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ORIGINAL RESEARCH

Acute toxicity, risk assessment and exposure of Nile tilapia larvae after stress to sub-lethal concentrations of oxytetracycline

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Abstract The present study evaluated the median lethal concentration (LC50-96h), risk assessment, biometrics of weight and length, and mortality of Nile tilapia *Oreochromis niloticus* larvae exposed to oxytetracycline (OTC). A total of 126 fish (mean weight: 32.25±3.74 mg; mean length: 13.01±0.64 mm; 7 larvae per aquarium) were utilized for the LC50-96h assessment. These fish were randomly distributed across 21 aquaria (1 L each) containing graded concentrations of OTC: 0.0, 2.01, 2.44, 3.31, 3.65, and 4.59 mg/L. Additionally, an acute stress test involving 180 fish (mean weight: 33.9±0.73 mg; mean length: 13.06±0.72 mm; 10 larvae per aquarium) was conducted, wherein the fish were subjected to air stress for 5 minutes before being randomly allocated to 18 aquaria (1 L each) and exposed to sub-lethal concentrations of OTC: 0.0, 0.03, 0.82, 1.65, 2.47, and 3.30 mg/L. Water quality parameters (temperature, pH, dissolved oxygen, conductivity, total ammonia, and total hardness) were monitored daily throughout the experiment. The LC50-96h for OTC in Nile tilapia larvae was estimated at 3.45 mg/L. Larval weight and length significantly decreased in both tests with increasing OTC exposure. Dissolved oxygen levels exhibited significant changes over 96 hours. The post-stress test revealed significant differences ($P < 0.05$) in pH, temperature, and dissolved oxygen. Notably, pre-exposure to air stress exacerbated the toxic effects of sub-lethal OTC levels. Risk quotient analysis indicated a high potential risk for Nile tilapia larvae exposed to therapeutic OTC concentrations.

Keywords Antibiotic . Aquaculture . Biosafety . Oreochromis niloticus . Toxicity

Introduction

Nile tilapia *Oreochromis niloticus* stands as one of the main species cultured worldwide, particularly prevalent in tropical and subtropical regions (FAO 2024). Currently, aquaculture systems are predominantly based on the use of high-stocking densities, such as those found in fish farming cages, where densities range from 80 to 120 kg/m^3 (Garcia et al. 2013). Consequently, this intensification leads to frequent disease outbreaks, making production reliant on chemical therapeutics (Monteiro et al. 2015).

Antimicrobials correspond to a class of drugs extensively applied in aquaculture, and which are available in both natural and synthetic forms, exerting either bactericidal or bacteriostatic effects that control the pathogenic growth (Mattioli et al. 2020; Cabello et al. 2013; Cãnada-Cãnada et al. 2009). Oxytetracycline (OTC), belonging to the tetracycline group, and its use is widespread for treating bacterial infections in fish, due to its broad spectrum, effectiveness, and low cost (Rigos and Troisi 2005). OTC acts bacteriostatically by inhibiting protein synthesis in gram-negative and gram-positive bacteria (Rigos et al. 2006).

The excessive use of antibiotics in aquaculture has raised global public health concerns potentially fos-

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tering the development of antibiotic resistance among commensal bacteria in human and animal intestines and facilitating the spread of resistant genes, particularly among pathogenic strains (Gastalho et al. 2014; Jijie et al. 2021). Antibiotics can induce environmental ramifications in aquatic ecosystems, through the development of antibiotic resistance to some aquatic bacteria and direct toxicity to microflora and microfauna, and pose risks to human health through potential ingestion of a non-contaminated target (Rigos et al. 2004). For the fish themselves, antibiotic misuse or overuse can cause immunosuppression, kidney damage (nephrotoxicity) (Islam et al. 2015), reduced phagocytic activity (Reda et al. 2013), oxidative stress, liver damage – hepatotoxicity (Guardiola et al. 2012), nervous system damage, cell degradation, and cell death (Rodrigues et al. 2018).

The impacts of antibiotic use on aquatic organisms require evaluation through ecotoxicological investigations across diverse species to ascertain substance effects or lethality (Fujimoto et al. 2012). Conversely, fish are exposed to stressful conditions during routine managements in fish farms, where stress manifests as a response to any situation disrupting their normal metabolism, eliciting physiological and biochemical alterations (Hoseini et al. 2016). Stocking density and low dissolved oxygen levels are primary stressors in fish farms, negatively affecting fish welfare, immune response, and growth (Yousefi et al. 2016; Ni et al. 2014).

Moreover, the presence of toxic compounds in aquatic environments alongside stressed animals impairs the restoration of homeostasis, as effects are potentiated, involving molecular, cellular, and biochemical responses (Faggio et al. 2014; Freitas et al. 2017; Ramya et al. 2023). Considering regular occurrence of management practices in fish farms, exposure to sub-lethal contaminant levels alongside stressful conditions can lead to mortality, especially in early stages (Chromcova et al. 2015; Chiste et al. 2021).

