# Genotoxicity of gemfibrozil in the gilthead seabream (*Sparus aurata*)

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**Abstract**

Widespread use of pharmaceuticals and suboptimal wastewater treatment have led to increased levels of these substances in aquatic
ecosystems. Lipid-lowering drugs such as gemfibrozil, which are among the most abundant human pharmaceuticals in the environment, may  have deleterious effects on aquatic organisms. We examined the genotoxicity of gemfibrozil in a fish species, the gilthead seabream (*Sparus aurata*), which is commercially important in southern Europe. Following 96-h waterborne exposure, molecular (erythrocyte DNA strand
breaks) and cytogenetic (micronuclei and other nuclear abnormalities in cells) endpoints were measured. Gemfibrozil was positive in both endpoints, at environmentally relevant concentrations, a result that raises concerns about the potential genotoxic effects of the drug in recipient waters.

**Keywords**

Gemfibrozil; Seabream; DNA damage; Comet assay; Erythrocyte abnormalities; Micronucleus assay

## 1. Introduction

The presence of human and veterinary pharmaceuticals in the environment is of increasing concern

[1], [2], [3], [4]. The environmental release of these substances and their metabolites, their persistence, and their bioactivities have led to their classification as emerging environmental contaminants of concern [2], [3], [4]. Lipid regulators, a group of human pharmaceuticals, are frequently reported in wastewater and surface waters, due to their increased use in recent years [2], [5], [6], [7], [8], [4]. The lipid regulator gemfibrozil (GEM) has been found in wastewater treatment plant effluents at levels as high as 2.1 μg/L in Canada [6] and 4.76 μg/L in Europe [2]. In surface waters, the highest concentrations of GEM were detected in North America and Europe, around 0.75 and 1.5 μg/L, respectively [5].

The risks to aquatic organisms associated with the presence of pharmaceuticals in the environment include behavioural alterations, genotoxicity, reduced pathogen resistance, and endocrine disruption [9], [10], [11]. Studies with GEM have shown that it affects feeding and attachment of the cnidarian *Hydra* attenuata [12]; growth of the alga *Chlorella vulgaris* [13]; decreases plasma testosterone levels in the goldfish (*Carassius auratus*) [14]; and activates antioxidant enzymes and interferes with metallothionein expression in the blue mussel (Mytilus edulis) [4] and zebra mussel (*Dreissena polymorpha*) [15]. Henriques et al. [16] showed that exposure to GEM affects the development and locomotor activity of zebrafish (*Danio rerio*) larvae. Only a few studies have evaluated its aquatic genotoxicity [17]. GEM can damage DNA in the zebrafish after 7-d exposure; [17] and in marine (*Mytilus* spp.) and freshwater (*Dreissena polymorpha*) mussels after 96-h exposure; [15], [4]. However, to our knowledge, no study has reported GEM effects on an estuarine/marine top-predator fish species.

Contaminants may interact with DNA directly or they may disrupt normal cellular processes, e.g. inducing oxidative stress [18]. Elevated
levels of reactive oxygen species (ROS) and/or depressed antioxidant defences may result in DNA oxidation and increased steady-state levels of unrepaired DNA leading to genotoxicity [19], [20], [21].

The comet assay is widely used in environmental toxicology for assessing DNA damage [22], [23], [24], [25] it combines the simplicity of biochemical techniques for detecting DNA single-strand

breaks (strand breaks and incomplete excision-repair sites), alkali-labile sites, and cross-linking, by measuring the migration of DNA from immobilized nuclear DNA, using the single-cell approach typical of cytogenetic assays [26], [27], [28], [29], [30]. The micronucleus (MN) assay, one of the most popular tests of environmental genotoxicity, is based on chromatid/chromosome fragments or whole chromatids/chromosomes resulting from DNA strand damage, which are not reincorporated into the daughter nucleus and are transformed into a MN [31], [32], [33], [34], [35]. MN may be induced by oxidative stress, by exposure to clastogens or aneugens, or by defects in cell-cycle checkpoints or DNA repair [33]. The simultaneous expression of other morphological nuclear abnormalities in addition to MN has proven to be a valuable tool in detecting genotoxicity of several contaminants at low concentrations [36], [37], [38], [39], [40], [41], [42]. We have tested the genotoxicity of waterborne GEM to a predatory fish species, *Sparus aurata*, following a 96-h exposure, by assessing damage with the comet assay and erythrocytic nuclear abnormalities (ENAs) assay.

