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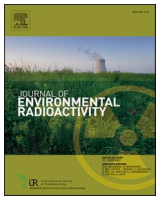

Prussian Blue to reduce radiocaesium accumulation in fish in lakes affected by the Chornobyl accident

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ABSTRACT

Fish in lakes situated within the Chornobyl exclusion zone have been highly contaminated since the accident and have not been utilized as food for humans. In the present study, field experiments with less-contaminated silver Prussian carp (*Carassius gibelio* (Bloch, 1782)) caged in contaminated lake within the Chornobyl exclusion zone was performed from June to October 2021 to investigate the effectiveness of clean feed containing potassium ferric hexacyanoferrate (KFCF) $KFe[Fe(CN)_6]$, a kind of Prussian Blue as a countermeasure to reduce ^{137}Cs accumulation in fish. The addition of clean feed containing 0.1% or 1% KFCF resulted respectively in 2.4 ± 0.4 or 4.2 ± 0.7 times lower activity concentration of ^{137}Cs in muscle tissue of the carp compared to control fish with clean feed without KFCF and in 7–16 or 12–27 times lower activities compared to fish without additional clean feeding. After 18 weeks exposure, ^{137}Cs levels in all the caged fish except for the control group were below the European permissible level (600 Bq kg^{-1}) for consumption. In contrast, KFCF did not affect the intake of ^{90}Sr in fish, although additional feed increased the growth rate and thereby accumulation in bone tissues. Hence, the use of clean feed containing KFCF is judged to be an effective and inexpensive countermeasure to reduce the ^{137}Cs contamination of edible fish muscles.

1. Introduction

Following the Chornobyl and Fukushima NPP accidents, the ^{137}Cs activity concentrations in fish from contaminated rivers and lakes in exclusion zone continue to exceed the permissible food intake levels (IAEA et al., 2006, 2015; Wada et al., 2019, 2023; Balonov et al., 2018; UNSCEAR 2020/2021 Report, 2022). Despite the activity concentrations of ^{137}Cs in lake waters being lower than the permissible drinking water level in Ukraine – 100 Bq L^{-1}, ^{137}Cs activity concentrations in fish could reach a factor of 10^3 higher than permissible levels for fish as food (150 Bq kg^{-1}), (Kaglyan et al., 2021; Teien et al., 2021). Consumption of lake water fish has been estimated to contribute up to 40–50% of the internal dose to inhabitants in some villages after the Chornobyl accident (Travnikova et al., 2004; IAEA et al., 2006).

Numerous international reports, articles and recommendations conclude that the most effective way to reduce the radiological impact on humans is the application of remediation actions/countermeasures (IAEA, 1994, 2006, 2012; Fesenko et al., 2006, 2007, 2021; European Commission, 2011; Nisbet and Watson, 2015). However, no effective countermeasures have been developed to reduce the levels of ^{137}Cs in fish for the purpose of radiation protection of the population (IAEA, 2012).

The uptake of radiocaesium in aquatic ecosystems is strongly influenced by potassium (K) concentration in water (Smith et al., 2000; IAEA, 2010; Khomutinin et al., 2011; Pinder et al., 2014; Konovalenko et al., 2016). The addition of potassium chloride (15 tons of KCl) to a contaminated lake after the Chornobyl accident resulted in a decrease in activity concentration of ^{137}Cs to approximately 40% of pre-countermeasure values in a number of different fish species (Smith et al., 2003). However, the application of KCl as a countermeasure for lakes was deemed ineffective due to subsequent remobilisation of ^{137}Cs from bottom sediments (IAEA, 2012).

Clean feeding to reduce activity concentrations of ^{90}Sr and ^{137}Cs in milk and meat has also been used to reduce the activity levels in farm

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animals, eggs and honey, and reduction factors of 2–5 times (ratio of activity concentration of radionuclides in the product before and after countermeasure application) have been reported (Fesenko et al., 2006, 2007; IAEA, 2012; European Commission, 2011; Nisbet and Watson, 2015). Since the major source of radiocaesium contamination of freshwater fish is feed and not water (Smith, 2006; Haque et al., 2017; Metian et al., 2019; Kashparova et al., 2020, 2022; Pavlenko et al.; Teien et al., 2021), replacement of natural food sources with uncontaminated feed to fish could reduce the ^{137}Cs concentrations in the tissues (IAEA, 1994). Recent field studies have demonstrated the effectiveness of using additional clean feed to counteract contamination of ^{137}Cs in the silver Prussian carp (*Carassius gibelio* (Bloch, 1782)) within the Glubokoye Lake situated in Chornobyl Exclusion Zone (ChEZ) (Pavlenko et al., 2021; Kashparova et al., 2023). The application of clean feed to contaminated fish under natural conditions resulted in 2–5 times lower activity concentration of ^{137}Cs in the fish muscle tissue, primarily due to the increase of fish mass and thereby biodilution. The biological half-life of ^{137}Cs activity concentration in muscle tissues from contaminated fish in Glubokoye Lake consuming both natural and additional clean feed was 115 ± 25 days (Pavlenko et al., 2021), in contrast to half-lives of 46–95 days obtained for fish consuming only clean food in clean water (Kashparova et al., 2019, 2022). There were no significant differences in the effectiveness of clean feed as a countermeasure when administered as floating compared to sinking clean feed to fish (Kashparova et al., 2023), and no differences between cages situated in the sediment or floating without any contact to contaminated sediments (Pavlenko et al., 2021). In contrast to ^{137}Cs, the increased mass due to intake of additional clean feed led to increased ^{90}Sr concentrations in fish bones compared to controls, by up to a factor of 5 (Pavlenko et al., 2021; Kashparova et al., 2023).