Despite the widespread use of OTC in aquaculture, there remains a significant dearth of primary data regarding its toxicity and associated risks for the early developmental stages of Nile tilapia. Such information is crucial for fostering the responsible application of this antibiotic and promoting the sustainable development of aquaculture. In the early developmental stages, fish are more sensitive to stressful conditions and exposure to contaminants, particularly in the post-hatching phase. Thus, the objective of the present study was to estimate the median lethal concentration (LC50-96h) of OTC for Nile tilapia larvae and biometrics of weight and length during this period, as well as evaluate the effect of sub-lethal dosages on the mortality of Nile tilapia larvae in post-stress conditions. Furthermore, it was determined the risk quotient (RQ) and possible effects of the evaluated drug concentrations on water quality parameters.

Materials and methods

Chromatography analysis

A commercial formulation of oxytetracycline (OTC) ($C_{22}H_{24}N_2O_9$) Zoetis Brazil (Terramycin® AG77; Campinas, Brazil) was used for the trials. OTC was dissolved in sterile distilled water with final concentrations of 4.59 and 3.30 mg/L for each experimental assay, which was used as the primary stock solution (Figure 1).

Water samples (n=108) were collected for OTC analysis at 0, 12, 24, 48, 72, and 96 h of each aquarium (three replicates) per treatment, and kept at -20 °C until analysis. Chromatographic analysis was performed at Central Analítica de Resíduos e Contaminantes (CRC), Embrapa Meio Ambiente, Jaguariúna – Brazil, using High-Performance Liquid Chromatography (1290 Infinity II LC System, Santa Clara, United States) with a diode array detector (UFLC Shimadzu LC20AT, Kyoto, Japan) coupled to the Amazon X Ion Trap (Bruker Daltonik GmbH, Bremen, Germany).

The samples were filtered through a Millex HV - PVDF- 0.45 µm filter and 13 mm in diameter. Mobile Phase: 0.1% Formic Acid: Acetonitrile; Isocratic: 40% 0.1% Formic Acid (A): 60% Acetonitrile (B); Flow rate: 0.5 mL/min; Injection Volume: 20 µL; Oven: 40 °C; Wavelength: 270 nm; Column: N°.: 85 - Agilent - Zorbax Eclipse - XDB - C18 – 5 µ - 150 x 2.1 mm; Running Time: 6 minutes; Retention Time: 0.87 min. A calibration curve (1) (µg/mL): 0.50, 0.75, 1.00, 1.50, 2.00, and 2.50. A calibration curve (2) (µg/mL) was: 2.50, 3.00, 3.50, 4.00, 4.50, 5.00, and 6.00.

Fish and experimental trials

The experimental trials were conducted at the Laboratório de Ecotoxicologia e Biossegurança (LEB), Em-

brapa Meio Ambiente, Jaguariúna – Brazil, and was approved by the Ethics Committee on the Use of Animals of Embrapa Meio Ambiente (Protocol 011/2018).

Fish were acquired from a commercial fish farm and were previously acclimatized for 48 h. pH, temperature, dissolved oxygen (DO), and conductivity were measured using a multiprobe U-50 Horiba (HORIBA Advanced Techno Co., Ltd. Kyoto - Japan). Physicochemical variables: pH: 7.0, temperature: 28 °C, DO >5 mg/L, conductivity 13.00 µS/cm. While total ammonia NH_4 <0.1 mg/L and total hardness <50 mg/L by colorimetric kit LabCon Test (Alcon® - Camboriú - Brazil). Larvae from each experimental unit were fed until apparent satiety with a commercial diet (Alisul Alimentos S.A., Brazil) 56% of crude protein before LC and stress tests four times a day (7, 10, 13, and 16h). Aquariums were siphoned daily to remove feces and feed waste.

LC50-96h test

Nile tilapia (seven-day post hatch) (n.:126; 32.25±3.74 mg and 13.01±0.64 mm) were used. The animals remained fasting, following the guidelines of the OECD (2019). The trials were conducted in a static system (without water renewal), with a photoperiod (12h light and 12h light-free). Seven larvae were distributed randomly per aquarium, totaling 18 experimental units (1 L useful volume), with three replicates. The experimental design was completely randomized with five experimental groups plus a control (without antibiotic). OTC concentrations of 0.0, 2.01, 2.44, 3.31, 3.65, and 4.59 mg/L were used for testing and calculating the LC50-96h. The higher concentration was stipulated considering an approximate average value of OTC recommendations for a therapeutic static bat of 10 mg/L (Rach et al. 2008). And 1mg/L as prophylactic during transportation (Klein et al. 2013). Mortality was registered at 24, 48, 72, and 96h of exposure to OTC.

Stress test

Nile tilapia (ten-day post hatch) (n.:180; 33.90±0.73 mg and 13.06±0.72 mm) were used for the stress test and exposure to sub-lethal concentrations of OTC for 48h.

