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Can spike fragments of SARS-CoV-2 induce genomic instability and DNA damage in the guppy, Poecilia reticulata? An unexpected effect of the COVID-19 pandemic

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HIGHLIGHTS
- Spike fragments of SARS-CoV-2 affect the health of P. reticulata adults.
- Fish exposed to Spike fragments present increase of erythrocytic nuclear alteration.
- Erythrocytic DNA damage is induced by Spike fragments of SARS-CoV-2 in P. reticulata.
- PSPD-2002 peptides induce oxidative stress in P. reticulata adults.

ABSTRACT
The identification of SARS-CoV-2 particles in wastewater and freshwater ecosystems has raised concerns about its possible impacts on non-target aquatic organisms. In this particular, our knowledge of such impacts is still limited, and little attention has been given to this issue. Hence, in our study, we aimed to evaluate the possible induction of mutagenic (via micronucleus test) and genotoxic (via single cell gel electrophoresis assay, comet assay) effects in Poecilia reticulata adults exposed to fragments of the Spike protein of the new coronavirus at the level of 40 μg/L, denominated PSPD-2002. As a result, after 10 days of exposure, we have found that animals exposed to the peptides demonstrated an increase in the frequency of erythrocytic nuclear alteration (ENA) and all parameters assessed in the comet assay (length tail, %DNA in tail and Olive tail moment), suggesting that PSPD-2002 peptides were able to cause genomic instability and erythrocyte DNA damage. Besides, these effects were significantly correlated with the increase in lipid peroxidation processes [inferred by the high levels of malondialdehyde (MDA)] reported in the brain and liver of P.
1. Introduction

Pandemically, COVID-19 (Coronavirus Disease-2019), caused by SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) has promoted unprecedented global impacts (Siddique et al., 2021), whether at an economic level (Maital and Barzani, 2020), public health (Sarkodie and Owusu, 2021) and social disruption (Viladrich, 2021). The United Nations University World Institute for Development Economics Research (UNU-WIDER) estimates that approximately 500 million people may succumb to poverty as a result of the new coronavirus (Summer et al., 2020). Data on the status of the pandemic in the world (obtained on 10 January 2022) record more than 305 million confirmed cases and more than 5 million deaths in 236 countries, areas, or territories (WHO, 2022).

As far as we know, the classic form of transmission of SARS-CoV-2 is by air and/or via contact with infected people (Meyerowitz et al., 2020; Harrison et al., 2020). However, other forms of transmission of the new coronavirus have been investigated due to the persistence of the virus in the environment for a few hours/day. In addition, one of these forms refers to possible contamination/transmission via the fecal-oral or fecal-nasal route (Giacobbo et al., 2021). In spite there is little concrete information on the subject, many researchers have warned about the possibility of infection through direct contact with domestic sewage or contaminated water (Westhaus et al., 2021; Gonçalves et al., 2021; Albastaki et al., 2021; Paul et al., 2021; Sangkhram, 2021; Baldwin et al., 2021; Shariif et al., 2021; Wu et al., 2022; Vo et al., 2022), with the aerosols generated in the systems of wastewater pumping and treatment (Gormley et al., 2020; Usman et al., 2022), with the aerosols generated in the systems of wastewater pumping and treatment (Gormley et al., 2020; Usman et al., 2022), and also via faulty connections of floor drains interconnected with the main piping of buildings/houses (Shi et al., 2021).

Regardless of whether these studies are still initially to epidemiological conclusions of definitive practical applications, the fact is that the new coronavirus or its fragments have already been identified in different fluvial systems and, therefore, constitutes a consolidated reality (Rimoldi et al., 2020; Guerrero-Latorre et al., 2020; Mahlknecht et al., 2021). As discussed by Guerrero-Latorre et al. (2020), in countries with a lack of basic sanitation, the spread of SARS-CoV-2 in freshwater environments may be even greater, considering, for example, that in numerous countries less than 30% of the sewage generated is treated before being discharged into the streams (Rodríguez et al., 2020). As a consequence, questions arise from this scenario about the extent to which the presence of the new coronavirus (or its fragments) in surface water represents an (eco)toxicological risk for non-target organisms.