## 2. Material and methods

### 2.1. Chemicals

All reagents used were analytical grade and acquired from Sigma-Aldrich. Gemfibrozil (GEM) (≥98%) was purchased from TCI and isotopically labelled d6-GEM was purchased from Santa Cruz Biotechnology (Dallas, USA). A stock solution (50 g/L) was prepared in dimethyl sulfoxide (DMSO). Exposure solutions of GEM (1.5; 15; 150; 1500; and 15,000 µg/L) were prepared by serial dilutions in artificial seawater.

### 2.2. Test animals and experimental design

All experimental procedures were carried out following the Portuguese and European legislation (authorization N421/2013 of the Portuguese legal authorities). Animal handling was performed by an accredited researcher.

Juvenile gilthead seabream (*Sparus aurata*), length 9 ± 0.9 cm, acquired from an aquaculture facility (Spain), were acclimated for 4 weeks in 220- L aquaria containing aerated and filtered artificial seawater (salinity, 35), under a controlled room temperature (20 °C) and natural photoperiod. During this period, the experimental fish (n = 55) were fed daily with commercial fish food (Sorgal, Portugal) at a ratio of 1 g per 100 g of fish and the water in the aquarium was renewed daily.

The procedures generally followed the OECD guidelines for fish acute bioassays [43]. The experiment was carried out in 80-L aquaria, under the conditions described for the acclimation period. Following acclimation, fish were randomly distributed into seven aquaria, with seven fish per aquarium. The experimental design included a negative control (seawater only), a solvent control (0.03% DMSO, the DMSO concentration used for the highest concentration of GEM) and five GEM concentrations: 1.5; 15; 150; 1500; and 15,000 µg/L. Fish were exposed for 96 h as recommended by the OECD guideline for fish acute toxicity testing (203), without feeding, with 80% medium renewal every 24 h, to prevent significant GEM degradation and to reduce the build-up of metabolic residues. Fish mortality, behavioural alterations, and water parameters (such as temperature, salinity, conductivity, pH, and dissolved oxygen) were monitored daily.

After 96 h exposure, the animals were anesthetized with tricaine methanesulfonate (MS-222) and a blood sample was collected from the posterior cardinal vein of each fish. For the comet assay, blood samples were diluted with saline phosphate buffer (2:2000, v/v) and used immediately. Blood smears were prepared for the assessment of MN and other erythrocytic nuclear abnormalities.

### 2.3. Quantitation of GEM in the test media

Water samples (10 mL) were collected each day (at 0 and 24 h) from each aquarium, and GEM was analysed by solid-phase extraction (SPE). Briefly, Strata X cartridges (200 mg, 3 mL) (Phenomenex, USA) were conditioned with 5 mL methanol and 5 mL ultra-pure water. Then, the water sample (10 mL) was percolated through the cartridge at a flow rate of 3–5 mL/min; the cartridge was rinsed with ultra-pure water

(5 mL), and dried under vacuum for 20 min. Finally, GEM was eluted with methanol, 10 mL. Extracts were evaporated until dryness under a gentle stream of nitrogen and reconstituted with acetonitrile/ultra-pure water (30:70, v/v, 1 mL). An aliquot (10 µL) of gemfibrozil-$d_6$ (5 mg/L) was added to the extract as internal standard before UHPLC–MS/MS analysis. GEM analysis was performed on a Nexera UHPLC system with a triple-quadrupole mass spectrometer detector LCMS-8030 (Shimadzu Corporation, Kyoto, Japan). Chromatographic separation was
achieved on a Kinetex C18 column (2.1 × 150 mm i.d., 1.7 µm particle size) from Phenomenex (USA); column temperature, 30 °C; autosampler temperature, 4 °C; injection volume, 5 µL. Elution conditions were: solvent A, 5 mM ammonium acetate/ammonia buffer (pH 8); solvent B, acetonitrile; flow rate, 0.22 mL/min. Gradient program was as follows: initial conditions: 30% B; 0–2.0 min, 30%–100% B; 2.0–4.5 min, maintained at 100% B; 4.5–5.5 min, return to initial conditions; from 5.5–9.5 min, re-equilibration of the column.