Fish (n=10) were subjected to air exposure on a drying paper for five minutes. After the acute stress by air exposure, fish were randomly distributed into 18 tank with a volume of 1 L (ten fish/aquarium), in a static system (without water renewal), where they were submitted to five concentrations plus a control (without

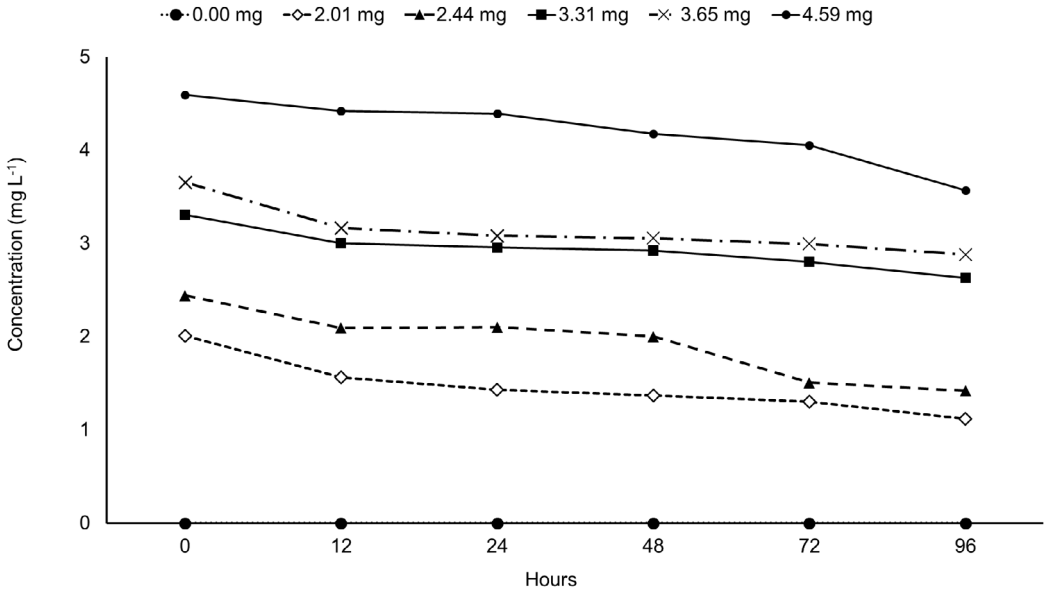

Fig. 1 Chromatographic analysis of concentration-time associated with degradation of oxytetracycline molecules in the experimental aquatic environment

antibiotic) of OTC: 0.0, 0.03, 0.82, 1.65, 2.47 and 3.30 mg/L, based on the previous LC50-96h test, totaling six treatments and three replicates. The choice of the test concentrations for the stress test was based on the use of a geometric series with a concentration in such a way the highest level is equivalent to the LC50-96h and the lowest concentration is equivalent to 1/100 LC50-96h (OECD 1984).

Mortality was accounted at 24 and 48 hours after exposure to the antibiotic. Temperature, DO, pH, conductivity, total ammonia, and total hardness were measured at 0, 24, and 48 h, following the same procedures of the acute test, using the same equipment and kit described previously.

Risk assessment

The adopted criterion for the interpretation of the risk quotient (RQ) establishing different levels was <0.01: insignificant risk; 0.01 - 0.1: low risk; 0.1 - 1: medium risk; >1: high risk (Díasz-Garduño et al. 2017; Hernando et al. 2006).

To evaluate the risk related to chronic effects, the estimated environmental chronic concentration ($EEC_{chronic}$) was calculated by the expression $ECC = RQ_{chronic}$ x NOEC (Dos Santos et al. 2015). The $RQ_{chronic}$ was considered in the low-risk value range (0.01 - 0.1). No observed effect concentration (NOEC) for long-term exposure was estimated based on the $EC_{50\text{-}96h\ /10}$ ratio (OECD 1995).

A safety factor equal to "3" was applied to the $EC_{50\text{-}96h}$ to prevent the risk in terms of acute toxic effects. At this assumption, the risk quotient related to acute effects (RQ_{acute}) was calculated by $EEC_{acute}/EC_{50\text{-}96h/3}$. Estimated environmental concentration to prevent acute effects EEC_{acute} was assumed to be the therapeutic concentration of OTC (4.59 mg/L).

Data analysis

Mortality was counted after 24, 48, 72, and 96 h of exposure to OTC, and in these periods, dead individuals were collected. These results were used to calculate the median lethal concentration (LC50-96h), with a 95% confidence interval. The software used was Stat graphics Centurion XIX, (2024), with the Probit Analysis method. The results of biometrics and water quality were subjected to the test of normality and homogeneity of variance, followed by the analysis of variance (ANOVA). If significant, the Tukey test was applied at a 5% probability. The data were analyzed using the Statistical Program R version 4.4.1 (2024b).

Results

OTC residues in the test solutions

The analyzed antibiotic concentrations during the acute test are shown in Table 1. After 96 h of exposure, the percentages of OTC recovery relative to the nominal concentrations were 55.72, 58.19, 79.46, 78.90, and 77.77 %, respectively to 1.12, 1.42, 2.63, 2.88 and 4.59 mg/L. The last three percentages were very close to the recommended percentage of chemical concentration to be maintained during the test (at least 80% of the nominal concentration (OECD 2019).

