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Chronic intake of an enriched diet with spirulina (*Arthrospira maxima*) alleviates the embryotoxic effects produced by realistic concentrations of tetracycline in *Danio rerio*

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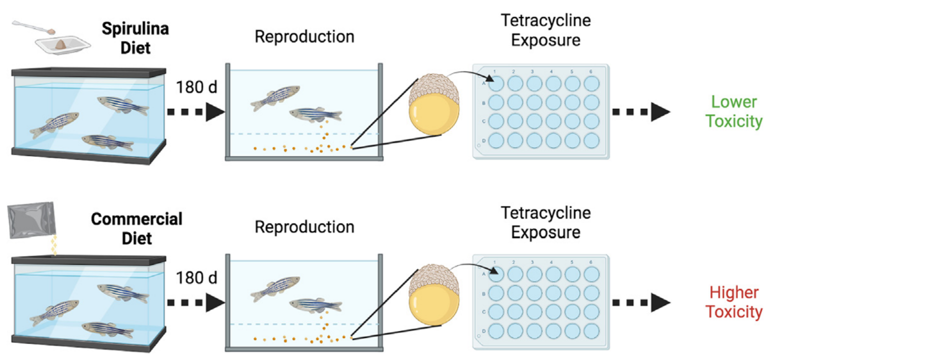
HIGHLIGHTS

- Tetracycline prompted the production of several malformations in *D. rerio* embryos.
- Realistic concentrations of tetracycline generated oxidative damage in the ELS of fish.
- Spirulina reduced the death and malformation rate of embryos exposed to tetracycline.
- Tetracycline-induced oxidative stress was alleviated by spirulina.

GRAPHICAL ABSTRACT



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ABSTRACT

Tetracycline (TC) is one of the most consumed antibiotics worldwide. Due to its high consumption, recent studies have reported its presence in aquatic environments and have assessed its effects on fish, algae, and daphniids. However, in most of those works, authors have tested TC toxicity at concentrations higher than the ones reported in the water matrix. Herein, we aimed to assess the likely embryotoxic and oxidative damage induced by environmentally relevant concentrations of TC in embryos of *Danio rerio*. Moreover, we seek to determine whether or not an enriched diet with spirulina can alleviate the embryotoxic damage produced by TC. Our findings indicated that TC at concentrations of 50 to 500 ng/L induced pericardial edema, tail deformities, and absence of head and fin in embryos after 96 h of exposure. Moreover, this antibiotic prompted the death of embryos in a concentration-dependent manner. According to our integrated biomarker response index, TC induced oxidative damage on *Danio rerio* embryos, as star plots showed a tendency to lipoperoxidation, hydroperoxides, and protein carbonyl content. Spirulina reduced the toxicity of TC by diminishing the levels of oxidative damage biomarkers, which resulted in a decrease in the rate of death and malformed embryos. Overall, TC at concentrations of ng/L prompted oxidative stress and embryotoxicity in the early life stages of *Danio rerio*; nonetheless, the algae spirulina was able to reduce the severity of those effects.

1. Introduction

Antibiotics are among the most widely used group of drugs on the planet. Their entry into the environment can be in an unaltered form or as metabolites present in the urine or excrement of patients, as well as

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from industrial, municipal, and hospital discharges (Kümmerer, 2009; Fatta-Kassinos et al., 2011).

Tetracycline (TC) is a widely consumed drug used to treat genitourinary, gastrointestinal, skin, respiratory, and other infections such as recurrent fever, Q fever, exanthemic typhus, endemic typhus, actinomycosis, and brucellosis (Lešnik et al., 2015). Its occurrence in worldwide water bodies is high, reaching concentrations from ng/L to μg/L (Campagnolo et al., 2002; Guerra et al., 2014; Tran et al., 2018). The presence of TC in the environment has gained concern in the scientific community as evidence suggests this drug prompts toxic responses in aquatic species (Ambili et al., 2013; Limbu et al., 2018; Rodrigues et al., 2018; Almeida et al., 2019; Yu et al., 2019; Escobar-Huerfano et al., 2020). Ambili et al., 2013 indicated that 20–120 mg/L oxytetracycline (OTC) altered several hematological and enzymatic parameters of the freshwater fish *Labeo rohita* after 25 days of exposure. Moreover, Rodrigues et al., 2018 pointed out that OTC (0.31–5.0 μg/L) affected the energetic metabolism and neurotransmission of *Oncorhynchus mykiss* after chronic exposure. Concordantly, Almeida et al., 2019 and Yu et al., 2019 established that OTC (0.1–10,000 μg/L) and chlortetracycline (CTC) (0.2–20 mg/L) reduced lipid levels, increased energy consumption, and induced oxidative damage in *Danio rerio*. Finally, Escobar-Huerfano et al., 2020 demonstrated that 90–900 μg/L of TC disrupted the normal development of *Danio rerio* after 96 h of exposure.

Today, the use of plants, their by-products or microalgae as dietary supplements in aquaculture has become increasingly popular due to their ability to enhance fish growth, confer immunity, promote disease resistance and reduce the toxicity of xenobiotic substances (Abdel Rahman et al., 2019a, 2019b, 2018; Ahmadzade-Nia et al., 2011; Cao et al., 2018). Spirulina (*Arthrospira maxima*) used as a dietary supplement has shown to have effects on reducing toxicity induced by Pb, As, and Cu, improving the immune system, reducing stress hormone cortisol in species such as *Cirrhinus mrigala*, *Oreochromis niloticus*, *Clarias batrachus*, and *Danio rerio* (Evaz-Zadeh Samani et al., 2017; James et al., 2009; Sayed et al., 2017, 2015). Nowadays, no evidence has demonstrated the protective effects of spirulina against drugs such as TC.

