Does long-term radiation exposure in Chornobyl impact the reproductive structures of Nuphar lutea (Linné) Smith?

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Abstract

The 1986 Chornobyl Nuclear Power Plant accident caused radioactive contamination of water bodies within the Pripyat River floodplain, resulting in the accumulation of radionuclides by macrophytes, which are fundamental species in water ecosystems. Yellow water lily *Nuphar lutea* (L.) Smith., a macrophyte playing a significant role in the formation of vegetation cover in aquatic ecosystems, is commonly considered as a bioindicator of water pollution. In this study, we investigate the potential of *N. lutea* as an indicator of radionuclide contamination in water bodies, particularly through changes in its reproductive structures, such as pollen viability, morphology of pollen grains, seeds, and fruits. Our findings reveal that pollen viability remains stable at total absorbed dose rates below 14.4 μGy/h, with only 1-4% of sterile grains. However, beyond this threshold the percentage of sterile grains increases nearly fivefold, pointing to high internal plant

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exposure to ^{90}Sr. A similar trend was observed in the allometry and size of pollen grains, where small and flattened grains are formed in the reservoir with the highest external radiation dose rate (≥ 14.4 μGy/h). On the other hand, while morphometric parameters of fruits are influenced by radiation, their variation appears to be the result of a combination of physicochemical factors and the trophic status of a water body. Our research highlights the adverse impact of long-term radiation exposure on the male reproductive system of *N. lutea* and shows the potential of using the pollen grains sterility as an indicators of heavily radionuclide-contaminated water bodies. Additionally, we observed gradual changes in a pollen allometric length-to-width coefficient as the radiation dose level increases.

Keywords: Chornobyl exclusion zone, water bodies, *Nuphar lutea,* radionuclide contamination, absorbed dose rate, pollen grains, dose-dependent changes

1. Introduction

Following the Chornobyl Nuclear Power Plant (CNPP) accident, the floodplain of the Pripyat River experienced substantial radionuclide contamination. The Chornobyl Exclusion Zone (CEZ), an area of about 2,600 km² surrounding CNPP, encompasses diverse water bodies, including rivers, lakes, backwaters, streams, and canals. These shallow aquatic environments are characterized by the prevalence of aquatic macrophytes – large, visible plants that primarily grow in permanently or periodically submerged areas (Thomaz et al., 2009). Due to their significant capacity for absorbing radioactive substances, macrophytes play a pivotal role in the biogeochemical cycling and redistribution of radionuclides within aquatic ecosystems (Fukuda et al., 2014; Ganzha et al., 2014, 2020; Gudkov et al., 2002; Gudkov et al., 2003; Klochenko et al., 2008). Additionally, they are commonly employed in monitoring studies to assess the contamination of water bodies by radionuclides (Gudkov et al., 2006; Gudkov et al., 2003).

Aquatic plants exhibit a remarkable ability to adapt their traits to changing habitats, resulting in a high degree of phenotypic plasticity and local adaptations (Dorken and Barrett, 2004; Keddy et al., 2000; Puijalon and Bornette, 2004; Santamaría, 2002; Titus and Gary Sullivan, 2001; Yuan et al., 2024; Wells and Pigliucci, 2000). Long-term radiation exposure within the CEZ elicits responses in aquatic plants, indicating damages or alterations at multiple organizational levels (Iavniuk et al., 2020; Nurgudin et al., 2009; Shevtsova and Gudkov, 2009; Tsyusko et al., 2006; Yavnyuk et al., 2009). The extensive effects of chronic ionizing radiation exposure (Gudkov et al., 2006; Gudkov et al., 2016; Mousseau and Møller, 2020) underscore the importance of investigating responses dose-dependent reactions in aquatic macrophytes.

Nuphar lutea (L.) Smith, commonly known as the yellow water-lily, is a fundamental species shaping the vegetation cover of water bodies within the CEZ (Zub et al., 2023). Monodominant communities of *N. lutea* are widespread in the shallow waters of large and shallow lakes, while ephemeral communities associated with *Stratiotes aloides* L. can be found in abandoned channels with muddy sediments along the shores (Zub et al., 2023). In addition, *N. lutea* metal absorption capacity (Polechońska et al., 2022) highlights its potential use in biomonitoring studies of radionuclide-contaminated water bodies.

One of the key factors determining a species' resilience to adverse environmental conditions is the viability of its reproductive structures, specifically pollen grains. The male gametophyte is highly sensitive to various environmental pollutants (De Storme and Geelen, 2014; Hjelmroos, 2000; Jefferies, 1977; Kurtar, 2009; Mičieta and Murín, 1996; Mulcahy, 1981; Murin and Micieta, 1998), particularly radionuclides resulting in chronic radiation exposure for plants (Blasco et al., 2016; Møller et al., 2016; Møller and Mousseau, 2017). This vulnerability is evidenced by an increase in the proportion of nonviable pollen grains and a decrease in their fertility. Exposure to adverse conditions can also lead to observable variations in the morphology of pollen grains and other reproductive structures in aquatic plants, serving as indicators of environmental toxicity.

The objective of this study is to investigate the effects of long-term ionizing radiation on the fertility and pollen morphology of *N. lutea*, as well as the traits of its seeds and fruits. Although seed dispersal is not a critical factor for *N. lutea* reproduction, we hypothesized two key points: (i) the sensitivity of its male gametophyte is dose-dependent concerning radioactivity, and (ii) fruit morphology and seed quality may serve as indicators of the absorbed dose rate experienced by the plant.

2. Material and methods

2.1. Biological and aquatic research objects

The investigation involved *N. lutea* specimens from five reservoirs within the CEZ, each characterized by varying absorbed dose rates. Additionally, we examined a reference reservoir with a low background level of radioactive contamination. This study was centered on the analysis of pollen grains, fruits, and seeds from *N. lutea*. Specimens were collected from five reservoirs in the CEZ between 2020 and 2021. Specifically, our study encompassed the following locations: Hlyboke (formerly referred to as Glyboke or Glubokoye) (51°26'37.24"N, 30°3'48.72"E), Vershyna (51°25'58"N, 30°4'23"E), Daleke (51°25'46"N, 30°6'10"E), Plyutovyshche (51°15'12"N, 30°16'24"E), and Yanivskiy Backwater (51°24'28"N, 30°5'5"E). To serve as a reference, we selected a floodplain reservoir of the same type (50°20'3"N, 30°35'1"E) with minimal background radionuclide contamination. This reservoir is situated within the estuary of the Konyk River, a right tributary of the Dnipro River, and falls within the local landscape reserve (LR) known as “Zhukiv Ostriv“ (referred to as LR “Zhukiv Ostriv“ Lake in the text) (Fig. 1). Located within a protected area, this reservoir is still almost untouched by human activity and shares similar hydrological and hydrochemical characteristics with other water bodies under study.

