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# Effects of Bisphenol A on redox balance in red blood and sperm cells and spermatic quality in zebrafish *Danio rerio*

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**Abstract**

Bisphenol-A (BPA) is a potential endocrine disruptor besides being associated with oxidative damage in several vertebrate classes. In the present study we investigated oxidative effects in erythrocytes and sperm cells as well as spermatic quality in *Danio rerio* exposed to 14 days at BPA concentrations of 2, 10 and 100 μg/L. Organelles structure, reactive species of oxygen (ROS) and lipoperoxidation (LPO) on erythrocytes and sperm cells were measured by flow cytometry and spermatic parameters were analyzed by the computer-assisted sperm analysis (CASA) system. For both cell types, when compared with control BPA treatment induced a significant increase in ROS and LPO production causing the membrane fluidity disorder, loss of membrane integrity and mitochondrial functionality. Furthermore, it was found a significant increase in DNA fragmentation in erythrocytes of zebrafish BPA exposed. Regarding the spermatic quality, results showed lower sperm motility in animals exposed to BPA, and alterations on velocity parameters of spermatozoa. Thus, the present study concludes that BPA affects the oxidative balance of both cell types, and that can directly affects the reproductive success of the adult *Danio rerio*. The sensitivity of erythrocytes to oxidative damage induced by BPA was similar to sperm cells, indicating a potential use of blood cells as indicators of oxidative damage present in fish sperm.

**Keywords** Bisphenol-A · Blood cells · *Danio rerio* · Oxidative stress · Reproduction · Sperm quality

## Introduction

Bisphenol-A (BPA) is an industrial chemical, which has been widely used in the manufacture of several products, such as polycarbonates, epoxy resins, thermal papers and dental sealants (Bermudez et al. 2010; Riu et al. 2011). It is one of the chemicals with the highest volume of production worldwide, according to estimates will be produced around 8.4 million tons of BPA by 2018 (Gran View Research 2015) and over 100,000 tons of this compound are released annually into the environment (Myridakis et al. 2016). BPA migrates to the environment mainly through processes such as the manufacture of plastics, incomplete removal during treatment of wastewater and leaching of waste discarded of BPA-based materials (Im and Löffler 2016). As a result, BPA has been detected in several environmental matrices such as soil, sediments, groundwater, surface waters, atmosphere and food (Kang et al. 2006; Careghini et al. 2015). In China, BPA was detected in rivers, groundwater and even seawater samples at concentrations up to 16 μg/L (Huang et al. 2012). Some authors defined about 12 μg/L or lower as the environmentally relevant concentration in surface waters (Flint et al. 2012) but in a review of data reported in Europe, Asia and North America, it was revealed that BPA was found in surface water at an average 56 μg/L (Corrales et al. 2015). BPA levels in hazardous waste landfill leachates in Japan were between 1.3 and 17,200 μg/L; mean concentration was 269 μg/L (Yamamoto et al. 2001). Thus, the concentrations used in the present study were chosen according with concentrations of this contaminant found in the environment. Studies have shown that BPA is an endocrine disruptor in several vertebrate classes (Vandenberg et al. 2009; Bhandari et al. 2015) and also is capable of generating oxidative stress by increasing the amount of reactive oxygen species (ROS) in the brain, liver, testis and epididymis and of reducing mitochondrial