Even though the scientific community has assessed the ecotoxicological impact of TC on aquatic species, recent data suggest information gathered is still in the early stage as most of the studies evaluating TC's detrimental effects used high exposure doses (Xu et al., 2021). Therefore, more toxicity data on TCs on different species and using environmentally relevant concentrations are paramount to understanding the ecological risk of this drug. Herein, we aimed to assess whether or not environmentally relevant concentrations of TC (50,100, 150, 250, 300, 350, 400, 450, and 500 ng/L) might disrupt the growth and development of *Danio rerio* from 0 hpf to 96 hpf. Furthermore, in this study, we also pursue to prove whether or not an enriched diet with spirulina is likely to alleviate the embryotoxic effects of TC at those concentrations. We hypothesized that the abovementioned environmentally relevant concentrations of TC will prompt malformations and dead in *Danio rerio* embryos, but the spirulina-enriched diet will decrease the toxicity of antibiotics.

2. Materials and methods

2.1. Reagents

Tetracycline hydrochloride standard (98 %, $C_{22}H_{24}N_2O_8 \cdot HCl$) and all other reagents were acquired from Sigma-Aldrich (St. Louis, MO). Spirulina algae (*Arthrospira maxima*) was purchased from Spiral Spring (Puebla, Mexico).

2.2. Diet preparation

All ingredients, shown in Table 1, were homogenized in a grain mill for 5 min or until their total incorporation. Afterward, the mixture was added with alginate at 1 % and sieved with mesh # 80. Upon sifting, we spread the mixture into trays and placed them in ovens at a temperature of 55 °C for 12 h. The proximate composition of both the basal and commercial diet was determined using the Association of Official Analytical Chemists International (AOAC methods). Our diet covers the recommended nutrient requirements of fish according to National Research Council (NRC) (n.d.).

Table 1
Composition and proximate chemical composition (% on dry matter basis) of the basal diet.

	Functional diet (g)	Formulation (%)
Fish meal (*Engraulis encrasicolus*)	50	67.1
Fish muscle (*Oreochromis* sp.)	10	13.4
Fish oil	1.4	1.9
Soybean meal	10	13.4
Arthrospira maxima	1	1.3
Vitamin and mineral premix[a]	2.1	2.9
Total dry matter contains	74.5	100
Calculated chemical analysis	**Functional diet**	**Commercial diet**
Dry matter (%)	100	100
Crude protein	40.2	43.0
Crude fibre	15.9	3.0
Carbohydrates	24.8	40.0
Ash	12.0	n.a.
Lipid	4.0	5.0
Moisture	3.1	9.0
Grass energy (Kcal 100 g^{-1} diet)	555.9	377.0

[a] Composition of vitamins and minerals premix g 100 g^{-1}: Ascorbic acid 23.7 g; Biotin 7.5 mg; Boron citrate 63.6 mg; Calcium 5.7 g; Choline (bitartrate of choline) 6.3 g; Chromium (III) nicotinate 6.8 mg; Copper gluconate 45.5 mg; Magnesium 6.1 g; Manganese gluconate 45.4 mg; Molybdenum Citrate 1.1 mg; Potassium 0.6 g; Potassium iodide 9 mg; Selenium 4.5 mg; Silicon 90.9 mg; Sodium 0.3 g; Tocopherol mixture 2 g; Vanadium citrate 0.9 mg; Vitamin A 26.2 mg; Vitamin B1 0.7 g; Vitamin B2 0.7 g; Vitamin B3 1 g; Vitamin B5 2 g; Vitamin B6 0.8 g; Vitamin B9 15.1 mg; Vitamin B12 1.2 mg; Vitamin D3 1 mg; Vitamin E (D-alpha tocopherol succinate) 4.1 g; Vitamin K1 1.5 mg; Vitamin K2 1.5 mg; Zinc 20.6 mg. **Flavonoids mixture**: *Camellia sinensis* extract 1.7 g; Hesperidin 1 g; Quercetin hydrochloride 5 g; Resveratrol (*Polygonum cuspidatum*) 1 g; Rutin 1 g. **Carotenoids mixture**: Alpha-carotene, gamma-carotene 10.1 mg; Curcumin 1.4 g; Lutein 15.1 mg; Lycopene 25.2 mg. **Antioxidants**: Alpha-lipoic acid 2.5 g; Coenzyme Q10 0.3 g; Inositol 3.2 g; N-Acetyl L-Cysteine 3.6 g.

2.3. Zebrafish maintenance and reproduction

In two 100 L tanks provided with UV-sterilized and charcoal-filtered water (28 ± 1 °C, 14–10 h light/dark cycles), we allocated two hundred adult zebrafish (AB strain). Along 180 days, we fed half of the organisms with spirulina and the other half with a commercial diet (Lomas Wardley). Every other day, we renew the medium of both thanks. Oviposition and fertilization of eggs were done naturally with the onset of light in the morning and following the protocols established by Elizalde-Velázquez et al., 2021a. Briefly, six males and three females, each 4–7 cm in size, were placed into two reproduction tanks equipped with a spawning tray. One reproduction tank contained organisms fed with the commercial diet and the other with the spirulina-enriched diet. To ensure high embryo viability, we used three reproduction tanks with the same ratio of organisms for each diet. After spawning, oocytes from each tank were collected and placed in Petri dishes filled with water added with 60 μg of Instant Ocean® salts/mL. With the help of a stereomicroscope, we selected medium blastula stage oocytes and used them for subsequent experiments.