Our research group recently reported some effects arising from the exposure of amphibians, fish, and invertebrates to direct protein fragments of the Spike protein of SARS-CoV-2 (Charlie-Silva et al., 2021; Mendonça-Gomes et al., 2021; Malafaia et al., 2022). Initially, from a systemic approach (including the synthesis, cleavage, purification, and alignment of three peptide fragments of the SARS-CoV-2 Spike protein, as well as the exposure of neotropical Physalaemus cinerii tadpoles to these fragments) we gathered evidence that confirms the toxicity of the viral constituents in the evaluated animal model. The increase in several biomarkers predictive of oxidative stress and the alteration in acetylcholinesterase (AChE) activity demonstrated that the short exposure (24 h) to these peptides was sufficient to affect the health of tadpoles (Charlie-Silva et al., 2021). In the study by Mendonça-Gomes et al. (2021), we showed for the first time that short-term exposure (48 h) of PSPD-2002 and PSPD-2003 peptides (at 40 μg/L) induced alterations in the locomotor system and in the olfactory behavior of Culex quinquefasciatus larvae, which were associated with increased production of reactive oxygen species (ROS) and AChE activity. In Malafaia et al. (2022), we show that exposure to the aforementioned peptide fragments can also alter the behavior of fish (Poecilia reticulata), induce redox imbalance, as well as affect the growth and development of animals. Therefore, these studies "shed light" on the (eco)toxicological potential of peptide fragments of SARS-CoV-2 in aquatic biota, going beyond the works that have focused on the susceptibility of different mammalian species to viral infection and their roles in the dissemination of COVID-19 (e.g.: Tiwari et al., 2020; Delahay et al., 2021; Audino et al., 2021).

In this regard, any definitive conclusions about the ecotoxicological impacts caused, particularly, by the presence or dispersion of SARS-CoV-2 (or its protein fragments) in aquatic environments are very incipient, as well as on how much this can enhance the already known impacts on aquatic and terrestrial species. There are many gaps to be filled, and our understanding of the scope of their effects on other faunal species and their mechanisms of action is very limited. Thus, in the present study, we aimed to evaluate the potential mutagenic and genotoxic effects on erythrocyte of Poecilia reticulata (a model system traditionally used in ecotoxicological studies) induced by exposure to one of the previously synthesized peptide fragments (Charlie-Silva et al., 2021). In this study, we determined the potential association between mutagenic and genotoxic effects with the induction of redox imbalance in various organs/tissues of the evaluated animals. Importantly, our motivation for conducting this study is based on recent studies that demonstrated that SARS-CoV-2 infection induces the formation of micronuclei and the activation of DNA damage pathway [in syngtia and Hela-ACE2 cells Ren et al., 2021] and DNA damage response in Vero E6 cells (Víctor et al., 2021). It is therefore questionable whether similar effects are observed in non-target organisms (P. reticulata) when exposed to peptide fragments of SARS-CoV-2 dispersed in water. We believe our findings help to explain the ecotoxicological effects of SARS-CoV-2 at cellular and molecular levels, providing new potential targets for an investigation into the impacts of COVID-19 on wild freshwater ichthyofauna.