Acute toxicity test

The LC50-96h of OTC for the Nile tilapia larvae was 3.45 mg/L, with a lower limit of 3.14 mg/L and an upper limit of 3.80 mg/L (Figure 2). In the control treatment, no mortality occurred, according to observa-

Table 1 Mean values (± standard deviation) of analyzed test-solutions (n=3) after several exposure periods of Nile tilapia larvae to OTC

Time (h)	0.00	2.01	2.44	3.31	3.65	4.59
12	0.00±0.00	1.56±0.46	2.09±0.11	3.00±0.22	3.16±0.49	4.42±0.33
24	0.00±0.00	1.43±0.26	2.10±0.24	2.95±0.09	3.08±0.012	4.39±0.26
48	0.00±0.00	1.37±0.09	2.00±0.10	2.92±0.20	3.05±0.26	4.17±0.17
72	0.00±0.00	1.30±0.34	1.51±0.28	2.80±0.25	2.99±0.32	4.05±0.30
96	0.00±0.00	1.12±0.17	1.42±0.19	2.63±0.07	2.88±0.14	3.57±0.18

tion. Nevertheless, at the highest concentrations mortality reached 90.5%.

The LC50-96h was also calculated using the mean geometrics values of the measured concentrations within test solutions (OECD 2013). This resulted in a value of 2.90 (2.58-3.28) mg/L, which indicates no statistical difference (P >0.05) against the above LC50-96h value since the confidence limits overlap with each other (Bejgarn et al. 2015).

Variables of biometrics are shown in Table 2. The final average weight and the final average length did not present differences (P >0.5), but there was a trend for lower values with an increase in OTC. At the highest concentration (4.59 mg/L), the final weight was 25% lower compared to the initial weight and 18.7% inferior to the control, evidencing the acute toxic effect of OTC on tilapia larvae at the levels assessed in the study.

The water quality parameters, pH (6.94±0.03), total ammonia (0.29±0.04 ppm), conductivity (0.11±0.02 mS/cm), total hardness (50.0±0.5 mg/L), and temperature (26.47±0.06 °C), did not show statistical differences and are in accordance to the production of freshwater fish (encompassing several species) (Boyd and

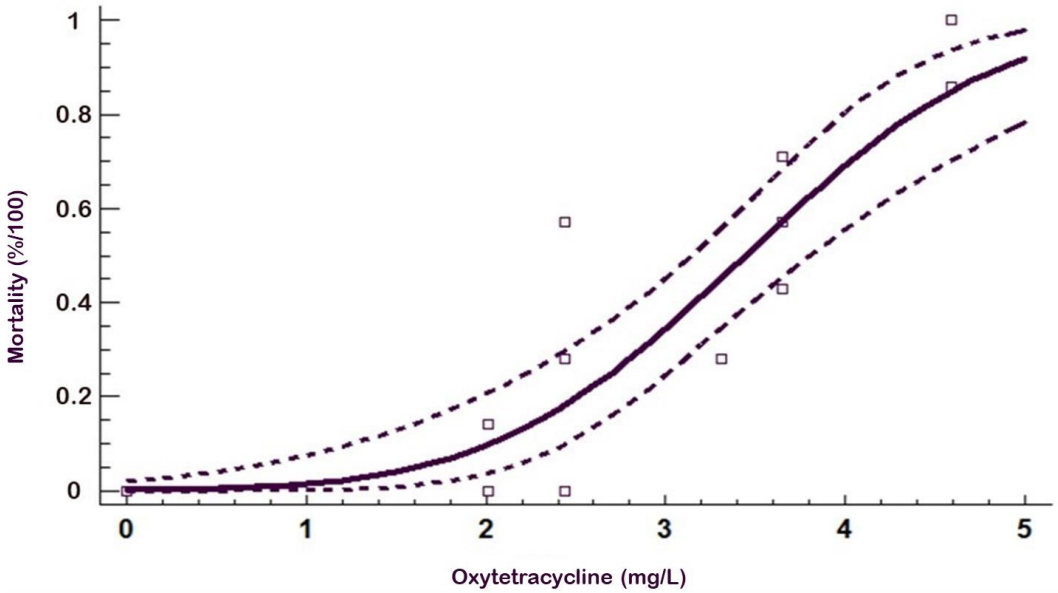

Fig. 2 Median lethal concentration (LC50-96h) of oxytetracycline of OTC for Nile tilapia larvae

Table 2 Mean values (± standard deviation) of the biometric parameters for Nile tilapia larvae

Treatments mg/L	Final weight (mg)	Final length (mm)
0	29.97±1.58	12.67±0.58
2.01	28.27±3.08	12.33±0.58
2.44	26.30±2.46	12.67±0.58
3.31	26.30±2.46	12.33±0.58
3.65	26.77±0.85	12.00±1.00
4.59	24.37±0.93	11.67±0.58

Table 3 Mean values (± standard deviation) obtained from dissolved oxygen (mg/L) of the physical-chemical parameters of the water of theLC50-96h experiment with oxytetracycline. Different letters indicate significant differences by Tukey test (P < 0.05)