2.4. FET assay

Medium blastula stage zebrafish oocytes were exposed to environmentally relevant concentrations of TC (0, 50, 100, 150, 250, 300, 350, 400, 450, and 500 ng/L) in 24-well plates as described in Test No. 236: Fish Embryo Acute Toxicity (FET) Test, 2013. Three 24-well plates full of *Danio rerio* oocytes, one embryo per well, were used per each concentration of TC. Three 24-well plates full of *Danio rerio* oocytes, one embryo per well, were used per concentration of TC; thus, we used seventy-two oocytes for each TC concentration. The TC concentrations used were environmentally relevant, considering the data reported in the influents and effluents of the wastewater treatment plants, raw wastewater, surface water, drinking water, and hospital effluents (Azanu et al., 2018; Gbylik-Sikorska et al., 2015; Li et al., 2014; Martínez-Alcalá et al., 2020; Proia et al., 2018; Wang et al., 2018). FET test got done using the oocytes from fish fed with both types of diet. After four days of exposure (96 h), we estimated the mortality and malformation rate of embryos, and with collected data, we calculated the teratogenic index of TC, LC_{50}, and EC_{50} of malformations. Using IBM SPSS Statistics 22 software, we carried out a concentration-response curve with the malformations induced by TC in *Danio rerio*. We calculated the malformation rate as the percentage of embryos with at least one malformation compared to the control group.

2.5. Oxidative stress test

0.5 g of medium blastula stage zebrafish oocytes were exposed to each of the eleven TC concentrations in 4 L tanks as previously established Elizalde-Velázquez et al., 2021b. Half of the organisms were sampled and homogenized in 1 mL of PBS (pH 7.4) at 72 hpf while the other at 96 hpf. We choose those endpoints because the antioxidant system of embryos is already working at those times (Timme-Laragy et al., 2018). Homogenate got split into two Eppendorf tubes and centrifuged for 15 min at 4 °C. Tube 1, enclosing 300 μL of TCA (trichloroacetic acid) and 300 μL of homogenate, was centrifuged at 13307*g*. Meanwhile, tube 2, containing the remaining homogenate (700 μL), was centrifuged at 15722*g*. Using tube two and following the protocols of Misra and Fridovich (1972), Radi et al. (1991), and Gunzler and Flohe-Clairborne (1985), we evaluated the activity of superoxide dismutase (SOD), catalase (CAT), and (glutathione peroxidase) GPx, respectively. Meanwhile, using tube one and keeping track of guides published by Buege and Aust (1978), Levine et al. (1994), and Jiang et al. (1992), we assessed the levels of lipoperoxidation (LPX), protein carbonyl content (POX), and hydroperoxides content (HPX), respectively. The total protein content of each sample was determined through the Bradford (1976) method and used to express the results of oxidative stress biomarkers. For an in-depth description of the methods employed, see Table 1S in the Supplementary material.

2.6. IBR index

For IBR estimation, first, we split the oxidative stress results of all biomarkers (Xi) from each concentration against the control group (Xo). Next, we log-transformed the product to reduce variance (Yi). Afterward, we subtract the mean of each value from the Yi value and split that result against the deviation standard of each biomarker (Zi). Finally, the deviation biomarker index value (A) got calculated by abstracting Zo from Zi. All A values were depicted in star plots and summed up to get IBR values.

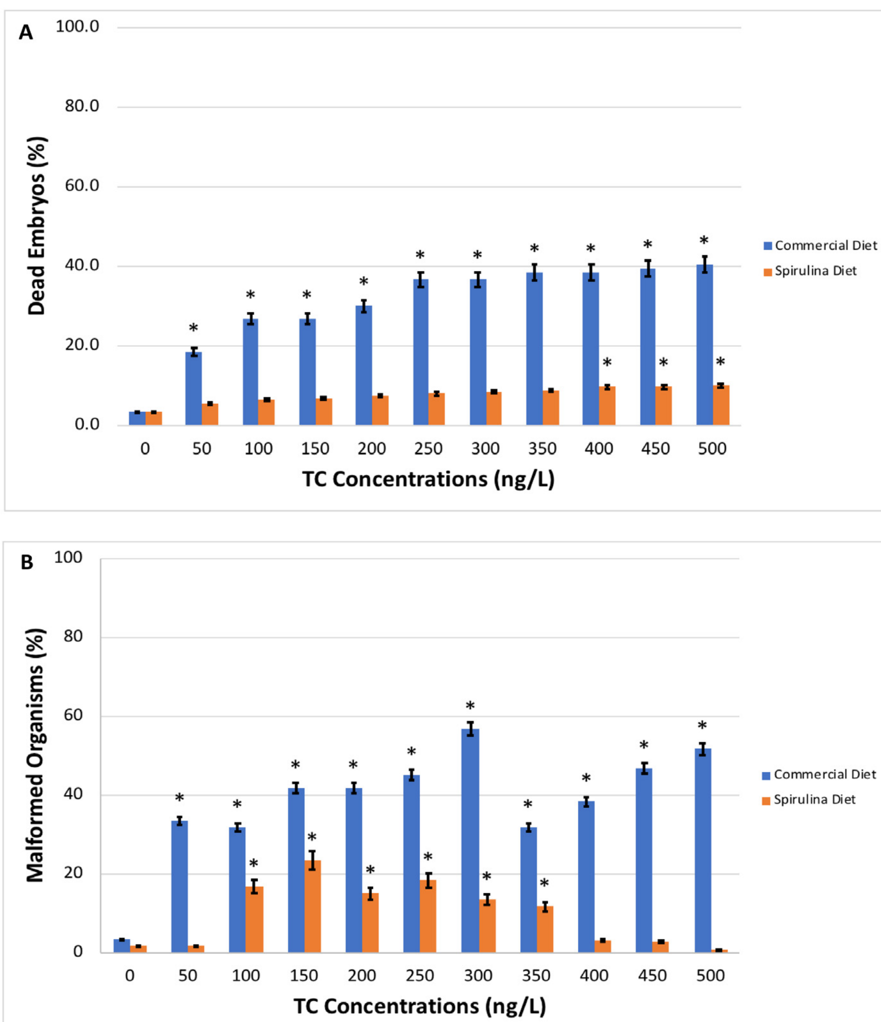

Fig. 1. Mortality and malformations rate in *Danio rerio* embryos exposed to environmentally relevant concentrations of TC (ng/L). **A** represents the average of the mortality percentages obtained for each concentration of TC tested in embryos from fed parents with a commercial diet and a diet enriched with spirulina; **B** represents the average of the percentages of malformations obtained for each concentration of TC tested in embryos from fed parents with commercial diet and a diet enriched with spirulina.