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function in mammals (Nakagawa and Tayama 2000; Bindhumol et al. 2003; Chitra et al. 2003). The mechanism of action of this compound in oxidative damage is not entirely clear, but livers from rats exposed for 30 days to BPA (0.2, 2 and 20 µg/kg/day) demonstrated a decrease in important antioxidant enzymes such as superoxide dismutase-1 (SOD-1) and catalase (CAT) (Bindhumol et al. 2003). In fish, BPA was also related to oxidative stress via reduction of antioxidant defenses, even though further studies of the activity of this compound as a pro-oxidant in these animals are needed (Hulak et al. 2013). In general, studies of fish exposed to BPA at concentrations found in the environment are still scarce; thus, there is little information on the reproductive development and cellular stress at these concentrations in fish. *Danio rerio* fish has an interesting model in ecotoxicology, since it has been used over the years due to its advantages as a fast reproductive cycle and easy maintenance (Shrader et al. 2003; Coe et al. 2009; Bambino and Chu 2017). Sperm cells are vulnerable to the action of endocrine disrupters, since fish spermatozoa, for example, have limited ability to adjust to physicochemical changes in their external environment (Lahnsteiner et al. 2004). Similar to mammalian spermatozoa, fish spermatozoa contain high levels of polyunsaturated fatty acids, which are particularly susceptible to ROS-induced lipid peroxidation (Vernet et al. 2004). In mammals the effect of ROS on spermatozoa is well characterized, it may cause lipid peroxidation of spermatozoa membranes, loss of motility and infertility (Sikka 2001). Thus, the oxidative stress in fish is able to cause irreversible reproductive damage, like loss of viable spermatozoa and sperm motility (Li et al. 2010). In this way, studies of sperm parameters and oxidative stress against environmental contamination are necessary, since changes in these parameters can lead to infertility and cause important reproductive problems in affected populations (Schulz et al. 2010). A comparative study of proteomics in fish has shown that, in contrast to mammals, most proteins present in seminal plasma are blood proteins (Dietrich et al. 2014). However, these cells have completely different metabolisms and functions, which can lead to a difference in sensitivity to different contaminants including BPA. The analysis of oxidative parameters in blood cells after exposure to contaminants has been a good biomarker for several species, since they perform key functions such as transport of nutrients and oxygen and immune defense (Shimada and Yamauchi 2004; Çimen 2008; Cocci et al. 2017). In vertebrates, the most abundant cell types in the blood are erythrocytes, which are nucleated cells in fish and most vertebrates, except for mammals. Some studies analyzing erythrocytes from BPA exposure were recently performed with humans and demonstrated that there was an increase in ROS, lipid peroxidation and changes in the main enzymes of the antioxidant defense system (Maćczak et al. 2017a, 2017b). Considering the potential of blood cells as biomarkers of several environmental contaminants, together with the sensitivity of the gonadal cells to endocrine disrupters, they were chosen as a study model to verify the action of BPA (2, 10 and 100 µg/L) on the animals' redox balance. This study aimed at analyzing comparatively ROS and organelle alterations in erythrocytes and spermatic cells. In addition, knowing that oxidative stress can cause severe reproductive damage, was analyzed the spermatic quality of zebrafish *Danio rerio* exposed to BPA, so as to generate a discussion about reproductive changes caused by this compound and its impact on the wildlife.

## Methodology

### Exposure and sampling

Bisphenol-A exposure was performed at the laboratory of Toxicology at the Federal University of Rio Grande FURG (Rio Grande, RS, Brazil). Photoperiodic lighting was 12-h light and 12-h dark, at 26 ± 1 °C and 70% oxygen saturation. The male adults zebrafish were fed twice times a day with commercial feed (Supervit®) ad libitum. For the exposure, the animals (weight of 0.5826 ± 0.1161 g) were randomly divided into four groups respecting the proportion of 1 g animal per liter of water: a control group and three groups at BPA (Sigma-Aldrich Co., St. Louis, MO, USA) concentrations of 2, 10 and 100 µg/L (or 0.008, 0.04 and 0.4 µM). In each group, the fish were arranged in triplicate with 12 animals in each replicate, totaling 36 animals per experimental group. Fish were exposed to BPA for 14 days, in agreement with the guidelines issued by the OECD (OECD 1993), through continuous flow regulated by programmed peristaltic pumps. After two-week exposure, all animals were euthanized according Experimental Animal Ethic Committee (CEUA – FURG—Process number 23116.000355/2016-72). The tail was sectioned posteriorly to the genital orifice, and blood was placed in 500 µL of fetal bovine serum (FBS) and the gonads were also stored for flow cytometer.

### Flow cytometer analysis

Attune® Acoustic Focusing Flow Cytometer (Applied Biosystems) was used to detect the cells population. For the erythrocytes and sperm cells, removal cell debris and other cellular types was based on the FSC x SSC scatter plots (Petrunkina et al. 2005; Piehler et al. 2006) and was eliminated by staining cells with Hoechst 33342 at concentration of 16.2 µM (Sigma-Aldrich Co., St. Louis, MO, USA). A total of 20,000 erythrocytes and 10,000 spermatozoa per sample with flow of 200 event/s were analyzed.

**Concentration of reactive oxygen species (ROS)** ROS concentration was determined by the fluorescent dye 2'7-dichlorofluorescein diacetate at final concentration of 1.0 µM, which emits green fluorescence when oxidized by intracellular ROS and IP (7.3 µM final concentration). Only the median intensity of green fluorescence of the erythrocytes and living sperm (IP-) was used as measurement (Domínguez-Rebolledo et al. 2011).

