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Reproductive and endocrine-disrupting toxicity of *Microcystis aeruginosa* in female zebrafish

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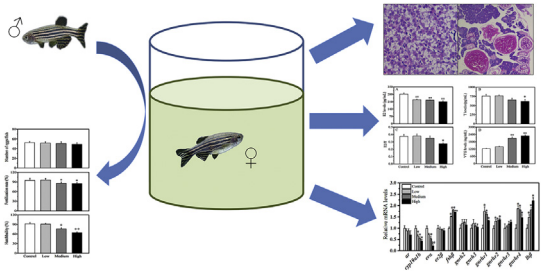
HIGHLIGHTS

- *M. aeruginosa* caused histological lesions in liver and gonad of female zebrafish.
- *M. aeruginosa* impaired reproductive capacity and caused transgenerational effects.
- *M. aeruginosa* exposure altered the level of plasma hormones in female zebrafish.
- *M. aeruginosa* changed the transcriptions of genes involved in endocrine system.
- *M. aeruginosa* can disrupt reproductive and endocrine system in fish.

GRAPHICAL ABSTRACT



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ABSTRACT

Microcystis aeruginosa, a primary species in cyanobacterial blooms, is ubiquitously distributed in water. Microcystins (MCs) purified from *M. aeruginosa* can exert reproductive toxicity in fish. However, the effects of *M. aeruginosa* at environmentally relevant levels on the reproductive and endocrine systems of zebrafish are still unknown. The present study investigated the reproductive and endocrine-disrupting toxicity of *M. aeruginosa* on female zebrafish (*Danio rerio*) by short-term exposure (96 h). After exposure, marked histological lesions in the liver or gonads, such as nuclear pyknosis and deformation, were observed, and the fertilization rate and hatchability of eggs spawned from treated females were both significantly lower than they were in females in the control group, suggesting the possibility of transgenerational effects of *M. aeruginosa* exposure. Moreover, *M. aeruginosa* exposure decreased the concentration of 17β-estradiol (E2) and testosterone (T) in female zebrafish. Interestingly, the *vtg1* transcriptional level significantly decreased in the liver, whereas plasma vitellogenin (VTG) protein levels increased. The present findings indicate that *M. aeruginosa* could modulate endocrine function by disrupting transcription of hypothalamic-pituitary-gonadal-liver (HPGL) axis-related genes, and impair the reproductive capacity of female zebrafish, suggesting that *M. aeruginosa* causes potential adverse effects on fish reproduction in *Microcystis* bloom-contaminated aquatic environments.

1. Introduction

Due to a combination of factors, including eutrophication and warming, cyanobacterial blooms frequently occur in freshwater

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and marine ecosystems and have been causing global public health concerns with increasing frequency (Carvalho et al., 2013). These blooms have caused serious problems in food webs, drinking water safety, water and habitat quality, and the sustainability of freshwater ecosystems (Paerl et al., 2011; Paerl and Otten, 2013; Song et al., 2017). Approximately 60,000 cases of human poisoning resulting from cyanobacterial blooms are reported each year, with an overall mortality rate of approximately 1.5% (Ajani et al., 2017; You et al., 2017). Cyanobacterial blooms can cause substantial economic losses. For instance, the cost of cleanup efforts for Taihu Lake, China, has exceeded US$ 16 billion since 2007 (Zhang et al., 2016).

Microcystis aeruginosa is one of the primary species responsible for cyanobacterial blooms. Microcystins (MCs) are the most commonly occurring toxic metabolites synthesized by *Microcystis*, and more than 100 structural variants of MCs have been described, of which microcystin-LR (MC-LR), microcystin-RR (MC-RR), and microcystin-YR (MC-YR) are the most extensively studied (Lawton et al., 1994; Puddick et al., 2014). These cyanotoxins can accumulate in fish tissue (Zimba et al., 2001) and cause developmental toxicity (Qi et al., 2016), neurotoxicity (Jonas et al., 2015), immune toxicity (Chen et al., 2016a), hepatotoxicity (Chen et al., 2016b), cytotoxicity (Dias et al., 2009), and endocrine disruption (Botha et al., 2004; Prieto et al., 2006).

In addition to the above-mentioned toxins, *Microcystis* cells and lysates have been shown to contain numerous other bioactive substances. For example, *Microcystis* can also produce many peptides classified as aeruginosins, micropeptins, and microviridins that have diverse biological functions (Chen et al., 2017). These metabolites may also lead to various negative health effects on organisms (Jonas et al., 2015). A few studies have demonstrated that crude extracts of cyanobacterial biomass or *Microcystis* lysates have greater effects on organisms than purified cyanotoxins (Oberemm et al., 1997; Palíková et al., 2007). Esterhuizen-Londt et al. (2016) found that crude cyanobacterial extracts elicited higher oxidative stress in *Daphnia pulex* compared to purified MCs or even MC mixtures, which implies that unidentified compounds in crude cyanobacterial extracts may enhance the toxic effects of MCs synergistically.

