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Exposure to ionizing radiation and liver histopathology in the tree frogs of Chornobyl (Ukraine)

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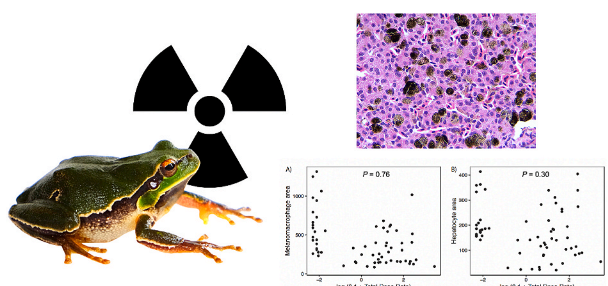
HIGHLIGHTS

- The study examined the effects of radiation on the liver of Chornobyl tree frogs.
- Melanomacrophage and hepatocyte morphology are good proxys of condition in tree frogs.
- Absorbed dose rates did not alter melanomacrophage area or hepatocyte morphology.
- Dorsal skin coloration was not correlated with the area of melanomacrophages in liver.
- Current radiation levels did not induce damage in the liver of Chornobyl tree frogs.

GRAPHICAL ABSTRACT



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ABSTRACT

Ionizing radiation has the potential to damage organic molecules and decrease the health and survival of wildlife. The accident at the Chornobyl Nuclear Plant (Ukraine, 1986) led to the largest release of radioactive material to the environment. Among the different organs of a vertebrate, the liver plays a crucial role in detoxification processes, and has been used as a biomarker to investigate cellular damage in ecotoxicological research. Here, we examined the impact of the exposure to the current levels of ionizing radiation present in the Chornobyl Exclusion Zone on the liver of Eastern tree frogs (*Hyla orientalis*). We quantified the area of melanomacrophage cells and morphological variables of hepatocytes, two cell types often used to estimate damage caused by pollutants in vertebrates. First, we investigated whether these hepatic parameters were indicative of frog (individual) condition. Then, we analyzed the effect of individual absorbed dose rates and ambient radiation levels on frog livers. Most of the studied parameters were correlated with individual body condition (a good predictor of amphibian fitness and survival). We did not detect marked morphological lesions in the liver of frogs captured in medium-high radiation environments. The area occupied by melanomacrophages and the morphology of hepatocytes did not change across a gradient of radiocontamination covering two orders of magnitude. Once

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accounting for body condition and sampling locality, the area of melanomacrophages was lower in areas with high radiation levels. Finally, the area occupied by melanomacrophages was not linked to dorsal skin coloration. Our results indicate that current levels of radiation experienced by tree frogs in Chornobyl do not cause histopathological damage in their liver. These results agree with previous physiological work in the species in the Chornobyl area, and encourage further molecular and physiological research to fully disentangle the current impact of the Chornobyl accident on wildlife.

1. Introduction

Ionizing radiation has the potential to damage organic molecules (Mothersill and Seymour, 2014; Alizadeh et al., 2015). Damage can occur directly through the impact of radiation on biomolecules mostly creating single, double or multiple DNA breaks, or indirectly via the interaction of radiation with water molecules and the subsequent overproduction of reactive oxygen species (Santivasi and Xia, 2014; Desouky et al., 2015). Organisms have repair mechanisms to handle this type or damage, although repair can be energetically costly or imperfect under high radiation conditions, thus leading to mutations or even to cell and organismal death (Willers et al., 2004). The extent of damage caused by ionizing radiation at the cellular and tissue level depends on the amount of radiation received and the duration of the exposure.

All living organisms are exposed to, at least, low levels of ionizing radiation coming from cosmic and solar rays or from naturally occurring radioactive materials, known as background radiation (Sohrabi, 2013). Apart from background radiation, much higher levels of ionizing radiation can be found in ecosystems as a consequence of human activities. This is the case of the accidents at the nuclear power plants of Chornobyl (Ukraine) and Fukushima (Japan). In particular, the accident at the Chornobyl Nuclear Power Plant on the April 26, 1986 led to the largest release of ionizing radiation to the environment in human history (UNSCEAR, 1988). Studies reported an initial impact of the acute exposure to high levels of radiation on the abundance, physiology and genetics of many species (Beresford et al., 2016; Møller and Mousseau, 2006; Sazykina and Kryshev, 2006). However, more than three decades after the accident, studies suggest contrasting effects of current radiation levels in Chornobyl; still detecting some negative effects of radiation exposure (e.g. Møller and Mousseau, 2006, 2015) but also revealing the existence of abundant and diverse communities of wildlife in the area (Deryabina et al., 2015; Schlichting et al., 2019), the lack of effects of current levels of radiation in several species (Bonisoli-Alquati et al., 2018; Lerebours et al., 2018; Burraco et al., 2021a), and even the existence of adaptive responses to radiation (Kovalchuk et al., 2004; Galván et al., 2014; Jernfors et al., 2018; Burraco and Orizaola, 2022). Further research is clearly needed to better understand the long-term effects of the exposure to ionizing radiation on Chornobyl wildlife (Beresford et al., 2020a).