**Lipoperoxidation (LPO)** Analysis of erytrocytes and spermatozoa lipoperoxidation was evaluated with final concentration of 1 µM of lipid peroxidation sensor Bodipy C11 (Hagedorn et al. 2012) in 100 µL sample. It was incubated for 2 h at room temperature (20 °C). The rate of lipoperoxidation was calculated by the median intensity of green fluorescence (peroxidized lipid)/median green fluorescence intensity + median red fluorescence (non-peroxidized lipid) * 100.

**Fluidity of plasma membrane** Plasma membrane fluidity was analyzed by hydrophobic merocyanine 540 dye (M540) at final concentration of 2.7 M (Sigma-Aldrich Co., St. Louis, MO, USA) and YO-PRO, which fluoresces green, at final concentration of 0.1 M (Invitrogen, Eugene, OR, USA). Only live cells (YO-PRO negative) were selected and classified into high fluidity cells (high M540 concentration) and low fluidity cells (low M540 concentration) (Gillan et al. 2005).

**DNA fragmentation** Only for the erythrocytes, DNA integrity was assessed by the chromatin structure assay (SCSA). To verify this parameter, 10 µL of FBS blood cells was added to 5 µL of TNE (0.01 M Tris-HCl, 0.15 M NaCl, 0.001 M EDTA, pH 7.2), 10 µL 1X Triton (Triton X-100, 1%) (v/v) at 30-second intervals. The acridine orange dye was added and incubated for 30 s not exceeding 2 min to read at room temperature (22 °C). The erythrocyte cells were classified as whole (green) and fragmented (orange/red) DNA. The DNA fragmentation index was calculated by median red/medium fluorescence green + red fluorescence.

### Assessment of sperm motility by computer-assisted semen analysis (CASA)

To analyze sperm motility, semen diluted in Milli-Q water was placed on slides under coverslips and analyzed by CASA (Chyb et al. 2001). Resulting images were reproduced and efficiently and objectively analyzed by the Sperm Class Analyzer (SCA) software to assess overall motility parameters, progressive motility, straight line velocity (VSL), curvilinear velocity (VCL) and VAP (Verstegen et al. 2002). Determination of time of motility after sperm activation was based on the time of complete arrest of the progressive movement of the spermatozoa, in agreement with the method described by (Sorensen 1979). Every image ($n = 10$) was analyzed using the standard settings for fish by SCA. Sperm was considered immotile when velocity was <10 m/s. Although SCA simultaneously assessed more than 15 sperm motility end points, for brevity only VCL, VSL, and VAP were considered for further analysis, since similar effects were observed for all end points. To determine these velocities, every individual sperm cell ($n =$ at least 1000 sperm) was followed throughout the 10 images and a sperm trajectory was calculated.

### Statistical analysis

In this study, descriptive data mean ± SEM were generated for every dependent variable: ROS, LPO, plasma membrane fluidity, DNA fragmentation, total sperm motility and progressive sperm motility. For all dependent variables, normality was tested by the Shapiro–Wilk test. Subsequently, the Kruskal Wallis test for nonparametric data was used because no variable exhibited normal distribution. Statistix® 2009 software was used for the analyses.

## Results

Throughout the experiment, there was no mortality of animals, a fact that indicates that BPA concentration was sublethal. Moreover, neither behavioral not feeding abnormalities were observed in the animals. This study found alterations in erythrocytes and sperm cells, especially at concentrations of 10 and 100 µg/L of BPA. A significant increase in the ROS in erythrocytes of the animals exposed to 10 and 100 µg/L ($P < 0.05$) and in sperm cells only to 10 µg/L ($P < 0.05$) was observed (Fig. 1a, b). Besides increase of lipoperoxidation was demonstrated at concentrations of 10 and 100 µg/L ($P < 0.05$) in both cell types, when compared with the control groups (Fig. 2a, b). However, fish erythrocytes exposed to 100 µg/L of BPA had higher levels ($P < 0.05$) of LPO than those treated with 10 µg/L of BPA. In contrast, the sperm cells presented more LPO at the concentration of 10 µg/L than in 100 µg/L ($P < 0.05$).