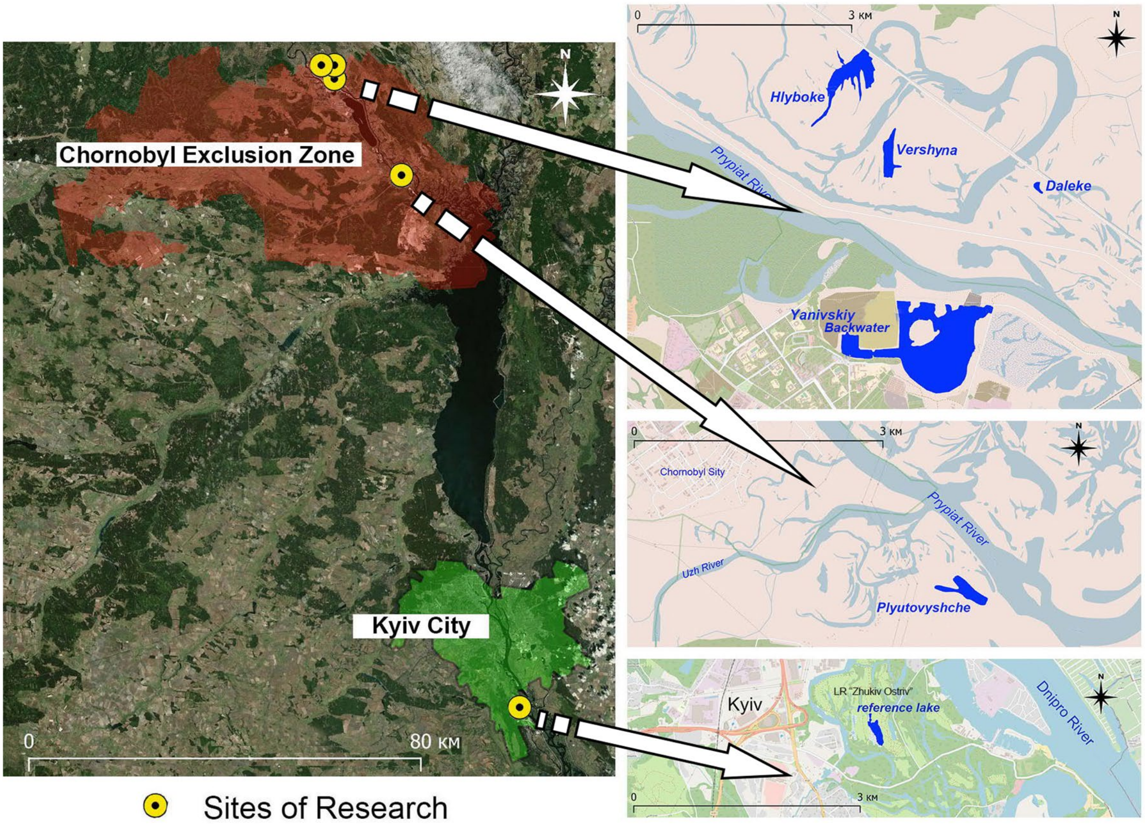

Fig. 1. Scheme of the location of research reservoirs.

The studied water bodies are exposed to a wide range of radionuclide contamination, resulting in varying radiation absorbed dose rates for aquatic macrophytes. These differences primarily arise from their geographical positioning withing the CEZ, their proximity to the destroyed CNPP unit, and the intensity of radioactive fallout in the surrounding area during the active phase of the accident in 1986.

Yanivskiy Backwater, Daleke, Vershyna and Hlyboke lakes are all situated within the 10-km exclusion zone at a distance 3.2, 4.6, 5.3 and 6.7 km from the CNPP, respectively. These water bodies were directly exposed to the north-western radioactive emission during the accident at the 4th unit. Yanivskiy Backwater located on the right bank of the Prypiat River, had been connected to the Pripyat River before the accident. However, due to extensive radiactive contamination it was separated from the Pripyat riverbed using sandbags and later by a dike. Nonethless, compared to the lakes of the left bank of the Pripyat River floodplain, the hydrological connection between the backwater and the river has been substantially higher, resulting in a faster cleanesing of radionuclides from the water and leading to a relatively low dose rate for plants during the study period.

Vershyna and Hlyboke lakes are situated in the dammed section of the left-bank floodplain of the Prypiat River and experienced the highest level of radionuclide deposition, while Plyutovyshche Lake, located 24 km from the CNPP, has the lowest levels of radionuclide contamination.

2.2. Radiation dose rate assessment

The assessment of the total radiation dose rate for *N. lutea* was conducted based on calculated external and internal exposure of studied plants (see Table 1). For external radiation dose rate measuments, we used Na-I field radiometer SRP-68-03 (Rompribor, Russia) for performing studies in aquatic environments. Measurements were carried out at the surface, in different layers of the water column, and also up to 10 cm deep into bottom sediments. Internal radiation dose rate calculations were based on the activity concentration of the main dose-contributing radionuclides within the plant tissues – ^{137}Cs and ^{90}Sr. For these purposes plants were sampled as a whole (a rhizome fragment 30-40 cm long with leaves and peduncles) in the littoral zone of water bodies up to 25 m long from a depth of 50-80 cm. To measure the radionuclide content, 10-15 plant samples were used for each reservoir. The concentration of ^{137}Cs was measured using a γ-spectrometry complex: PGT IGC-25 detector (France), Nokia LP 4900 B analyser (Nokia, Finland), low-voltage power source, NIM BIN crate, amplifier NU-8210 (Elektronicus Merokeszulekek Gyara, Hungary) and 100mm-thick lead shield.

Table 1

Components of the average total absorbed dose rate to *Nuphar lutea* in studied water bodies, μGy/h.

Water body	External	Internal ^{90}Sr	Internal ^{137}Cs	Total
Vershyna Lake	7.49±3.01	6.84±0.89	0.06±0.01	14.39±3.04
Hlyboke Lake	8.14±3.06	2.31±1.28	0.30±0.09	10.75±3.15
Daleke Lake	1.43±0.27	1.32±1.15	0.22±0.08	2.97±1.10
Yanivskiy Backwater	1.65±0.65	0.27±0.13	0.03±0.01	1.95±0.48
Plyutovyshche Lake	0.19±0.08	0.039±0.009	0.004±0.001	0.233±0.075
LR "Zhukiv ostriv" Lake	0.08±0.01	0.001±0.0003	0.0003±0.0001	0.081±0.010

The concentration of ^{90}Sr was measured on a low-background NRR-610 β-radiometer (Tesla, Czech). Minimal detectable activity was 0.04 Bq after 1000 s of counting. The measurement results are given in Bq/kg at natural humidity; the error of estimated radionuclide concentration fell within 15–25%. The average activity concentrations for both radionuclides on whole body of *Nuphar lutea* within the studied reservoirs are given in Table 2.

Table 2

Average activity concentration of radionuclides in *Nuphar lutea* in studied water bodies, Bq/kg at natural humidity.

Water body	Activity concentration	
	^{90}Sr	^{137}Cs
Vershyna Lake	14100±1609	484±117
Hlyboke Lake	4712±2599	2409±654
Daleke Lake	2679±2285	1851±1292
Yanivskiy Backwater	556±320	254±167
Plyutovyshche Lake	81±18	33±11
LR “Zhukiv ostriv” Lake	2.1±0.6	2.4±0.7

Dose rate from incorporated radionuclides using ERICA Assessment Tool 2.0. The integrated approach seeks to combine exposure/dose/effect assessment with risk characterization and managerial considerations (ERICA Assessment Tool 2.0, 2023)

2.3. Reproductive structures study

Pollen analysis included the assessment of fertility and sterility in *N. lutea* pollen, coupled with a comparison of morphometric parameters of pollen grains across water bodies exposed varying degrees of radioactive contamination. Pollen samples were collected during the peak flowering period. Fertility assessments were conducted using the iodine method, a technique for detecting starch in mature pollen grains (Jefferies, 1977). Fertile pollen grains exhibited dark purple coloration, while sterile pollen grains remained uncoloured, as they either contained no starch or only traces of it (Fig. 2 B).

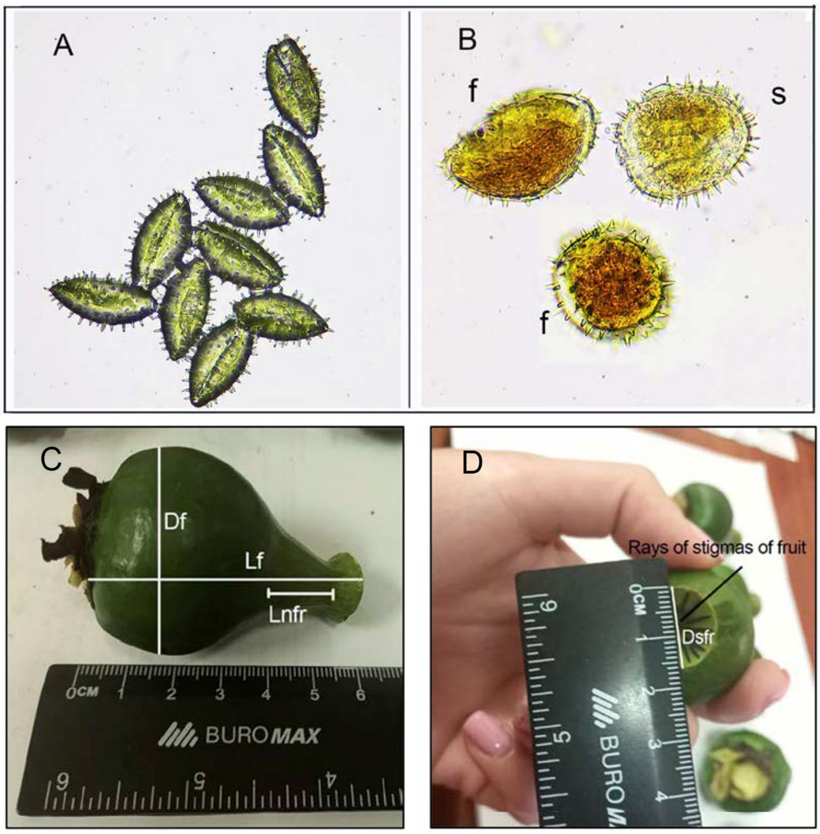

Fig. 2. View of *Nuphar lutea* pollen under a microscope (40x magnification): A – general view of pollen grains, B – stained pollen (where: f – fertile grains, s – sterile grains) C, D – the main morphometric parameters of the fruits that were measured.