In the field, application of additional clean feed did not lead to a significant decrease in the intake of ^{137}Cs contaminated feed by fish. Therefore, the radiological effectiveness of clean feed as countermeasure could be increased by adding selective Cs sorbents such as hexacyanoferrate (IAEA, 1997; Fesenko et al., 2007). Hexacyanoferrate compounds (also known as Prussian blue or ferrocyn) are highly effective chelates of radiocaesium ions, changing the physico-chemical form from simple cations to large molecular mass chelates and thereby reducing the bioavailability of radiocaesium. By adding Prussian blue to clean feed, gut absorption would be reduced and thereby the transfer of radiocaesium across membranes to muscles and organs could be inhibited (IAEA, 1997, 2012).

Following the Chornobyl accident, the Prussian blue compounds most commonly used for remediation of farm animals and poultry were ammonium ferric hexacyanoferrate (AFCF) $NH_4Fe[Fe(CN)_6]$, potassium ferric hexacyanoferrate (KFCF) K $Fe[Fe(CN)_6]$ and ferric hexacyanoferrate (FCF) $Fe_4[Fe(CN)_6]_3$ (IAEA, 1997, 2006, 2012; Fesenko et al., 2006, 2007). Application of Prussian blue with relatively small amounts for daily consumption (1–40 mg kg^{-1} live body weight of the animal) for sheep, goats, dairy cows, bull calves, pigs and chickens resulted in reduction factors of 3–8 times without any negative influence on animal or human health (Pearce, 1994; Fesenko et al., 2007; IAEA, 1997, 2012). On October 14, 2001 permanent authorisation was given by the European Communities for AFCF to be used as a feed additive for the purposes of binding radiocaesium (Regulation, 2013/2001) (European Commission, 2011). Prussian blue application is also a cost effective option and was one of the most effective management options used in Norway and the former USSR after the Chornobyl accident (Brynildsen et al., 1996; Jacob et al., 2009; IAEA, 2012; Labunska et al., 2018).

Knowledge on the effectiveness of remediation actions to reduce radioactive accumulation in fish following nuclear accidents is important for radiation protection of the population (IAEA et al., 2006; 2012). Therefore, the main objective of the present study was to investigate the influence of, and estimate reduction factors for, clean feed containing different concentrations of KFCF, to reduce the ^{137}Cs transfer to the silver Prussian carp under natural climate conditions for commercial fish farming in cages with clean feeding supply in contaminated lakes.

2. Material and methods

The uptake of ^{137}Cs and ^{90}Sr in silver Prussian carp fed clean feed containing different KFCF concentrations were investigated in fish contained in cages in the Glubokoye Lake (51.444796° N, 30.063938° E), the most contaminated lake within the Chornobyl exclusion zone (see Fig. S1 in SI) (Kashparov et al., 2020; Teien et al., 2021; Kashparova et al., 2023). Experiments were carried out between June and October 2021, using the same control fish as described previously (Kashparova et al., 2023). Ethical approval of the experiments (to maintain and expose fish) was given by the NUBiP Commission of Ukraine in compliance with the requirements of the Law of Ukraine "On protection of animals from cruel treatment" of 21.02.2006 N№ 3447-IV.

2.1. The feed properties and production method

Commercial feed for pond carp, made by "Skaliaria" (Rivne, Ukraine, https://skaliaria.rv.ua/), was used as feed with size 3 mm (30% protein, 10% fat, 3% cellulose, vitamins A, D3, E, C). In the process of preparing feed, 10 g of KFCF (0.1% of feed weight) was added to 10 kg of feed ingredients during mixing and 100 g of KFCF (1% of feed weight) was added to another 10 kg of feed, in accordance with IAEA recommendations for small animals – 10–40 mg (day kg)$^{-1}$ of animal live weight (IAEA, 1997). After that, the feed was granulated into pellets (see Fig. S2 in SI).

Sorption testes with KFCF treated feed were performed to test whether KFCF increase the ^{137}Cs contamination in feed, thereby influence the ^{137}Cs uptake in fish. The sorption of waterborne ^{137}Cs to clean feed containing different KFCF concentrations was studied in laboratory experiments, three replicate tests with 10 g of feed each, without and with 0.1% and 1% of KFCF, were exposed for different time periods (1, 5, 10, 15, 30, 60 and 120 min at the water temperature 20°C) in a plastic mesh bag containing 1000 ml of water from Glubokoye Lake with the addition of 1 mL ^{137}CsCl solution. The resultant activity concentration of ^{137}Cs in the water was 1.0 ± 0.1 kBq L^{-1}. At the end of the designated time in the water, the feed was weighed and activities of ^{137}Cs were measured in the feed. In addition, the activity concentrations of ^{137}Cs in the water were determined at the beginning and at the end of each experiment.

2.2. Fish species and experimental design

The study (Table 1) included wild Prussian carp of the age 1+ years with an average body weight of 16–20 g that were caught from a lake close to Kyiv with activity concentrations of ^{137}Cs and ^{90}Sr in fish less than 10 Bq kg^{-1}, i.e., three orders of magnitude lower than the levels of radioactive contamination of native fish in Glubokoye Lake (Teien et al., 2021; Kaglyan et al., 2021; Kashparova et al., 2023). The activity concentration of ^{137}Cs for 12 native carp (320–1700 g) caught in Glubokoye Lake in March 2019 was on average 6.7 ± 1.2 kBq kg^{-1} in muscle (Teien et al., 2021; Kashparova et al., 2023) and similar to levels reported Kaglyan et al. (2021). The activity concentration of ^{90}Sr was 0.5 ± 0.3 kBq kg^{-1} in muscle and 63 ± 16 kBq kg^{-1} in bone of the native carp.