In vertebrates, the liver is an organ involved in processes such as protein and carbohydrate synthesis, blood-forming, and detoxification of both endogenous and exogenous substances. Due to the particular role of the liver in the transformation of toxins (a process with potential systemic implications), this organ has been the subject of many studies on the ecotoxicological effects of pollutants (Newman and Clements, 2008). Detoxification is undergone by the two main liver cells: melanomacrophages and hepatocytes (Fenoglio et al., 2005; Steinel and Bolnick, 2017). Melanomacrophages are pigmented cells involved in the phagocytosis of a broad range of substances. Previous studies have reported an overall increase in the area occupied by melanomacrophages in liver in response to the presence of pollutants (Steinel and Bolnick, 2017). Hepatocytes have also a pivotal role in metabolism and detoxification (Klover and Mooney, 2004; Schulze et al., 2019). Ionizing radiation can affect liver function and morphology, including the damage of hepatocytes (Toesca et al., 2018). Studies conducted shortly after the Chornobyl accident reported a general impact in the morphology of the liver of rats living in areas affected by radioactive fallout (Pinchuk et al., 1991). Small rodents collected across the Chornobyl Exclusion Zone shortly after the accident (1987) showed alterations in the morphology of hepatocytes as well as changes in the magnitude of antioxidant activity of lipids, the composition of phospholipids, and the activity of dehydrogenases in the liver (Shishkina et al., 1992). Nowadays, radiation levels within the Chornobyl Exclusion Zone have dropped around two orders of magnitude since the accident, all short-lived radioisotopes (e.g. ^{131}I) have disappeared, and the amount of radiation currently present in the area represents less than 10% of the radiation released in 1986 (Intelligence Systems, 2011). Recent research has reported shorter telomeres and higher telomerase expression in the liver of Chornobyl bank voles (Kesäniemi et al., 2019), whereas no relationship was found between ionizing radiation levels and the relative liver mass in the same species (Kivisaari et al., 2020). Since the conditions of Chornobyl radiation have changed substantially since the accident, remaining low levels may have limited effects on wildlife. In this line, recent studies have shown the lack of effects of the current exposure to ionizing radiation in blood parameters linked to tissue damage, including some common markers of liver and kidney damage (Burraco et al., 2021a).

Amphibians are adequate study subjects to evaluate the impact of radioactive contamination in wildlife. The life cycle of most amphibians includes life stages linked both to the terrestrial and aquatic environment (Wesner et al., 2020) which exposes them to a broad range of radioactive scenarios. Also, their short dispersal capacity facilitates the design of field-based research and the estimation of absorbed radiation rates in the wild (Cayuela et al., 2020; Orizaola, 2022). Similar to other vertebrates, the liver of amphibians is a key organ under polluted scenarios, which is confirmed by many studies reporting how chemical pollutants can induce molecular, biochemical and cellular effects on this organ (e.g. Bernabò et al., 2017; Çakıcı, 2015; de Gregorio et al., 2016; Regnault et al., 2014; Pérez-Iglesias et al., 2016; de Oliveira et al., 2016). Morphological parameters of hepatocytes (such as hepatocyte and nuclear areas) can be used to measure cytotoxic effects of distinct environmental stressors (Araújo et al., 2020; Franco-Belussi et al., 2021). Alterations on the hepatocytes parenchymal area can be indicative of metabolic changes or even degeneration processes (Ostaszewska et al., 2018), and nuclear area alterations usually indicate cytoplasm degeneration or even apoptotic events (Ostaszewska et al., 2018). Thus, such biomarkers can also act as useful tools to evaluate radiotoxicity.

The amphibian liver also contains melanomacrophage cells, which are essential during immunological and detoxification processes and have been used as biomarkers for evaluating the impact of diverse pollutants (De Oliveira et al., 2017; Curi et al., 2021; Pinto-Vidal et al., 2021) and infectious diseases (Salla et al., 2020). Many studies have reported higher densities of melanomacrophages in the liver of amphibians exposed to different aquatic and terrestrial pollutants (e.g. Çakıcı, 2015; de Gregorio et al., 2016; Pérez-Iglesias et al., 2016; Bach et al., 2018; Franco-Belussi et al., 2021; Alnoaimi et al., 2021; Şişman et al., 2021). Among other functions, melanomacrophages synthesize melanin (Agius and Roberts, 2003), a compound able to neutralize free radicals and mitigate the impact of ionizing radiation in cells (Cordero and Casadevall, 2020). A previous study has reported the existence of much darker tree frogs within the Chornobyl Exclusion zone, a pattern likely explained by the role of melanin in buffering the damage of radiation on cells (Burraco and Orizaola, 2022).

Here, we examined the effects of the chronic exposure to ionizing radiation on the liver of tree frogs living across a gradient of radioactive contamination inside the Chornobyl Exclusion Zone. Using a histological approach, we estimated the area occupied by melanomacrophages centres, as well as the morphology of hepatocytes. We first investigated the relationship between liver parameters and frog body condition, which is often considered as a good proxy for individual health and fitness in amphibians and other ectotherms. Then, we assessed the relationship between individual absorbed dose rate and liver parameters linked to the organ's health. We also explored the liver morphology comparing populations with low and medium-high ambient radiation levels. Additionally, we examined the link between skin coloration and the density of melanomacrophages in liver, since previous studies have found a positive relationship between internal and external coloration in frogs (Franco-Belussi et al., 2016). We hypothesized that the chronic exposure to higher ionizing radiation levels would lead to histological changes in the liver of Chornobyl frogs, in the form of a larger area occupied by melanomacrophages or changes in the size and shape of hepatocytes and their nuclear areas. However, considering the reduction in radiation levels within the Chornobyl Exclusion Zone, current radiation levels may be insufficient to generate damage in the structure of the liver in Chornobyl tree frogs.

2. Materials and methods

2.1. Field work

We examined liver histology on the Eastern tree frog (*Hyla orientalis*), a cryptic species of the European tree frog (*Hyla arborea*) group that inhabits from the Caspian to the Baltic Sea. Females start to breed at 2–3 years of age (Özdemir et al., 2012), thus 10–15 generations have passed since the Chornobyl accident in 1986. The Eastern tree frog is one of the ten amphibian species living in the Chornobyl area and has been recently used as a study model to examine the long-term effects of Chornobyl radiation in a wild vertebrate (Burraco et al., 2021a,b; Burraco and Orizaola, 2022; Car et al., 2022).