Treatments mg/L	0h	24h	48h	72h	96h
0	8.20 ± 0.10^{a}	8.03 ± 0.06^{a}	8.10 ± 0.10^{a}	7.23 ± 0.32^{b}	7.03 ± 0.06^{b}
2.01	8.03 ± 0.06^{a}	7.97 ± 0.06^{a}	7.93 ± 0.11^{ab}	7.57 ± 0.25^{b}	7.03 ± 0.15^{c}
2.44	8.10 ± 0.10^{a}	7.93 ± 0.11^{a}	8.00 ± 0.26^{a}	7.57 ± 0.30^{ab}	7.07 ± 0.11^{b}
3.31	8.20 ± 0.18^{a}	7.97 ± 0.06^{ab}	8.00 ± 0.005^{ab}	7.77 ± 0.15^{b}	7.00 ± 0.10^{c}
3.65	7.99 ± 0.02^{a}	7.93 ± 0.06^{a}	7.92 ± 0.10^{a}	7.83 ± 0.06^{a}	7.10 ± 0.10^{b}
4.59	8.19 ± 0.21^{a}	8.10 ± 0.26^{a}	7.99 ± 0.02^{a}	7.90 ± 0.10^{a}	7.10 ± 0.26^{b}

Tucker 1998). The DO data, however, were different (P <0.05) over the experimental period (Table 3).

Stress test

A complementary test was carried out to simulate the effect of OTC contamination in water at sub-lethal doses associated with practical stress conditions arising through the management performed in fish farming. The mortality of Nile tilapia larvae, after managing air exposure and sub-lethal concentrations of OTC, was recorded at 24h and 48h (Figure 3). In the 24h period, mortality was more accentuated and increased (P <0.05) linearly with the rising OTC (y = 15.142x + 9.9565; R^2 = 0.9186), reaching 57% at its highest level (3.30 mg/L). In the 48h period, mortality was also responsive to OTC levels (y = 16.504x + 9.1039; R^2 =

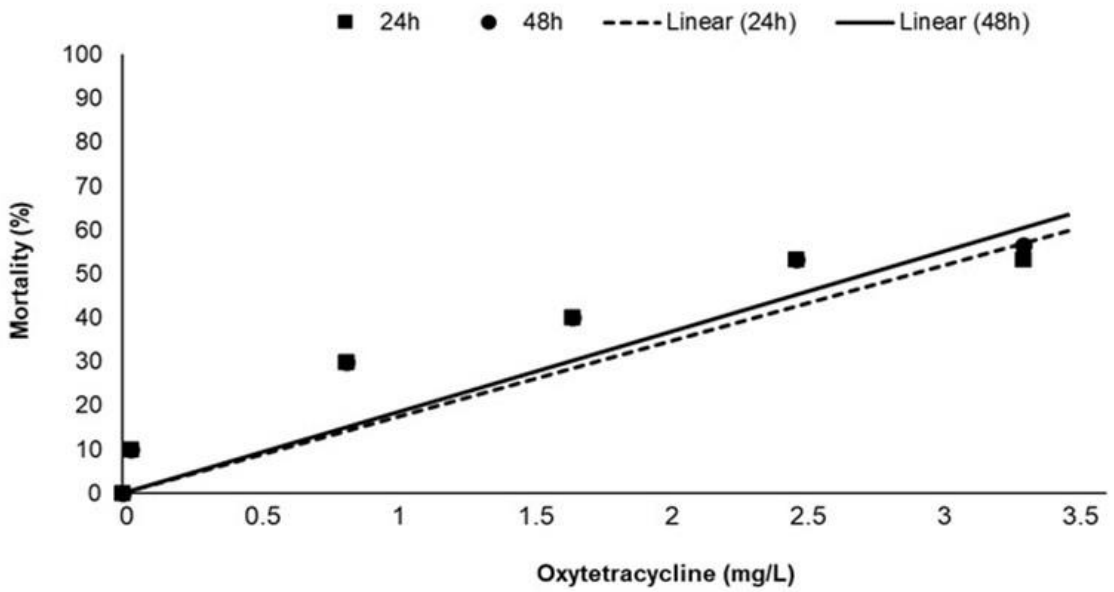

Fig. 3 Mortality of Nile tilapia larvae after acute stress and exposed to oxytetracycline for 24 and 48 hours (h)