Cages of 1 × 1x1m were covered with plastic netting with a mesh size of 1 cm (Teien et al., 2021; Pavlenko et al.; Kashparova et al., 2023). In total, 8 separate cages were included in the study (see Fig. S2 in SI): 2 control cages (1 and 2) without additional clean feed; 2 cages (3 and 4) with clean feeding without KFCF, 2 cages (5 and 6) with clean feeding with 0.1% KFCF and 2 cages (7 and 8) with clean feeding with 1% KFCF (Table 1, Table S1 in SI). All floating cages were located at a distance of 6–10 m from the shore of Glubokoye Lake at a depth of 1.3–1.6 m, without contact with bottom sediments (0.3–0.6 m above sediments), meaning that 10–15 cm of the cages was above the water surface. Cages

Table 1
Average body weight, activity concentrations of ^{137}Cs in muscle and ^{90}Sr in bone tissue of fish (WM) and whole-body radionuclide activity in fish received feed with different concentration of potassium ferric cyanoferrate (KFCF) (Mean ± SE, N = 7). Values are from the end of the experiments on October 5, 2021.

Cage#			Body weight, g		Activity concentration, kBq kg^{-1}		Activity whole-body of fish, Bq	
No	Feeding		Beginning	End	^{137}Cs in muscle	^{90}Sr in bone	^{137}Cs	^{90}Sr
	Additional clean feed	+KFCF, %						
1	No	0	20 ± 1	20 ± 2	2.7 ± 0.2	14.5 ± 1.3	36 ± 4	150 ± 23
2	No	0	16 ± 1	17 ± 1	3.1 ± 0.2	15.5 ± 1.1	38 ± 3	134 ± 11
3	Yes	0	20 ± 1	44 ± 2*	0.91 ± 0.05*	26.8 ± 0.9*	33 ± 2	602 ± 42*
4	Yes	0	16 ± 1	42 ± 2*	1.1 ± 0.1*	28.7 ± 1.6*	34 ± 5	602 ± 58*
5	Yes	0.1	20 ± 1	46 ± 3*	0.43 ± 0.05**	24.9 ± 1.6*	15 ± 3**	583 ± 73*
6	Yes	0.1	16 ± 1	42 ± 2*	0.40 ± 0.05**	28.4 ± 2.0*	12 ± 2**	598 ± 63*
7	Yes	1	20 ± 1	51 ± 2*	0.25 ± 0.02**	29.6 ± 1.1*	9 ± 1**	751 ± 42*
8	Yes	1	17 ± 1	44 ± 3*	0.24 ± 0.03**	26.4 ± 2.1*	7 ± 1**	598 ± 87*

* - Significant different from control cages 1 and 2 without additional feed, $p < 0.01$.
** - Significant different from cages 1–4 without KFCF, $p < 0.01$.

3–8 with sinking clean feeding (i.e., sinking feed with different KFCF content) were located at a distance of 20–30 m from the two control cages (see Fig. S2 in SI). During summer the activity concentration ^{137}Cs in muscle tissue of fish introduced to the contaminated water increased to about 5.8 kBq kg^{-1}, i.e., similar to levels observed in several years old native silver Prussian carp (6.7 ± 1.2 kBq kg^{-1}) (Teien et al., 2021). Therefore, in addition to the fish without additional feeding (cages # 1–2), wild native carp were used as a control group (Kashparova et al., 2023).

Two automatic fish feeders (Aqua Nova, Poland) were placed on top of each cage (3–8), supplying the same volume of clean sinking feed to the cages two times every day, at 12:00 and 18:00 h. The supplied daily feed mass (30 g per day, approx. 8–20% of total fish body weight) always exceeded the required daily ration of 1–2% of the fish body weight (Craig et al., 2009). The feed passed from the feeder through a guide pipe to a special plate in the center of the cages to prevent loss through the plastic mesh at the bottom of the cage (see Fig. S2a in SI). To control the amount of feed used, the weight of feed added to the feeder was measured every month, with an accuracy of ±1 g.

Seven tagged carps were added to each cage on June 1, 2021. The passive integrated transponder (PIT) tag (8 mm × 1.4 mm FDX-B "Skinny", Oregon RFID, USA) embedded in the abdominal cavity allowed the identification of fish, enabling changes in mass in individual fish to be followed during the experimental period.

2.3. Water and fish sampling

Monthly water samples were collected from Glubokoye Lake to characterize the water quality and obtain information about the dissolved fraction of radionuclides in 0.45 μm filtered water samples (Teien et al., 2021; Kashparova et al., 2023). Water temperature loggers (Onset HOBO UA-001-64) were placed in the central part of a cage.

The ^{137}Cs activity of 7 tagged fish was measured on July 13, September 1 and October 5, 2021 during the experiment by live *in situ* monitoring to demonstrate the dynamics of contamination. Both weight and length of each fish were recorded at each time of live monitoring.

Individual muscle and bone tissue samples from each fish, together with a composite sample of the intestine contents from the 7 fish in each cage, were collected at the end of the experiment on October 5, 2021 after whole body live monitoring. Fish were euthanized by a blow to the head. Masses and total lengths were measured followed by dissection and collection of muscle and bone tissue samples in accordance with the EMERGE sampling protocol (Rosseland et al., 2001; Teien et al., 2021; Kashparova et al., 2023). Samples of muscle without skin, bone tissue, as well as the intestine content samples were kept in a freezer at −20 °C prior to analysis (Teien et al., 2021; Kashparova et al., 2023).