2. Material and methods

2.1. Peptide fragments of the SARS-CoV-2 spike protein

The synthesis, cleavage, purification, and characterization of the peptides from the SARS-CoV-2 Spike protein used in our study were performed according to methods described in detail by Charlie-Silva et al. (2021). Briefly, the synthesis of the Spike S protein was conducted using the solid phase peptide synthesis method (SPPS) following the Fmoc strategy (Raibaut et al., 2014; Behrendt et al., 2016). The resins used in this process were Fmoc-Thr-Wang and Fmoc-Asn-Wang for the PSPD-2002 (sequence: Gln-Cys-Val-Asn-Leu-Thr-Thr-Arg-Thr-COOH; MW: 1035.18 g/mol) and PSPD-2003 (sequence: Asn-Asn-Ala-Thr-Asn-COOH; MW: 532.51 g/mol), respectively. At the end of the synthesis, these resins made it possible to obtain peptides with the carboxylated C-terminal end. After coupling all the amino acid residues of the peptide sequences, the chains were removed from the solid support using acid cleavage using trifluoroacetic acid (TFA), similarly to Guy and Fields (1997). The crude compounds were purified by high-performance liquid chromatography (HPLC) with a reverse-phase column using different purification methods according to the retention time obtained in a gradient program of 5% to 95% in 30 min (exploration gradient) in Analytical HPLC [similarly to Klaassen et al. (2019)]. Only compounds with purity equal to or greater than 95% were considered for in vivo evaluation, following the rules determined by the National Health Surveillance Agency (ANVISA/Brazil) and Food and Drug Administration (FDA/USA). The similarities between the peptides PSPD-2002 and PSPD-2003 were evaluated using the CLUSTAL W software version 1.83 [Higgins et al., 1996; Pai et al., 2014 - http://www.ebi.ac.uk/clusterw/].
2.2. Animals and experimental setup

We used in our study individuals of the species *Poecilia reticulata* (Cyprinodontiformes: Poeciliidae) (wild strain), commonly known as ‘guppy’, and considered native to northwestern South America (Bisazza, 1993). This species was selected based on its wide distribution in neotropical regions (CABI, 2021), in which it can inhibit strongly impacted aquatic environments where few species can occur (Araújo et al., 2009), as well as its previous use in different ecotoxicological studies (Aich et al., 2015; De-Lima Faria et al., 2021; De-Souza-Trigueiro et al., 2021).

Preliminary, females were captured in a natural environment (municipality of Urutaí, GO) (license SISBIO/ICMBio/MMA/Brasil n. 73342-1), taken to the laboratory, and kept in an aquarium (60 L) containing dechlorinated water and constant oxygenation, under room temperature (25–26 °C) and photoperiod controlled (12–12 h light: dark cycle). After 60 days of acclimatization, 16 non-pregnant females of *P. reticulata* were separated and distributed into two experimental groups (four replicates/group). The group “PSPD-2002” was composed of *P. reticulata* exposed (for 10 days) to the peptides at a concentration of 40 μg/L, diluted in water. Such concentration is considered predictive, as SARS-CoV-2 particles have been identified and quantified via RT-qPCR assays applied for SARS-CoV-2 RNA detection. Therefore, the units of measurement are not comparable or convertible into “μg/L”. The control group consisted of fish kept in dechlorinated water (naturally) free of viral peptides. Each replica consisted of two animals kept in cylindrical aquariums with 2.2 L of dechlorinated water (under constant oxygenation), without using filters or substrates. The temperature (25–26 °C) and luminosity (12–12 h light: dark cycle) conditions were properly controlled. Every three days there was a complete renewal of the exposure waters, and, at the end of the experiment, the animals were submitted to different evaluations, as described below.

![Fig. 1](image_url)

**Fig. 1.** Frequency of micronucleus (MN test) and others erythrocytic nuclear alterations (ENA) in adult females *Poecilia reticulata* exposed or not to PSPD-2002 peptide fragments (at 40 μg/L). (A) Total ENA, kidney-shaped nucleus, blebbed nucleus, nuclear constriction, and multi-lobed nucleus; (B) displaced nucleus, notched nucleus, binucleated cell, micronucleus, and nuclear vacuole. The bars indicate the mean ± SD of the data, which were submitted to Student’s *t*-test (if parametric) or Mann-Whitney *U* test (if non-parametric) (see the statistical summary at the top of the graphs). Different lowercase letters indicate significant differences between experimental groups. *n* = 8 animals/group.
2.3. Toxicity biomarkers

2.3.1. Micronucleus test and other erythrocytic nuclear alteration

The possible mutagenic effect of exposure to PSPD-2002 peptides was evaluated using the frequency of micronucleus test (MN test) and other erythrocytic nuclear alteration (ENA), as described by Carrasco et al. (1990) and modified by Guimarães et al. (2021). Briefly, 5 μL of blood (collected via cutting the caudal peduncle – after the animals were deeply anesthetized in ice-cold water) was deposited on a previously sanitized glass slide to form a thin smear, which was dried at room temperature. Next, slides were fixed in 100% (v/v) cold methanol and stained with Panoptic Rapid® (Laborclin®, Paraná, Brazil, code no. 620529), based on Pavan et al. (2021) and Estrela et al. (2021). One thousand erythrocytes were analyzed per fish [according to Bolognesi and Hayashi, 2011], with 400 × magnification and evaluated for the presence of MN and others ENA that manifested as changes in the typical elliptical nuclear shape of erythrocytes.