The zebrafish is an important model organism for biological research (Engeszer et al., 2007). Although several studies have demonstrated the endocrine-disrupting activity of single purified MCs in zebrafish, the effects of *M. aeruginosa* on the hypothalamic-pituitary-gonadal-liver (HPGL) axis remain to be elucidated. The purpose of the current study was to assess the toxic effects of *M. aeruginosa* at environmental levels on the reproductive and endocrine systems of zebrafish. Changes in reproductive capacity, tissue histology, plasma sex steroid hormone level, and transcription levels of key genes along the HPGL axis were examined after 96 h of exposure. This study will contribute to a better understanding of *M. aeruginosa* reproductive toxicity and relevant endocrine disruption potential in adult fish.

2. Materials and methods

2.1. Algal cultures

The freshwater unicellular cyanobacterium *M. aeruginosa* (FACHB-905) was obtained from the Institute of Hydrobiology, Chinese Academy of Sciences (Wuhan, China). The algae were cultivated in an incubator (Laifu Company, China) with a light irradiance of 46 μmol photons m^{-2} s^{-1} held at a constant temperature of 25 ± 0.5 °C and a 12 h:12 h light-dark cycle in 2 L beakers containing 1 L of BG-11 medium (pH = 7.1). Algal cell density was monitored at 685 nm by optical density values (UV/Visible spectrophotometer, Shimadzu). When the culture reached the middle of the exponential growth phase (approximately 3.7×10^5 cells/mL), the algae were used in for subsequent experiments.

2.2. Zebrafish maintenance, exposure, and sample collection

Healthy five-month-old adult zebrafish (*Danio rerio*) from the Institute of Hydrobiology of the Chinese Academy of Science were acclimated for two weeks in housing tanks containing BG-11 medium (12 h:12 h light/dark cycle, 25 ± 0.5 °C). The fish were fed two times daily with brine shrimp during the acclimation period, which stopped 24 h before the start of the toxicity tests.

Female zebrafish were randomly distributed into the glass tank containing 40 L of *M. aeruginosa* culture (35 fish/tank). The initial concentrations of algae corresponded to an optical density (OD) of 0.02, 0.04, and 0.08, hereafter referred to as low, medium, and high, respectively. Triplicate tanks were used for each treatment level. Control tanks with the same amount of BG-11 medium were also established. After 96 h, algal cell density ultimately reached approximately 4.4×10^5, 7.2×10^5, and 10.0×10^5 cells/mL in the low, medium, and high concentration tanks, respectively.

After 96 h exposure, five females were collected randomly from each tank and paired with five males from the untreated group. Eggs were immediately collected from each tank, and the fertilization rate was calculated. The eggs were then cultured in Ringer's solution at 28 °C, and hatchability was determined after 72 h. The rest of the fish in the control and treated groups were anaesthetized in MS 222 (Tricaine, Sigma-Aldrich) simultaneously for body weight measurements and blood collection. Blood was collected from the caudal vein using a heparinized syringe and maintained on ice until centrifugation (1200*g*, 15 min). The separated plasma collected from fish of each tank was pooled as one sample and stored at −80 °C for later analysis. The brain, gonads, and liver from each fish were dissected and weighed, and the brain-somatic index (BSI = 100 × [brain weight (g)/body weight (g)]), hepatosomatic index (HSI = 100 × [liver weight (g)/body weight (g)]), and gonadosomatic index (GSI = 100 × [gonad weight (g)/body weight (g)]) were calculated. The gonads and liver were then rinsed with cold phosphate-buffered saline (PBS, pH 7.0) and divided into two parts: one part was snap-frozen in liquid nitrogen and preserved at −80 °C for gene expression analysis, and the other part was fixed for histological analysis. All brain samples were preserved at −80 °C for gene expression analyses.

2.3. Histological analysis

For histopathological analysis of liver and brain tissue sections, samples were fixed in 10% buffered formalin for 24 h at 4 °C, dehydrated in a graded series of ethanol, immersed in xylol, and embedded in paraffin wax. Samples were cut to a thickness of 3–5 μm, deparaffinized, and rehydrated by immersion in a sequence of xylene, 100% alcohol, 70% alcohol, 50% alcohol, and finally water, stained with hematoxylin and eosin (H&E), and observed under a light microscope.

2.4. E2, T, and VTG determination

17β-estradiol (E2), testosterone (T), and vitellogenin (VTG) levels in the plasma were determined using sex steroid hormones detection kits (Yuanye, Shanghai, China) and VTG detection kits (Abebio, Wuhan, China), which were based on the enzyme-linked immunosorbent assay (ELISA). The standard curves of these three detection kits were obtained to determine the amount of each plasma sample, with a satisfactory linearity of $R^2 > 0.99$. E2, T and

VTG concentrations were normalized to the volume (mL) of the corresponding plasma sample, and the detection limits of the E2, T and VTG detection kits were 1.0 pg/mL, 1.0 pg/mL and 60 ng/mL, respectively. All samples and standards were run in triplicate.