2.4. Determination of the activity concentration of ^{137}Cs and ^{90}Sr

Determination of ^{137}Cs activity concentrations in water samples and fish tissues samples were based on measurements using a low background γ-spectrometric complex with multi-channel analyser ASPEC-927 (software GammaVision 32) and a detector of high purity germanium GEM-30185 «EG & G ORTEC» (USA) with an energy resolution of 1.78 keV at the ^{60}Co line of 1.33 MeV in low-background passive protection. The minimum detectable activity of ^{137}Cs was 0.1 Bq (detailed protocols have been described previously in Teien et al., 2021; Kashparova et al., 2023).

In situ measurements of ^{137}Cs activity in each group of 7 live fish were carried out in the field (51.466762° N, 30.020235° E, 3.9 km from the Glubokoye Lake with minimal gamma radiation background <5 Bq) in Marinelli vessel (1 L) with "clean" water to demonstrate the dynamics of ^{137}Cs contamination of fish without and with additional clean feed containing different KFCF concentrations. Measurement of ^{137}Cs activity was carried out for 600–1000 s on a scintillation gamma-ray spectrometer (SEG-05, Ukraine; software LSRM) with NaI(Tl) 63 × 63 mm detector in passive protection (detailed protocols have been described previously in Teien et al., 2021; Kashparova et al., 2023). Results from whole body measurements are presented as ^{137}Cs activity concentration in muscle tissue, calculated using the muscle tissue to whole body ratio determined at the end of the experiment. The ratio of muscle tissue to whole-body activity concentration of ^{137}Cs in silver Prussian carp was 1.4 ± 0.1 in this and in other similar experiments (Teien et al., 2021; Kashparova et al., 2023).

The ^{90}Sr activity in fish bone tissues was determined after ashing in a muffle furnace at a temperature of 550 °C using the direct method on a SEB-01-70 beta spectrometer (AKP, Ukraine) (Courti et al., 2002). The minimum detectable activity of ^{90}Sr in bone ash on a beta spectrometer was 1 Bq. The ash content of silver Prussian carp bones was 20 ± 3% on a wet weight basis (N = 35). The ratio of bone tissue to whole-body activity concentration of ^{90}Sr in silver Prussian carp was 2.0 ± 0.4 (Gudkov et al., 2008; Teien et al., 2021; Kashparova et al., 2023).

The activity concentrations of radionuclides in fish tissues are given as wet mass weight (WM). To assess the reduction factors of clean feed, only results from laboratory measurements of the activity concentration of ^{137}Cs in individual muscle tissue samples from each tagged fish by gamma-spectrometry at the end of the experimental period (on October 5, 2021) were used.

2.5. Data analysis

The time dependent changes of the activity concentration of radionuclides in the body of fish $C_f(t)$ could be described by linear differential equation (1) (Smith, 2006; Teien et al., 2021):

$$\frac{dC_f}{dt} = (k_f + k_w)C_w - (k_b + \lambda)C_f, \quad (1)$$

where $C_w(t)$ and $C_f(t)$ are activity concentrations of the radionuclides in water and fish tissues (Bq kg^{-1}), respectively, at time t (days); k_f and k_w are the rate of uptake of the radionuclides in fish by diet and water (day^{-1}), respectively; k_b is the rate of depuration of radionuclides from fish (day^{-1}); λ is decay constant 6.6·10^{-5} day^{-1} for ^{90}Sr and 6.3·10^{-5} day^{-1} for ^{137}Cs. This model is based on the following assumptions: linear dependence between contamination of food and water; fish growth is neglected.

In the case of transferring a "clean" fish ($C_f(0) = 0$ Bq kg^{-1}) into a radioactive contaminated lake with water activity concentration (C_w, Bq kg^{-1}), the solution of equation (1) has the form of equation (2):

$$C_f(t) = \frac{(k_f + k_w) \bullet C_w}{(k_b + \lambda)}(1 - exp(-(k_b + \lambda)t)) \quad (2)$$

Initially at small values, $((k_b + \lambda) \bullet t) < 0.5$ (for ^{137}Cs when $\boldsymbol{k_b} < 0.01$ day^{-1} $\boldsymbol{t} < 50$ day and for ^{90}Sr when $\boldsymbol{k_b} < 0.001$ day^{-1} $\boldsymbol{t} < 500$ day (Teien et al., 2021)), and equation (2) can be approximated by a linear dependency shown in equation (3):

$$C_f(t) \cong (k_f + k_w) \bullet C_w \bullet t \quad (3)$$

Using measured concentrations of radionuclides in fish ($C_f(t)$) and water (C_w), equation (3) was used to determine the uptake rates ($\boldsymbol{k_f} + \boldsymbol{k_w}$) of radionuclides in fish.

2.6. Statistical analysis

Raw data such as tissue activity concentrations, whole-body activity and weight of fish were either collected from individuals or as an average of the seven fish in each cage at the day of measurements (Table S1 in SI). For the statistical analysis, each individual fish was assigned a random normal value in Bq kg^{-1} and Bq. The measured activity concentration value was used as the mean and the uncertainty value was used as the variance as input for the random number generator. Individual random numbers were created regardless of whether the measured value was from individual fish or from cage averages.

Initial fitting of the ^{137}Cs-data with many linear and non-linear models showed that the One Compartment Oral Dose model achieved the smallest Akaike's Information Criterion (AIC) because there was an apparent decrease in the ^{137}Cs activity after day 42 among the KCFC treated fish (Table S2 in SI). However, from day 42 on, linear models were well suited for comparing the feeding regimes. Since we only had cage means from the live measurements of ^{137}Cs, a repeated measurement analysis on the individual fish was not possible. Effects due to differences in feeding regimes on ^{137}Cs activity concentrations were assessed by comparing the slopes for Bq kg^{-1} on days exposed. All models were run 2500 times with new random normal values generated for each individual fish and the mean p-value estimates are reported. Analyses were done in JMP Pro 17.0.0 (SAS Institute, Cary, NC, USA) and JASP 0.17.1 (JASP Team (2023), Amsterdam, The Netherlands).