For the plasma membrane fluidity, both cell types were changed, where there was an increase in fluidity in erythrocytes at concentrations of 10 and 100 µg/L ($P < 0.05$). Already for the lower concentration in the erythrocytes (2 µg/L), there was a decrease of the plasma membrane fluidity, by comparison with the control group ($P < 0.05$). For sperm cells, there was an increase in membrane fluidity in relation to the control, but these cells were shown to be less sensitive to this parameter when compared to blood cells ($P < 0.05$) (Fig. 3a, b). DNA fragmentation analysis

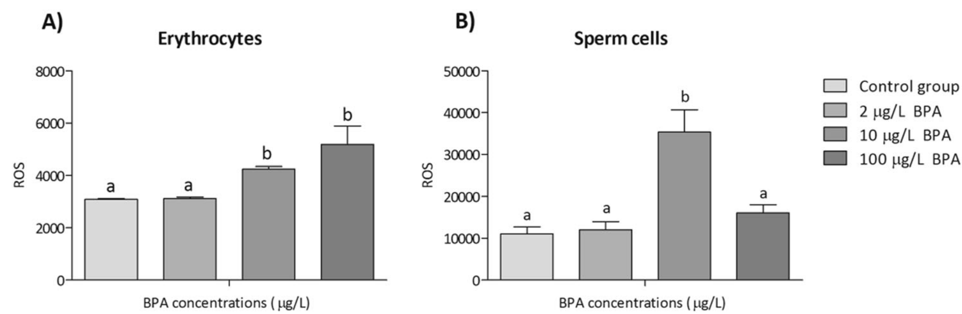


**Fig. 1** Production of reactive oxygen species (ROS) in erythrocytes (**a**) and sperm cells (**b**) of *Danio rerio* exposed for 14 days to BPA (2, 10 and 100 μg/L) and the respective control groups (0.0 μg/L). The values are means ± SEM ($n = 12$). The different letters represent significant differences among treatments at the same exposure periods ($P < 0.05$)

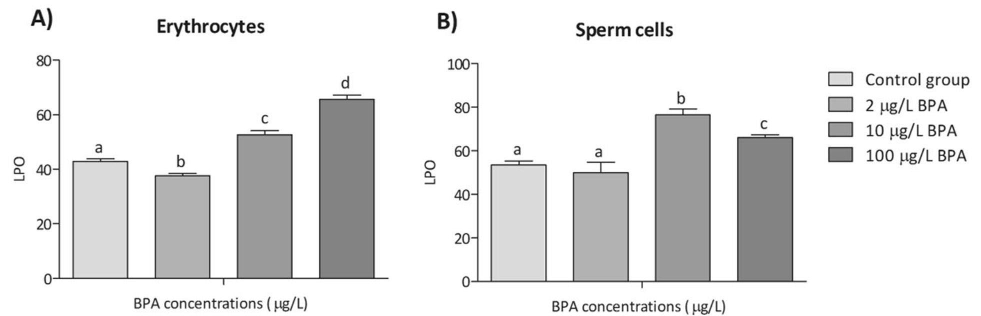


**Fig. 2** Lipoperoxidation (LPO) in erythrocytes (**a**) and sperm cells (**b**) of *Danio rerio* exposed for 14 days to BPA (2, 10 and 100 μg/L) and the respective control groups (0.0 μg/L). The values are means ± SEM ($n = 12$). The different letters represent significant differences among treatments at the same exposure periods ($P < 0.05$)

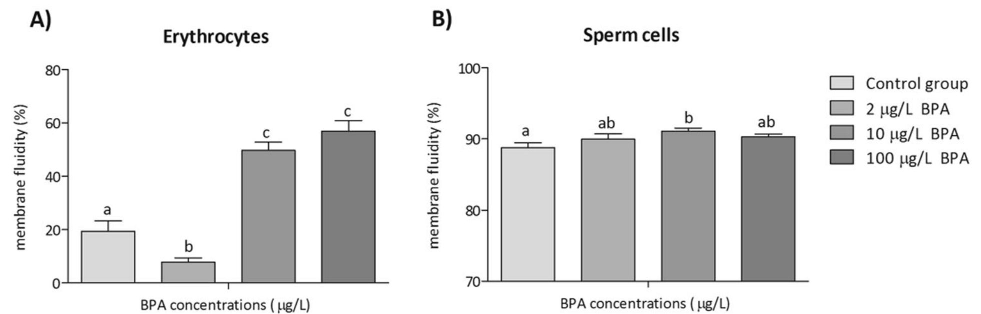


**Fig. 3** Membrane fluidity (%) in erythrocytes (**a**) and sperm cells (**b**) of *Danio rerio* exposed for 14 days to BPA (2, 10 and 100 μg/L) and the respective control groups (0.0 μg/L). The values are means ± SEM ($n = 12$). The different letters represent significant differences among treatments at the same exposure periods ($p < 0.05$)

