± 11.5	0.01 ± 0.009	0.003 ± 0.002	0.04 ± 0.06	0.01 ± 0.003
Yanovsky (H)	0.7 ± 0.2	117.5 ± 6.8	0.008 ± 0.009	0.005 ± 0.0007	0.02 ± 0.03	0.2 ± 0.1
Cooling P. (H)	0.8 ± 0.1	188.4 ± 14.2	0.002 ± 0.001	0.004 ± 0.0006	0.1 ± 0.3	0.6 ± 0.06
Svyatoye (M)	0.3 ± 0.001	64.4 ± 1.1	nd	0.01 ± 0.0009	nd	0.0006 ± 0.00005
Stoyacheye (L)	0.7 ± 0.05	61.5 ± 2.9	0.001 ± 0.001	0.006 ± 0.0002	nd	0.03 ± 0.003
Dvoriche (L)	0.9 ± 0.05	71.4 ± 1.8	0.0004 ± 0.002	0.02 ± 0.0007	0.01 ± 0.028	0.003 ± 0.0006
Gorova (L)	0.9 ± 0.2	159.8 ± 5.7	0.002 ± 0.001	0.002 ± 0.0007	0.006 ± 0.014	0.2 ± 0.03

Table S4

Lake (H)	^{241}Am Activity concentration (Bq/kg w.w.) 2015				^{239,240}Pu Activity concentration (Bq/kg w.w.) 2015				^{238}Pu Activity concentration (Bq/kg w.w.) 2015			
	Roach		Perch		Roach		Perch		Roach		Perch	
	Liver	Muscle	Liver	Muscle	Liver	Muscle	Liver	Muscle	Liver	Muscle	Liver	Muscle
Glubokoye	31.2 ± 4.1	4.6 ± 0.3	43.9 ± 8.4	4.1 ± 0.9	15.0 ± 4.4	1.8 ± 0.2	22.7 ± 4.0	1.2 ± 0.9	5.5 ± 2.2	0.7 ± 0.1	8.4 ± 2.0	0.5 ± 0.4
Yanovsky	33.1 ± 14.5	3.0 ± 0.03	22.1 ± 2.5	3.0 ± 0.03	12.5 ± 4.4	0.09 ± 0.04	8.4 ± 1.6	0.06 ± 0.03	4.6 ± 1.9	0.03 ± 0.01	3.2 ± 0.8	0.02 ± 0.01
Cooling Pond	23.9 ± 12.6	3.0 ± 0.04	23.2 ± 3.9	3.0 ± 0.03	9.5 ± 8.2	0.08 ± 0.05	9.3 ± 2.5	0.09 ± 0.03	3.7 ± 3.3	0.02 ± 0.01	3.3 ± 1.1	0.04 ± 0.01

Table S5.

		Glubokoye (H)	Yanovsky (H)	Cooling P. (H)	Svyatoye (M)	Stoyacheye (L)	Dvoriche (L)	Gorova (L)
Fulton condition factor	Perch	1.2 ± 0.4	1.2 ± 0.1	1.2 ± 0.2	1.2 ± 0.1	1.3 ± 0.1	1.2 ± 0.1	1.1 ± 0.1
	Roach	1.1 ± 0.1	1.0 ± 0.1	1.1 ± 0.0	1.1 ± 0.1	/	1.2 ± 0.1	1.1 ± 0.1
Hepatosomatic index	Perch	0.9 ± 0.3	1.4 ± 0.4	1.3 ± 0.4	0.8 ± 0.3	1.2 ± 0.5	0.9 ± 0.3	1.0 ± 0.3
	Roach	1.0 ± 0.2	0.9 ± 0.2	1.4 ± 0.1	0.7 ± 0.2	/	0.9 ± 0.1	1.2 ± 0.4
Body weight (g)	Perch	74 ± 38	88 ± 32	81 ± 20	99 ± 29	85 ± 14	70 ± 26	70 ± 16
	Roach	115 ± 23	80 ± 16	69 ± 13	105 ± 14	/	96 ± 16	77 ± 21
Total length (cm)	Perch	18.0 ± 2.4	19.4 ± 1.9	19.1 ± 1.6	20.0 ± 1.9	18.5 ± 1.1	17.9 ± 2.5	18.4 ± 1.4
	Roach	21.7 ± 1.5	20.2 ± 1.1	18.6 ± 1.3	21.1 ± 0.8	/	20.2 ± 1.0	19.1 ± 1.6
Gonad weight (g)	Perch	1.7 ± 1.2	2.0 ± 1.7	2.0 ± 1.4	2.5 ± 1.2	1.9 ± 0.8	2.3 ± 0.9	3.0 ± 1.0
	Roach	6.3 ± 2.0	2.8 ± 0.9	1.4 ± 0.2	3.2 ± 1.1	/	5.3 ± 1.2	3.9 ± 1.5
Age (yr)	Perch	5.1 ± 0.6	5.3 ± 0.7	4.5 ± 0.6	/	/	/	5.6 ± 0.8
	Roach	5.3 ± 0.5	6.9 ± 0.7	4.0 ± 0.0	/	/	/	4.9 ± 1.0

Table S6.

	Svyatoye (M)	Stoyacheye (L)	Dvoriche (L)	Glubokoye (H)	Yanovsky (H)	Cooling Pond (H)	Gorova (L)
Perch	0.3 ± 0.2	0.4 ± 0.2	0.3 ± 0.1	0.3 ± 0.3	0.2 ± 0.3	0.2 ± 0.1	0.1 ± 0.1
Roach	0.6 ± 0.2	/	0.6 ± 0.2	0.5 ± 0.1	0.1 ± 0.1	/	0.5 ± 0.1