In other figures, mean ± standard error (SE) values are reported. Statistical significance at $p < 0.05$ is used if no other information is given.

3. Results and discussion

3.1. Sorption of ^{137}Cs and ^{90}Sr in clean feed

The activity concentrations of ^{137}Cs and ^{90}Sr in the Glubokoye Lake water were 4.0 ± 1.0 Bq L^{-1} and 100 ± 10 Bq L^{-1}, respectively, while potassium concentration was 1.2 ± 0.1 mg L^{-1} and calcium concentration 30 ± 2 mg L^{-1} (Kashparova et al., 2023).

The transfer of ^{137}Cs from contaminated laboratory water ($\boldsymbol{C_w}$ = 1.0 ± 0.1 kBq L^{-1}) into the feed after 1 min contact ($\boldsymbol{C_{feed}/C_w}$ = 0.3–0.5) was most probably due to absorption of water by the feed, resulting in a 1.5 times increase in feed wet mass (Fig. 1). The activity concentration of ^{137}Cs in wet feed without KFCF showed no further change and remained a factor of 2 lower than the activity concentration of ^{137}Cs in the water after 120 min (Fig. 1). Laboratory experiments demonstrated, however, that prolonged contact time up to 120 min resulted in some adsorption of ^{137}Cs to feed containing KFCF, where the activity concentration of ^{137}Cs in wet feed with KFCF increased with a factor of 2 compared to the contaminated water activity concentration (Fig. 1).

Field experiments also showed that sorption of ^{137}Cs and ^{90}Sr from Glubokoye Lake water to sinking clean feed without KFCF was insignificant (Kashparova et al., 2023). The obtained values were one-two orders of magnitude lower than the activity concentration of ^{137}Cs and ^{90}Sr in the intestine content of fish previous reported in Glubokoye Lake (Gudkov et al., 2008). Thus, water contamination of the clean feed of ^{137}Cs and ^{90}Sr is judged to be insignificant with respect to radionuclide uptake in fish.

3.2. Clean feed effect on growth and ^{137}Cs activity concentration

Fig. 2 shows the temperature, dynamics of average fish weight and the specific activity of ^{137}Cs in muscle tissue from fish given clean feed with different KFCF concentrations and from control fish (no additives) (see Table S1)). The average water temperature in the Glubokoye Lake was 23 ± 4°C and varied between 11 and 30°C during the experimental period from June 1 to October 5, 2021 (Fig. 2a).

The fish weight increased most rapidly at the beginning of the experiment from 01.06.21 to 13.07.21 at the water temperature 18–30°C (Fig. 2a); the average fish weight gain rate was 0.08 ± 0.01 g day^{-1} over the first six weeks for carp without additional clean feed (cage 1–2) and significantly different ($p < 0.01$, Table S1, Figs. 2b and 3) from 0.44 ± 0.01 g day^{-1} for carp receiving clean feed without and with KFCF (cage 3–8). Maximum fish weight was reached after the initial 12 weeks of exposure at the water temperature >18°C, decreasing slightly in the last 4 weeks (Figs. 2b and 3 and Table S1). By autumn, the growth rate of fish in all cages slowed down, which probably was due to seasonality with variable activity of silver Prussian carp, differences in metabolism as well as in nutrition due to the reduction in water temperature during September from 21°C to 11°C (Fig. 2a) (Teien et al., 2021).

By September 1, the body weight of carp receiving clean feed had increased by a factor of 2.6 ± 0.2 while the control group had increased by only a factor of 1.2 ± 0.1, relative to the initial body weight of fish (Fig. 2b and Table S1). The content of KFCF in feed showed no statistically significant influence on fish growth ($p < 0.05$). Weight curves showed a best fit with a quadratic term to the linear model because

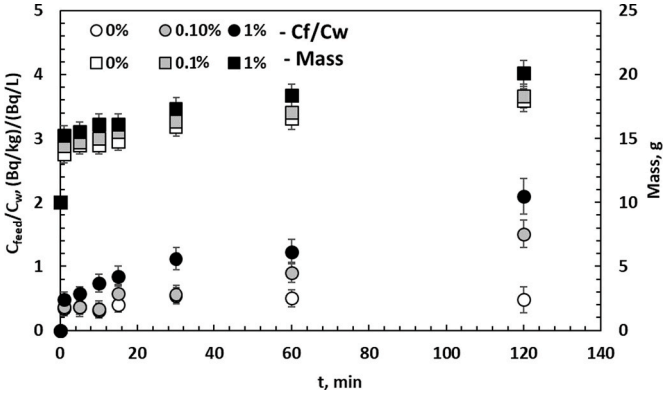

Fig. 1. Dynamics of the activity concentrations of ^{137}Cs in wet feed ($\boldsymbol{C_{feed}/C_w}$, $\boldsymbol{C_w}$ = 1.0 ± 0.1 kBq L^{-1}) and wet mass of feed (dry mass was 10 g) without KFCF (0%) and containing 0.1% KFCF and 1% KFCF relative to water ($\boldsymbol{C_w}$), as a function of contact time.