2.7. Statistical analysis

Differences between mean were assessed and established employing Dunnett's multiple comparison test. Moreover, a two-way ANOVA test was applied to determine the significance of differences between concentrations. Normality and homoscedasticity were evaluated and verified with the Shapiro-Wilk and Bartlett tests, respectively. All oxidative stress biomarkers passed the normality test. Embryotoxic and teratogenic effects results were appraised by Fisher's exact test. Significance was acknowledged when $p < 0.05$, using SigmPlot 12.3 software.

3. Results

3.1. Mortality and malformation rate

The percentage of dead embryos exposed to TC augmented depending on the concentration, reaching a maximum of 40 % (Fig. 1A–B). Nonetheless, we also observed that parents' treatment with spirulina significantly reduced the mortality rate of embryos exposed to TC by up to 30.3 %. Concordantly to the mortality rate, the malformation rate of embryos exposed to TC increased, reaching a maximum of up to 58 % (Fig. 1A–B). It is paramount to indicate that the concentration of 300 ng/L was the one that produced more deaths in the embryos. A decrease in the percentage of malformed embryos, up to 94.3 %, got observed in fish nourished with spirulina compared to those fed with a commercial diet. In agreement with the above-mentioned, Fig. 2 shows that the number of normal embryos decreased in a significant manner in organisms exposed to TC. However, in the case of fish nourished with spirulina, the ratio of dead and malformed organisms decreased as the number of normal ones augmented.

3.2. Evaluation of embryonic development

According to the scores obtained after the evaluation of Hermsen et al. (2011) and Kimmel et al. (1995), embryos not exposed to TC and that came from parents feeding with both diets did not show any alterations and reached the maximum score (Fig. 3A–B). Nevertheless, embryos from parents fed with the commercial diet and exposed to TC reached the lowest development scores due to the incidence of malformations in them (Fig. 3A). On the other hand, embryos coming from parents nourished with spirulina did not present too many malformations, and consequently, their scores did not fluctuate (Fig. 3B). Thus, the spirulina-enriched diet protects embryos against TC toxicity compared to the commercial diet.

Pericardial edema, without tail, tail malformation, and chorda malformation were the alterations with the highest incidence in all the embryos (Fig. 4). However, it is paramount to indicate that the incidence and severity of malformations in embryos from parents fed with spirulina were much lower than in organisms only exposed to TC. Concordantly, embryos from parents fed with the commercial diet and exposed to TC showed that as time elapsed, the severity and incidence of malformations increased (Fig. 4).

3.3. Oxidative stress test

Overall, embryos, no matter the diet, showed a significant increase in the activity of antioxidant enzymes and levels of oxidative damage biomarkers (Fig. 5). Nonetheless, there were significant differences between treatments. For example, it is paramount to stand out that embryos only treated with TC have more antioxidant activity than those from parents fed with spirulina. However, it is also true that oxidative damage of embryos from parents fed with spirulina was not as remarkable as in organisms only exposed to TC. We observed, for instance, that the levels of LPX, HPX, and POX were almost two times higher in embryos exposed to TC than in organisms from commercial diet-fed parents. Thus, TC at environmentally relevant concentrations disrupts the redox status of *Danio rerio* embryos. Moreover, spirulina might protect embryos against TC-induced oxidative stress. For all oxidative stress biomarkers, we found significant differences between treatment groups.

3.4. IBR index

Star plots show that the embryos exposed to TC and coming from parents fed with the commercial diet have a more remarkable tendency to oxidative damage biomarkers than to antioxidant enzymes (Fig. 6). Meanwhile, embryos from parents nourished with spirulina demonstrated a more significant deviation toward antioxidant enzymes than oxidative damage biomarkers. Thus, our IBR analysis indicates that the antioxidant activity of spirulina is more noteworthy than the oxidative stress activity produced by TC. Moreover, our star plots also depict that embryos exposed to environmentally relevant concentrations of TC suffer oxidative damage during their development.

4. Discussion

Tetracycline (TC) is one of the most consumed antibiotics around the globe, and consequently, it occurs in water bodies at concentrations

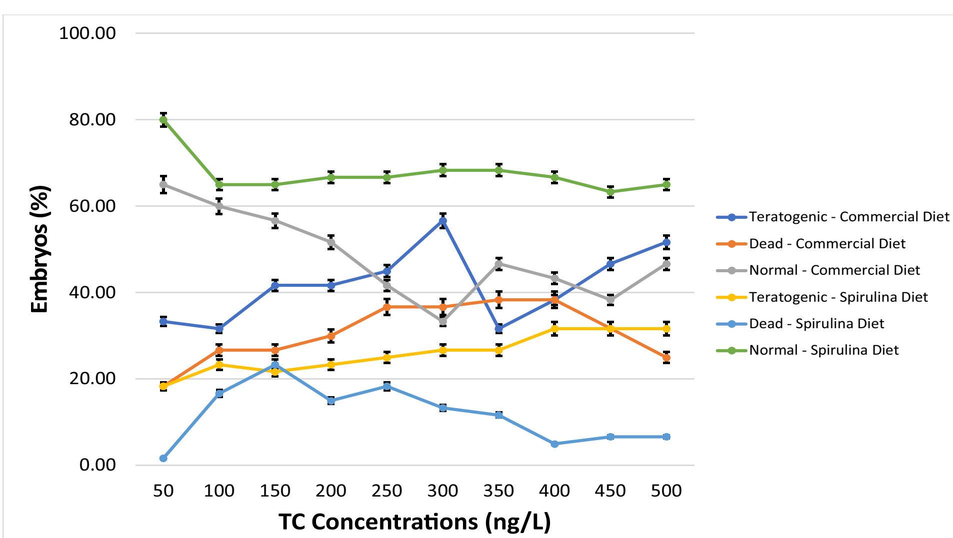

Fig. 2. Percentage of normal, dead and teratogenic embryos of *D. rerio* exposed to TC.