Flowers and fruits of *N. lutea* (10-15 samples from each water body) were collected from different parts of studied reservoir, including coastal shallow waters and central areas. We analysed 800-1000 pollen grains from each sample, using 70-80 repetitions of the field of view with an Ulab XY-B2N LED microscope. To facilitate morphometric studies of pollen grains, seeds, and fruits, images acquisition, processing, and analysis were provided using AxioVision Rel.4.8 software. Specifically, we measured the dimentions of pollen grains (length Lp and width Wp), seeds (Ls, Ws), as well as fruits length (Lfr), diameter (Dfr), neck length (Lnfr), stigma diameter (Dsfr) (Fig. 2 C), and the number of stigma rays (Nsr) (Fig. 2 D). Additionally, we calculated the number of seeds (Nsfr) in each pod. Fruit morphometry was carried out for lakes Hlyboke, Daleke, Plyutovyshche, Yanivsky Backwater and the reference "Zhukiv Ostriv" Lake. However, it's worth noting that due to the limited population size of *N. lutea* in Vershyna Lake the detection of pods was not feasible, and consequently, the fruit morphometric study was not provided for this particular reservoir.

To analyse the environmental conditions and determine the trophic status of the reservoirs, we conducted hydrochemical studies, focusing on the content of key nutrients (NO_2^-, NO_3^-, NH_4^+, PO_4^{3-}) and the assessment of total mineralisation of the reservoir (Table 3). Biogens analysis was performed by the colorimetric method using the DR/890 Colorimetre. Water samples were collected during the summer month within the *N. lutea* communities.

Table 3

Results of the hydrochemical analysis of the water in the studied reservoirs (Standard photometric deviation ±0.005).

Water object	Date	NH_4^+, mgN/dm^3	NO_2^- mgN/dm^3	NO_3^-, mgN/dm^3	PO_4^{3-} mgP/dm^3	Total mineralisation
Vershyna Lake	22.08.2020	0.076	0.090	0.18	0.57	157.93
Hlyboke Lake	21.08.2020	0.010	0.071	0.28	2.36	148.70
Daleke Lake	22.08.2020	0.020	0.023	0.49	0*	135.53
Yanivskiy Backwater	24.08.2020	0.050	0.010	0.74	0.32	143.18
Plyutovyshche Lake	25.08.2020	0.020	0.010	0.25	0	97.70
LR “Zhukiv ostriv” Lake	25.08.2020	0.260	0.001	0.09	1.47	195.36

* - below the analised level

2.4. Statistical data processing

To analyse pollen morphology, we employed centered principal component analysis (PCA) and one-way multiple analysis of variance (MANOVA). When dealing with mising values in morphometric data, we substituted them with the site-mean values to facilitate PCA.

To assess the relationships between environmental variable and morphological parameters in studied water bodies, Coinertia Analysis was carried out based on the two tables obtained in PCAs applied to respective datasets (Doledec and Chessel, 1994). PCA was performed on pollen data collected from all six water bodies, while CoA was conducted on pollen, seed, and fruit measurements from five of the studied water bodies as the fruit morphometric data was absent in the Yanivsky reservoir.

Following MANOVA, we conducted post-hoc univariate one-way Analysis of Variance (ANOVA) test to identify the variables that contributed to the overall difference among sites. Subsequently, Tukey pairwise comparisons test was performed to determine specific differences between pairs of sites. We applied Bonferroni multiple testing correction in multiple post-hoc examinations. To compare the proportion of fertile and sterile pollen at studied sites we used the two-sided exact Fisher's test. We utilised R-software's 'ade4' and 'car' packages for PCA/CoA and MANOVA.

3. Results and discussion

A preliminary analysis of the results showed relative resistance of pollen grains to radiation exposure. Only in the reservoir with the highest absorbed dose rate (14.39 μGy/h) – Vershyna Lake – the rate of sterile grains was 18%, while in the rest of the reservoirs it did not exceed 1-4% (Fig. 3, Table S1). The result of the Fisher's exact test (p>0.001) says the six sites have the different pollen viability.

Only one species of *Nuphar*, *N. lutea*, is represented in all reservoirs in the study area. This is why we rejected the hypothesis of hybridisation affecting pollen grain diversity, as noted by other researchers (Arrigo et al., 2016; Padgett et al., 1998). *N. lutea* is one of the macrophytes that can concentrate radionuclides in significant quantities, primarily ^{137}Cs (second-order concentrator) (Gudkov et al., 2003; Gudkov et al., 2002). This is due to the ability of the species to accumulate relatively large amounts of potassium, which is a chemical analogue of Cs, in its tissues – up to 30.02 g/kg (rhizomes and roots) 29 g/kg (leaves) of dry plant mass (Tomaszewicz and Ciecierska, 2011). The ability of pollen grains to accumulate Ca in their shells is important in terms of forming a dose to plant reproductive structures (Butowt et al., 1997; Tian et al., 1998). Ca is a chemical analogue of Sr, and its radioactive isotope (^{90}Sr) forms the overwhelming part of the radionuclide contamination and internal dose to macrophytes in the CEZ (Gudkov et al., 2009, Gudkov et al., 2002; Kashparov, 1998).

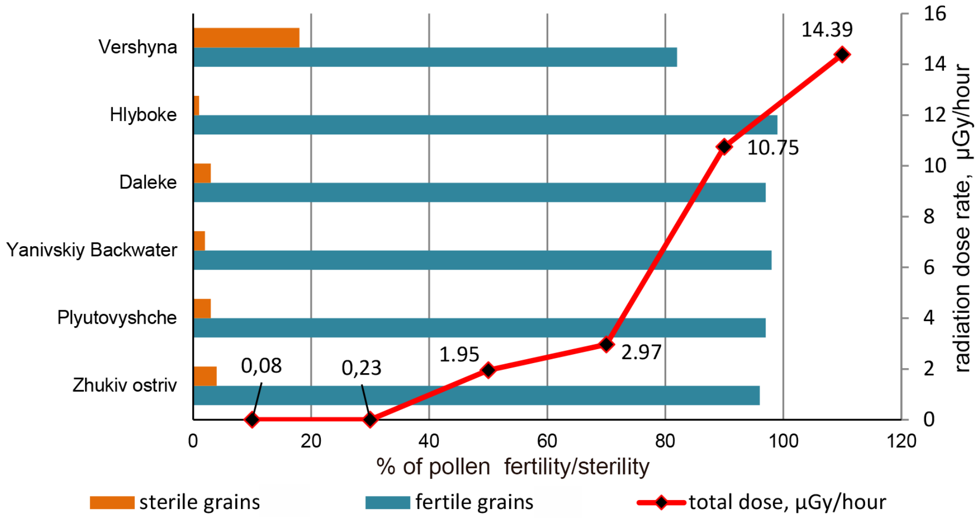

Fig. 3. Viability of *Nuphar lutea* pollen grains in the gradient of the absorbed dose rate.

The total absorbed dose rate of radiation exposure does not significantly affect the range of pollen grains dimentions. Nevertheless, we observed the smallest pollen grains, both in terms of length and width, within the reservoir characterised by the highest external radiation dose rate – Vershyna Lake (Fig. 4, Table S1). In contrast, in the other reservoirs the grains were 10–14% larger; their dimensions were similar. Across all water bodies, we documented a wide range of values in the morphometric indices of pollen grains, with some variations reaching nearly double the size, e.g., Yanivskiy Backwater Lp_{max}=74,43 µm, Lp_{min}=37,05 µm; Wp_{max}=48,23 µm, Wp_{min}=25,16 µm.