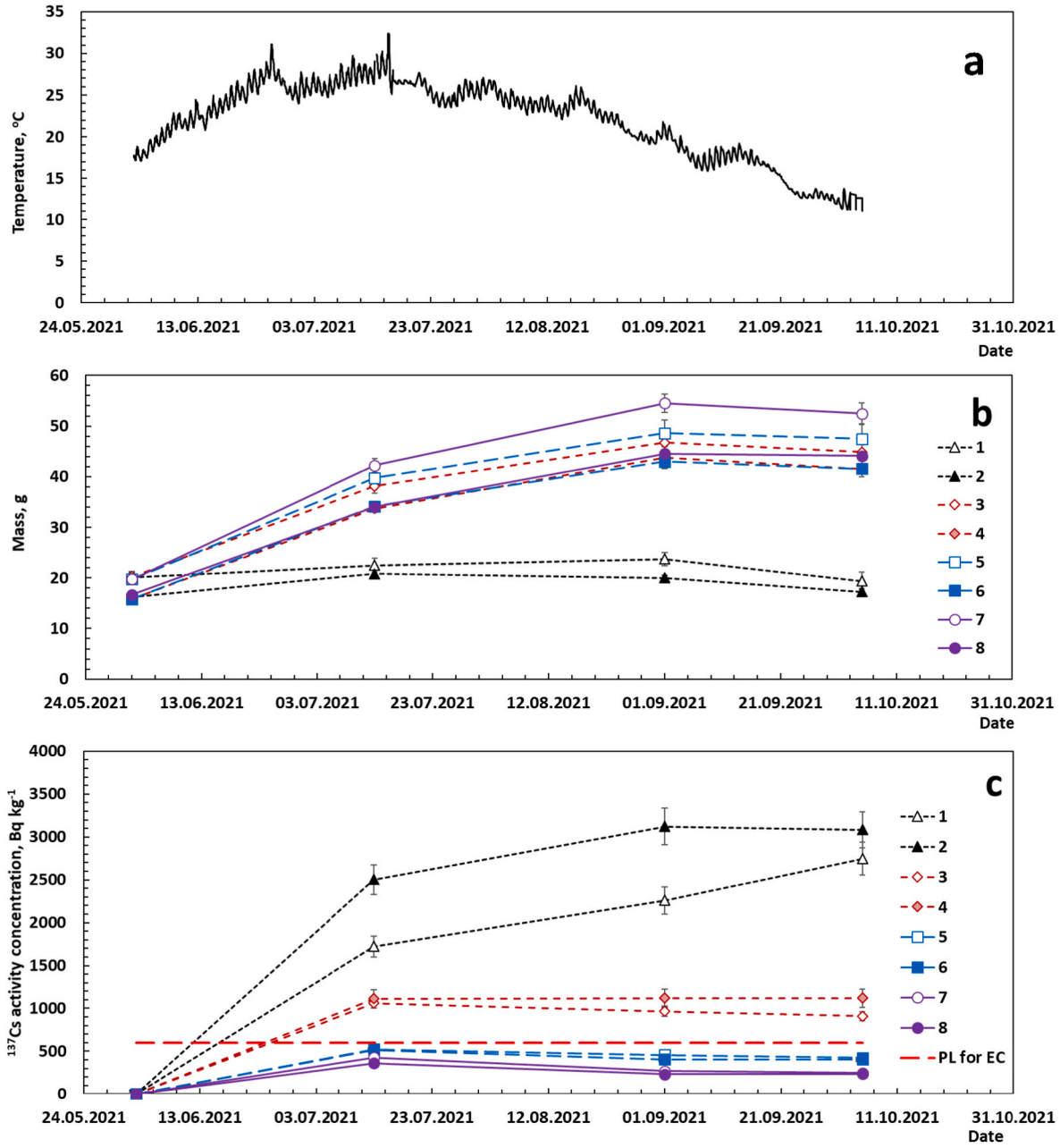

Fig. 2. a) Variations in water temperature during the feeding experiment in field; b) variation in average weight of silver Prussian carp; c) ^{137}Cs activity concentrations in muscle tissues, 1 and 2 - controls without additional clean feeding; 3 and 4 - with additional sinking clean feed without KFCF; 5 and 6 - with additional sinking clean feed containing 0.1% KFCF; 7 and 8 - with additional sinking clean feed containing 1% KFCF. The red line shows the European permissible level (PL) for food consumption 600 Bq kg^{-1}. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

average body weights had decreased at the last sampling (Fig. 3).

Figs. 2c and 3 show the dynamics of the activity concentration of ^{137}Cs in the muscle tissue of silver Prussian carp during the experiment. For control fish (cages 1 and 2), the activity concentration of ^{137}Cs in the muscle tissue increased during the 18 weeks of exposure from <0.01 to 2.7–3.1 kBq kg^{-1} (Table 1, Fig. 2c), and reached 50% of the ^{137}Cs activity concentration observed in native carp from the lake (Kaglyan et al., 2021; Teien et al., 2021; Kashparova et al., 2023). The activity concentrations of ^{137}Cs in muscle tissues of fish that received additional clean feed without KFCF (cages 3 and 4) also increased, but only up to 0.9–1.1 kBq kg^{-1} (Table 1, Fig. 2c). However, the average whole body ^{137}Cs activity of fish (33–38 Bq) at the end of the experiment did not differ significantly between control fish and those given additional clean feed without KFCF (Table 1, Fig. 3). Slightly lower ^{137}Cs content in whole-body of fish with additional clean feeding without KFCF (33–34 Bq) compared to control (36–38 Bq) may be due to slightly reduced fish intake of natural food. Thus, additional clean feed given to fish did not significantly reduce the uptake of ^{137}Cs associated with natural diet into the fish body, but decreased the activity concentrations of ^{137}Cs in the muscle tissue due to biodilution; the increase of fish mass in clean feed fish being a factor of 2.4 ± 0.2 compared to control (Fig. 2b and c). Similar results were obtained in experiments performed during 2018–2020 (Pavlenko et al., 2021; Kashparova et al., 2023).