GEM was analysed in the negative ionization mode and quantitation was performed in multiple reaction monitoring mode (MRM) using two transitions between the precursor ion and the most abundant fragment ions. A summary of individual MS/MS parameters is shown in Table S1 (Supplementary Material). Quantitation was performed by the internal standard calibration method. The method detection limit (MDL) for GEM in water was 4.0 ng/L. Detailed QA/QC information is given in the Supplementary Material (Table S2).

### 2.4. Evaluation of genetic damage

#### 2.4.1. Comet assay

The alkaline comet assay was conducted according to the method of Singh et al. (1988) with some modifications. To prevent UV-induced DNA damage, the procedure was conducted under yellow light. Briefly, diluted blood samples (20 µL) were added to 1% (w/v) low-melting-point agarose, 140 µL (at 40 °C) and the mixtures applied to microscope slides pre-coated with 1% (w/v) normal-melting-point agarose. A coverslip was added to each slide, which was then placed on ice for agarose solidification; then, the coverslips were carefully removed and the slides immersed, for 1 h at 4 °C, in lysis solution (2.5 M NaCl, 100 mM EDTA and 10 mM Tris, pH 10.0), containing freshly added 1% Triton X-100. Slides were incubated in alkaline buffer (300 mM NaOH and 1 mM EDTA, pH > 13) for 10 min for DNA denaturation and unwinding.
Electrophoresis was performed using the same buffer, for 30 min at 300 mA and 20 V. Note: State the field strength in 0.83 V/cm. After electrophoresis, slides were neutralized in 400 mM Tris buffer (pH 7.5), dehydrated with absolute ethanol for 10 s, and left to dry for 1 d in the dark. Slides were stained with ethidium bromide (20 µL/mL, 100 µL), covered with a coverslip, and analysed using a fluorescence microscope (Olympus BX41TF) at 400X magnification. To verify that the electrophoresis conditions were adequate, negative (blood from fish maintained in an aquarium with seawater only) and positive (blood from fish treated with 25 µM $H_2O_2$ for 10 min) controls were included in each electrophoresis run. $H_2O_2$ was used as a model genotoxic agent since it produces both single-strand breaks and oxidative DNA damage [80] and has been used routinely as a positive control in the comet assay [44], [45]. To avoid bias, slides were randomly analysed, counting one hundred randomly selected cells from each slide. Cells were scored visually, according to tail length, into five classes: class 0 – undamaged, without a tail; class 1 – with a tail shorter than the diameter of the head (nucleus); class 2 – with a tail length 1–2 times the diameter of the head; class 3 − with a tail longer than twice the diameter of the head; class 4 – comets with no heads [19]. A damage index (DI) expressed in arbitrary units was assigned to each replicate (for 100 cells) and consequently for each treatment, using the formula:

$$\mathrm{DI} = (0 \times \mathrm{n0}) + (1 \times \mathrm{n1}) + (2 \times \mathrm{n2}) + (3 \times \mathrm{n3}) + (4 \times \mathrm{n4})$$

where: *n* = number of cells in each class.

DI can range from 0 to 400 [46]. The percentage of DNA damage relative to the control was calculated.

#### 2.4.2. Erythrocytic nuclear abnormalities (ENAs) assay

This assay was carried out in mature peripheral erythrocytes according to the procedure of Pacheco

and Santos [47]. Blood smears were fixed in methanol during 10 min and stained with Giemsa (5%) for 30 min. The nuclear abnormalities were randomly scored under a light microscope in 1000 intact erythrocytes per fish. Nuclear lesions were scored as: micronuclei, lobed nuclei that display a nucleus with a small evagination of the nuclear membrane with euchromatin; segmented nuclei, symmetrical or asymmetrical hourglass-shaped nuclei; kidney- shaped nuclei, nuclei with a kidney-shaped profile and vacuolated nuclei showing central vacuoles in the nucleus without nuclear material. Blebbed, lobed, and notched nuclei were considered in a single category – lobed nuclei – and not scored differentially, as suggested by other authors, due to some ambiguity in their distinction [48], [40]. In general, blebbed nuclei have a relatively small evagination of the nuclear envelope, lobed nuclei present evaginations largest than the blebbed nuclei and the notched nuclei have an appreciable invagination [40].
Results were expressed as the percentage mean value for erythrocytic nuclear abnormalities (ENAs) using the equation:

$$ENAs\,(\%) = \frac{\mathrm{NumberofcellscontainingENAs}}{\mathrm{Totalnumberofcellscounted}} \times 100$$

### 2.5. Statistical analysis

Prior to parametric analyses, data were tested for normality (Shapiro-Wilk test) and homogeneity of variance (Levene's test), using the SPSS 21 software package. Differences between controls (negative and solvent) were evaluated using the Student *t*-test ($p < 0.05$). Data from treatments and controls were compared using one-way analysis of variance (ANOVA), followed by Dunnett's comparison post-hoc test whenever applicable ($p < 0.05$).

## 3. Results and discussion

We assessed the genotoxic effects of waterborne GEM in an important commercial species (*S. aurata*), at a wide range of concentrations, including environmentally relevant concentrations.

### 3.1. Quantitation of GEM in the test media

Spiked nominal concentrations of GEM were generally lower than the observed concentrations; this difference varied from 6 to 37% (Table 1). In a study with zebrafish, Henriques et al. [16] similarly reported that nominal concentrations of GEM differed from 3 to 30% of the added amount, as detected by chemical analysis. After 24 h, the decrease of GEM was most evident at the lowest concentrations (about 60% at
1.5 μg/L and 16% at 15 μg/L). At the highest concentrations, the decrease of GEM amount was <2% (Table 1). In a study with goldfish (*Carassius auratus*), a decrease of GEM concentration was detected in water at the end of the exposure (14 d), also at the lowest concentrations [14].

Table 1. Nominal and measured concentrations (μg/L) of gemfibrozil from water samples collected at time 0 and after 24-h of spiking.

| Nominal concentrations (μg/L) | | 1.5 | 15 | 150 | 1500 | 15,000 |
|---|---|---|---|---|---|---|
| Measured concentrations (μg/L) | 0h | 1.6 | 23.7 | 237.7 | 1973.5 | 18824.6 |
| | 24h | 0.6 | 19.9 | 234.8 | 1958.8 | 18530.9 |

### 3.2. Evaluation of genetic damage

DNA integrity loss was measured with the comet assay in *S. aurata* erythrocytes after DMSO exposure (t-test, $p < 0.05$). The negative control displayed a damage index around 77, corresponding to 19.25% DNA damage, whereas the solvent control showed a damage index around 150, corresponding to 37.5% damage. There is limited and contradictory information available on the possible genotoxic effects of DMSO and no study was found concerning the genotoxicity of this solvent to fish. Although some studies have described DMSO as not genotoxic, other studies have

shown the importance of studying possible genotoxic effects of DMSO in various organisms [49]. A decrease in DNA integrity
was reported for the digestive gland of blue mussel *Mytilus* spp. [4] and for the visceral mass of zebra mussel *Dreissena polymorpha* [15] following exposure to 0.2% DMSO. The concentration of DMSO used in the current study (0.03%) was nearly one order of magnitude lower than the concentrations used in earlier studies with GEM (i.e., 0.1 and 0.2% DMSO) [13], [12], [15], [4]. The concentration chosen here was a compromise, considering the limits recommended in the guidelines [50], [51], [52], the results of previous studies [14], [81], [13], [12], [15], [4], [53], and the aim of the present work.

The differences between the negative and solvent control groups seen in the comet assay were not seen in the ENAs assay. DNA damage corresponds to a very early signal of stress in the cell. Some authors also reported loss of DNA integrity in marine mussels (*Mytilus* spp.) after exposure to 0.2% DMSO, but other biomarkers, such as lipid peroxidation levels and glutathione transferase activity, were not affected [4].