2.3.2. Single cell gel electrophoresis assay (comet assay)

The potential damage to erythrocyte DNA induced by exposure to PSPD-2002 peptides was evaluated by the comet assay, similarly to the methodology adopted by Estrela et al. (2021), with minor modifications. Briefly, after the step described in the previous item, the animals were transferred to conical bottom microtubes containing 250 μL of phosphate-buffered saline (PBS, pH 7.2, 4 °C) and kept for 5 min. Afterward, the blood samples were centrifuged (6000 rpm, 5 min, 4 °C) for subsequent disposal of the supernatant and homogenization of the pellet. Then, 2 μL of the pellets were mixed with 120 μL of low melting point agarose (0.5%) at 37 °C and then placed on the cover slides (previously prepared using

Fig. 2. Parameters evaluated in the single-cell gel electrophoresis assay (comet assay) in erythrocytes of adult female Poecilia reticulata exposed or not to PSPD-2002 peptide fragments (at 40 μg/L). (A) Tail length; (B) %DNA in tail; (C) Olive tail moment (OTM); (D–E) representative images of nucleoids from animals in the control group and PSPD-2002, respectively; and (F) correlation analysis between the total ENA and the parameters evaluated in the comet assay. In “A, B, and C”, the bars indicate the mean ± SD of the data, which were submitted to the Student’s t-test (if parametric) or Mann-Whitney U test (if non-parametric) (see the statistical summary at the top of the graphs). Different lowercase letters indicate significant differences between experimental groups. n = 8 animals/group.
normal agarose at 1.5% in PBS) and, later, covered with a glass coverslip. After incubation at 4°C for 10 min, the coverslips were removed and the slides were submerged in lysis solution (NaOH, Na2EDTA, Tris-HCl, NaOH, purified water, Triton X-100, and DMSO) for 2 h at 4°C - it was protected from light. Later, the slides were introduced into the electrophoresis vat containing a buffer solution (NaOH, Na2EDTA, and purified water), which remained at rest for 30 min. Then, the slides were electrophoresed at 300 mA and 25 V (0.90 V/cm) for 30 min under no light.

After electrophoresis, the slides were placed in a staining tray, covered with neutralization buffer (Tris-HCl, pH 7.5) and kept for 5 min, then dried at room temperature, fixed in ethanol P.A. (for 10 min), and stained with ethidium bromide to 10 μg/mL (in purified water). Using a fluorescence microscope, the slides were photographed, and 50 nucleoids/animal were evaluated using the comet assay software (CaspLab®), according to the procedure also performed by Kaur et al. (2021) and Mehra and Chadha (2021). The following parameters were used to assess the possible damage to erythrocyte DNA induced by exposure to viral peptides: (i) tail length (TL), (ii) DNA percentage in the tail (% DNA), and (iii) Olive tail moment (OTM), as described by Collins (2004).

2.3.3. Biochemical biomarkers (oxidative stress and antioxidant activity)

Aiming to associate the possible mutagenic/genotoxic effects to the induction of a redox imbalance, different biochemical toxicity biomarkers were evaluated. For this, after blood collection, the animals were deeply anesthetized and subsequently euthanized in ice-cold water, and fragments of the brain, liver, muscle, and gills of *P. reticulata* were collected, macerated in 500 μL of PBS (pH 7.2). Then, the samples were centrifuged (10,000 rpm, 5 min, 4°C) and the supernatants were used for the biochemical evaluation. Malondialdehyde (MDA), a by-product of the lipid peroxidation (LPO) reaction (Yaman and Ayhanci, 2021), were used as oxidative stress biomarkers, as used in other studies – Tan et al. (2019), Patel et al. (2021), Issac et al. (2021), and Rangasamy et al., 2022. For this, we adopted the procedures described in detail in the study by Sachett et al. (2020). In addition, we evaluated the activity of superoxide dismutase (SOD) [according to Del Maestro and McDonald, 1985] and catalase (CAT) [as proposed by Sinha, 1972], considered as enzymes that make up the organisms’ first line of antioxidant defence (Ighodaro and Akinloye, 2018). The results of the analysis of all biomarkers were expressed proportionally to the concentration of total proteins, evaluated according to the instructions of the commercial kit used [Commercial kit (Reference number: BT1000900)].