During the breeding season of 2017 (8th-14th May, Table 1), we collected 65 *H. orientalis* adult males in five sites located across a gradient of radioactive contamination within the Chornobyl Exclusion Zone (Northern Ukraine; Fig. 1, Table 1). Localities differed in their ambient radiocontamination levels up to two-orders of magnitude (0.07–7.61 μSv/h; Table 1): three localities were located in areas of medium-high radiation (AZ, NT, DO in Fig. 1) and two in areas of low (background) radiation (GL and RA in Fig. 1, Table 1). At each locality, we estimated ambient dose rate using a radiometer MKS-AT6130 to measure gamma dose rate (μSv/h) in five different points along the shoreline of each sampling locality (see Burraco et al., 2021b for more details). We captured frogs that were actively calling from 10 p.m. to 1 a.m., placed them in plastic bags and transported to our laboratory within the Chornobyl Exclusion Zone. Once in the laboratory, we kept frogs overnight individually in small plastic containers with perforated lids to allow oxygen diffusion and containing ca. 3 cm of water. On the next morning, we recorded the morphological measurements (snout-to-vent length, body depth and width) using a calliper to the nearest 1 mm. We also weighted each individual using a precision balance to the nearest 0.01 g. Frog coloration was previously quantified by estimating the dorsal skin luminance of each frog through pictures and colour analysis using the software ImageJ (see Burraco and Orizaola, 2022 for methodological details and full dataset). Once morphometric measurements were recorded and pictures were taken, we euthanized frogs by pithing without decapitation (AVMA, 2020), collected the liver and stored it in 70% ethanol.

Table 1
Geographic coordinates (latitude and longitude), number of sampled frogs, sampling date, and current levels of environmental radiation (i.e. ambient dose rate) of the locations included in the study.

Location	Code	GPS coordinates	Sampled frogs (n)	Sampling date	Radiation (μSv/h)
Azbuchin	AZ	51.4047, 30.1044	12	May 12, 2017	7.61
Northern Trace	NT	51.4567, 30.0486	18	May 14, 2017	2.51
Dolzhikovo	DO	51.4256, 30.1161	15	May 14, 2017	1.09
Glinka	GL	51.2300, 29.9250	13	May 08, 2017	0.10
Razjezzheie	RA	51.2786, 29.9050	10	May 08, 2017	0.07

2.2. Liver histological analysis

Liver samples were processed by the laboratory technicians of the CABIMER-Molecular Biology and Regenerative Medicine Centre (Seville, Spain). Livers were dehydrated in alcohol series and embedded in paraffin (ca. 16 h). Samples were then cut with a microtome to create 6 μm sections. All the sections were dried overnight at 37 °C and then dewaxed and hydrated before staining with hematoxylin-eosin (Fig. 2). After staining, sections were dehydrated again and mounted on glass slides with DPX mounting medium. We examined slides under a Euromex bscope microscope, at 400x, and took 13–18 photos per slide and individual, which aimed to cover the distribution of cells within a liver.

We first examined all the slides in order to detect histopathologic lesions (vacuolization of hepatocytes, leucocyte infiltration, or cell necrosis; see e.g. Franco-Belussi et al., 2021). Then, we estimated the area occupied by melanomacrophages (15 micrographs per individual, per locality, expressed as μm^2). The morphometric analysis was performed using Image Pro Plus 6.0 software (Media Cybernetics), based on differences in pixels intensity (Pérez-Iglesias et al., 2016; Salla et al., 2020). To evaluate hepatocyte morphometry, we measured the cell and nucleus perimeter (in mm) and area (in mm^2) in 50 hepatocytes per individual (10 hepatocytes on 5 different photos per individual). We then estimated hepatocyte nucleus volume (Vn) via the equation: $Vn = (4/3\,\pi\, r^3)$, where r is the nuclear radius, and hepatocyte volume (in μm^3) with the formula (Vh): Vh = (hepatocyte area * nucleus volume)/nucleus area (Leão et al., 2021). All measures were assessed by a single observer (RFS) blindly on regard to the correspondence of each slide to any sample characteristic (e.g., sampling locality or individual absorbed radiation). We used the software Image ProPlus 6.6 (Media Cybernetics) for estimating all the morphological parameters indicated above.

2.3. Exposure to ionizing radiation

To quantify the total dose rates absorbed by each frog (in μGy/h), we first estimated the total activity of ^{90}Sr and ^{137}C, i.e., the two main isotopes currently contributing to radiation exposure in Chornobyl amphibians (Beresford et al., 2020b), in the bones and muscles of tree frogs. Taken into account the relative mass of bones (10%) and muscles (69%; Barnett et al., 2009), and also the body mass of each frog, we estimated the internal activity concentrations of each individual. We used dose coefficients to transform radionuclide activity concentrations in frogs, soil, and water (in Bq/kg or Bq/L) into dose rates (in μGy/h). Dose coefficients for *H. orientalis* internal and external exposure were calculated using EDEN v3 IRSN software by applying a theoretical habitat use scenario for the species during the breeding season. For each frog, total individual dose rate was calculated by adding internal and external dose rates. Full details of this methodology can be found in Burraco et al. (2021b).

2.4. Statistical analysis

Parametric assumptions were evaluated using Breusch-Pagan test for heteroscedasticity and Kolmogorov-Smirnov test for normality. We estimated body condition as the residuals of the regression between snout-to-vent-length and body mass (Green, 2001). To explore the link