Taking into account the observed differences between the seawater control and the solvent control, the effects of GEM treatments were compared to the solvent control. Overall, GEM displayed genotoxic potential, assessed by DNA strand breakage at environmentally relevant concentrations (Fig. 1). The damage index increased with GEM concentration, with a maximum of 316 in organisms exposed to 15,000 µg/L (15 mg/L). In terms of damage classes, the two most abundant classes in the negative control group (seawater only), which displayed little or no DNA migration, were class 0 and 1 damage, unlike the solvent control, where classes 1 and 2 were the most abundant (Table 2). At all GEM treatment exposures, when compared to the solvent control, significantly lower class 0 and 1 cells were seen, with increased class 2 at 1.5 µg/L,
class 3 at 15 µg/L, and classes 3 and 4 at 150, 1500, and 15,000 µg/L. The ability of GEM to decrease DNA integrity has been previously reported in studies with aquatic organisms: in *Danio rerio* erythrocytes after 5 d exposure to 380 ng/L [17], in *Mytilus* spp. digestive gland [4], and in *Dreissena polymorpha* visceral mass [15] after 24 and 96-h exposure to 1 and 1000 µg/L. The mechanism of GEM-dependent DNA integrity loss in aquatic organisms is not yet clear. GEM, classified under the generic designation of fibrates, is a potent peroxisome proliferator (PPs) [54]. Fibrates are characterized by the pronounced induction of hepatic peroxisome proliferation, mediated via peroxisome proliferator-activated receptor alpha (PPAR**α**), increasing the number and size of peroxisomes in the liver [55]. PPAR**α**-induced oxidative stress may contribute to cell proliferation via increased signalling or may damage DNA, initiating carcinogenesis. However, data for peroxisome proliferator-induced
DNA damage are conflicting [56], [57]. In mammals, GEM may be metabolized to reactive acyl glucuronide metabolites which may react with
nucleophilic centres in DNA via a Schiff base mechanism [58]. GEM may also cause DNA strand breaks via oxygen-radical generation. In addition, perturbed DNA repair may lead to formation of mutagenic and clastogenic lesions [59].

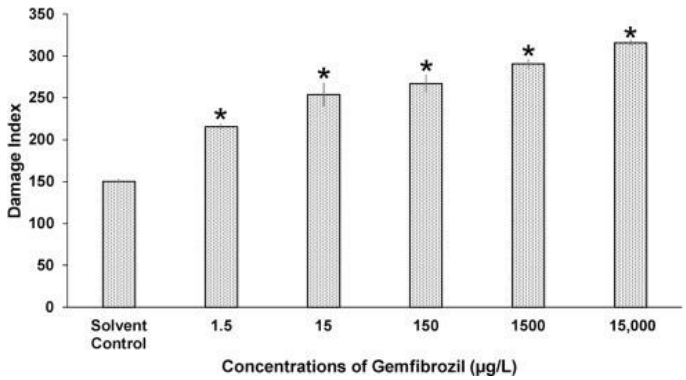


Fig. 1. DNA damage index of peripheral blood cells from *Sparus aurata* exposed for 96-h to gemfibrozil. * Significant diferences to solvent control (Dunnett's test, $p < 0.05$); data are presented as mean and standard error.

Table 2. DNA damage classes, measured by the comet assay, of peripheral blood cells of *Sparus aurata* exposure to gemfibrozil for 96 h.

*Significant diferences to solvent control (Dunnett's test, $p < 0.05$); data are presented as mean ± standard error.

| Treatment group (µg/L) | Damage classes (%) | | | | | Damage index |
|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | |
| Negative Control | 40.8 ± | 44.8 ± | 11.8 ± 4.0 | 1.8 ± 2.2 | 0.8 ± 1.3 | 77.0 ± 3.0 |
| Solvent Control | 9.8 ± 0.9 | 43.2 ± | 34.6 ± 3.0 | 10.0 ± 0.5 | 2.0 ± 0.9 | 150.4 ± 3.2 |
| 1.5 | 1.6 ± | 9.0 ± | 65.2 ± | 21.2 ± 9.9 | 3.2 ± 2.5 | 215.8 ± 14.2* |
| 15 | 1.2 ± | 5.0 ± | 43.0 ± 8.2 | 40.2 ± | 10.6 ± 3.7 | 254.0 ± 10.7* |
| 150 | 0.4 ± | 4.2 ± | 42.4 ± 6.6 | 41.8 ± | 13.2 ± 2.9* | 267.2 ± 5.9* |
| 1500 | 0.6 ± | 1.4 ± | 43.2 ± 3.9 | 41.8 ± | 13.0 ± 3.2* | 290.6 ± 3.4* |
| 15,000 | 0.2 ± | 0.8 ± | 8.8 ± 3.2* | 63.2 ± | 27.0 ± 3.5* | 316.0 ± 13.0* |