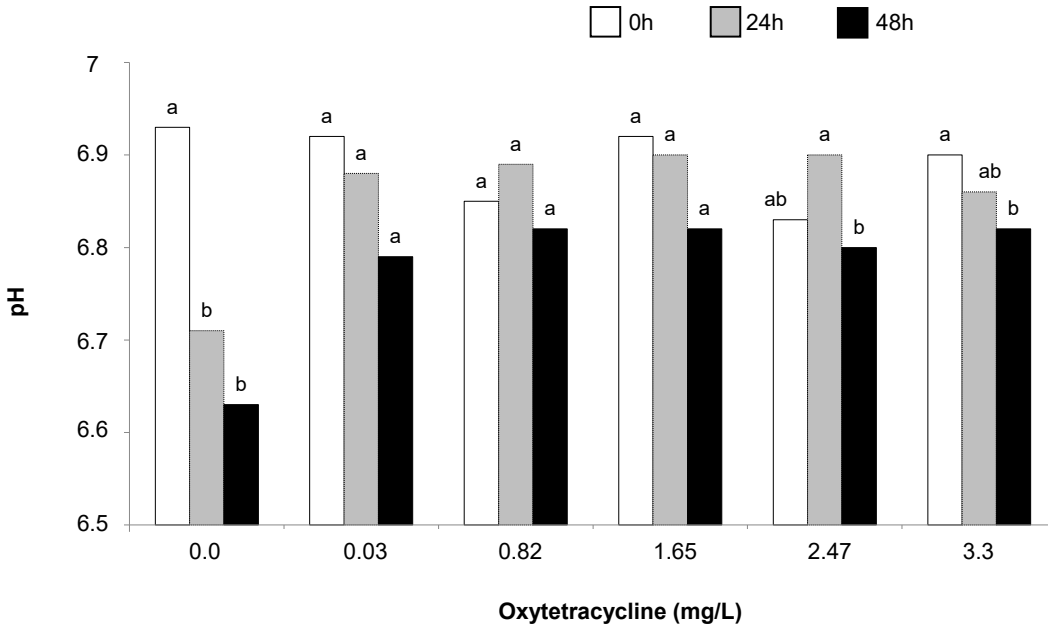

Fig. 4 pH values in oxytetracycline sub-lethal exposure test for 0, 24 and 48 hours (h), respectively. Different letters indicate significant differences by Tukey test (P < 0.05)

0.9447). There was no mortality in the control group (0.0 mg/L).

The pH values for water during the stress test are shown in Figure 4, exposing significant effects ($P < 0.05$) between 0 to 48 hours for the control: 2.47 and 3.30. The water temperature (Figure 5) indicated a statistical difference in the concentrations of 2.47 and 3.30 mg/L. The dissolved oxygen (DO) from the control treatment (0.00 mg/L and 3.30) decreased ($P < 0.05$) throughout the experimental period (Figure 6). At 0.03 mg/L, there was a slight reduction ($P < 0.05$) in the first 24 h, which differed statistically from that of the initial period.

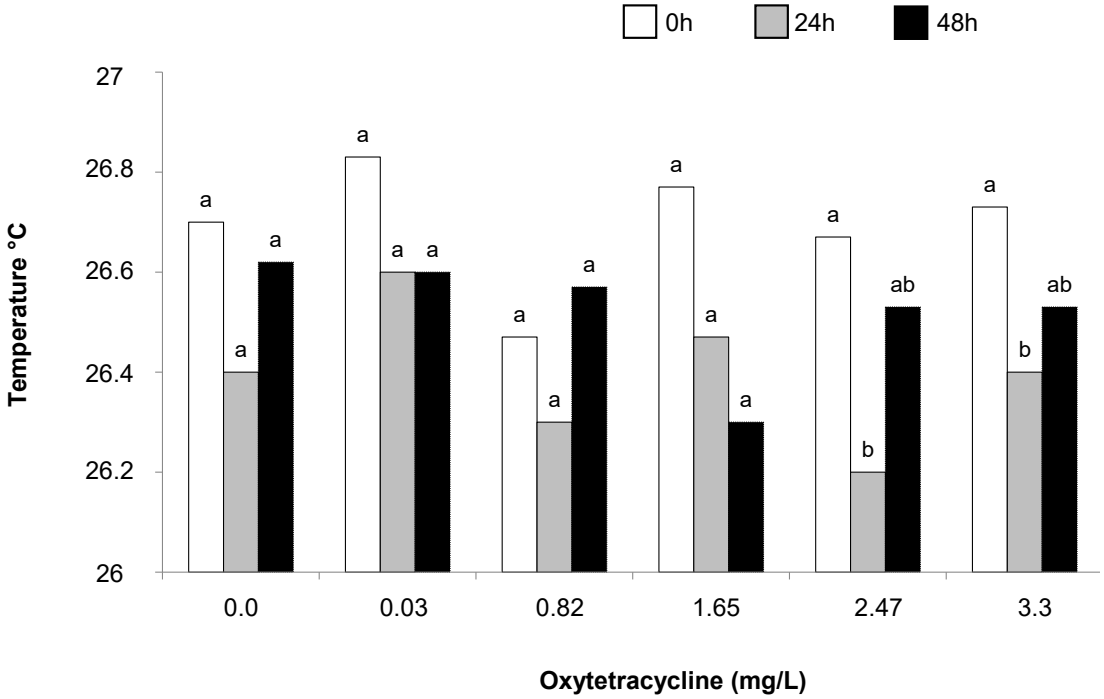

Fig. 5 Temperature values (°C) in oxytetracycline sub-lethal exposure test for 0, 24 and 48 hours, respectively. Different letters indicate significant differences by Tukey test ($P < 0.05$)