2.4. Statistical analysis

All data obtained were evaluated regarding the assumptions for using parametric models. For this, we used the Shapiro-Wilk test to assess the distribution of residual data and the Bartlett test was used to assess the homogeneity of variances. Afterward, the means were compared by Student’s t-test (if parametric) or Mann-Whitney U test (if non-parametric). Additionally, correlation analyses were performed using Pearson’s (for parametric data) or Spearman’s (for non-parametric data) correlation coefficients, as well as linear regression analysis. For all analyses, we considered a significance level of 95% (*p* ≤ 0.05), using the GraphPad Prism software (version 7.0).

3. Results

We did not record any deaths during the exposure period, and, at the end of the experiment, several ENA were recorded (kidney-shaped nucleus, blebbed nucleus, multi-lobed nucleus, nuclear constriction, displaced nucleus, notched nucleus, binucleated erythrocytes, MN, and nuclear vacuole) in both experimental groups (Fig. 1A–B). However, we found that in animals exposed to PSPD-2002 peptides, the total ENA was higher than that observed in non-exposed animals, whose increase was greater than 70%

![Fig. 3.](image-url) (A) Malondialdehyde (MDA) levels and (B) superoxide dismutase (SOD) and (C) catalase activity in the brain, liver, muscle, and gills of adult female *Poecilia reticulata* exposed or not to PSPD-2002 peptide fragments (at 40 μg/L). The bars indicate the mean ± SD of the data, which were submitted to Student’s t-test (if parametric) or Mann-Whitney U test (if non-parametric) (see the statistical summary at the top of the graphs). Different lowercase letters indicate significant differences between experimental groups. *n* = 8 animals/group.
In addition, all parameters evaluated in the comet assay (TL, % DNA, and OTM) were superior in these animals when compared to the control group (background) (Fig. 2A–C), whose values were positively correlated with the total ENA (Fig. 2F).

From these data, we evaluated the possible relationship between the observed mutagenic/genotoxic effects and a possible redox imbalance induced by exposure to PSPD-2002 peptides. As seen in Fig. 3, the levels of MDA in the brain and liver of animals exposed to the peptides were higher than those observed in the control group (Fig. 3A), and the liver levels of this biomarker were correlated positively with all parameters evaluated in the single-cell gel electrophoresis assay (comet assay) (Fig. 4A–C) and with the total ENA (Fig. 4D). On the other hand, the suppression of SOD and CAT activity observed in the liver of animals exposed to PDPPD-2002 (Fig. 3B, respectively) was negatively correlated with all parameters evaluated in the comet assay (Fig. 5).

4. Discussion

Comprehension of the environmental/ecological impacts caused by the dispersion of peptide fragments of the new coronavirus (SARS-CoV-2) in freshwater ecosystems invariably depends on the development of studies that assess the risk of exposure to these particles causing damage to the biology of aquatic organisms. In this sense, our study not only confirms previous studies on the toxicity of PSPD-2002 peptides in non-target aquatic animal models (Charlie-Silva et al., 2021; Malafaia et al., 2022; Mendonça-Gomes et al., 2021), as well as providing insight into how exposure to these fragments can impact the health of *P. reticulata*. We observed that exposure to the peptides induced genomic instability (inferred by the MN test and ENA) (Fig. 1) and erythrocyte DNA damage (inferred by the comet assay) (Fig. 2) of *P. reticulata*.