The rate of ^{137}Cs uptake in muscle tissues of fish ($k_f + k_w$) without additional clean feed (cages 1 and 2) was 10–15 day^{-1} and for fish with additional clean feed without KFCF (cages 3 and 4) was 6–7 day^{-1} (Table S1, for Fig. 3 ($k_f + k_w$) = β/C_w).

Based on measurement of weight and activity concentrations of

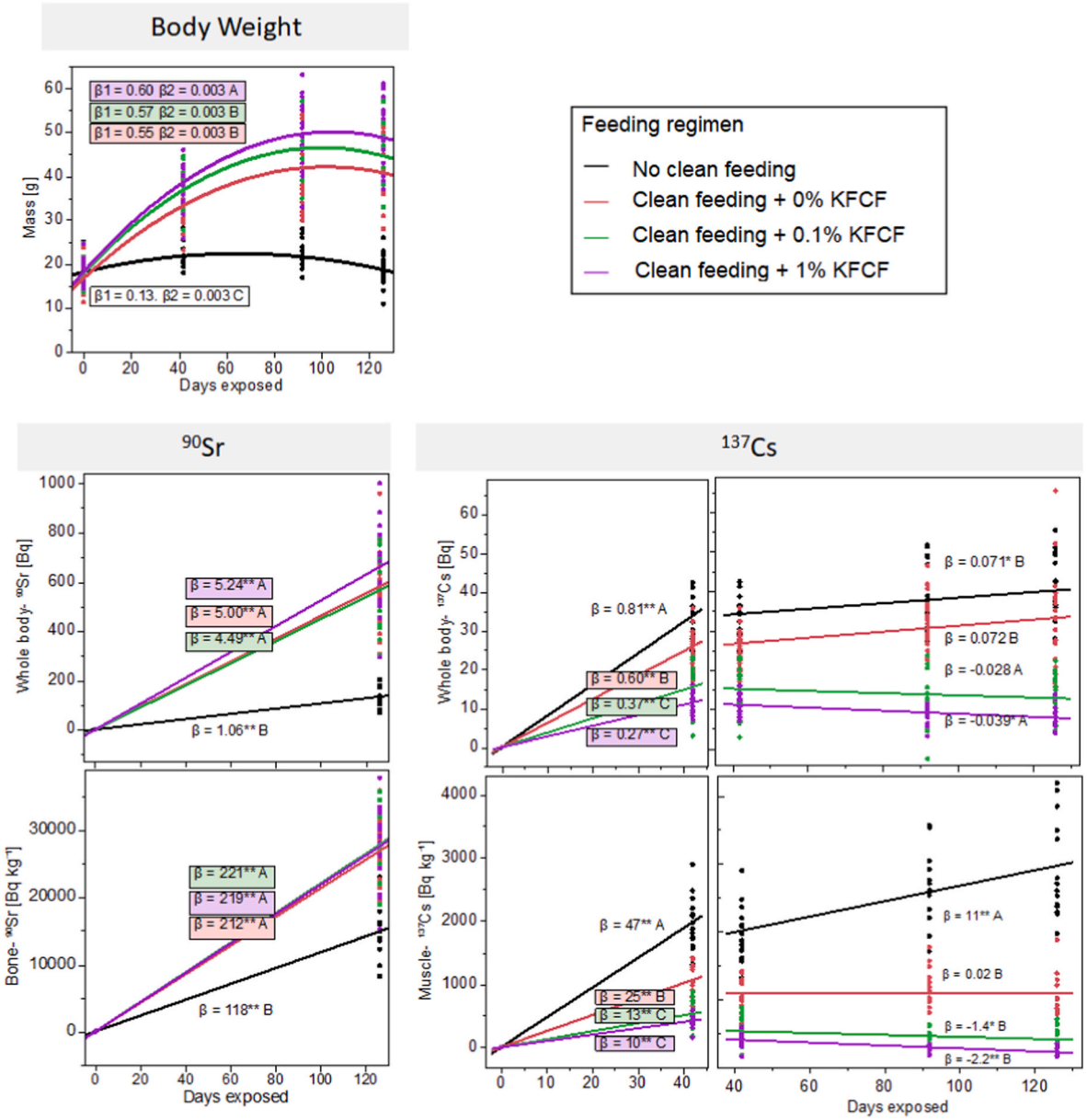

Fig. 3. Whole-body radionuclide activity in fish (Bq), activity concentration of ^{90}Sr in bone and ^{137}Cs in muscle tissue and body weights during experimental feeding. Betas are slopes for each treatment group with 95% confidence intervals (β-s are marked with *p < 0.05, **p < 0.01 when different from zero. β-s not sharing capital letters (A, B, C) have significantly different slopes at p < 0.05). Power analysis and details for the slope estimates for the ^{137}Cs data from day 42 onwards are presented in the simulation results table (Table S2 in SI).

radionuclides in fish at the end of the experiment, the caging effect was judged to be of minor importance (Fig. 2b and c and Table 1), as shown in previous experiments performed during 2016–2020 (Teien et al., 2021; Kashparova et al., 2023).