In the current study, no micronuclei were detected in control fish (both seawater and solvent controls), in agreement with the study of Bolognesi et al. [60], where micronuclei baseline frequencies of 0.012% were reported for *S. aurata* captured in a reference area. The 96-h exposure of *S. aurata* to 1500 and 15,000 µg/L GEM led to significantly higher MN frequencies (Fig. 2). However, if all ENAs were considered, significant differences to control would have been noted for all GEM concentrations (Fig. 3). An analysis of the different types of anomalies suggested that events leading to segmented and vacuolated nuclei occurred both at lower and higher concentrations of GEM, with differences to control detected even at the lowest tested concentration (1.5 µg/L). Kidney-shaped and lobed nuclei were mostly detected in fish exposed to concentrations >150 µg/L (Table 3).

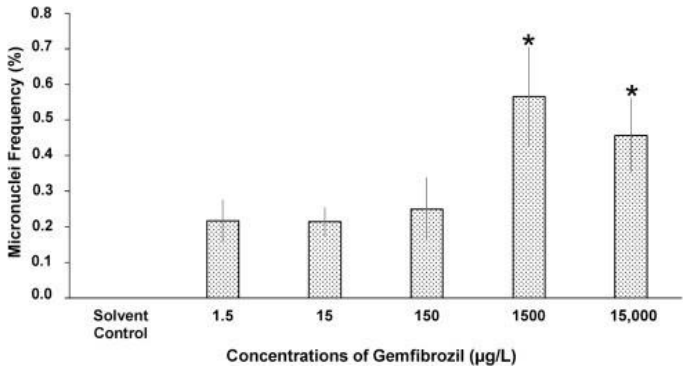


Fig. 2. Micronuclei in mature peripheral erythrocytes of *Sparus aurata* after 96-h exposure to gemfibrozil. *Significant differences to solvent control (Dunnett's test, $p < 0.05$); data are presented as mean percentage and standard error.

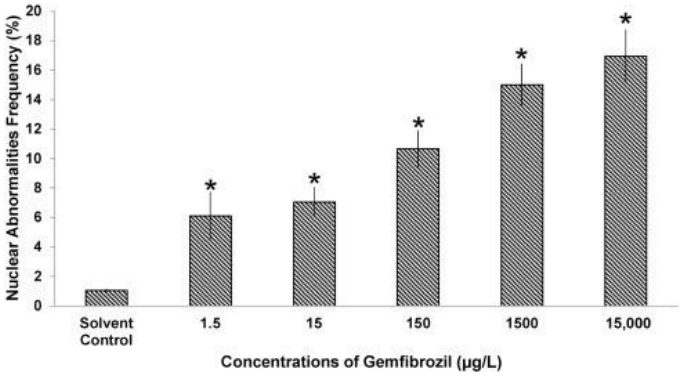


Fig. 3. Erythrocytic nuclear abnormalities in *Sparus aurata* after 96-h exposure to gemfibrozil. *Significant differences to solvent control (Dunnett's test, $p < 0.05$); data are presented as mean percentage and standard error.