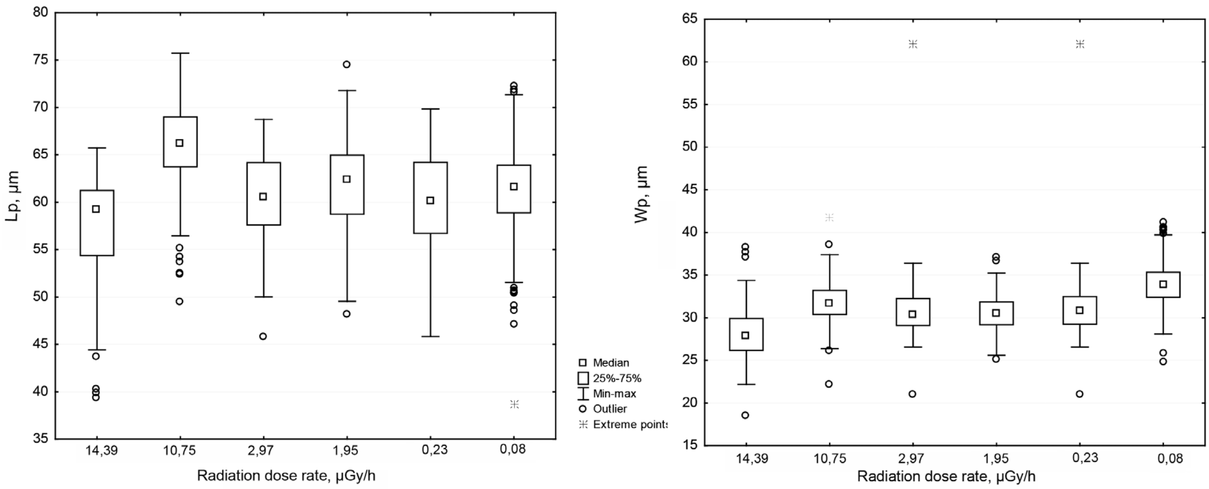

Fig. 4. Diagrams of the range of morphometric parameters of *Nuphar lutea* pollen grains in gradient of radation dose rate: left side – length of pollen grains, right side – width of pollen grains.

Fig. 5 shows the results of PCA applied to pollen morphology and fertility data. In the correlation circle (left side of a figure), the first axis reflects the gradient in the Lp/Wp ratio, with its highest values set toward the left side. The second axis mainly associated with a gradient of fertility (Fert), but it also related to Lp and Wp separated by the first axes.

The factor map (Fig. 5, right side) shows the groupping of the six water bodies marked by inertia ellipses. Vershyna Lake, with the the highest radioactive contamination, stands at the most distinct position. Its projection toward the bottom left part of the factor map corresponds to high values of Lp/Wp ratio and low fertility. Having the second highest exposition level, Hlyboke Lake sets at the top left part of the map implying high pollen fertility. “Zhukiv Ostriv” sets a position at the right side, indicating higher Lp/Wp ratio. The projections of the other sites cluster around the center of coordinates, showing their relatively average parameters values among all sites.

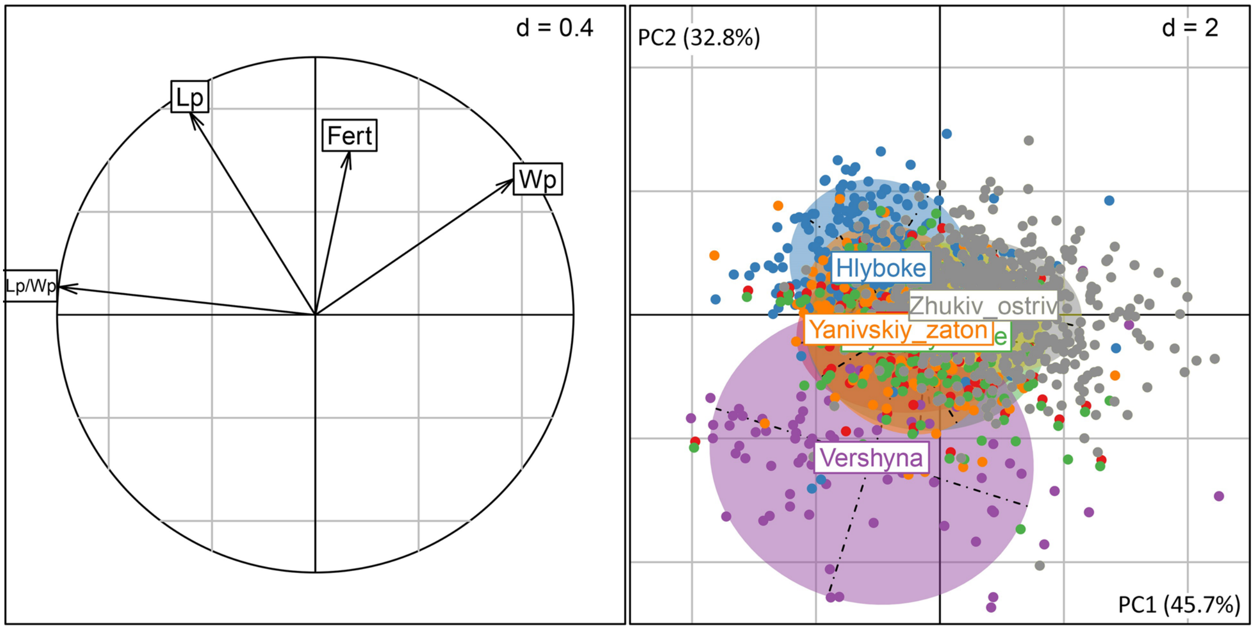

Fig. 5. PCA of the pollen morphology and fertility data. Left: parameters correlation circle. Right: PCA sites factor map, with samples grouped for each water body. Ellipses are inertia ellipses highlighting ~68% samples of each group projection on axes (Lp is the length of a grain; Wp is the width of grain; Lp/Wp is the length-to-width ratio; Fert is the pollen grains fertility).

The results of MANOVA confirmed the significant differences (p-value < 0.001) among the sites. Subsequent post-hoc tests showed there was a significant difference (p-value < 0.05) in each studied pollen parameter between the sites. Further pairwise comparison revealed that the most prominent differences were observed between Vershyna Lake, Hlyboke Lake, and "Zhukiv Ostriv" when compared to other sites (Table S1).

Pollen shape index (Lp/Wp) showed gradually grows with the increase of the radiation dose rate (Fig 6). A strong relationship (R^2=0.86) was found between the size of pollen grains and the total absorbed dose rate, suggesting the dose-dependent influence of radioactive contamination on the reproductive structures of plant. With the increase of absorbed dose rate, the size of pollen grains decreases (the value of Lp/Wp for the control is 1.81, while for the most contaminated water body – Vershyna Lake – it was 2.04). Thus, pollen grains have the smallest size in the water body with the highest dose rate to the plants.

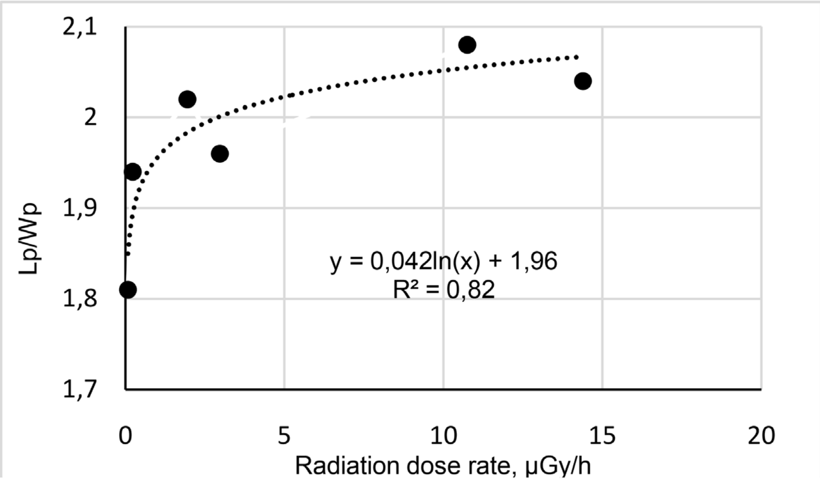

Fig. 6. The dependence between total radiation dose rate and pollen grain length-to-width ratio (average values for water bodies).