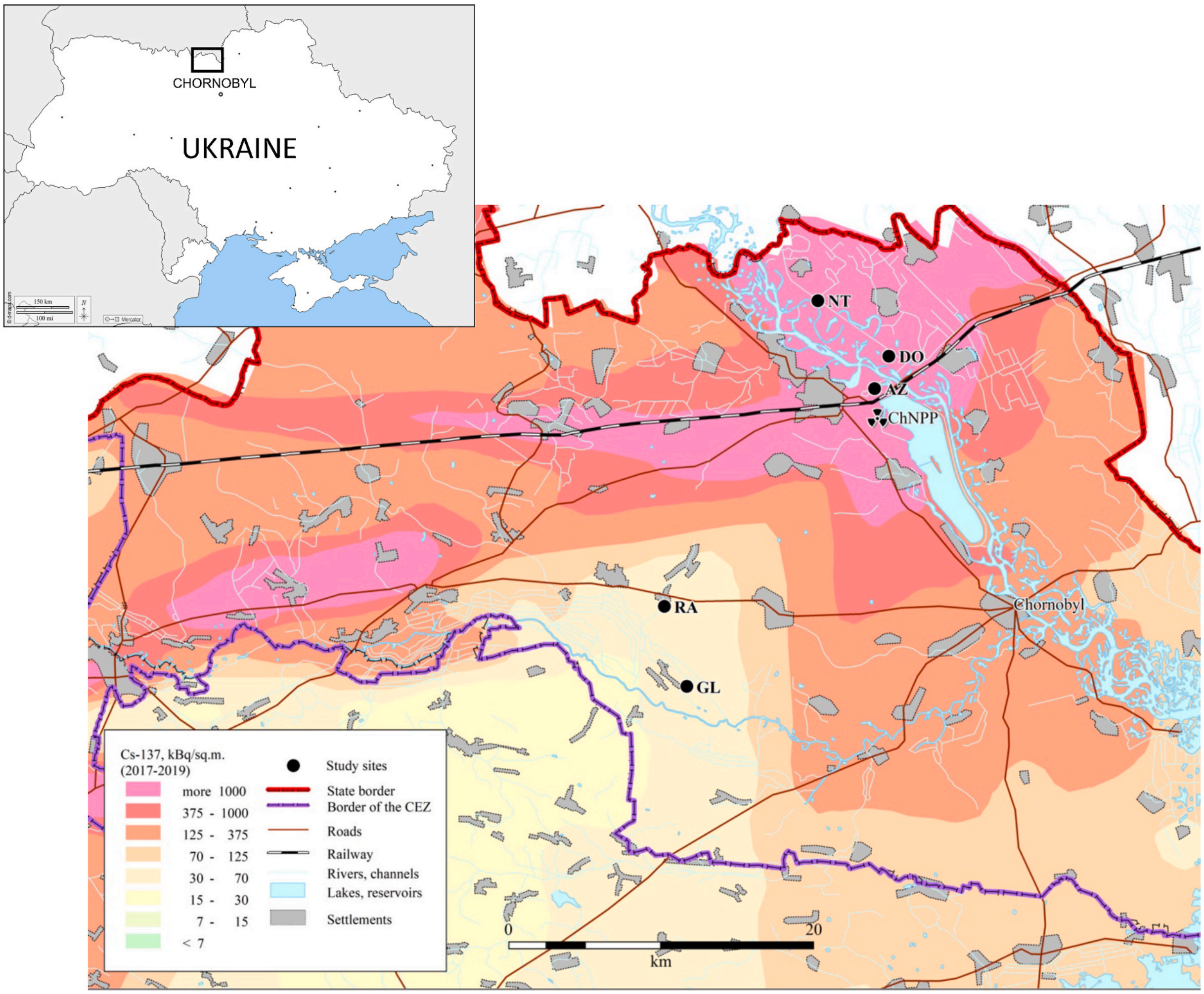

Fig. 1. Map of the studied area in Ukraine showing the Eastern tree frog (*Hyla orientalis*) locations (see also Table 1). The underlying ^{137}Cs soil data (decay corrected to spring 2017) is derived from the Atlas of Radioactive Contamination of Ukraine (Intelligence Systems, 2011).

between the liver parameters and frog body condition, we conducted linear mixed regressions between those variables, including sampling locality as a random factor. We explored the relationship between individual absorbed dose rate or radiation category, and body condition, through mixed models including sampling locality as a random factor. We also conducted linear mixed regressions between individual dose rate and liver parameters, and we included body condition as a covariate and sampling locality as a random factor in those models. To address whether the levels of liver parameters varied between radiation categories, we conducted mixed models including body condition as a covariate, and sampling locality as a random factor. Finally, we explored the link between melanomacrophage area and dorsal skin luminance with the help of a linear mixed regression that included sampling locality as the random factor. Individual dose rates were log-transformed after adding 0.1 to each data. All liver parameters were log-transformed to improve parametric assumptions. All analyses were conducted in R version 3.6.1.

3. Results

Total individual absorbed dose rates of the studied frogs ranged from below detection limits (i.e., 0.01 μGy/h) to 31.92 μGy/h (see Supplementary data). Individuals with a higher body condition had a larger area occupied by melanomacrophages ($P < 0.01$; Fig. 3A, Table S1). Higher body condition was also linked to a higher hepatocyte cell area and a higher hepatocyte nucleus area and volume ($P < 0.04$, in all cases; Fig. 3, Table S1). Overall, individual dose rates did not significantly affect frog body condition ($P = 0.22$, Chi-q = 1.48; d.f. = 1). Frogs inhabiting highly contaminated areas had a lower body condition than those from areas with low radiation levels ($P = 0.04$, Chi-q = 4.11; d.f. = 1). A histological structure considered as healthy was observed in the livers of most frogs examined, based on the existing knowledge on amphibian liver histology (Akiyoshi and Inoue, 2012). Histopathological lesions (e.g. vacuolization, leucocyte infiltration; Fig. 2) were detected only in six out of 65 frogs, and they were not linked to high exposure to radiation (4 out of 6 were frogs experiencing background radiation levels and with absorbed dose rates below the detection limit; Table 2).

The area occupied by liver melanomacrophages was not affected by the amount of ionizing radiation absorbed by a frog ($P = 0.76$, see Table S2; Fig. 4A), although melanomacrophage area was on average 72.9% higher in frogs collected in localities with low ambient radiation levels ($P < 0.01$; Table S2; Fig. 5A). We did not detect any significant effect of absorbed dose rates (Fig. 4B–E) or locality radiation category (medium-high radiation vs. low radiation; Fig. 5B–E) in hepatocyte morphology (total area or volume, nucleus area or volume; $P > 0.07$, in all cases, Table S2). Dorsal skin coloration did not correlate with the area of liver melanomacrophages (Chi-sq. = 2.43, $P = 0.12$).