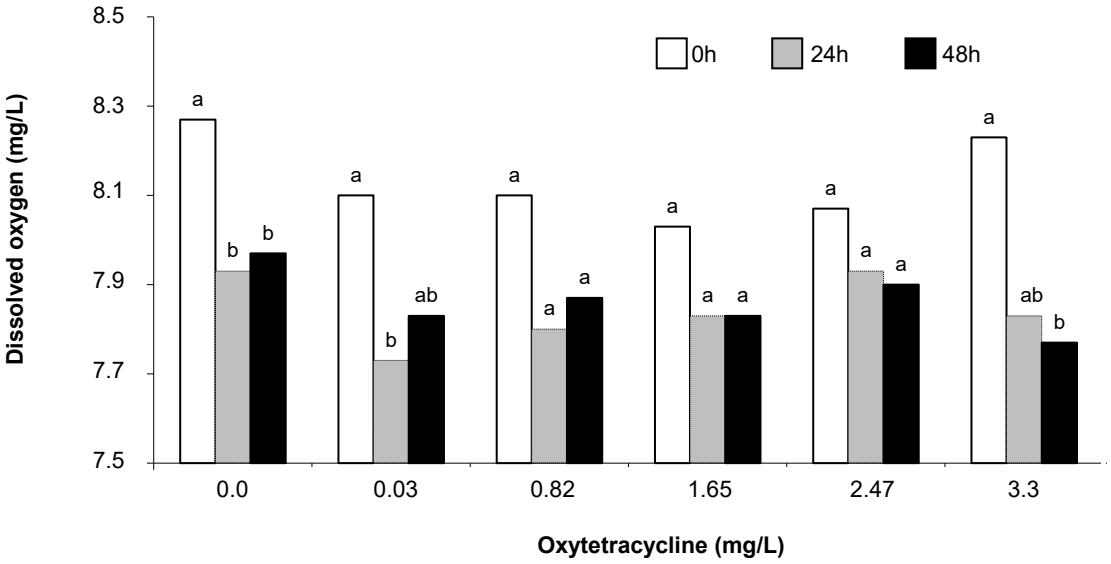

Fig. 6 Dissolved oxygen values in oxytetracycline sub-lethal exposure test for 0, 24 and 48 hours (h), respectively. Different letters indicate significant differences by Tukey test ($P < 0.05$)

Acute and chronic risk evaluation

To estimate the risk for preventing acute effects, an RQ_{acute} was calculated to equal 3.48 derived from the relation 4.59 mg/L / (3.45 mg/L / 3). This represents a high risk associated with larvae exposed to the therapeutic concentration of OTC (4.59 mg/L) over a period of 96h. Therefore, in the use of such a concentration, careful handling is recommended, with short periods of exposure in the immersion baths or during transportation to prevent acute effects.

Discussion

A significant reduction in the recovery of oxytetracycline (OTC) was observed at low test concentrations, measuring 55.72% and 58.19% for concentrations of 1.12 mg/L and 1.42 mg/L, respectively. This phenomenon can be elucidated by the absorption of the antibiotic by the fish and the subsequent adsorption of the molecules onto the walls of the test aquariums (Jonsson et al. 2017). However, it is noteworthy that the recovery percentage permissible for chromatographic analyses typically ranges up to 70% (Zhong et al. 2019; Queiroz et al. 2004; Shabi 2003). This inherent limitation likely contributed to the observed decline in measured residues.

Several studies have determined an LC50-96h of OTC for different fish species: 1000 mg/L for *Danio rerio* larvae (Barros et al. 2012); 597 mg/L for *Morone saxatilis* juveniles (2.2±0.9g) (Bumguardner and King 1996); 5.49 mg/L for *Hyphessobrycon eques* (0.22±0.2g) juveniles (Fujimoto et al. 2012); 62.5-75 mg/L for *Morone saxatilis* larvae (5–7 days post-hatching) (Hughes 1973) and 110.1 mg/L for *Oryzis latipes* juveniles (10–14 days post hatching) (Park and Choi 2008). On the other hand, an LC50-96h of OTC for Nile tilapia juveniles (0.5-1.0 g) was estimated at 6.92 mg/L (Machado et al. 2016). The LC of the present study indicates that Nile tilapia larvae are more sensitive to the toxic effects of OTC compared to other species and other development stages. However, it is essential to consider that such differences are attributed to the intrinsic characteristics and resilience of each species, as well as their size and the duration of exposure.

Antibiotic residues contaminate aquatic environments through leaching, uneaten feed (Rigos et al. 1999), and excretion (Rogstad et al. 1991). Fish absorb OTC at a minimal rate, with over 90% being excreted through gills and feces (Cravedi et al. 1987), leading to the accumulation of OTC in sediment and effluents of fish farms (Rico et al. 2012; Bebak-Williams 2002; Smith et al. 1994). Consequently, elevated residual levels of OTC persist in aquatic environments throughout the drug administration period, potentially affecting other aquatic organisms in natural ecosystems and fish reared in neighboring farms through cross-contamination.