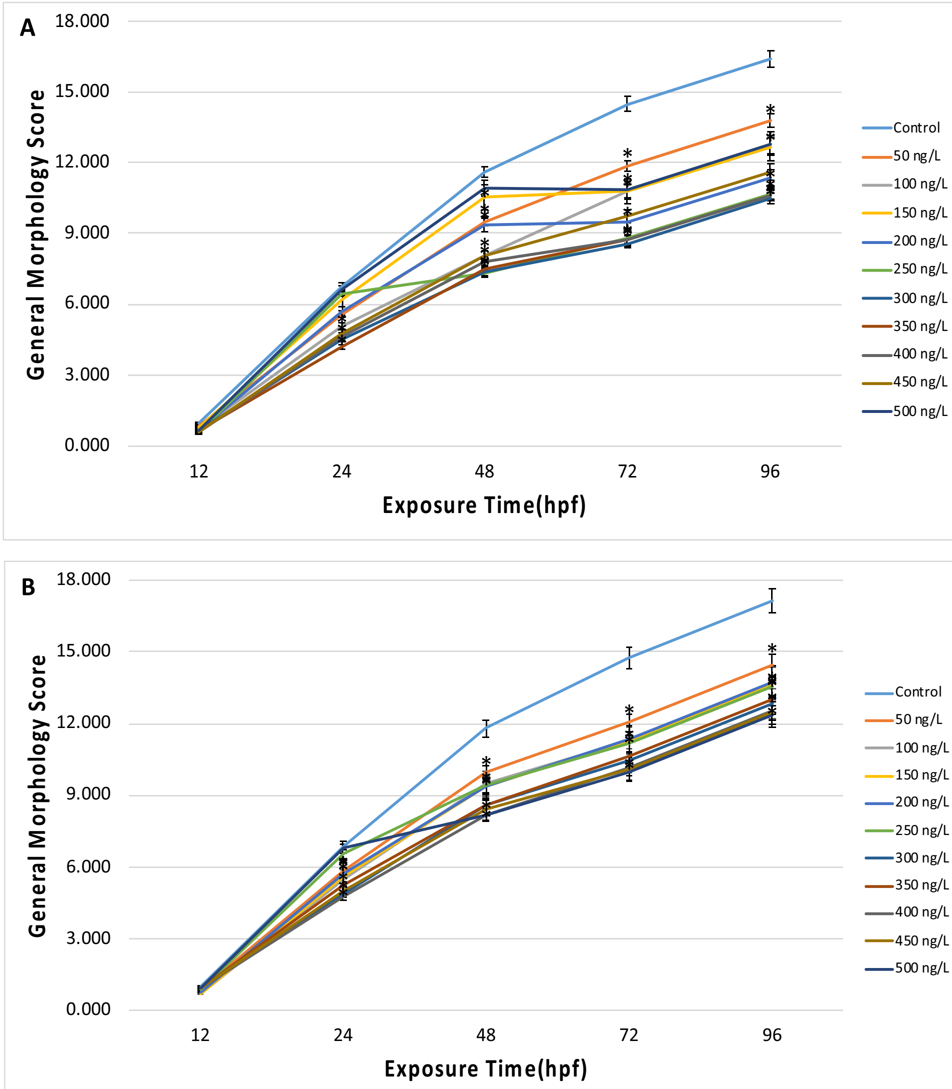

Fig. 3. Concentration-response curves of TC in *D. rerio* embryos. **A** represents embryos from parents fed with a commercial diet, and **B** represents embryos from parents fed with spirulina enriched diet.

of ng/L to μg/L. Upon its discharge into surface water, TC represents a threat to aquatic organisms as evidence suggests it produces DNA damage, REDOX status- and gut microbiota-alterations, embryotoxicity, and hematotoxicity in several fish species, such as *Oncorhynchus mykiss*, *Labeo rohita*, *Oreochromis niloticus*, and *Danio rerio* (Almeida et al., 2019; Ambili et al., 2013; Limbu et al., 2018; Rodrigues et al., 2018; Yu et al., 2019). Nevertheless, the concentrations used to demonstrate the effects were above the limits found in the aquatic environment. Herein, we aim to determine whether or not TC at concentrations of ng/L is likely to alter the embryonic development of *Danio rerio*. Moreover, we also seek to prove whether or not an enriched diet with spirulina can mitigate the embryotoxic effects of TC at those concentrations. Our findings indicated that TC at concentrations that range from 50 ng/L to 500 ng/L generated several malformations in *Danio rerio* embryos, such as chord malformation, craniofacial and eye malformation, hatching retardation, pericardial edema, tail malformation, yolk sac bleeding, and yolk sac hyperpigmentation. In agreement with our results, two previous studies also indicated that TC ~~also~~ disturbed the development of early life stages (ELS) in fish; but, in those works, authors exposed fish to higher proportions of this drug. Zhang et al., 2015, for instance, referred that 20 μg/L of TC generated phenotypes of developmental delays, such as hatching delay, body shortening, tail deformation, and yolk sac deformation in *Danio rerio* embryos. Moreover, Escobar-Huerfano et al., 2020 indicate that exposure to TC (90–900 μg/L) prompted the generation of yolk-sac bleeding, pericardial edema, scoliosis, tail malformation, delayed hatching, and hyperpigmentation in *Cyprinus carpio* embryos. Differences in results with Zhang et al., 2015 can be due to different embryonic stages to which organisms were exposed. They exposed organisms after 4.0 hpf, and we did it at 2.5 hpf. Previous studies have pointed out that the structure of the chorion changes as time elapses, triggering the toughness of the embryo membrane to rise and consequently producing a low permeability (Elizalde-Velázquez et al., 2021a). Authors related the identified aberrations with the production of reactive oxygen species (ROS) and cellular apoptosis.