Undoubtedly, proposing any mechanisms that explain these effects will demand further investigation. However, our data suggest that the redox imbalance observed in animals exposed to PSPD-2002 peptides (inferred by increased brain/liver MDA levels (Fig. 3A) and suppression of the antioxidant activity of hepatic SOD and CAT (Fig. 3B–C, respectively)) was associated for the mutagenic and genotoxic alterations reported in our study. Several studies point to oxidative stress as a factor inducing ENA formation and erythrocyte DNA damage (Antunes et al., 2016; Hathout et al., 2021; El-Garawani et al., 2021; Costa, 2021), corroborate our hypothesis. Moreover, the induction of oxidative stress (inferred by MDA levels - in the brain and liver) observed in our study is in line with previous reports involving not only the exposure of non-target models to peptide fragments of SARS-CoV-2 (Charlie-Silva et al., 2021; Malafaia et al., 2022; Mendonça-Gomes et al., 2021), as well as corroborating investigations describing the important role of Spike protein constituents in the induction of a disproportionate cellular antioxidant-oxidant balance in SARS-CoV-2-infection (Nyong-Pono, 2020; Delgado-Roche and Mesta, 2020; Cecchini and Cecchini, 2020; Suhail et al., 2020).

Furthermore, in our study, we recognize that *P. reticulata* were not experimentally infected with SARS-CoV-2, but it is tempting to speculate on the occurrence of processes that relate the absorption of protein fragments to the observed effects. One possibility concerns the absorption of PSPD-2002 peptides in the intestine of animals, after involuntary ingestion of fragments dispersed in water. This hypothesis is especially supported by studies that point to the possibility of small protein fragments (and not just free amino acids) being transported through the gastrointestinal epithelium to the bloodstream via PepT1-mediated permeation, paracellular transport via tight junctions, transcytosis, and/or passive transcellular diffusion (Adibi, 2003; Moss et al., 2018; Xu et al., 2019; Sun et al., 2020). Once in the hepatic portal circulation, these peptides may have reached...
the liver and triggered reactions that culminated in the increased production of free radicals inducing oxidative stress, which would explain the elevated levels of MDA in the liver (Fig. 3A). In this case, it is plausible to suppose that resident macrophages, such as Kupffer cells, would have led to a respiratory burst in response to the presence of SARS-CoV-2 Spike protein peptides and may also induce ROS production, which is in line with recent studies that point to the important role of macrophages in COVID-19 (Bangash et al., 2020; Knoll et al., 2021; Meidaninikjeh et al., 2021; Ristic-Medic et al., 2021). Alternatively, the peptide fragments dispersed in the exposure water may have reached the bloodstream via absorption by the gill epithelial cells, since this organ is the main absorption route of waterborne chemical compounds in aquatic organisms, especially due to the wide contact with seawater and a high exchange rate of solutes between gills and blood/hemolymph (Hayton and Barron, 1990; Erickson and McKim, 1990; Thurston, 1996). On that account, it is plausible to suppose that PSPD-2002 peptides would also have crossed the blood-brain barrier and induced an increase in LPO processes in the brain, which would explain the high levels of brain MDA (Fig. 3A). The recent study by Rhea et al. (2021), in particular, demonstrates that S1 protein of SARS-CoV-2 can cross the blood-brain barrier in mice, reinforcing this hypothesis. Hence, further advanced investigations are needed to confirm this hypothesis and to better understand the mechanisms intrinsic to the effects observed in our study.

Regardless of how exposure to PSPD-2002 peptides induced an increase in the frequency of ENA, as well as damage to the erythrocyte DNA of *Poecilia reticulata*, such effects could have dramatic consequences for the health of animals. Continuous/chronic exposure to peptides can lead, for example, to the accumulation of DNA strand breaks, since the DNA repair capacity