As shown in Fig. 6, when the Lp/Wp ratio exceeds >1.9, it indicates the radioactive contamination of the environment. This increased ratio signifies a reduction in the reproductive capacity of local populations resulting from radiation pollution. Notably, the Lp/Wp ratio is sensitive to low radiation doses, with discernible changes in pollen grains observed in Plyutovyshche Lake at a dose as low as 0.233 μGy/h.

The results of the Coinertia Analysis are shown in Fig. 7. The projection of the first axes of the two separate PCAs for environmental variables and morphological datasets onto the coinertia axes reflects relationships between the ain structures of each dataset and co-structure obtained in Coinertia Analysis. The coinertia axes are not equivalent to the axes of both the environmental and morphological PCAs, necessitating an axis rotation (as indicated by the circles in the top left of Fig.8).

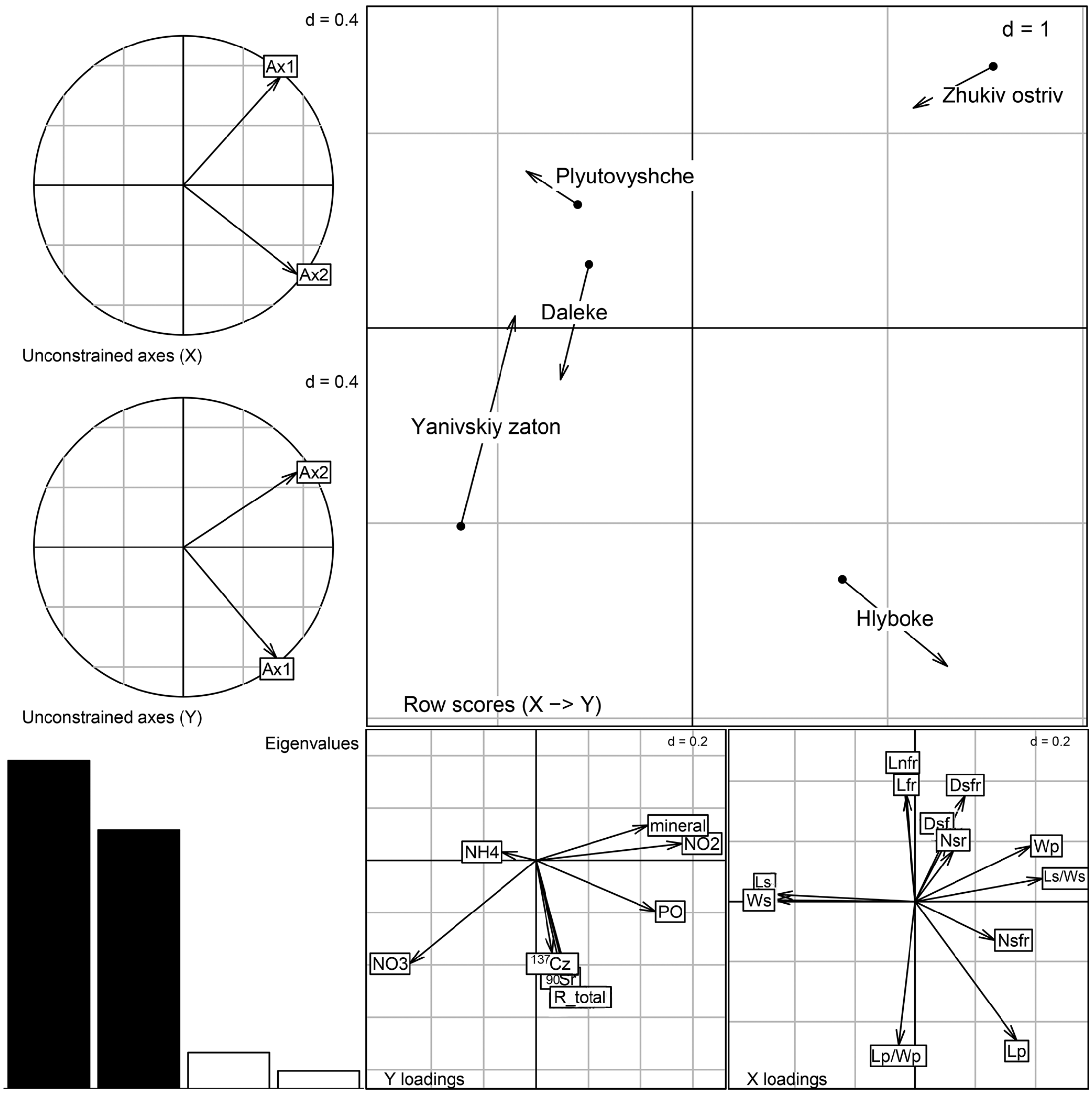

Fig. 7. Composite plot of Coinertia Analysis performed for environmental variables and pollen, fruits, seeds morphology datasets. The projection of the first axes of the two simple PCA for environmental variables and morphological data onto the coinertia axes at the top left. The combined plot of site's scores of environmental conditions (arrows) and morphological dataset (points). The barplot of eigenvalues at the bottom left. Coinertia Analysis coefficients for environmental variables (Y loadings) and morphological parameters (X loadings) at the two graphs at the bottom right.

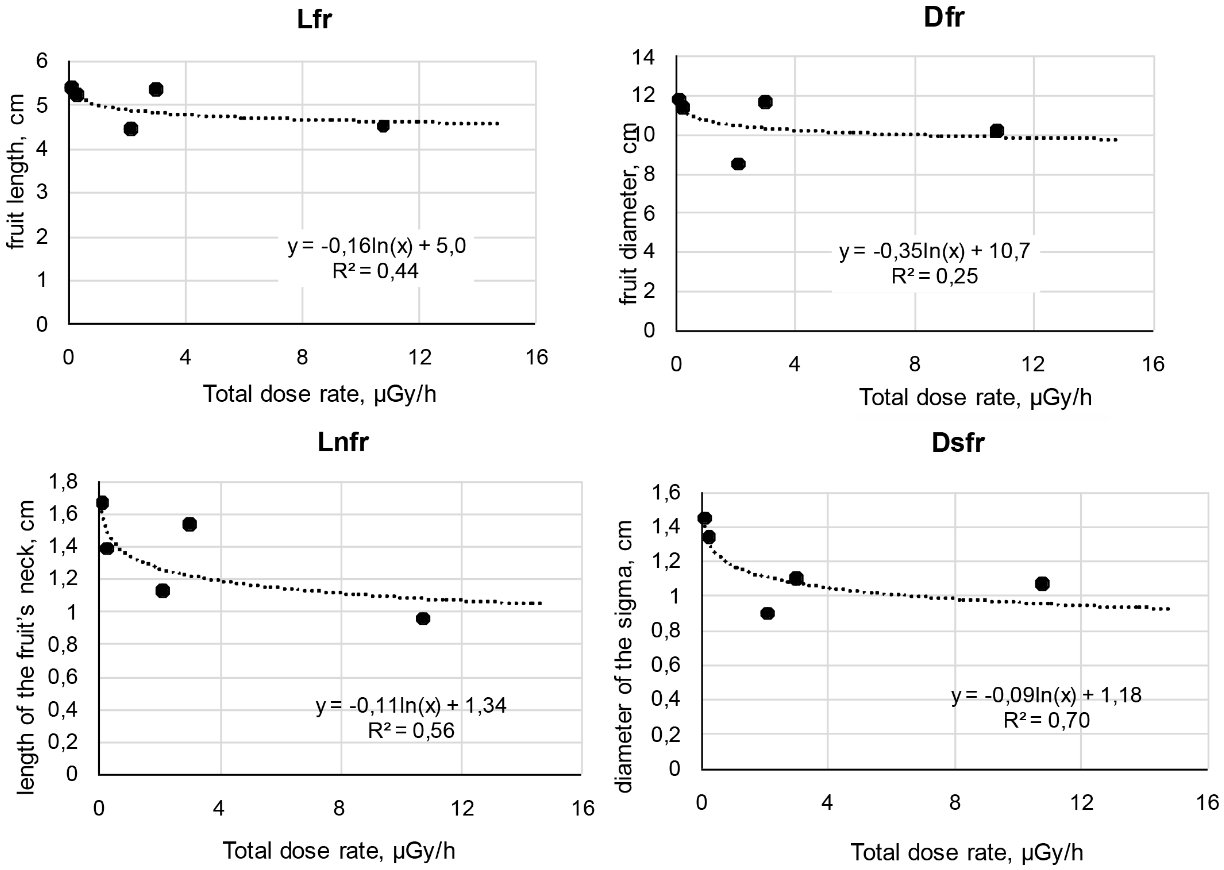

Fig. 8. Average morphological parameters of *Nuphar lutea* fruits in gradient of radiation dose rate: Lfr – fruit's length, Dfr – fruit's width, Lnfr – length of the fruit's neck, Dsfr – width of the fruit's neck.