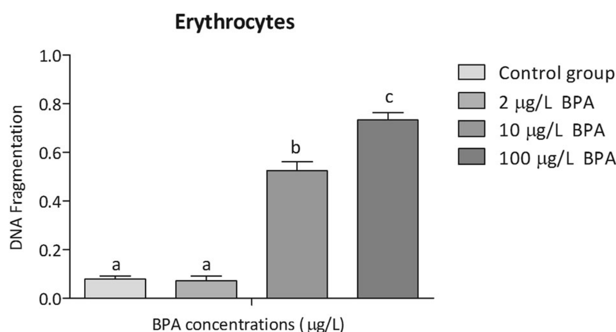


**Fig. 4** DNA fragmentation in erythrocytes of *Danio rerio* exposed for 14 days to BPA (2, 10 and 100 μg/L) and the respective control groups (0.0 μg/L). The values are means ± SEM ($n = 12$). The different letters represent significant differences among treatments at the same exposure periods ($p < 0.05$)

was performed only for erythrocytes, and an increase was observed to 10 and 100 μg/L in comparison to the control group ($P < 0.05$) (Fig. 4).

The sperm quality data analyzed by the computer-assisted sperm analysis (CASA) system showed that total motility and progressive motile spermatozoa were decreased at BPA concentrations of 2, 10 and 100 μg/L ($P < 0.05$) (Fig. 5a, b). The analysis also showed that distance average path (DAP), curved line distance (DCL), straight line distance (DSL), mean velocity (VAP), linearity (LIN), equilibrium (WOB) and flagellar beating frequency (BCF) had significantly higher values at concentrations of 10 and 100 μg/L ($P < 0.05$) by comparison with the control (Table 1).

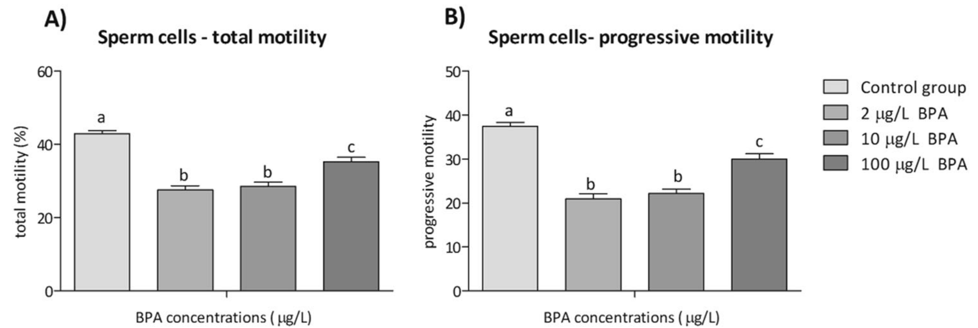


**Fig. 5** Sperm motility total (**a**) and progressive (**b**) in *Danio rerio* exposed for 14 days to BPA (2, 10 and 100 µg/L) and the respective control groups (0.0 µg/L). The values are means ± SEM ($n = 12$). The different letters represent significant differences among treatments at the same exposure periods ($P < 0.05$)

**Table 1** *Danio rerio* spermatozoa exposed to BPA at concentrations of 2, 10 and 100 µg/L for 14 days, evaluated in relation to mean distance average path (DAP), distance curved line (DCL), mean velocity average path (VAP), linearity (LIN), balance (WOB), amplitude lateral head displacement amplitude (ALH) and flagellar beat frequency (BCF), by CASA