4. Discussion

Current levels of ionizing radiation experienced by breeding males of

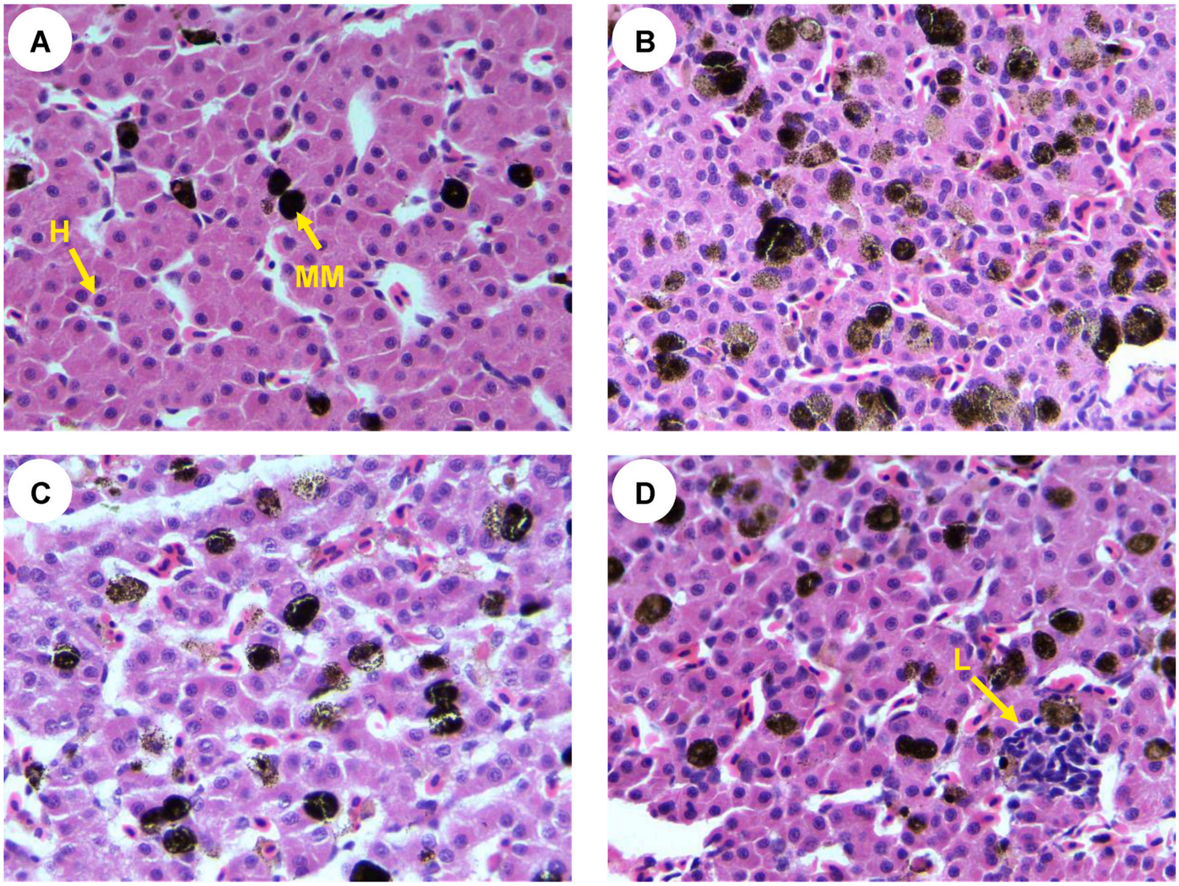

Fig. 2. Histological sections of the liver of Eastern tree frog (*Hyla orientalis*) males, showing hepatocytes (H), melanomacrophages (MM). A) section with typical structure of hepatocytes and melanomacrophages; B) section with melanomacrophages with very clear melanophores, and hepatocytes with reduced cytoplasm; C) section showing moderate vacuolation; D) section with leucocyte (L) infiltration.

the Eastern tree frog (*Hyla orientalis*) within the Chornobyl Exclusion Zone had no effect on their liver morphology. Although the studied liver parameters correlated with frog body condition (a proxy for health and fitness in post-metamorphic amphibians; Earl and Whiteman, 2015) hence confirming their link with organismal performance, we did not found significant histopathologic lesions in frogs living within the Chornobyl area, nor liver damages associated to individual absorbed dose rates. Contrary to our predictions, melanomacrophage area was not larger in individuals with higher absorbed dose rates. Actually, when considering only environmental radiation levels, frogs living in medium-high radiation localities had a lower area occupied by melanomacrophages in the liver. Overall, current exposure to ionizing radiation did not affect the body condition of the examined frogs, although individuals from highly contaminated areas showed lower body condition on average. Our study shows that the exposure to current levels of ionizing radiation within the Chornobyl Exclusion Zone has no significant effect on the liver histology of adults of an ectothermic vertebrate. These results agree with studies conducted in the same species and other vertebrates, and may contribute to explain the current abundance and diversity of wildlife in the area (e.g. Deryabina et al., 2015; Schlichting et al., 2019).

Liver melanomacrophages are often used as a proxy for the capacity to detoxify exogenous substances and for hepatic immune status (de Oliveira et al., 2016). Increases in the melanomacrophage area can be also linked to damage in hepatic tissue resulting from oxidative stress and increased activation and recruitment of immune cells (Da Silva et al., 2012). However, whereas most of this knowledge comes from fish studies, the impact of environmental conditions on melanomacrophage areas has not been broadly addressed in amphibians (Steinel and Bolnick, 2017; Campos Gutierrez et al., 2018). In our study, the liver area covered by melanomacrophages was overall lower than the area reported in a previous study for control individuals (i.e., non-exposed to pollutants) of the amphibians *Physalaemus albonotatus* and *Xenopus laevis* (Salla et al., 2020), and similar to that found in *Physalaemus nattereri* (Franco-Belussi et al., 2016). Previous studies have shown that the exposure to UV radiation induce a rapid increase (within 3 h) in the area occupied by melanomacrophages in the liver of *Physalaemus nattereri*, although this increase appeared to be attenuated with longer exposure periods (Franco-Belussi et al., 2016). In our study, we did not find any correlation between individual absorbed dose rates and melanomacrophage area, which suggests that current radiation levels do not induce an increase in detoxification processes in the liver of frogs. We detected higher melanomacrophage area in individuals with higher body condition, which may indicate a higher constitutive detoxifying ability in those frogs, in line with their expected higher performance. When considering environmental radiation categories within Chornobyl Exclusion Zone (medium-high versus low-background), we detected a lower melanomacrophage area in individuals living in medium-high radiation localities. This pattern may be counterintuitive, although a reduction in melanomacrophage areas has been previously reported in fish exposed to other stressful conditions (e.g. infection; Kranz, 1989) or in salamanders exposed to triazine (Johnson et al., 2004). The potential costs associated to this response should ideally be further explored. Also, melanomacrophages are known to participate in functions such as cell turnover and by secreting growth factors, therefore the interaction between radiation exposure and organismal processes may have also mediated the observed differences (Geissmann et al., 2010). Finally, contrary to previous studies (Franco-Belussi et al., 2016), we did not