An increase in ROS during embryonic development can trigger adverse effects in organisms, such as affecting the p53 gene that acts as a modulator and coordinator of the cell cycle (Li et al., 2011). Furthermore, the increase in ROS can also lead to the generation of pro-oxidant conditions in the cell, favoring lipid peroxidation, DNA damage, alteration of different signaling cascades, disruption in gene expression, and alteration of proteins (Elizalde-Velázquez et al., 2022). Herein, our results demonstrated TC at concentrations of 50–500 ng/L disrupted the REDOX status of *Danio rerio* embryos, leading to an oxidative stress response. Moreover, previous

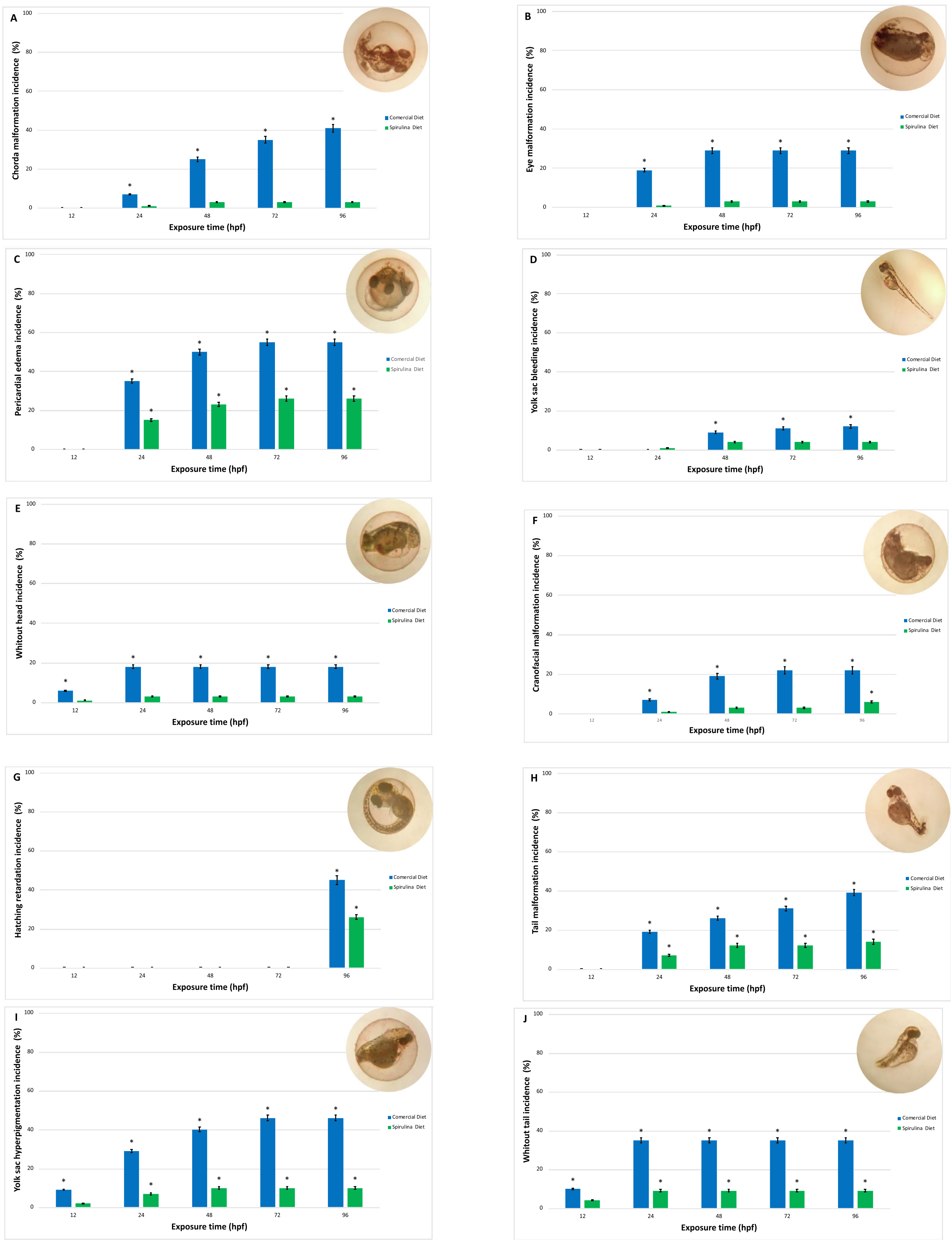

Fig. 4. More severe and frequent malformations induced by exposure to TC concentrations in *D. rerio* embryos from parents fed with the two diets tested.