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Fig. 5. Correlation analysis between the activity of (A-C) superoxide dismutase (SOD) and (D-F) catalase (CAT) and the different parameters evaluated in the single-cell gel electrophoresis assay (comet assay) performed in erythrocytes of exposed adult female *Poecilia reticulata* or not to PSPD-2002 peptide fragments (at 40 μg/L). n = 8 animals/group.
of the fish cell is low as compared to other species (Kienzler et al., 2013). This can lead to interruption of the erythrocyte cell cycle, dysregulation of gene expression and if accumulation exceeds its elimination by DNA repair mechanisms, cellular senescence or apoptosis will occur and this may contribute to the increase in cellular dysfunctions and their negative impacts on the physiology of animals. On the other hand, the increase in the total EAN in fish exposed to PSPD-2002 is suggestive of the occurrence of genetic alterations arising from chromosomal and/or damage to the mitotic apparatus, which constitute a risk for the development of different types of cancers (Tucker and Preston, 1996; De-Campos-Junior et al., 2020). Clearly, such consequences illustrate just a few examples of the impacts that exposure to PSPD-2002 peptides can cause on individuals, which are not restricted to indirect effects associated with chromosomal alterations (inferred from the total EAN) and at the DNA level (inferred by the commit assay). Moreover, the increase in LPO processes observed in the brain and liver of P. reticulata exposed to viral peptides can trigger several harmful effects to animals, including changes of a neurological nature (with behavioral changes) to metabolic/endocrine, motivated by dysfunctions in these organs.

Conclusively, it must be recognized that our study is not exhaustive and, therefore, constitutes only the “tip of the iceberg” that represents the possible (eco)toxicological effects associated with the presence of SARS-CoV-2 particles in freshwater ecosystems. Not only do the biological mechanisms affected by exposure of P. reticulata to PSPD-2002 peptides need to be better investigated, but also the more systemic impact of mutagenic and genotoxic effects, and redox imbalance (in brain and liver) observed in our study. At the individual level, assessments using predictive biomarkers of gene dysregulation and histopathological, behavioral, and hormonal effects are important investigative perspectives for a better understanding of how viral peptide fragments can affect animal survival. Furthermore, at the population level, it is questioned, for example, to what extent the exposure of animals to these fragments can affect their social interactions, reproduction, and the dynamics of their populations in natural environments.

5. Conclusion

In conclusion, our study confirmed the hypothesis that exposure of P. reticulata adults to PSPD-2002 fragments dispersed in water can induce genomic instability and DNA damage in circulating erythrocytes, which were in correlation with a redox imbalance marked by increased MDA levels in the liver and brain, as well as by suppressing the antioxidant activity of hepatic SOD and CAT. Consistently, our study reinforces the ecological and environmental importance of evaluating the presence of SARS-CoV-2 fragments in freshwater environments, as well as their impacts on fish health. Through the development of more studies involving this theme, it will be possible to understand the real magnitude of the impacts caused by the COVID-19 pandemic on wild ichthyofauna.

Ethical aspects

All experimental procedures were performed by the ethical standards for animal experimentation and meticulous efforts were made to ensure that the animals suffered as little as possible and to reduce external sources of stress, pain, and discomfort. The current study has not exceeded the number of animals needed to produce reliable scientific data. This article does not refer to any study with human participants performed by any of the authors.

CRediT authorship contribution statement

Sandy de Oliveira Gonçalves: study conception and design, data collection, analysis, and interpretation of results, and draft manuscript preparation.
Thiarelen Marinho da Luz: data collection.
Abner Marcelino Silva: data collection.
Sindoal Silva de Souza: data collection.
Higgins, D.G., Thompson, J.D., Gibson, T.J., 1996. Using CLUSTAL for multiple sequence alignment. Methods Enzymol. 266, 383–402.

Suhail, S., Zajac, J., Fossum, C., Lowater, H., McCracken, C., Severson, N., ... Bhattacharyya, S.K., Shurtleff, S.A., 2021. Tissue based SARS-CoV-2 RNA detection in wastewater: a potential supplementary system for monitoring COVID-19 transmission. PloS one 16 (1), e0245603.

Mendoca-Gomes, J.M., Charlie-Silva, I., Guimarães, A.T.B., Estrela, F.N., Calmon, M.F., Meyerowitz, E.A., Richterman, A., Gandhi, R.T., Sax, P.E., 2020. Transmission of SARS-CoV-2 on the heat of the neo-tropical fish, Poecilia reticulata. Aquat. Toxicol. 245, 106140. https://doi.org/10.1016/j.aquatox.2022.106140.

Mehta, S., Chaudha, P., 2021. Naphthalene-2-sulphonate induced toxicity in blood cells of freshwater fish Channa punctatus using comet assay, micronucleus assay and ATR-FITR approach. Chemosphere 265, 129147.