| Parameter/[BPA] | Control group - mean ± *SEM* | 2 µg/L BPA – *mean ± SEM* | 10 µg/L BPA – *mean ± SEM* | 100 µg/L BPA – *mean ± SEM* |
|---|---|---|---|---|
| **DAP** | 19,690 ± 0,3968 | 21,012 ± 0,6890 | 24,475* ± 0,6408 | 26,822* ± 0,6872 |
| **DCL** | 22,759 ± 0,3945 | 24,488 ± 0,7296 | 27,770* ± 0,6426 | 29,934* ± 0,6596 |
| **DSL** | 17,231 ± 0,3931 | 18,588 ± 0,6728 | 22,146* ± 0,6111 | 24,457* ± 0,6876 |
| **VAP** | 43,037 ± 0,8722 | 45,423 ± 1,4684 | 52,810* ± 1,3466 | 57,741* ± 1,4657 |
| **LIN** | 0.7462 ± 5,251E-03 | 0,7431 ± 8,754E-03 | 0,7834* ± 6,778E-03 | 0,7983* ± 7,355E-03 |
| **WOB** | 0.8571 ± 3,687E-03 | 0,8485 ± 7,132E-03 | 0,8694* ± 5,388E-03 | 0,8823* ± 5,479E-03 |
| **ALH** | 1,2038 ± 0,0285 | 1,1783 ± 0,0369 | 1,1713* ± 0,0426 | 1,0668* ± 0,0254 |
| **BCF** | 25,814 ± 0,1989 | 26,752 ± 0,4995 | 27,650* ± 0,3780 | 28,661* ± 0,3051 |

Asterisks represent differences among treatments in the same exposure period ($P < 0.05$)

## Discussion

The constant presence of Bisphenol A (BPA) in different types of samples in humans (Calafat et al. 2008) and its short half-life in the organism indicates that human exposure to BPA is permanent (Huang et al. 2012) Bisphenol A in addition to its endocrine-disrupting chemical (EDC) properties is also a non-persistent organic chemical (NPOC) that disturbs semen quality (see review by Dziewirska et al. 2018). Erythrocytes are considered important biological models for toxicity screening, since they play a key role in the transport of O2 and CO2, essential for respiration and maintenance of nutrient metabolism in fish (Çimen 2008; Farag and Alagawany 2018). Hence, non nucleated red blood cells (mammals) and nucleated red blood cells (non-mammal vertebrates) are used to study the effect of oxidative stress caused by endocrine disruptors and other environmental pollutants (Shimada and Yamauchi 2004; Maćczak et al. 2017a; Cocci et al. 2017). Consequently, carry out research on the effects of chemicals on red blood cells can be an advantageous model because the cells are relatively easy to obtain, can also be used in vitro or in vivo research and are sensitive biomarkers to chemical-induced damage. The chemical used in this study was BPA in three concentrations (2, 10 and 100 µg/L or 0.0087, 0.0438 and 0.438 µM) which we consider to be environmentally relevant. We denominate environmentally relevant concentrations those that are close to the average of the actual concentrations found in the environment (Flint et al. 2012).

In the present study we observed in the red blood cells a significant oxidative stress response and DNA fragmentation. In the same way, we studied the sperm cells of these fish and then were observed a response quite similar to what we had recorded in the red blood cells. Spermatogenesis is a complex process controlled from higher centers, Hypothalamus and Pituitary; and locally by the Sertoli and Leydig cells, among others (Schulz et al. 2010). Considering this complexity can deduce that exogenous chemicals acting in one or more these places can cause spermatogenesis impairment. In fact, several studies have shown that BPA at environmental concentrations caused reproductive toxicity in males of *Danio rerio* (Chen et al. 2015; Li et al. 2016) as well as similar effects were recorded in other classes of vertebrates and is therefore characterized as a potential EDC (Vandenberg et al. 2009).

It is known that BPA is a chemical capable of generating oxidative stress by reducing antioxidant defenses in various tissues and in different classes of vertebrates (Chitra et al. 2003; Kourouma et al. 2014), which may explain the generation of ROS in the erythrocytes and sperm cells of fish exposed to concentrations of 10 and 100 µg/L of BPA. In aerobic organisms, reactive oxygen species (ROS) are formed as natural products of oxygen metabolism and have