Table 3. Erythrocytic nuclear abnormalities detected in *Sparus aurata* after 96-h exposure to gemfibrozil. *Statistically significant differences to solvent control (Dunnett's test, $p < 0.05$); data are presented as mean ± standard error.

| Treatment group (µg/L) | Frequency | | | | |
|---|---|---|---|---|---|
| | K | S | L | V | MN |
| Solvent Control | 0.7 ± 0.1 | 0.4 ± 0.1 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| 1.5 | 3.1 ± 0.9 | 1.7 ± 0.5* | 0.3 ± 0.1 | 0.9 ± 0.2* | 0.2 ± 0.1 |
| 15 | 3.0 ± 0.7 | 2.3 ± 0.4* | 0.5 ± 0.1 | 1.0 ± 0.2* | 0.2 ± 0.0 |
| 150 | 6.3 ± 0.9* | 2.5 ± 0.4* | 0.8 ± 0.2* | 1.0 ± 0.3* | 0.3 ± 0.1 |
| 1500 | 9.0 ± 1.2* | 3.3 ± 0.6* | 0.8 ± 0.2* | 1.1 ± 0.2* | 0.6 ± 0.3* |
| 15,000 | 9.4 ± 1.1* | 3.3 ± 0.8* | 2.5 ± 0.4* | 1.3 ± 0.4* | 0.5 ± 0.1* |

K – kidney-shaped nuclei; S – segmented nuclei; L – lobbed nuclei; V – vacuolated nuclei; MN – micronuclei.

To our knowledge, GEM induction of ENAs in aquatic organisms has not been reported previously. However, quantitation of ENAs has been used detect genotoxicity in fish species such as golden grey mullet (*Liza aurata*) [61], [48], European seabass (*Dicentrarchus labrax*) [62], [63], Nile tilapia (*Oreochromis niloticus*) [64], European minnow (*Phoxinus phoxinus*) [65], and European eel (*Anguilla anguilla*) [66], [67], [68]. Although the mechanisms responsible for nuclear abnormalities are not completely understood, some nuclear abnormalities (such as lobed and segmented nuclei) may be interpreted as nuclear lesions analogous to MN that may be induced by genotoxic compounds even if MN *per se* are not induced [65], [48], [69], [42]. According to some authors, nuclear buds (lobed, blebbed, and notched nuclei) may be caused by problems in segregating tangled and attached chromosomes or by gene amplification via the breakage-fusion-bridge cycle, during the elimination of amplified DNA from the nucleus [70], [48]. Segmented cells contain two nuclei, possibly due to blocking of cytokinesis or cell fusion [71]. With respect to vacuolated nuclei, these have been proposed to be a result of aneuploidy leading to MN formation [40], [69], [42]. Concerning kidney-shaped nuclei, such as nuclear invagination, for some authors, these are considered to have a cytological cause [60], whereas by others, they are ascribed to genotoxic origin [40], [69]. Although, at lower concentrations, GEM did not significantly induce the formation of MN, other nuclear abnormalities related to genotoxicity events were induced, namely high frequency of vacuolated and

segmented nuclei. At the highest concentrations, vacuolated and segmented nuclei continued to be present but GEM also induced the formation of MN, kidney- shaped and lobed nuclei.

The ENAs and comet assay proved to be sensitive tools for detection of GEM genotoxicity, supporting their use as biomarkers in water quality monitoring and risk assessment [72]. As with the present study, several studies already used these assays simultaneously to understand the genotoxic effects of contaminants in fish species [73], [74], [27], [75], [76], [77], [72].

Overall, our data suggest that GEM may represent a hazard to aquatic organisms. The detected genotoxicity is cause for concern, taking into account that the concentrations were environmentally relevant and that, in the environment, fish are exposed to a variety of contaminants, including pharmaceuticals sharing the toxicological properties of GEM (e.g., other peroxisomal proliferators). The mechanism of GEM genotoxicity is probably multifactorial, based on the available studies with mammals; the effects may be due to oxidative stress or formation of reactive metabolites binding to DNA and causing adduct formation [78]. Considering that the responses of fish to contaminants, in terms of genetic damage, are similar to those of other vertebrates, including humans [79], fish species may be useful model organisms for risk- assessment studies.

## 4. Conclusions

Our study highlights the potential consequences of the release of pharmaceuticals in the environment. Gemfibrozil caused both molecular and cytogenetic effects in *Sparus aurata* erythrocytes after 96-h waterborne exposure. The findings are ecologically relevant as gemfibrozil induced genotoxic effects at a concentration observed in the environment (1.5 μg/L). Our results should be considered in the management of aquatic environments and in regulation of pharmaceuticals.

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