2.5. Gene expression analyses

Total RNA was extracted from the brain, gonads, and liver using RNAiso Plus (Takara, Dalian, China), and cDNA synthesis and SYBR Green Real-Time PCR were performed using commercial kits (Toyobo, Tokyo, Japan) according to protocols we have previously described (Chen et al., 2016c). Three biological replicates were used for every treatment level. In total, 23 genes related to the HPGL axis, including two gonadotropin-releasing hormone genes (*gnrh2*, *gnrh3*), four gonadotropin-releasing hormone receptor genes (*gnrhr1*, *gnrhr2*, *gnrhr3*, *gnrhr4*), the follicle stimulating hormone beta gene (*fshβ*), the follicle-stimulating hormone receptor gene (*fshr*), the luteinizing hormone beta gene (*lhβ*), the luteinizing hormone receptor gene (*lhr*), two hydroxymethylglutaryl CoA reductase genes (*hmgra*, *hmgrb*), steroidogenic acute regulatory protein genes (*star*), four cytochrome P450 genes (*cyp11a*, *cyp17*, *cyp19a1a*, *cyp19a1b*), two hydroxysteroid dehydrogenase genes (*3βhsd*, *17βhsd*), two estrogen receptor genes (*erα*, *er2β*), the androgen receptor gene (*ar*), and the vitellogenin 1 gene (*vtg1*), were selected. Glyceraldehyde-3-phosphate dehydrogenase (*gapdh*) was used as an internal control gene to normalize the expression profiles. The primer sequences for these genes are listed in Table S1.

2.6. Statistical analyses

Statistical analyses were performed using SPSS 18.0 (SPSS Inc., Chicago, IL, USA). After assessment of normality and homogeneity of variance, a one-way analysis of variance (ANOVA) followed by Tukey's multiple range test was used to evaluate differences between the control and exposure groups. Data are presented as the mean value ± standard error (SE). Differences between the control and *M. aeruginosa* treatment groups were considered to be statistically significant at $p < 0.05$.

3. Results

3.1. Effects of M. aeruginosa exposure on the BSI, HSI and GSI of female zebrafish after 96 h

Microcystis aeruginosa had no effect on the body weight of female zebrafish after 96 h of exposure (data not shown). Similarly, no effects were observed on the BSI and HSI in treated zebrafish (Fig. 1). However, the GSI significantly decreased by 25.8% and 29.8% in the medium and high groups, respectively, whereas no difference was observed in the low group when compared with the control (Fig. 1).

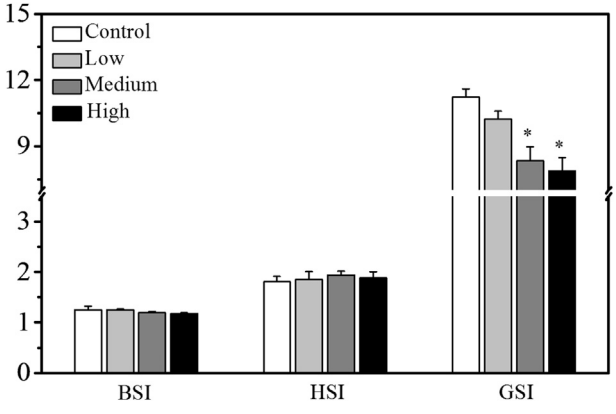

Fig. 1. Brain index (BSI), hepatosomatic index (HSI), and gonadosomatic index (GSI) of female zebrafish exposed to *M. aeruginosa* for 96 h.

3.2. Reproductive capacity of female zebrafish after 96 h of M. aeruginosa exposure

The number of eggs spawned by treated females was slightly lower than that by control females, but this difference was not statistically significant. However, compared with the control group, the fertilization rate of eggs significantly decreased by 9.5% and 10.5% in the medium and high groups, respectively. Additionally, hatchability of eggs in the medium and high groups significantly decreased by 8.2% and 21.6%, respectively, compared to the control group (Fig. 2).