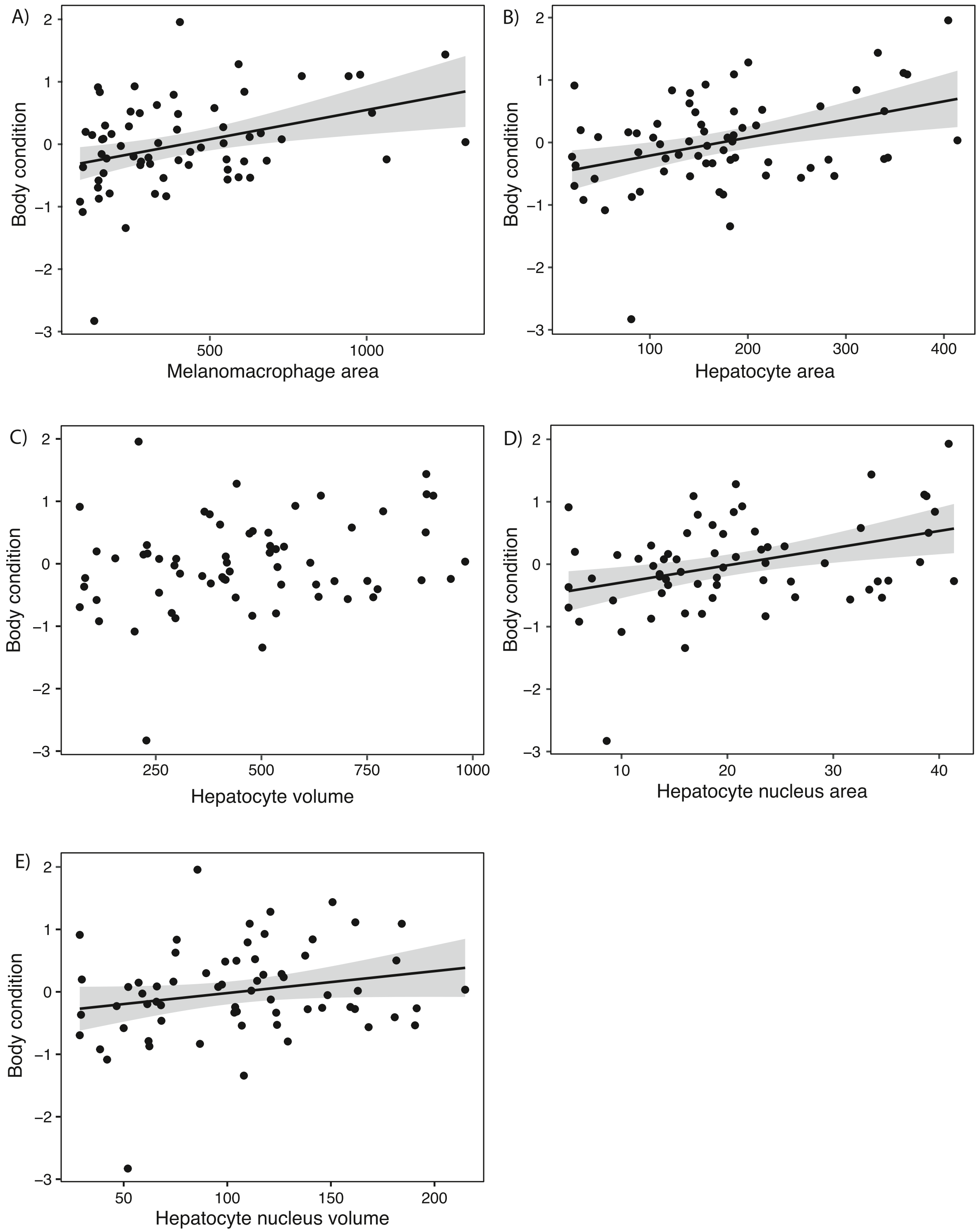

Fig. 3. Regressions between body condition index and, A) liver melanomacrophage area (in μm^2), B) hepatocyte area (in μm^2), C) hepatocyte estimated volume (in μm^3); D) hepatocyte nucleus area (in μm^2), and E) hepatocyte nucleus estimated volume (in μm^3) in Eastern tree frog (*Hyla orientalis*) males sampled across a gradient of ionizing radiation within the Chornobyl Exclusion Zone.

detect any correlation between external coloration (i.e. dorsal skin coloration) and internal coloration, the latter measured as the area occupied by melanomacrophages. A recent study has shown that melanin-based coloration in Chornobyl tree frogs seems to be conditioned mainly by the historical exposure to high radiation levels shortly after the accident (Burraco and Orizaola, 2022). It its plausible that melanomacrophage density acts as a short-term response uncoupled from the pattern of coloration in these populations, and/or linked to other functions rather than the protective role of pigmentation in the liver of frogs.

The morphology of hepatocytes has been used as a biomarker of liver damage and/or malfunction in different species (Opute and Oboh, 2021; Saha et al., 2021). An increase in the cytoplasm or nuclear areas is often suggested as an indication of cell degradation or hypertrophy, whereas a reduction may be related to cellular damage and cell death (Thoolen et al., 2010). In our study, we did not detect any effect of individual dose

Table 2
Histopathology lesions in Eastern tree frog (*Hyla orientalis*) breeding males sampled in areas of medium-high and low-background radiation within Chornobyl Exclusion Zone (Ukraine).