As illustrated in the eigenvalues barplot, the first two Coinertia axes suffice to describe the relationship between the structures of the studied morphological parameters and environmental variables. Quantitatively, this relationship is expressed by the RV coefficient, which was calculated to be 0.65.

Several groups of morphological parameters are identified at the bottom right plot, X loadings, in Fig 7. The first two groups stretch along the first axis and consist of Ls/Ws ratio, Wp, and Nsfr on the right side and Ws and Ls on the left side. High values of Nsfr also rose with PO_4^{3-} content growth in the waterbodies. High values of morphological parameters at the right side of the first axis are related to high NO_2^-

content and water mineralization level (see Y loadings in Fig.7) and probably with low NO_3^- content, while the second group has an inverse relationship. The third group consists of Dfr, Dsfr, and Nsr, which tend to be higher in water bodies with low content of NO_3^-. The fourth group of parameters are Lnfr and Lfr that opposite the sixth group, i.e., Lp and Lp/Wp ratio, which are associated with high and low levels of radiation dose parameters respectively.

The studied water bodies are represented in the right top plot (Row scores (X –> Y) in Fig. 7). The short arrows show a concordance between structures of environmental composition and morphological parameters in each waterbody. The Hlyboke Lake takes a separate position enabled by high radiation dose and PO_4^{3-} content and is characterised by long Lp, large Lp/Wp ratio (the longest and one of the widest pollen grains) and short Lnfr and Lfr (the smallest fruits with the shortest fruit necks). These parameters have the highest loadings in the second component and could be the indicator of radiation impact. In general, fruits dimensions did not show a significant correlation with the total radiation exposure (Fig. 8).

Among the morphological parameters of fruits, only Lnfr and Dsfr exhibited a dependence on the radiation dose rate, although other physicochemical properties of water may have an effect. The morphological characteristics of a plant greatly vary across their distribution range and depending on their habitat conditions (Latowski et al., 2014). Therefore, direct comparisons with data from other studies were not provided. Nevertheless, *N. lutea* fruit morphology in the CEZ's reservoirs varies in a much wider range than those observed in previous research (Gudkov et al., 2003). Aquatic macrophytes in the most heavily contaminated water bodies within the CEZ have displayed remarkable resilience despite nearly four decades of continuous exposure to ionizing radiation. There have been no substantial alterations observed in their plant community composition (Zub et al., 2023). Changes in composition of macrophyte communities inhabiting the shallow waters of CEZ reservoirs is predominantly influenced by hydrological factors, including degree of reservoir isolation, water depth, the presence of watercourses, and the rates of water exchange (Gudkov et al., 2003). Various morphological and physiological effects in macrophytes have been attributed to long-term exposure to low doses of ionising radiation (Gudkov et al., 2006; Gudkov et al., 2016; Iavniuk et al., 2020; Nurgudin et al., 2009; Yavnyuk et al., 2009).

Furthermore, radiation levels of up to 80 Gy that were reached in heavily contaminated water bodies immediately after the accident (Belyaev et al., 2020, 2023), may have induced genetic and chromosomal disorders in exposed plant cells. These instabilities could persist through many replication cycles, increasing the frequency of genetic alterations in subsequent generations. Cytogenetic investigations revealed an increased incidence of chromosomal mutagenesis in the cells of aquatic plants and animals in the CEZ (Car et al., 2023, 2022; Gudkov et al., 2016; Gudkov et al., 2009; Kesäniemi et al., 2018; Shevtsova and Gudkov, 2013; Tsyusko et al., 2006). Additionally, epigenetic regulations of genes expressions changes may also play a significant role in the response of plants to radiation exposure (Shevtsova and Gudkov, 2009).

Compared to other aquatic plants, *N. lutea* is characterised by low phenotypic variability (Henriot et al., 2019; Schoelynck at al., 2014). However, determining the plant's growth and size is challenging due to the effect of water depth and abundance of clones, attributed to their substantial rhizomes. Hence, researchers usually focus on traits independent of water depth and long-term individual development. These traits typically pertain to recent plant growth, providing insights into the conditions preceding sampling, particularly reproductive indicators.

The reproductive system of *N. lutea* shows a height sensitivity to environmental stressors, rendering it more susceptible to external influences than the plant as a whole (Mičieta and Murín, 1996). Thus, key indicators of a species' sustainability encompass pollen viability and seed quality (Hjelmroos, 2000). Our study further supports the significance of pollen fertility as an indicator of heavily radionuclide-contaminated water bodies. Additionally, our results suggest that the allometric Lp/Wp ratio is sencitive to relatively low-dose radiaton, bringing new perspectives in the assessment of radoactively contaminated waterbodies.

The low fertility of pollen is evident solely in Vershyna Lake characterised by absorbed dose rate, exceeding 14 μGy/h, which is most likely due to increased ^{90}Sr incorporation in plants' tissues. The proportion of sterile grains in the samples here was approximately 4.5 times higher than in the reference water body, while at other sites we did not observe a significant lowering of pollen viability (see Fig. 4). This implies that the reproductive system of *N. lutea* is relatively resistant to long-term radiation exposure.

Vershyna Lake is situated at the center of the dammed area of the left-bank floodplain of the Pripyat River. This region has the highest activity concentration of ^{90}Sr in the CEZ, which is caused by the rewetting and waterlogging of the floodplain. Biologically available forms of ^{90}Sr are transferred from the soil of adjacent territories to the water bodies, followed by their accumulation in aquatic organisms (Gudkov et al., 2003; Gudkov et al., 2009).

The studied water bodies disappplayed more pronounced variations in the chemical composition of water and sediments than in the samples of *N. lutea* leaves, rhizomes, and roots (Tomaszewicz and Ciecierska, 2011). This inference is further supported by *N. lutea's* capability to accumulate higher concentrations of elements such as Ca, an analog of ^{90}Sr in a physiological context, in its organs than in surrounding water (Mazej and Germ, 2009; Polechońska et al., 2022; Tomaszewicz and Ciecierska, 2011).

In contrast, the allometric coefficient Lp/Wp increased gradualy in response to rising absorbed dose rates. In heavily contaminated reservoirs (Vershyna, Hlyboke, and Daleke lakes), this indicator exceeded the control sample by 10-13% (see Fig. 6).

As radioactive contamination increased, the size of *N. lutea* fruits and their morphological structures decreased. However, these parameters, showing a correlation with radiation dose rates, may be influenced by other physicochemical parameters of the water, particularly the content of biogens. For instance, Klok and van der Velde (2017) reported a significant positive relationship between water phosphorus content and the number of flowers and seeds in *N. lutea*. These findings indicate that the reproductive success and productivity of the species tend to decline under nutrient-deficient conditions. In our study, we observed a substantial impact of the biogenic stressors on the reproductive structures of *N. lutea*, with approximately 65% of the variation in morphological parameters being attributable to environmental factors.

In cases where water bodies had higher PO_4^{3-} content, there was an associated increase in the number of seeds per capsule. Conversely, in water bodies with lower NO_3- content, larger seed capsules were formed, characterised by larger fruit diameters, stigma sizes, and the number of stigma rays.