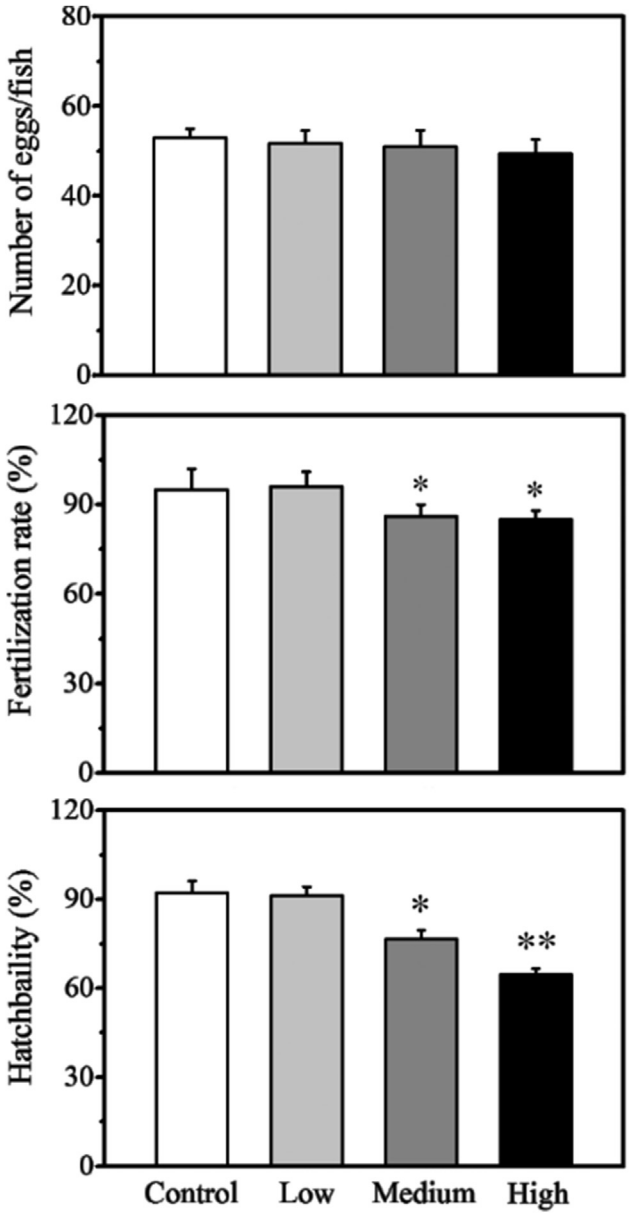

Fig. 2. Effects of *M. aeruginosa* on the reproductive capacity of female zebrafish.

3.3. Histological analysis of the liver and ovaries of female zebrafish

The hepatocytes of control females were densely interconnected, and the cord-like parenchymal structure with central spherical nuclei was clear. The cytoplasm of these hepatocytes contained various types of inclusions (Fig. 3A). However, *M. aeruginosa* induced hepatocellular vacuolization in a dose-dependent manner, and the spatial community and intercellular spaces were optically empty. Some cytoplasmic inclusions were no longer detectable and nuclei appeared pyknotic or deformed and parenchyma disorganized indicating partial necrosis of the liver (Fig. 3B–D).

Histopathological study of the control group showed normal development of gametogenic populations. Oocyte/vitelline membranes in the vitellogenic stage appeared regular, and oocytes were connected by a gonad somatic tissue stained red by eosin. However, in the ovaries of treated fish, loss of contact between the oocyte cell membranes and the follicular cell layer was observed. Irregularity in cell layers was seen in the treatment groups (Fig. 3G and H), and atretic oocytes were observed in oocytes at mature stages (Fig. 3K and L). The number of early-stage follicles increased in the treatment groups compared with the control. In addition, increased oocyte atresia and degenerating vitellogenic oocytes were found in treated female fish (Fig. 3E–H).

3.4. Changes in plasma hormones

Compared with the control, plasma E2 levels were significantly decreased in the low, medium, and high groups by 18.6%, 20.1%, and 25.6%, respectively, following 96 h of *M. aeruginosa* exposure (Fig. 4A). Plasma T concentrations significantly decreased (−18.8%) in the high group, and no change was found in the low and medium groups (Fig. 4B). The E2/T ratio only significantly decreased in the high group (−26.7%) compared with that of the control (Fig. 4C). Plasma VTG levels in treatment groups increased in dose-dependent manner compared with that of the control group, and in the medium and high groups, this increase was significant (68.6% and 84.7% greater than the control group, respectively) (Fig. 4D).

3.5. Transcriptional changes in genes of the HPGL axis

In the brain, transcript levels of *gnrhr1*, *gnrhr2*, and *gnrhr4* in the treated groups all increased significantly to more than 1.3 fold higher than the levels in the control after 96 h of exposure, whereas expression of *gnrh2*, *gnrh3*, and *gnrhr3* did not change. Significant upregulation of *fshβ* and *lhβ* was observed. In the low, medium, and high groups, *fshβ* was upregulated by 52.0%, 81.3%, and 71.5% of the control, respectively; and *lhβ* expression increased to 1.5 fold, 1.8 fold, and 2.1 fold higher than that in the control, respectively. Significant downregulation of *cyp19a1b* and *erα* was observed in the treated groups. In the three treated groups, *cyp19a1b* expression decreased to 65.7%, 83.8%, and 42.8% of the levels of control, respectively, and *erα* expression decreased to 71.1%, 53.6%, and 22.4% of the levels in the control. However, expression of *ar* and *er2β* did not change (Fig. 5).