3.3. KFCF effect on transfer of ^{137}Cs in fish

At end of the experiment, the activity concentrations of ^{137}Cs in the intestine content of fish given additional clean feed with different KFCF concentrations (0.8 ± 0.1 kBq kg^{-1}) showed no difference between groups (cages 3–8), but was a factor of 2 lower than the activity concentrations of ^{137}Cs in the intestine content from control fish (cages 1–2) without additional clean feed (1.6 ± 0.4 kBq kg^{-1}). Fish given additional clean feed containing 1% KFCF showed an increase in activity concentration of ^{137}Cs in muscle tissues during the first 5 weeks of exposure in the contaminated lake (June 1 to July 13, 2021), followed by decrease in activity concentrations of ^{137}Cs during the remaining experimental period (July13 to October 5, 2021) (Figs. 2c and 3, Tables S1 and S2). The decrease in activity concentration during this 12-week period, from 395 ± 57 Bq kg^{-1} to 242 ± 19 Bq kg^{-1} ($p < 0.01$), corresponded to a biological half-life for ^{137}Cs of 105 ± 12 days. This biological half-life corresponded to the biological half-life of ^{137}Cs observed in silver Prussian carp muscle tissues after placing the fish in clean water (Kaglyan et al., 2018; Kashparova et al., 2019, 2022; Teien et al., 2021) suggesting that KFCF successfully prevent uptake ^{137}Cs and its elimination was due to the excretion and biological dilution (Fig. 3).

Comparing whole-body fish activity concentrations after 18 weeks of exposure, the application of additional clean feed containing 0.1% or 1% KFCF significantly reduced the uptake of ^{137}Cs activity by a factor of 2.7 ± 0.9 and 4.4 ± 0.3 ($p < 0.01$), respectively, compared to control fish and to fish given additional clean feed without KFCF (Table 1, Fig. 3). Taking biodilution into account, the radiological efficiency (reduction

factor) of the countermeasure in reducing activity concentrations of ^{137}Cs in fish muscle tissues was a factor of 7–16 and 12–27, respectively, for the application of clean feed containing 0.1% ($(k_f + k_w) = 3.1 \pm 0.6$ day^{-1}) and 1% ($(k_f + k_w) = 2.3 \pm 0.5$ day^{-1}) KFCF, compared to the control groups without additional clean feeding (cages 1 and 2) and native carp, and a factor of 2.4 ± 0.4 and 4.2 ± 0.7, respectively, compared to fish with additional clean feed without KFCF (cages 3 and 4) (Table 1).

Implementation of the present countermeasure would increase feeding costs by 1% and 10%, respectively, due to the addition of 0.1% and 1% of KFCF to feed, as the cost of KFCF is about 10 Euro per kg (Ulanovsky et al., 2011; Labunska et al., 2018), compared to present costs of fish feed in Ukraine of about 1 Euro per kg (https://skaliaria.rv.ua/). But given the high reduction factors obtainable, the countermeasure could certainly be economically justified in terms of radiation protection and fish food production.

3.4. Clean feed on transfer of ^{90}Sr in fish

As demonstrated in previous experiments (Kashparova et al., 2023), the addition of clean feed resulted in an increase in the transfer of ^{90}Sr to fish bones. Without additional feed (cages 1and 2 in Table 1), the total activity in fish and the activity concentrations of ^{90}Sr bone tissues at the end of the experiment were 142 ± 25 Bq and 15 ± 1 kBq kg^{-1} ($(k_f + k_w) = 1.2 \pm 0.1$ day^{-1}), respectively (cages 1 and 2 in Table 1). The activity concentration of ^{90}Sr for fish with additional feed showed a statistically significant increase of 620 ± 60 Bq and 27 ± 2 kBq kg^{-1} ($(k_f + k_w) = 2.1 \pm 0.2$ day^{-1}) ($p < 0.01$), while no statistically significant difference was found between groups receiving additional feed with and without KFCF (Table 1, Fig. 3).

Since KFCF in feed affected neither fish weight gains nor ^{90}Sr uptake to fish (Fig. 3), the increased ^{90}Sr levels in fish bones receiving additional clean food, could be attributed to the significant increase in bone tissue mass due to the additional diet compared to the control, as the uptake of ^{90}Sr in fish comes directly from the contaminated water. This is consistent with previously obtained results (Pavlenko et al., 2021; Kashparova et al., 2023).

4. Conclusions

Feeding experiments with less-contaminated silver Prussian carp caged in contaminated lake water within the Chornobyl exclusion zone have shown that additional clean feed containing KFCF is an effective and cheap countermeasure to reduce the ^{137}Cs accumulation of fish muscle tissues.

Clean feed without KFCF reduced the ^{137}Cs activity concentration in muscle by a factor of 2.6 ± 0.3 during summer, due to increased mass growth (in 2.6 ± 0.2 time) and subsequent biodilution. Application of additional clean feed containing 0.1% or 1% KFCF led to additional reduction in ^{137}Cs in fish tissues of 2.7 ± 0.9 and 4.4 ± 0.3 times, respectively, compared to fish without and with additional clean feed without KFCF. Taking biodilution into account, the radiological efficiency (reduction factor) of applying additional clean feed containing 0.1% and 1% KFCF to reduce the activity concentrations of ^{137}Cs in fish muscle tissue of fish was a factor of 7–16 and 12–27, respectively, compared to the control groups without additional clean feeding. In contrast to ^{137}Cs, additional clean feed with KFCF did not affect the uptake of ^{90}Sr. Compared to control, however, the accumulation of ^{90}Sr in fish bone tissues increased due to the addition of clean feed, followed by increase in fish mass and the intake of ^{90}Sr directly from the water. However, the radiological risk related to dietary intake is low since the activity concentration of ^{90}Sr in muscle tissue is low, about 1% of the activity concentrations in bone tissues (Teien et al., 2021).

Presently, KFCF application (0.1–1%) would enable the ^{137}Cs contamination levels in fresh water fish to be reduced and below the permissible level in almost all water bodies of Ukraine outside the ChEZ. Furthermore, the European permissible level (600 Bq kg^{-1}) would not be exceeded even in the most radioactively contaminated water bodies of the ChEZ, including the Glubokoye Lake.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvrad.2023.107282.

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