The toxic effects of OTC include immunosuppression, manifested by reduced antibody production, and circulating leukocyte levels such as neutrophils, decreasing the phagocytic index (Reda et al. 2013). Additionally, OTC exposure leads to nephrotoxicity (Islam et al. 2015), liver damage (Guardiola et al. 2012) and vertebral column deformities (Toften and Jobling 1996). Furthermore, histological examination revealed moderate to severe morphological changes in the liver, kidney, and gill tissues of *O. mykiss* (7.75 – 9.24±0.38 g) exposed to OTC (Rodrigues et al. 2017). These adverse effects influenced fish mortality in this study, indicating the high toxicity of OTC.

Dissolved oxygen (DO) oscillation in an acute toxicity test may result from OTC molecule degradation of the OTC and fish consumption (respiration) (Kramer 1987). Moreover, organic matter generated from fish metabolism is oxidized by bacteria that consume oxygen, and potentially contributing to decreased DO levels (Lopes et al. 2022).

After the stress test (stress by air exposure) and submission to stress and sub-lethal levels of OTC, fish showed irregular swimming behavior and increased opercular movement, corroborating the behavior demonstrated for Nile tilapia juveniles (weight between 0.5 and 1.0 g) (Machado et al. 2016). In studies involving pollutants, these signs of stress and toxicity are commonly reported as responses aimed at relieving respiratory stress (Shrivastava et al. 2011).

The decrease in pH during stress tests results from increased H^+ ion concentration due to carbonic acid (H_2CO_3) dissociation, linked to fish metabolism and organic matter decomposition (Zhou et al. 2015). According to Boyd and Tucker (1998), the optimal pH for the aquaculture exclude species intended for human consumption in fresh waters varies from 6 to 8, aligning with the pH values observed in this study.

The temperature range suitable for Nile tilapia culture is 24.0 - 32.0 °C (El-Sayed and Kawanna 2008), consistent with the values obtained in this study. Temperature significantly influences fish activity, behavior, nutrition, growth, and reproduction, with metabolic rates increasing with rising temperatures (Lall and Tibbetts 2009). On the other hand, a decrease in DO was to be expected, due to the fish's consumption and decomposition of metabolic organic matter (feces). Despite the decrease in DO levels, the values presented in this study were remained within the comfort range established for the species (Tran-Ngoc et al. 2016).

Machado et al. (2016) calculated an RQ_{acute} of 1.82 for Nile tilapia larvae based on environmental chronic concentration (EEC) of 10 mg/L. Similarly, Carraschi et al. (2018) estimated an RQ_{acute} of 6.58 for *Piaractus mesopotamicus* juveniles with EEC of 50 mg/Kg. These findings from studies with different species highlight the significant environmental risk associated with OTC exposure in fish. This information underscores the importance of implementing robust biosafety protocols in aquaculture practices to minimize risks of cross-contamination and detrimental effects on fish at varying developmental stages within the same farm or neighboring areas.

Considering that the aquatic compartments surrounding areas of aquaculture systems may be contaminated with effluents containing OTC and with that lixiviated from the medicated feed, the range of $EEC_{chronic}$ in these compartments was estimated to obtain a low risk ($RQ_{chronic}$ = 0.01-0.1). This concentration ranges from 0.00345 to 0.0345 mg/L ($EEC_{chronic}$ = 0.01 or 0.1 x 3.45/10) and might be used to prevent long-term deleterious effects in larvae when exposed to OTC. Leal et al. (2019) reported some studies on OTC toxicity and show that by-products formed early on in treatment cause increased toxicity, but toxicity decreases in line with more extensive treatment times.

While oxytetracycline (OTC) serves as a commonly employed antibiotic in aquaculture, there remains a notable absence of specific information regarding its toxicity and risk assessment for early-stage Nile tilapia, particularly at post-hatch stage. Our findings offer valuable insights into the necessary precautions for managing fish diseases and establishing maximum permissible levels within aquatic ecosystems. They emphasize the importance of exercising caution in OTC utilization and underscore the significance of maintaining low antibiotic concentrations to mitigate the potential for chronic effects on Nile tilapia larvae. Additionally, our study advocates for the responsible use of OTC, thereby contributing to the reduction of its environmental impact.

Conclusions

The median lethal concentration LC50-96h of oxytetracycline for larvae of Nile tilapia was 3.45 mg/L. The observed toxic effects of oxytetracycline impaired the development of these larvae. Exposure to air was identified as a stressor for Nile tilapia larvae, and when combined with sub-lethal doses of oxytetracycline, it exacerbated its toxic effects. Furthermore, a risk quotient analysis indicated that therapeutic concentrations of oxytetracycline presented a high risk to tilapia larvae, underscoring the necessity for stringent measures to mitigate the potential for chronic effects. Specifically, to maintain low-risk conditions, it is imperative to maintain an environmental concentration of oxytetracycline below 0.0345 mg/L. However, considering the observed declines in oxytetracycline levels in test solutions (with the lowest recuperation rate measured at 55.72%), it is advisable to apply a safety factor of 0.56 to this concentration value.

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Competing interests The authors declare that they have no competing interests.

Data availability statement The data supporting the findings of this study are available from the corresponding author, H. Hisano, upon reasonable request.

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