important functions in cellular signaling systems (Devasagayam et al. 2004). However, excess ROS can overcome the levels of antioxidant defense and cause damage in macromolecules, impairing cellular homeostasis (Li et al. 2010). The presence of contaminants such as BPA in the cell can trigger oxidative stress directly, through its metabolites, through interaction with transcription factors, among other pathways (Hassan et al. 2012). However, the mechanism of action by which BPA can generate ROS increase is unclear despite its action (in vitro and in vivo) as an oxidizing agent in several studies and in different cell types (Hulak et al. 2013; Hamed and Abdel-Tawwab 2017). In abalone *Haliotis diversicolor*, BPA was able to alter signaling pathways related to energy metabolism and stress responses, impairing its metamorphosis (Liu et al. 2011). Furthermore, in common carp, for example, similar concentrations of BPA resulted in ROS generation in the liver, as well as disturbances in their immune response (Qiu et al. 2016). Larvae of rare minnow *Gobiocypris rarus* exposed to BPA for seven days presented inflammatory effects resulting from increased ROS production, accompanied by immunosuppression (Tao et al. 2016). (Michałowicz et al. 2015) demonstrated that exposure to BPA in human blood cells is capable of causing decrease in cell viability due to ROS generation and consequent lipoperoxidation in cells exposed to this chemical. The LPO results observed in our study showed significant oxidative damage in erythrocytes and sperm cells of fish exposed to 10 and 100 μg/L of BPA when compared to the control group. Besides that, in erythrocytes the LPO displayed a significant decrease $P < 0.05$) in fish exposed to 2 μg/L of BPA. This pattern of response fits into the non-monotonic curve response characteristics that are observed after exposure to various hormones and endocrine disrupters (Vandenberg et al. 2013).

Thus, the increase of lipoperoxidation in exposed fish may be a consequence of the high levels of ROS generated by the presence of the compound. Red blood cells are cells that are relatively vulnerable to lipid peroxidation, which makes them a good biological membrane model to analyze oxidative stress and to lipoperoxidation against different xenobiotics (Farag and Alagawany 2018). Increased levels of lipoperoxidation may be responsible for decrease in plasma membrane integrity, which in turn affects cell permeability and may cause enzymatic inactivation and DNA damage (Domínguez-Rebolledo et al. 2011). In the present study an increase in membrane fluidity in erythrocytes and sperm cells exposed to BPA was found. Changes in membrane fluidity are generally related to changes in lipid composition caused by various types of cell injury such as membrane lipid peroxidation or internal cell damage (Sergent et al. 2009). Besides that, the sperm plasma membrane, as the first barrier, may also be more sensitive to different types of pollutants. This idea was demonstrated by (Harayashiki et al. 2013; Lopes et al. 2014) in *Poecilia vivipara* and *Danio rerio* spermatozoa, respectively, treated by different glyphosate concentrations. Finally, several studies have shown that BPA has a genotoxic and mutagenic potential, and may lead to DNA molecule breakdown, DNA adduct formation among other similar effects (Tiwari et al. 2012; Jalal et al. 2018). In the present study, an increase in the DNA fragmentation erythrocytes of the animals exposed to 10 and 100 μg/L of BPA was observed in relation to the control group. The DNA fragmentation caused by exposure to BPA can be explained mainly by the generation of oxidative stress which in turn can damage the DNA molecule, as observed in other studies (Lv et al. 2017; Gao et al. 2018). Moreover, it is known that some BPA metabolites are capable of interacting with the DNA and consequently generate damage that can lead to the breakdown of the molecule (Mokra et al. 2017; Zhao et al. 2018). In vitro studies with acute exposure to low doses of BPA also altered DNA integrity and promoted the generation of ROS in a fish cell line (Hulak et al. 2013). Other DNA damage, such as methylation, was found in rare minnow exposed to relatively low concentrations of BPA (Zhang et al. 2017). Other contaminants, such as endosulfan, cause DNA damage as well as generation of ROS in *Danio rerio* (Shao et al. 2012). Fragmentation or loss of DNA integrity may induce chromosomal aberrations and mutations, and in the long run cause more severe effects such as apoptosis, cancer and irreversible toxic changes (Rocco et al. 2012). Thus, the results obtained showed that the environmentally relevant concentrations of BPA can generate excess ROS and consequent effects on the plasma membrane and blood and sperm cells, and DNA damage on erythrocytes. From this, parameters related to sperm quality were also evaluated in order to evaluate the possible impact of these alterations on the performance of fish reproductive system. Semen quality is considered a biomarker of early warning to reproductive disorders and within its various parameters, sperm motility is one of the most important characteristics to be evaluated because it is a prerequisite for fertilization (Rurangwa et al. 2004). Findings of this study showed that a decreased of sperm motility, total and progressive, on zebrafish exposed to 10 and 100 μg/L of BPA. When male fathead minnows, *Pimephales promelas*, were exposed to BPA concentrations of 16 μg/L or higher ones, their gonads significantly reduced the number of mature spermatozoa and increased the number of immature spermatozoa in seminiferous tubules (Sohoni et al. 2001). A study of goldfish, *Carassius auratus*, also found decrease in motility in sperm exposed to BPA concentrations, similar to the one described by this study after a 90-day exposure period (Hatef et al. 2012). Parameters related to spermatic velocity, straightness, linearity, balance, lateral displacement amplitude and frequency of flagellar beats were significantly