In the ovary, expression of *cyp19a1a* decreased to 52.0%, 38.1%, and 20.6% of the levels in the control in the low, medium, and high groups, respectively, and expression of *cyp17* decreased to nearly half that of that in the control in the three treated groups. However, expression of *cyp11a* did not change. The expression of *hmgra* was

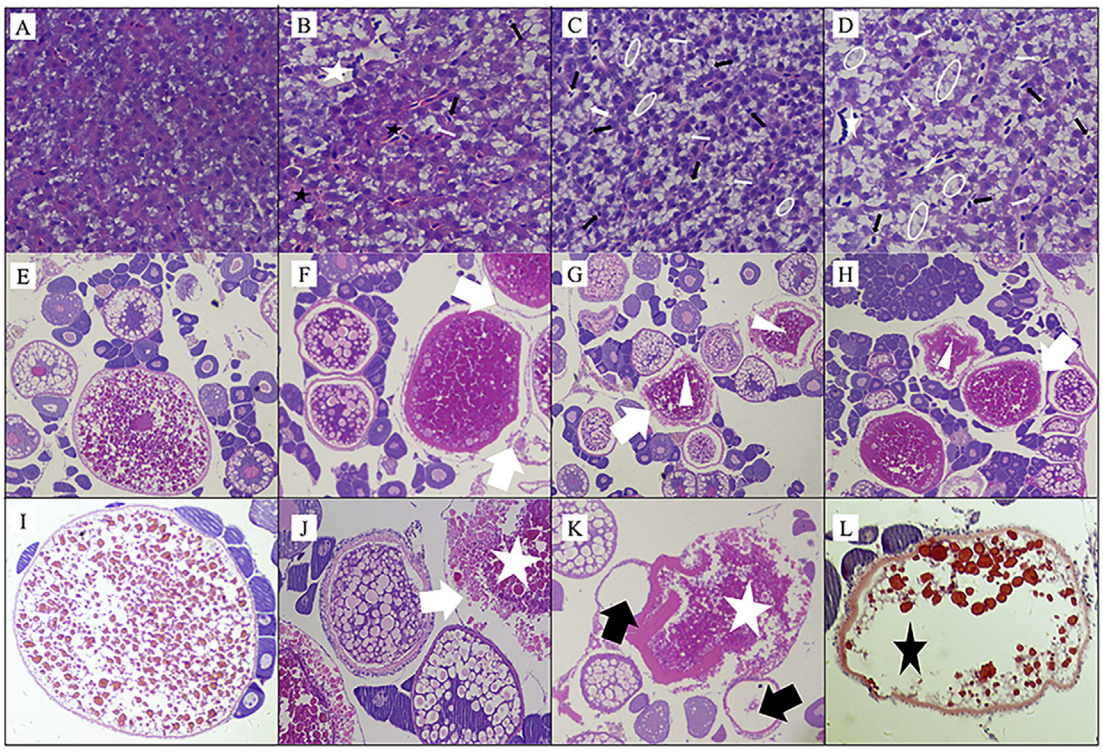

Fig. 3. Histological changes in the liver (A–D) and ovary (E–L) of zebrafish exposed to *M. aeruginosa* for 96 h. Microphotographs show liver and ovary sections (5 μm) stained with hematoxylin and eosin (H&E). The original magnification was ×400 (A–D), ×100 (E–H), ×200 (I–L). (A–D): H&E-stained liver section. A: Control group. B: Low group. C: Medium group. D: High group. Black asterisk: hyperemia. White asterisk: dilatation of sinusoid. Black arrow: pyknotic or deformed nucleus. White arrow: vacuolation. White ellipse: parenchyma disorganization. (E–L): H&E-stained ovary section. E, I: Control group. F, J: Low group. G, K: Medium group. H, L: High group. White arrow: the loss of contacts (ellipses) between the oocyte cell membranes and the follicular cell layer. Black arrow: the vacuolation of the gonadosomatic tissue. White triangle: Irregularity in cell layers. Black asterisk: oocyte atresia. White asterisk: degenerating vitellogenic oocytes.