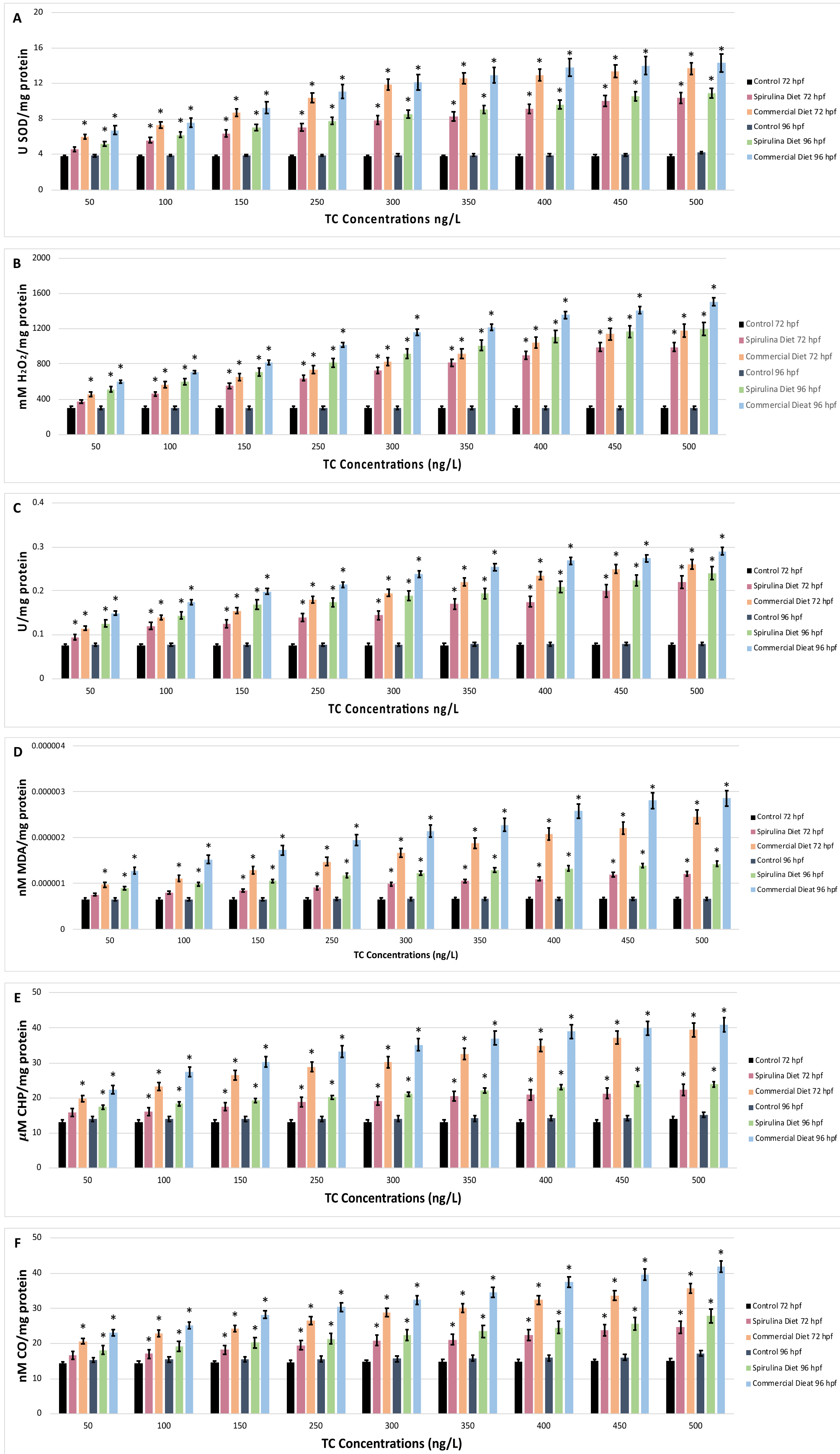

Fig. 5. Oxidative stress TC-induced in embryos coming from parents of both diets. **A** SOD. **B** CAT. **C** GPX. **D** LPX. **E** HPX. **F** POX.