4. Conclusions

This study revealed that reduced pollen viability and an increased pollen grain length-to-width ratio serve as indicators of high radiation exposure in *N. lutea*. Although pollen grains demonstrate relative resistance to long-term radiation exposure, in a water body with a radiation dose rate 176 times higher than the reference water body, we observed a 18% pollen sterility rate, which is 4.5-fold higher than in reference samples. A similar trend in pollen grain sizes was observed: the smallest pollen grains (with both length and width averaging lower values) were found in the reservoir with the highest external radiation dose rate, whereas in the remaining reservoirs, pollen grains were approximately 10-14% larger and exhibited relatively consistent sizes. Conversely, the length-to-width ratio exhibited a distinct, gradual shift in response to increasing absorbed dose rates. In water bodies with substantial radioactive contamination, this indicator exceeded the control sample by 10-13%. The increase in radioactive contamination coincided with a reduction in fruit size and its associated morphological features in *N. lutea*. However, changes in fruit morphometric parameters were less dependent on the level of radiation exposure and probably influenced by a combination of factors which should be investigated further.

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References

Arrigo, N., Bétrisey, S., Graf, L., Bilat, J., Gerber, E., Kozlowski, G., 2016. Hybridization as a threat in climate relict Nuphar pumila (Nymphaeaceae). Biodivers Conserv 25, 1863–1877. https://doi.org/10.1007/s10531-016-1165-z

Belyaev, V.V., Volkova, O.M., Gudkov, D.I., Prishlyak, S.P., Skyba, V.V., 2023. Radiation dose reconstruction for higher aquatic plants and fish in Glyboke Lake during the early phase of the Chernobyl accident. J Environ Radioact 263, 107169. https://doi.org/10.1016/j.jenvrad.2023.107169

Belyaev, V.V., Volkova, O.M., Gudkov, D.I., Pryshlyak, S.P., 2020. Reconstruction of the absorbed dose of ionizing radiation for helophytes in the water bodies of the near emergency zone at the Chornobyl NPP. Nuclear Physics and Atomic Energy 21, 338–346. https://doi.org/10.15407/jnpae2020.04.338

Blasco, M., Badenes, M.L., del Mar Naval, M., 2016. Induced parthenogenesis by gamma-irradiated pollen in loquat for haploid production. Breed Sci 66, 606–612. https://doi.org/10.1270/jsbbs.16021

Butowt, R., Rodriguez-Garcia, M.I., Alchi, J.D., Gorska-Brylass, A., 1997. Calcium in electron-dense globoids during pollen grain maturation in Chlorophytum elatum R.Br. Planta 203, 413–421. https://doi.org/10.1007/s004250050208

Car, C., Gilles, A., Armant, O., Burraco, P., Beaugelin-Seiller, K., Gashchak, S., Camilleri, V., Cavalié, I., Laloi, P., Adam-Guillermin, C., Orizaola, G., Bonzom, J., 2022. Unusual evolution of tree frog populations in the Chernobyl exclusion zone. Evol Appl 15, 203–219. https://doi.org/10.1111/eva.13282

Car, C., Gilles, A., Goujon, E., Muller, M.-L.D., Camoin, L., Frelon, S., Burraco, P., Granjeaud, S., Baudelet, E., Audebert, S., Orizaola, G., Armengaud, J., Tenenhaus, A., Garali, I., Bonzom, J.-M., Armant, O., 2023. Population transcriptogenomics highlights impaired metabolism and small population sizes in tree frogs living in the Chernobyl Exclusion Zone. BMC Biol 21, 164. https://doi.org/10.1186/s12915-023-01659-2

De Storme, N., Geelen, D. The impact of environmental stress on male reproductive development in plants: biological processes and molecular mechanisms. Plant Cell Environ. 2014 Jan;37(1):1-18. doi: 10.1111/pce.12142.

Doledec, S., Chessel, D., 1994. Co-inertia analysis: an alternative method for studying species-environment relationships. Freshw Biol 31, 277–294. https://doi.org/10.1111/j.1365-2427.1994.tb01741.x

Dorken, M.E., Barrett, S.C.H., 2004. Phenotypic plasticity of vegetative and reproductive traits in monoecious and dioecious populations of *Sagittaria latifolia* (Alismataceae): a clonal aquatic plant. Journal of Ecology 92, 32–44. https://doi.org/10.1111/j.1365-2745.2004.00857.x

ERICA Assessment Tool 2.0,2023. The integrated approach seeks to combine exposure/dose/effect assessment with risk characterization and managerial considerations (http://www.erica-tool.com)

Fukuda, S., Iwamoto, K., Atsumi, M., Yokoyama, A., Nakayama, T., Ishida, K., Inouye, I., Shiraiwa, Y., 2014. Global searches for microalgae and aquatic plants that can eliminate radioactive cesium, iodine and strontium from the radio-polluted aquatic environment: a bioremediation strategy. J Plant Res 127, 79–89. https://doi.org/10.1007/s10265-013-0596-9

Ganzha, Ch., Gudkov, D., Ganzha, D., Klenus, V., Nazarov, A., 2014. Physicochemical forms of 90Sr and 137Cs in components of Glyboke Lake ecosystem in the Chornobyl exclusion zone. J Environ Radioact 127, 176–181. https://doi.org/10.1016/j.jenvrad.2013.03.013

Ganzha, Ch.D., Gudkov, D.I., Ganzha, D.D., Nazarov, A.B., 2020. Accumulation and distribution of radionuclides in higher aquatic plants during the vegetation period. J Environ Radioact 222, 106361. https://doi.org/10.1016/j.jenvrad.2020.106361

Gudkov, D.I., Derevets, V.V., Kuzmenk, o M.I., Nazarov, A.B., 2003a. Radioactive contamination of aquatic ecosystem within the Chernobyl NPP exclusion zone: 15 years after accident., in: Protection of the Environment from Ionising Radiation. IAEA-CSP-17. IAEA, Vienna, pp. 224–231.

Gudkov, D. I., Kuzmenko, M.I., Derevets, V. V., Nazarov, A.B., 2003. Radioactive contamination of aquatic ecosystem within the Chernobyl NPP exclusion zone: 15 years after accident, in: Protection of the Environment from Ionising Radiation. IAEA-CSP-17. IAEA, Vienna. Vienna, pp. 224–231.

Gudkov, D.I., Kuzmenko, M.I., Kireev, S.I., Nazarov, S.I., Klenus, A.B., Kaglyan, V.G., Kulachinsky, A.V., Zub, L.N., 2006. Radionuclides in components of aquatic ecosystems of the chernobyl accident restriction zone, in: E.B. Burlakova, V.I. Naidich (Eds.), 20 Years after the Chernobyl Accident: Past, Present and Future. Nova Science Publishers, Inc., New York, pp. 265–285.

Gudkov, D.I., Nazarov, A.B., Kaglyan, A.E., 2009. Change of radionuclide bioavailability in conditions of swamping territories within the Chernobyl accident Exclusion Zone. Radioprotection 44, 951–955. https://doi.org/10.1051/radiopro/20095170

Gudkov, D.I., Shevtsova, N.L., Pomortseva, N.A., Dzyubenko, E.V., Kaglyan, A.E., Nazarov, A.B., 2016. Radiation-induced cytogenetic and hematologic effects on aquatic biota within the Chernobyl exclusion zone. J Environ Radioact 151, 438–448. https://doi.org/10.1016/j.jenvrad.2015.09.004

Gudkov, D. I., Uzhevskaya, S.F., Nazarov, A.B., Kolodochka, L.A., Dyachenko, T.N., Shevtsova, N.L., 2006. Lesion in Common Reed by Gall-Producing Arthropods in Water Bodies of the Chernobyl NPP Exclusion Zone. Hydrobiological Journal 42, 82–88. https://doi.org/10.1615/HydrobJ.v42.i1.80

Gudkov, D.I., Zub, L.N., Savitskiy, A.L., 2002. Macrophytes of the Exclusion Zone of the Chernobyl Nuclear Power Station: the Formation of Plant Communities and Peculiarities of Radioactive Contamination of the Left-Bank Floodplain of the Pripyat River. Hydrobiological Journal 38, 17. https://doi.org/10.1615/HydrobJ.v38.i5.110

Henriot, C.P., Cuenot, Q., Levrey, L.-H., Loup, C., Chiarello, L., Masclaux, H., Bornette, G., 2019. Relationships between key functional traits of the waterlily *Nuphar lutea* and wetland nutrient content. PeerJ 7, e7861. https://doi.org/10.7717/peerj.7861

Hjelmroos, M., 2000. Interactions between Betula spp. Pollen and air pollutants, in: Mat. of II Symp. Vienna, pp. 0–703.