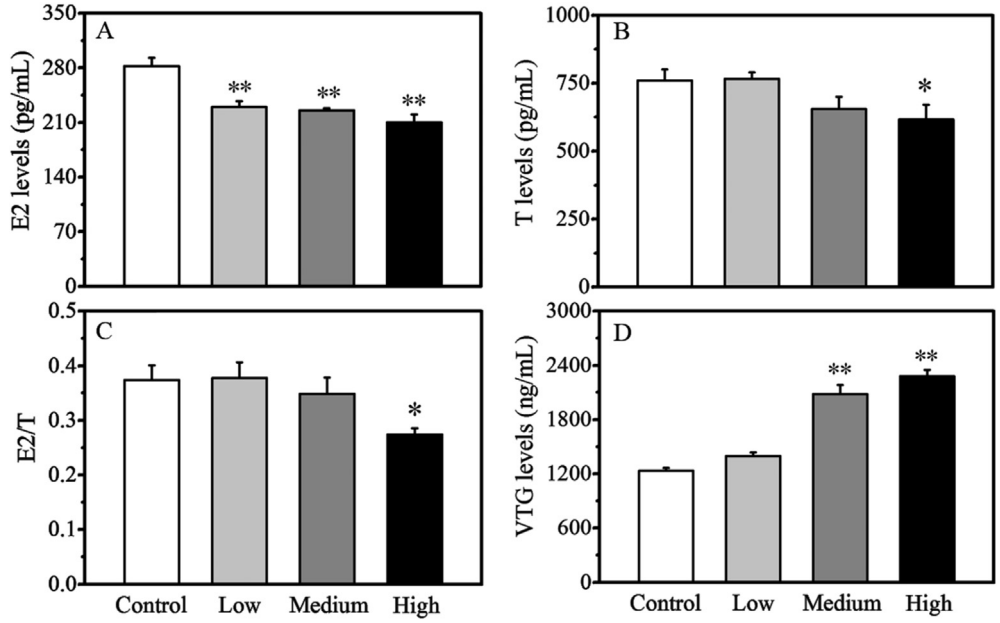

Fig. 4. Effects of *M. aeruginosa* on plasma levels of (A) 17β-estradiol (E2), (B) testosterone (T), (C) the E2/T ratio, and (D) vitellogenin (VTG) after 96 h of *M. aeruginosa* exposure.

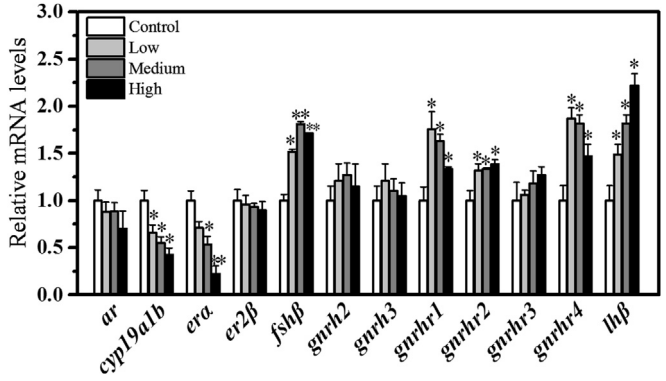

Fig. 5. Relative transcript levels of hypothalamic-pituitary-gonadal-liver (HPGL) axis genes in the brain of adult female zebrafish after exposure to *M. aeruginosa*.

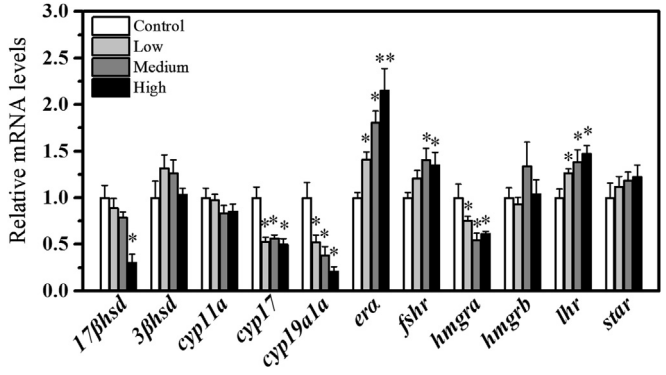

Fig. 6. Relative transcript levels of hypothalamic-pituitary-gonadal-liver (HPGL) axis genes in the ovary of adult female zebrafish after exposure to *M. aeruginosa*.

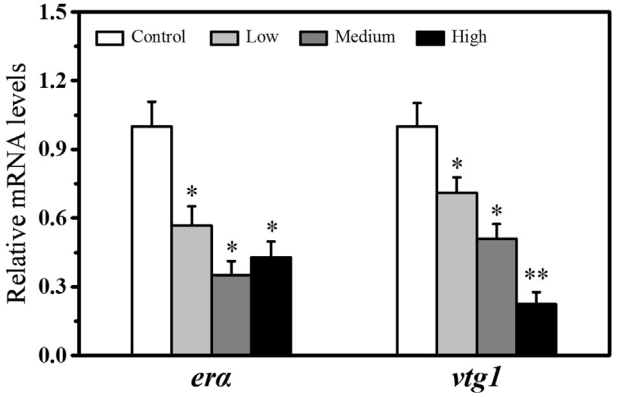

Fig. 7. Relative transcript levels of hypothalamic-pituitary-gonadal-liver (HPGL) axis genes in the liver of adult female zebrafish after exposure to *M. aeruginosa*.