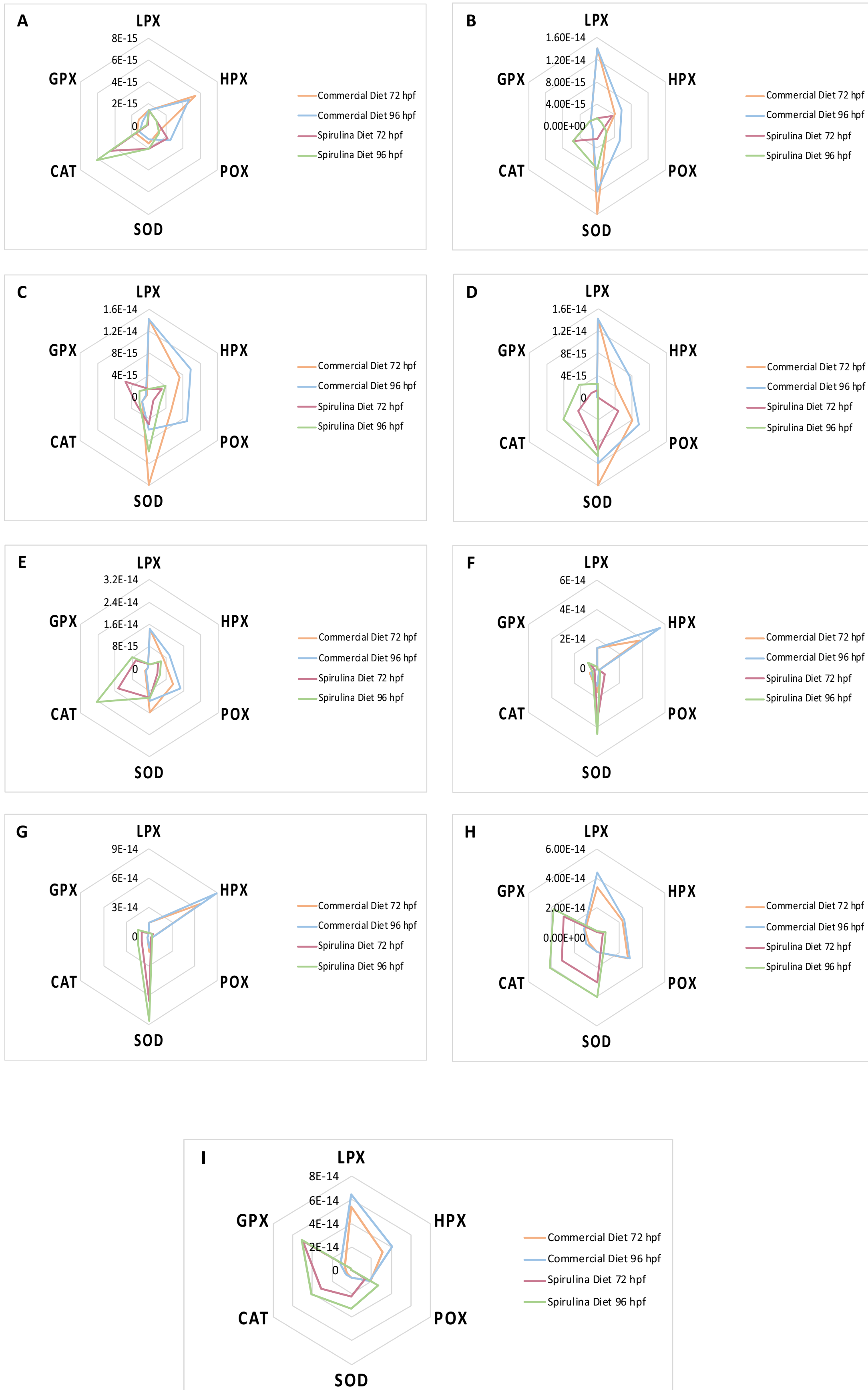

Fig. 6. Star plots of oxidative stress biomarkers from each of the TC concentrations. **A** 50. **B** 100. **C** 150. **D** 250. **E** 300. **F** 350. **G** 400. **H** 450. **I** 500.

studies established TC exposure results in significant increases in ROS production and cell apoptosis, mainly in the tail areas of *Danio rerio* embryos at 96 hpf (Zhang et al., 2015). REDOX signaling plays a fundamental role in the process of embryogenesis because organisms in this period are more susceptible to changes in redox potential, which in turn leads to modifications in cell density, such as proliferation in a state of reduction or necrosis in an oxidized state (Wang et al., 2020; Yang et al., 2020; Zhang et al., 2015). Therefore, alterations in the oxidative status of cells can modify the cell life cycle, affecting the embryonic development of fish (Dennery, 2007; Hansen, 2006; Schieber and Chandel, 2014; Timme-Laragy et al., 2018). Yolk sac hyperpigmentation and bleeding were the most prevalent alterations found in embryos exposed to TC, which might be due to the overproduction of ROS. ROS due to exposure to TC favors the alteration of the scl24a5 protein, generating, in turn, the accumulation of calcium that disrupts the transmembrane calcium channels and affects the melanogenesis process (Lamason et al., 2005; Mugoni et al., 2014). Moreover, yolk sac bleeding observed in embryos is also the result of an increase in superoxide anion and hydroxyl radical. Han et al., 2015 pointed out that both ROS can generate cardiovascular damage by developing thrombi and alterations in fibrinogen levels that favor hemorrhage. Besides yolk sac hyperpigmentation and bleeding, embryos exposed to TC showed pericardial edema and malformations in the tail, head, and fin. Pericardial edema might be a consequence of lipid peroxidation of TC, as it leads to cell membrane damage (Hollert et al., 2003). Cell membrane damage, in turn, triggers an osmotic disruption, generating the hyperaccumulation of liquids in the embryo. Pericardial edema is a malformation that compromises the integrity of the embryo since the accumulation of fluid in the interstitium of the pericardium increases the size of the heart, and its cardiac contraction, with the consequent death of the embryo (Zodrow et al., 2004). Regarding lack of fin, head, and tail development in embryos, TC can inhibit CYP26, which enzyme is involved in the migration, differentiation, and maturation of the cells of the neural crest. These cells are responsible for the formation of cartilage and bone in the head skeleton and the formation of the skeletal system in the fish (Hermsen et al., 2012; Menegola et al., 2006; Staal et al., 2018). Therefore, these findings could explain the alterations found in this study, such as without head, without tail, and head and tail malformations.

In addition to the insights generated about TC, we also demonstrated spirulina reduces the embryotoxic effects of this drug in *Danio rerio*. Spirulina is a raw material that has achieved high acceptance in the food industry due to its unbeatable properties for human and animal health and nutrition. One of the properties that make spirulina an ideal food is that it does not contain cellulose in its wall cell; instead, this algae has murein, a polymer effortlessly metabolized by the digestive enzymes of fish (Teimouri et al., 2013). Moreover, Spirulina can prevent cell damage because it contains antioxidant enzymes, such as superoxide dismutase, catalase, and glutathione peroxidase, as well as non-enzymatic defense systems that help counteract the effects of ROS and protect cells from them (Abd El-Baky et al., 2009; El-Tantawy, 2016; Kepekçi et al., 2013). The compounds with antioxidant activity in spirulina are carotenoids, tocopherols, ascorbic acid, and chlorophyll derivatives (Farag et al., 2016). The mechanism through which spirulina may exert its protective effects against TC toxicity is through its phycocyanin-C content. This phycobiliprotein has a scavenging action on ROS, especially on alkoxyl and hydroxyl radicals, in addition to the inhibition of 2,2-azobis-2-amidinopropane hydrochloride (HAAP), an initiator of free radical formation and lipid peroxidation. Moreover, Phycocyanin-C also can inhibit peroxynitrite formation (Bhat and Madyastha, 2000; Kumar et al., 2014; Yan et al., 2011).

5. Conclusions

According to the results obtained, we can conclude that TC harmed the health of *Danio rerio* and was capable of inducing alterations in embryonic development at environmentally relevant concentrations (ng/L). Chord malformation, craniofacial malformation, hatching retardation, and pericardial edema were the most frequent abnormalities found in embryos. Moreover, our findings indicate that ROS produced by TC was the principal mechanism by which TC prompted the formation of malformations in embryos. Feeding parents with a spirulina-enriched diet were able to counteract the adverse effects of TC. This study evidenced the hazardous effects of TC on fish species such as zebrafish. Further studies are encouraged to estimate the mechanism through which TC could exert its toxic effects.

CRediT authorship contribution statement

Paulina Tenorio-Chávez performed all the exposure experiments.

Leobardo Manuel Gómez-Oliván and Gustavo Axel Elizalde-Velázquez were involved in the conception.

Leobardo Manuel Gómez-Oliván, Gustavo Axel Elizalde-Velázquez, Paulina Tenorio-Chávez were involved in the design and interpretation of the data and the writing of the manuscript with input from María Dolores Hernández-Navarro.

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no conflict of interest.

Acknowledgment

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.159731.

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