Histopathology lesion	Medium-High Radiation	Low Radiation
No lesions	40	19
Vacuolization	0	4
Leucocyte infiltration	2	0

rate or environmental radiation category in the morphology of hepatocytes or hepatocyte nucleus. Individuals collected across a gradient of radioactive contamination comprising two orders of magnitude did not show any differences in the area and volume occupied by hepatocytes. The area of hepatocytes and their nucleus, as well as hepatocyte's nucleus volume, were higher in individuals with higher body condition, an interesting pattern that requires further exploration. Previous studies exposing amphibian larvae to microplastics or surfactants such as linear alkylbenzene sulfonate (LAS) showed that exposure to these substances

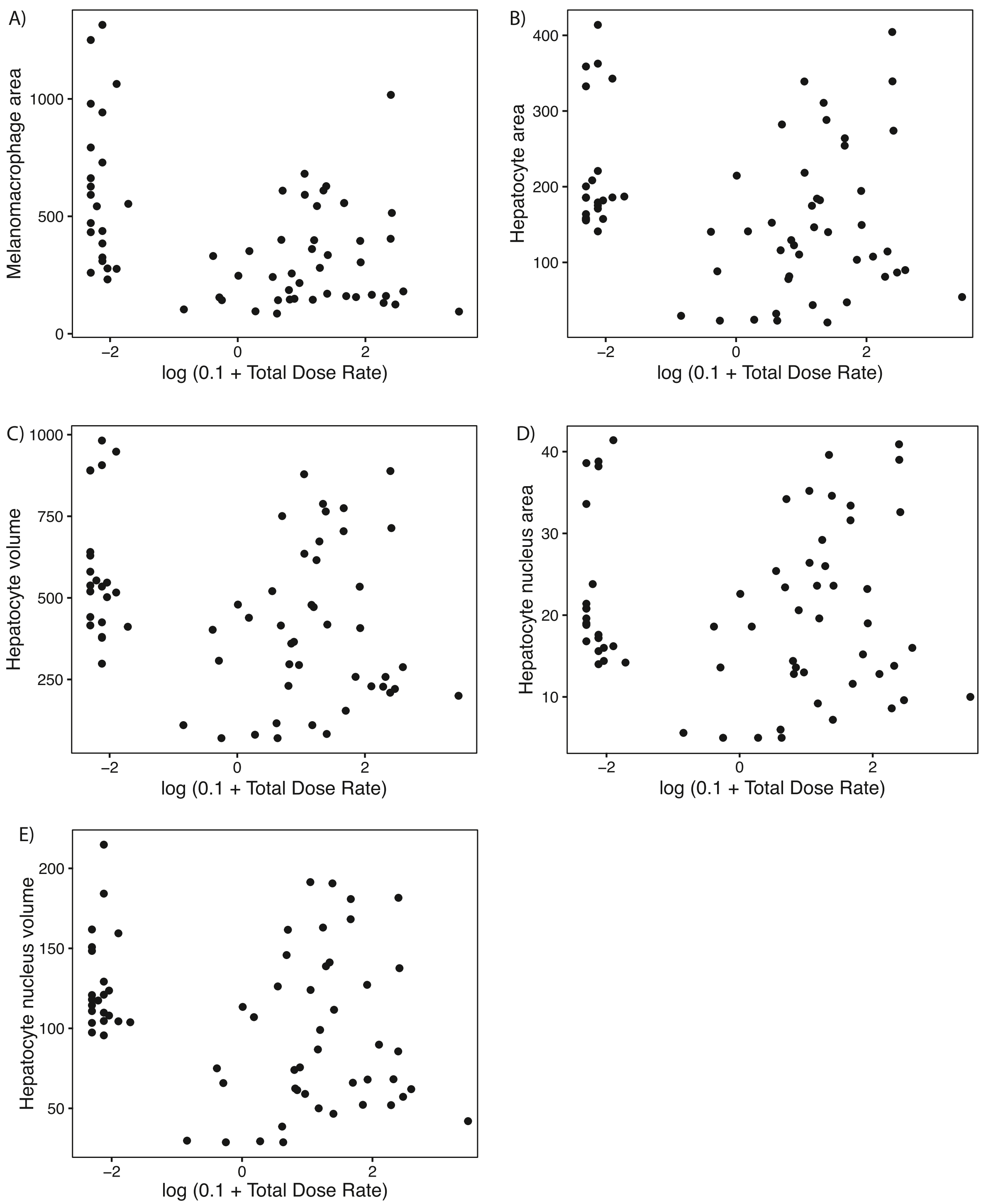

Fig. 4. Regressions between total individual dose rates (in μGy/h) and, A) liver melanomacrophage area (in μm^2), B) hepatocyte area (in μm^2), C) hepatocyte estimated volume (in μm^3); D) hepatocyte nucleus area (in μm^2), and E) hepatocyte nucleus estimated volume (in μm^3) in Eastern tree frog (*Hyla orientalis*) males sampled within the Chornobyl Exclusion Zone.