decreased to 55%–75% of that in the control in the three treated groups after 96 h of *M. aeruginosa* exposure, whereas transcript levels of *hmgrb* did not change. The expression of *17βhsd* in the low, medium, and high groups decreased to 58.8%, 68.6%, and 30.3% that of the control, respectively, whereas no change was found in the transcription of *3βhsd*. The estrogen receptor α, follicle-stimulating hormone receptor, and luteinizing hormone receptor genes in the treated groups exhibited different degrees of upregulation. In the three treated groups, expression of *erα* increased by 20.8%, 80.3%, and 115.0% compared to the control, respectively; that of *fshr* increased by 20%–40% compared to the control, and that of *lhr* increased by 26%–47% compared to the control. There were no changes in the transcript levels of *star* (Fig. 6).

In the liver of female zebrafish, transcript levels of *vtg1* exhibited a dose-dependent decrease following 96 h of treatment with *M. aeruginosa* and were 71.1%, 67.6%, and 22.4% of the control levels in the low, medium, and high groups, respectively. The transcription levels of *erα* decreased to 56.8%, 35.1%, and 42.8% of the control in the three treatment groups, respectively (Fig. 7).

4. Discussion

The concentrations of *M. aeruginosa* used in this study were over 100,000 cells/mL, which is similar to the environmental

cyanobacterial density during a bloom (Saker and Eaglesham, 1999). We measured the concentration of MC-LR, one of the most toxic and prevalent congeners among the variants of cyanotoxins, in the algal cells and the water at the end of the exposure period. In the low, medium, and high groups, the MC-LR concentration was 275.24 ± 8.04 (mean ± SE), 425.54 ± 18.08, and 571.95 ± 15.71 μg/L, respectively, in algal cells, whereas it was 24.53 ± 1.97, 41.85 ± 0.79, and 55.57 ± 5.34 μg/L, respectively in the water (unpublished data). Similar concentrations have commonly been encountered in cyanobacterial bloom events, indicating that this work has environmental significance (Kardinaal et al., 2007; Watson et al., 2017).

The results from both field and laboratory studies have shown that stress induced by toxic substances can impair the liver of fish, and the liver is the primary target organ for MCs (Chen et al., 2016b). In the present study, *M. aeruginosa* exposure caused a dose-dependent increase in hepatic histopathological lesions characterized by vacuolation and apoptosis of the hepatocyte accompanied by pyknosis in many of the necrotic cells. Similarly, severe hepatic injury, including dilatation of sinusoid and hyperemia, with blood stasis and inflammatory cells, were observed in adult zebrafish after exposure to 200 μg/L MC-LR for 96 h (Wei et al., 2017). Hepatocytes also exhibit different degrees of damage after being injected with crude extracts of cyanobacteria (Paulino et al., 2017).

Several biomarkers, such as GSI, fecundity rate, fertilization rate, and hatchability of eggs, in conjunction with more specific biomarkers, are widely used to examine possible adverse effects of environmental pollutants, including endocrine-disrupting chemicals, on reproduction (Xu et al., 2008; Naderi et al., 2014). Reproductive dysfunction in adult zebrafish caused by *M. aeruginosa* was observed in the present study, which manifested as decreased GSI, reduced fertilization rate, and inhibited hatchability. Moreover, a declining trend in the number of spawn was also observed, although the difference was not statistically significant. The decrease in GSI could be ascribed to pathomorphological changes in the ovaries (Wu et al., 2014), and histological lesions, such as vacuolation or irregularity in cell layers, were observed in gonadal tissue in the present study. The gonadosomatic tissue damaged could decrease the reproductive performance of fish. It has been reported that the reproductive performance of medaka fish, including the fecundity and the hatchability, was adversely affected by 5 μg/L MC-LR and *Microcystis* extract (5 μg/L of equivalent MC-LR) after 28 days of treatment (Qiao et al., 2016). Wu et al. (2015) also found that exposure to less than 40 μg/L MC-LR can lead to subfertility in female mice. Our results were in accordance with these previous studies, suggesting that *M. aeruginosa* inhibited the reproductive capacity of female zebrafish and may have transgenerational effects.