Hassan, F.A., Samoejden, G.C., Rutzikoski, C.F., Foladori, A., Da Fré, S.P., Müller, C., Hartmann, S.U., 2020. Presence and infectivity of SARS-CoV-2 virus in wastewaters and rivers. Sci. Total Environ. 743, 136257.

Malafaia, G., Ahmed, M.A.I., de Souza, S.S., Rezende, F.N.E., Freitas, Í.N., da Luz, T.M., ... da Costa Araújo, A.P., 2022. Toxicological impact of SARS-CoV-2 on the health of the neo-tropical fish, Poecilia reticulata. Aquat. Toxicol. 245, 106140. https://doi.org/10.1016/j.aquatox.2022.106140.

Mehta, S., Chaudha, P., 2021. Naphthalene-2-sulphonate induced toxicity in blood cells of freshwater fish Channa punctatus using comet assay, micronucleus assay and ATR-FITR approach. Chemosphere 265, 129147.

Hassan, F.A., Samoejden, G.C., Rutzikoski, C.F., Foladori, A., Da Fré, S.P., Müller, C., Hartmann, S.U., 2020. Presence and infectivity of SARS-CoV-2 virus in wastewaters and rivers. Sci. Total Environ. 743, 136257.

Malafaia, G., Ahmed, M.A.I., de Souza, S.S., Rezende, F.N.E., Freitas, Í.N., da Luz, T.M., ... da Costa Araújo, A.P., 2022. Toxicological impact of SARS-CoV-2 on the health of the neo-tropical fish, Poecilia reticulata. Aquat. Toxicol. 245, 106140. https://doi.org/10.1016/j.aquatox.2022.106140.

Mehta, S., Chaudha, P., 2021. Naphthalene-2-sulphonate induced toxicity in blood cells of freshwater fish Channa punctatus using comet assay, micronucleus assay and ATR-FITR approach. Chemosphere 265, 129147.
Sun, X., Acquah, C., Aluko, R.E., Udenigwe, C.C., 2020. Considering food matrix and gastrointestinal effects in enhancing bioactive peptide absorption and bioavailability. J. Funct. Foods 64, 103680.

Tan, K., Zhang, B., Ma, H., Li, S., Zheng, H., 2019. Oxidative stress responses of golden and brown noble scallops Chlamys nobilis to acute cold stress. Fish Shellfish Immunol. 95, 349–356.

Thurston, R., 1996. Water chemistry at the gill surfaces of fish and the uptake of xenobiotics. Toxicology of Aquatic Pollution: Physiological, Molecular And Cellular Approaches. 57, p. 1.

Tiwari, R., Dhama, K., Sharun, K., Iqbal Yatoo, M., Malik, Y.S., Singh, R., Rodriguez-Morales, A.J., 2020. COVID-19: animals, veterinary and zoonotic links. Vet. Q. 40 (1), 169–182.

Tucker, J.D., Preston, R.J., 1996. Chromosome aberrations, micronuclei, aneuploidy, sister chromatid exchanges, and cancer risk assessment. Mutat. Res./Rev. Genet. Toxicol. 365 (1–3), 147–159.

Usman, M., Farooq, M., Anastopoulos, I., 2021. Exposure to SARS-CoV-2 in aerosolized wastewater: toilet flushing, wastewater treatment, and sprinkler irrigation. Water 13 (4), 436.

Victor, J., Deutsch, J., Whitaker, A., Lamkin, E.N., March, A., Zhou, P., Chatterjee, N., 2021. SARS-CoV-2 triggers DNA damage response in Vero E6 cells. Biochem. Biophys. Res. Commun. 579, 141–145.

Usman, M., Farooq, M., Anastopoulos, I., 2021. Exposure to SARS-CoV-2 in aerosolized wastewater: toilet flushing, wastewater treatment, and sprinkler irrigation. Water 13 (4), 436.

Yaman, S.O., Ayhanç, A., 2021. Lipid Peroxidation. IntechOpen, pp. 1–11. https://doi.org/10.5772/intechopen.95802.