Iavniuk, A.A., Shevtsova, N.L., Gudkov, D.I., 2020. Disorders of the initial ontogenesis of seed progeny of the common reed (Phragmites australis) from water bodies within the Chernobyl Exclusion Zone. J Environ Radioact 218, 106256. https://doi.org/10.1016/j.jenvrad.2020.106256

Jefferies, C.J., 1977. Sequential Staining to Assess Viability and Starch Content in Individual Pollen Grains. Stain Technol 52, 277–283. https://doi.org/10.3109/10520297709116794

Kashparov, V.A., 1998. Bulletin of Ecological State of the Restriction Zone and the Zone of Compulsory (Mandatory) Evacuation.

Keddy, P., Gaudet, C., Fraser, L.H., 2000. Effects of low and high nutrients on the competitive hierarchy of 26 shoreline plants. Journal of Ecology 88, 413–423. https://doi.org/10.1046/j.1365-2745.2000.00456.x

Kesäniemi, J., Boratyński, Z., Danforth, J., Itam, P., Jernfors, T., Lavrinienko, A., Mappes, T., Møller, A.P., Mousseau, T.A., Watts, P.C., 2018. Analysis of heteroplasmy in bank voles inhabiting the Chernobyl exclusion zone: A commentary on Baker et al. (2017) "Elevated mitochondrial genome variation after 50 generations of radiation exposure in a wild rodent." Evol Appl 11, 820–826. https://doi.org/10.1111/eva.12578

Klochenko, P.D., Kharchenko, G. V., Klenus, V.G., Kaglyan, A.Ye., Shevchenko, T.F., 2008. 137Cs and 90Sr Accumulation by Higher Aquatic Plants and Phytoepiphyton in Water Bodies of Urban Territories. Hydrobiological Journal 44, 48–59. https://doi.org/10.1615/HydrobJ.v44.i1.50

Klok, P.F., van der Velde, G., 2017. Plant traits and environment: floating leaf blade production and turnover of waterlilies. PeerJ 5, e3212. https://doi.org/10.7717/peerj.3212

Kurtar, E.S., 2009. Influence of gamma irradiation on pollen viability, germination ability, and fruit and seed-set of pumpkin and winter squash. African Journal of Biotechnology 8, 6918–6926.

Latowski, K., Toma, C., Dąbrowska, M., Zviedre, E., 2014. Taxonomic features of fruits and seeds of Nymphaea and Nuphar taxa of the Southern Baltic region. Limnological Review 14, 83–91. https://doi.org/10.2478/limre-2014-0009

Mazej, Z., Germ, M., 2009. Trace element accumulation and distribution in four aquatic macrophytes. Chemosphere 74, 642–647. https://doi.org/10.1016/j.chemosphere.2008.10.019

Mičieta, K., Murín, G., 1996. Microspore analysis for genotoxicity of a polluted environment. Environ Exp Bot 36, 21–27. https://doi.org/10.1016/0098-8472(95)00050-X

Møller, A.P., Mousseau, T.A., 2017. Radiation levels affect pollen viability and germination among sites and species at Chernobyl. Int. J. Plant Sci. 178, 537–545.

Møller, A.P., Shyu, J.C., Mousseau, T.A., 2016. Ionizing Radiation from Chernobyl and the Fraction of Viable Pollen. Int J Plant Sci 177, 727–735. https://doi.org/10.1086/688873

Mousseau, T.A., Møller, A.P., 2020. Plants in the Light of Ionizing Radiation: What Have We Learned From Chernobyl, Fukushima, and Other "Hot" Places? Front Plant Sci 11. https://doi.org/10.3389/fpls.2020.00552

Mulcahy, D.L., 1981. Pollen Tetrads in the Detection of Environmental Mutagenesis. Environ Health Perspect 37, 91. https://doi.org/10.2307/3429255

Murin, G., Micieta, K., 1998. Pollen grains as an indicator of environmental stress. Plant Ontogenesis in Natural and Transformed Environments 271–273.

Nurgudin, M.A., Shevtsova, N.L., Gudkov, D.I., 2009b. Effects of chronic low-dose radiation on the common reed within the Chernobyl accident Exclusion Zone. Radioprotection 44, 941–944. https://doi.org/10.1051/radiopro/20095168

Padgett, D.J., Les, D.H., Crow, G.E., 1998. Evidence for the hybrid origin of *Nuphar* × *rubrodisca* (Nymphaeaceae). Am J Bot 85, 1468–1476. https://doi.org/10.2307/2446403

Polechońska, L., Klink, A., Golob, A., Germ, M., 2022. Evaluation of Nuphar lutea as bioindicator of metal pollution in freshwater ecosystems. Ecol Indic 136, 108633. https://doi.org/10.1016/j.ecolind.2022.108633

Puijalon, S., Bornette, G., 2004. Morphological variation of two taxonomically distant plant species along a natural flow velocity gradient. New Phytologist 163, 651–660. https://doi.org/10.1111/j.1469-8137.2004.01135.x

Santamaría, L., 2002. Why are most aquatic plants widely distributed? Dispersal, clonal growth and small-scale heterogeneity in a stressful environment. Acta Oecologica 23, 137–154. https://doi.org/10.1016/S1146-609X(02)01146-3

Schoelynck, J., Bal, K., Verschoren, V., Penning, W., Struyf, E., Bouma T., Meire, D., Meire, P., Temmerman, S. 2014. Different morphology of Nuphar lutea in two contrasting aquatic environments and its effect on ecosystem engineering. Earth Surface Processes and Landforms. 39. 10.1002/esp.3607.

Shevtsova, N.L., Gudkov, D.I., 2009. Cytogenetic effects of long-term radiation on higher aquatic plants within the Chernobyl accident Exclusion Zone. Radioprotection 44, 937–940. https://doi.org/10.1051/radiopro/20095167

Thomaz, S.M., Esteves, F.A., Murphy, K.J., Dos Santos, A.M., Caliman, A., Guariento, R.D., 2009. Aquatic macrophytes in the tropics: ecology of populationsand communities, impacts of invasions and human use. Tropical Biology and Conservation Management 4, 27–60.

Tian, H.Q., Kuang, A., Musgrave, M.E., Russell, S.D., 1998. Calcium distribution in fertile and sterile anthers of a photoperiod-sensitive genic male-sterile rice. Planta 204, 183–192. https://doi.org/10.1007/s004250050245

Titus, J.E., Gary Sullivan, P., 2001. Heterophylly in the yellow waterlily, *Nuphar variegata* (Nymphaeaceae): effects of [CO_2], natural sediment type, and water depth. Am J Bot 88, 1469–1478. https://doi.org/10.2307/3558455

Tomaszewicz, H., Ciecierska, H., 2011. Changes in macroelement content in Nuphar lutea (L.) Sibith. and Sm. during the growing season. Acta Societatis Botanicorum Poloniae 78, 151–165. https://doi.org/10.5586/asbp.2009.020

Tsyusko, O. V., Smith, M.H., Oleksyk, T.K., Goryanaya, J., Glenn, T.C., 2006. Genetics of cattails in radioactively contaminated areas around Chornobyl. Mol Ecol 15, 2611–2625. https://doi.org/10.1111/j.1365-294X.2006.02939.x

Wells, C.L., Pigliucci, M., 2000. Adaptive phenotypic plasticity: the case of heterophylly in aquatic plants. Perspect Plant Ecol Evol Syst 3, 1–18. https://doi.org/10.1078/1433-8319-00001

Yuan, G., Li Z., Deng, J., Pacheco, J.P. and Fu, H. 2024. The performance and adaptation of aquatic plants under global changes. Front. Plant Sci. 15:1380921. doi: 10.3389/fpls.2024.1380921

Yavnyuk, A.A., Efremova, N.N., Protsenko, O.N., Gudkov, D.I., Nazarov, A.B., 2009. Fluctuating asymmetry of zebra mussel (*Dreissena polymorpha* Pall.) and floating pondweed (*Potamogeton natans* L.) in water bodies within the Chernobyl accident Exclusion Zone. Radioprotection 44, 475–479. https://doi.org/10.1051/radiopro/20095088

Zub, L.M., Prokopuk, M.S., Gudkov, D.I., 2023. Long-Term Observations over the Structure of Macrophyte Communities in Floodplain Water Bodies of the Chornobyl Exclusion Zone. Hydrobiological Journal 59, 39–53. https://doi.org/10.1615/HydrobJ.v59.i2.30