higher at concentrations of 10 and 100 μg/L when compared with the control and the lowest BPA concentration (2 μg/L). Others endocrine disruptors, nonylphenol and atrazine, also increased sperm velocity parameters in mammals at environmental concentrations in which this contaminant is considered environmental (Duan et al. 2016; Saalfeld et al. 2018). Under physiological conditions and normal sperm density, fish semen has molecular inhibitors whose roles include regulation of spermatogenesis, removal of immature and damaged sperm and stimulation of sperm velocity (Inaba et al. 1998; Dzyuba and Cosson 2014). Results of increasing sperm velocity in animals exposed to BPA may be due to decrease in the number of viable cells at the 10 and 100 μg/L of BPA (decrease motility -%), and, consequently, dilution of inhibitors found in semen.

Although the evidence demonstrates adverse effects of BPA on sperm quality, their mechanisms are not well understood yet. Sperm maturation in fish, for example, is regulated by cell signaling with increased cAMP and pH (Cosson 2004) and sperm motility depends on the ATP content (Cosson 2010). The reduction in sperm motility after treatment with BPA can be explained in several ways. Most reported effects of BPA on vertebrates may be attributed to its activity as an estrogen receptor agonist, but this compound has also caused ROS generation and cellular oxidative stress (Crain et al. 2007). Sperm motility may be the most sensitive indicator of oxidative stress, since high ROS levels are able to inhibit one or more oxidative phosphorylation and/or glycolysis enzymes, limiting ATP generation (Lamirande and Gagnon 1995). In vitro studies of environmentally relevant BPA concentrations in fish spermatozoa demonstrated decrease in sperm motility and sperm velocity associated with increased ATP levels, oxidative stress and lipoperoxidation (Hulak et al. 2013). Therefore, more biochemical studies are needed, since oxidative stress may influence the sperm quality of these animals and reduce the motility of their spermatozoa. Toxin-induced oxidative stress is the most common cause of damage to sperm (Pasqualotto et al. 2000).

## Conclusion

Considering this scenario, our results allow us to conclude that zebrafish Danio rerio, exposed to environmentally relevant BPA concentrations, currently found in the aquatic environment, induced higher ROS amount, as well as structural damage caused by lipoperoxidation in both red blood cells and sperm cells indicating oxidative stress. Still, as a possible consequence of these cellular damages was observed a decrease in sperm quality, which can lead to important effects that compromise their reproductive success. The sensibility of red blood cells and sperm cells was similar, suggesting a potential use of the red blood cells as indicators of oxidative damages present in the sperm of the animal. Although wild fish are more genetically variable than the laboratory strains, it is known that this difference is small and does not invalidate the use of laboratory strains in ecotoxicological studies (Coe et al. 2009; Bambino and Chu 2017). Changes in key aspects of zebrafish sperm physiology from exposure to BPA suggest the possibility of reproductive disturbances which may affect the survival capacity of populations in BPA-contaminated environments. The ubiquity of BPA in different environments raises concern about the risks of preserving different populations multigenerational exposure to this chemical. Some studies have shown that gonadal damage in fish similar to the can lead to decrease in the fertility of the animals and consequent decrease in the population abundance (Baker et al. 2014; Akhter et al. 2018). de Kermoysan et al. (2013) demonstrated that exposure to BPA concentrations, similar of the present study, experiment caused damage to fish *Gasterosteus aculeatus* gonads as well as an increase in the proportion of immature males in subsequent generations in a chronic mesocosm assay. Thus, the results of present study indicate that BPA in environmentally relevant concentrations has the potential to cause oxidative damage in blood and sperm cells of zebrafish *Danio rerio*, which could in the long term provide irreversible changes in the fish populations.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted (Experimental Animal Ethic Committee CEUA – FURG Process number 23116.000355/2016-72).

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