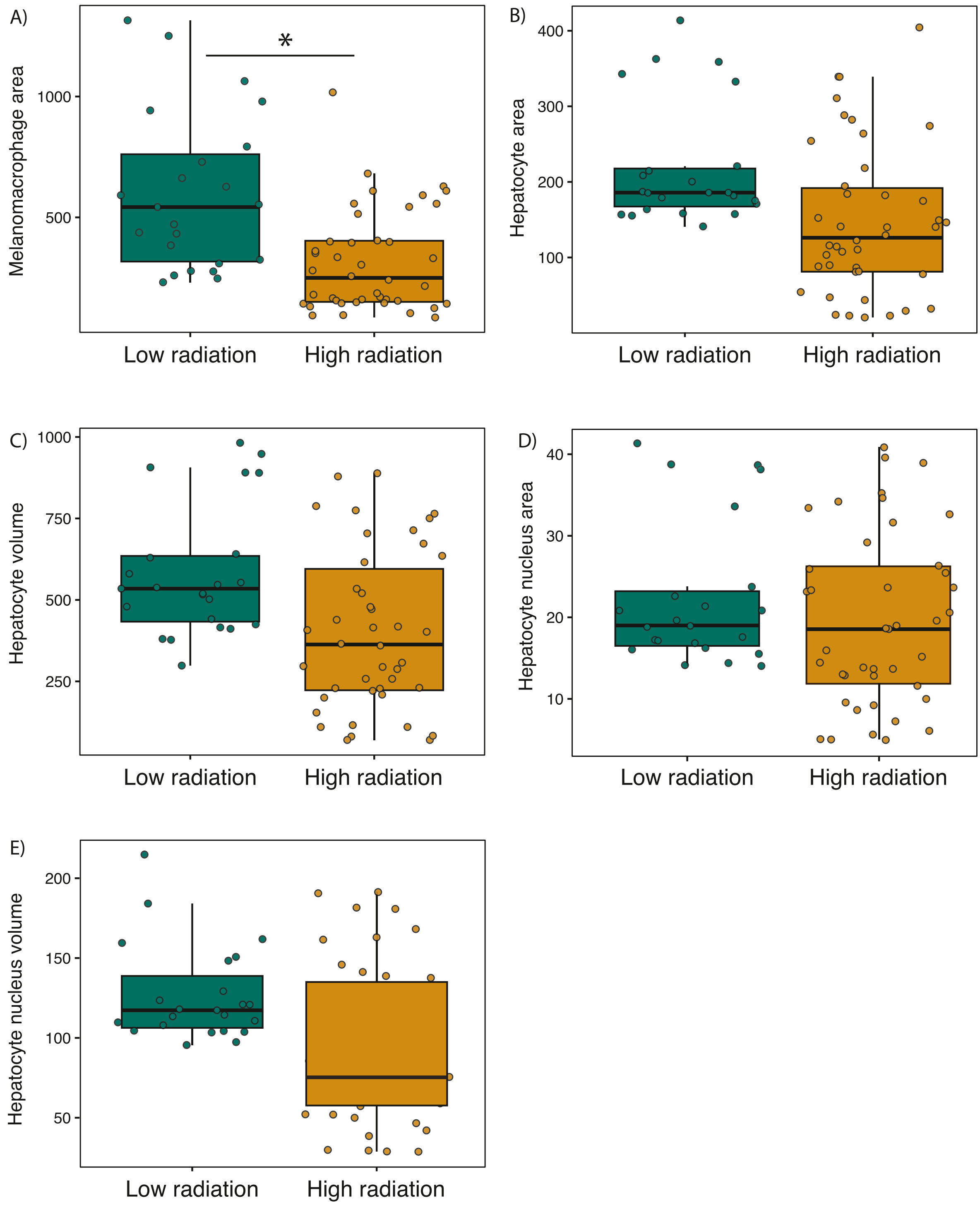

Fig. 5. Levels of A) liver melanomacrophage area (in μm^2), B) hepatocyte area (in μm^2), C) hepatocyte estimated volume (in μm^3); D) hepatocyte nucleus area (in μm^2), and E) hepatocyte nucleus estimated volume (in μm^3) in Eastern tree frog (*Hyla orientalis*) males sampled in areas with medium-high (>1 μSv/h) and low (<1 μSv/h) ambient radiation levels within the Chornobyl Exclusion Zone.

caused morphological alterations in hepatocyte morphology, mainly larger hepatocyte and nuclear areas indicative of cytotoxic effects (Araújo et al., 2020; Franco-Belussi et al., 2021). In our study, a lack of effect of the exposure to radiation on hepatocyte morphology suggests, once again, that current radiation levels experienced by tree frogs in Chornobyl are not causing noticeable effects at this physiological level.

Although the use of a small number of localities and few hepatic traits may be seen as a limitation of the study, our results agree with previous studies conducted on breeding males of the Eastern tree frog (*Hyla orientalis*) within the Chornobyl Exclusion Zone, indicating a lack of effect of current exposure to ionizing radiation levels on different blood biomarkers linked to kidney and liver malfunction, blood biochemical parameters commonly used as markers of overall physiological condition (Burraco et al., 2021a), or liver oxidative stress (Burraco and Orizaola, 2022). A previous work recently conducted on this species reported higher genetic diversity in Eastern tree frogs living in Chornobyl (Car et al., 2022), which may be associated to higher mutation rates, but also to the existence of diverse recolonization sources after the accident. It is important to notice that, since current radiation levels are not extremely high anymore, all frogs examined in the study had total absorbed dose rates that were below current international benchmarks supposed to induce biological damage in amphibians (i.e. 40 μGy/h; ICRP, 2008; Burraco et al., 2021b). Therefore, this and previous studies indicate that the ionizing radiation absorbed by Eastern tree frogs currently living within the Chornobyl Exclusion Zone are not compromising their health status.

5. Conclusion

We found that the current exposure to ionizing radiation within the Chornobyl Exclusion Zone has no significant effect on the liver morphology of Eastern tree frogs (*Hyla orientalis*). These results agree with previous work examining the health status of this and other vertebrate species in the Zone. However, more work is needed in early life stages (i.e. embryos and larvae; Orizaola, 2022) to comprehensively evaluate the long-term impact of the chronic exposure to radiation and the full validity of currently proposed benchmarks for radioprotection in amphibians (ICRP, 2008). Considering the rate of radioactive decay, the study of the effects of the chronic exposure to low-dose ionizing radiation across an organism lifetime is crucial for a correct assessment of the environmental effects of radioactive accidents.

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Ethical approval

All animals were collected, and experimental protocols conducted under permit and ethical approval of Ministry of Ecology and Natural Resources of Ukraine (licence No. 517, April 21, 2016). All methods were performed in accordance with the relevant ethical guidelines and regulations.

Author contributions

P.B. and G.O. conceived the study. P.B. and G.O. conducted field work and sample collection. R.F.S. analyzed the histological samples. P. B. performed statistical analyses. G.O. wrote the manuscript with significant contributions from P.B. and R.F·S.

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 available at https://figshare.com/articles/dataset/Dataset_of_Exposure_to_ionizing_radiation_and_liver_histopathology_in_Chornobyl_tree_frogs_/21782765

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2023.137753.

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