Sex steroid hormone measurements are useful for monitoring reproductive systems, as these hormonal changes play crucial roles in the reproduction of fish and correspond to alterations in steroidogenic gene transcription (Devlin and Nagahama, 2002; Ji et al., 2013). Exposure to *M. aeruginosa* significantly altered plasma sex hormone levels and the expression of genes involved in the steroidogenesis pathway. Changes in sex steroid hormone concentrations may result in subsequent reproductive dysfunction, including decreased fecundity rate, fertilization rate, and hatch success, as observed in this study. The activity and expression of steroidogenic genes control steroid hormone synthesis and metabolism (Hogg et al., 2013). For instance, in zebrafish gonads, *cyp17* catalyzes the conversion of 17-hydroxyprogesterone to androstenedione, which is catalyzed into T by *17βhsd* (Fernandes et al., 2011). Additionally, aromatase (*cyp19*) enzymes are known to catalyze the final conversion of androgen to estrogen (Trant et al., 2001), and in zebrafish, T is secreted from the follicle cell and finally converted to E2 by aromatase (Clelland and Peng, 2009). In the present study, *M. aeruginosa* exposure inhibited the expression of most steroidogenic genes (*cyp19a1a*, *cyp19a1b*, *17βhsd*, *cyp17*, and *hmgra*), corresponding to the changes of E2 and T levels in the plasma. For example, plasma T concentrations significantly decreased in the high group, but no change was found in the low and medium groups. Those changes were consistent with the concentration-dependent decrease of *17βhsd* gene expression. Moreover, decreased transcription of *cyp19a1a* in the ovary may inhibit the conversion of T to E2 such that the ratio of E2/T is reduced. This reduced ratio suggests that exposure to *M. aeruginosa* disrupts the balance of sex hormones in fish and results in adverse effects on oogenesis and reproduction (Orlando et al., 2004). Similar to our observations, Qiao et al. (2013) found that E2 levels were significantly decreased after exposure to 20 μg/L MC-LR, whereas no change in T was observed. When zebrafish were exposed to 50 μg/L MC-LR, both E2 and T levels were significantly decreased (Zhao et al., 2015).

In teleosts, the regulation of reproduction is primarily coordinated via interactions between genes along the HPGL axis in addition to genes involved in the steroidogenesis pathway (Hilscherova et al., 2004; Villeneuve et al., 2008). Studies in female zebrafish have revealed that FSH is primarily involved in vitellogenesis, whereas maturation of oocytes is primarily controlled by LH (Sarkar et al., 2014). In the present study, the transcription of *fsh* and *lh* genes in zebrafish after *M. aeruginosa* treatment increased significantly, suggesting possible promotion of oogenesis and maturation. However, these effects were not observed in the histological and reproductive examination. It is well known that endocrine-disrupting chemicals alter normal patterns of gene expression, by either steroid hormone receptor-mediated pathways or compensatory effects (Villeneuve et al., 2007). Insufficient E2 content could initiate a feedback mechanism that could stimulate the pituitary to respond, leading to increased secretion of gonadotropins (Hou et al., 2016). Therefore, the upregulation of *fshβ* and *lhβ* observed in this study indirectly indicates the existence of a feedback mechanism in the HPGL-axis. Other studies have noted that exposure to prochloraz decreases the plasma concentration of E2 and causes compensatory upregulation of genes of the HPGL in small fish (Ankley et al., 2009; Liu et al., 2010), strongly supporting our observations.

Changes in VTG levels in female fish have been identified as a sensitive endpoint for assessing the levels endocrine-disrupting chemicals (Dang et al., 2011). VTG is a yolk precursor protein that is synthesized by the liver in response to estradiol stimulation and is essential for vitellogenesis, oocyte maturation, and yolk biosynthesis (Aravindakshan et al., 2004; Yuan et al., 2013). Seven known *vtg* genes are located differently in the zebrafish genome, and *vtg1* is the most highly expressed (Tong et al., 2004; Wang et al., 2005). As the expression of *vtg* genes in the livers of female fish is modulated by E2 (Bemanian et al., 2004), the observed downregulation of the hepatic *vtg1* gene in the present study might have resulted from decreased E2 levels in the plasma. Liver lesions caused by *M. aeruginosa* in the present study may also have been responsible for the downregulation of *vtg1* mRNA expression. However, plasma VTG levels significantly increased in a dose-dependent manner in this study, which may be due to the impairment of the ovaries making it difficult for plasma VTG to incorporate into the oocytes, resulting in accumulation in the blood. Similarly, Qiao et al. (2013) also reported that MC-LR exposure decreased hepatic *vtg1* gene expression and increased VTG levels in female zebrafish with MC-LR exposure.

5. Conclusions

In summary, the present study demonstrated that short-term (96 h) exposure to *M. aeruginosa* could impair reproductive capacity and disrupt the endocrine system in female zebrafish, damaging the liver and gonadal tissue, altering plasma sex hormone levels, and changing the transcription of HPGL axis-related genes. Moreover, the decreased fertilization rate and hatchability of eggs spawned from treated females demonstrated the possibility of transgenerational effects of *M. aeruginosa* exposure. Future work is necessary to study the reproductive toxicology of *M. aeruginosa* in a systematic manner after long-term exposure to environmentally relevant concentrations of *M. aeruginosa*.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.chemosphere.2017.10.167.

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