Environmental Science and Pollution Research
https://doi.org/10.1007/s11356-022-23864-2

RESEARCH ARTICLE

Prolonged electrolysis injures the neural development of zebrafish (*Danio rerio*)

Chaoqun Zheng[1,2] · Yan Gao[3] · Jinling Zhu[1] · Lin Gan[4] · Mengmeng Wang[1] · Wen Zhang[1] · Shunqing Yang[1] · Liuyan Yang[1]

Received: 26 October 2021 / Accepted: 24 October 2022
© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2022

Abstract

Recently, electrolysis technology has been widely applied in nitrogen and phosphorus removal in river water due to its high efficiency, but its effects on aquatic animals, especially on their neurodevelopmental system, are still unclear. In this study, zebrafish (*Danio rerio*) embryos were used as model organisms and were put into an electrolytic reaction device with a Ti/IrO_2/RuO_2 mesh plate as the anode and a Ti mesh plate as the cathode to explore the effects of prolonged electrolysis on the nervous system. The neural development of zebrafish embryos was injured when the current density was greater than 0.89 A/m^2. Compared with the control group, the movement speed of zebrafish larvae (120 h postfertilization, hpf) was significantly reduced from 65.48 ± 23.69 to 48.08 ± 22.73 mm/min in a dark environment with an electric current density of 0.89 A/m^2 in the electrolysis group. In addition, the acetylcholinesterase activity of zebrafish larvae (120 hpf) gradually decreased from 7.60 ± 0.55 to 6.00 ± 0.01 U/mg prot and the dopamine concentration was reduced from 46.96 ± 0.85 to 40.86 ± 1.05 pg/mL with an electric current density from 0 to 0.89 A/m^2 in the electrolysis groups. Furthermore, the expression of nerve-related genes (*syn2a*, *mbp*, *nestin*, and *AChE*) was significantly inhibited when the current density was more than 0.89 A/m^2. However, there were few adverse effects on the neural development of zebrafish embryos when the current density was less than 0.86 A/m^2. Thus, a current density of 0.86 A/m^2 is a reference value to reduce the harm to the neural development of fish when electrolysis technology is used in river water pollutant treatment.

Keywords Zebrafish · No-observed-effect current density · Neural development · Electrolysis · Dopamine · AChE activity

Responsible Editor: Bruno Nunes

Highlights
- Electrolysis intensity over 0.86 A/m^2 affected motor behavior activity of zebrafish.
- AChE activity and dopamine concentration were reduced with increasing current density.
- The expressions of nerve-related genes of *syn2a*, *mbp*, *nestin*, and *AChE* decreased if the current density was more than 0.89 A/m^2.

✉ Liuyan Yang
yangly@nju.edu.cn

1 State Key Laboratory of Pollution Control and Resource Reuse, School of the Environment, Nanjing University, Nanjing 210023, People's Republic of China

2 School of Ecological Environment and Urban Construction, Fujian University of Technology, Fuzhou 350118, People's Republic of China

3 School of Environment Science and Engineering, Nanjing Tech University, Nanjing 211816, People's Republic of China

4 Nanjing Hydraulic Research Institute, Nanjing 210017, People's Republic of China

Introduction

With the rapid development of industry and agriculture, a large number of pollutants enter bodies of water, causing a decline in water quality. The pollutants mainly include nitrogen (N), phosphorus (P), oxygen-consuming substances, and heavy metals (Li et al. 2021; Smith and Schindler 2009). Aqueous environmental pollution has also brought many adverse effects on human production and life, leading to the application of new methods to remove water pollutants. Among them, electrolysis technology has been widely used in water treatment due to its diverse and highly efficient removal of pollutants. Yan et al. (2020) found that the introduction of electrolysis with a low current density (0.37 mA/cm^2) into ecological floating beds was beneficial for the removal of N and P in water. Moreover, electrolysis technology is also conducive to the degradation of refractory pollutants and heavy metals in water (Li et al. 2018; Wang et al. 2020b). Electrolysis can be applied for the deep purification treatment of polluted water bodies and has better

Published online: 08 November 2022

application prospects for rivers. However, the aquatic ecosystem is complex and includes aquatic animals and aquatic plants. Therefore, it is particularly important to study the effects of electrolysis on aquatic organisms.

To our knowledge, previous studies have focused on the effects of electrolysis on aquatic plants. Dannehl (2018) showed that electricity activated the stress response of plants and seeds, while a weak electric current promoted plant growth. Several studies have shown that low current stimulation enhances nutrient uptake and promotes seed germination and quality (Lee and Oh 2021). Otherwise, studies on the effects of electrolysis on aquatic animals have mainly focused on the adverse effects of electrofishing techniques on fish, including discoloration, muscle damage, skeletal injuries, nerve damage, reduced swimming stamina, reduced growth, and disturbances in physiology and behavior (Stewart 2014; Snyder 2003; Miller et al. 2021). However, electrolysis technology applied to rivers mainly utilizes lower current densities to act on pollutants for longer periods. At present, relatively few studies have examined the effects of low electric current density on aquatic animals, which could lead to potential safety hazards in the application of electrolysis technology and hinder its extensive popularization.

The fish nervous system is sensitive to electrolysis, and it is generally believed that the impact of high voltages is directly reflected in the nerves and behavior of fish (Miller et al. 2021). Therefore, in this study, we focused on the impact of electrolysis on the neural development of aquatic animals to assess the safety of electrolysis technology. A nonspecific and negative effect of electric field stimulation on neurons has been reported that a current density of approximately 100 μA/cm^2 can cause the blastopore of the *Xenopus* embryo to fall off (Robinson and Cormie 2008). Neurobehavior is a comprehensive response to many physiological and biochemical processes (Jia et al. 2020; Wang et al. 2015). It is known that acetylcholinesterase (AChE) is necessary for the development of zebrafish embryonic neurons (Chen et al. 2012). AChE activity is widely used as a sensitive biomarker of neurotoxins (Payne et al. 1996). Low AChE activity in zebrafish can lead to decreased muscle activity (Behra et al. 2002). In addition, dopamine plays an important role in regulating neurotoxic effects such as motor control and dopaminergic neuron development (Jia et al. 2020). Therefore, the effect of electrolysis on the nervous system should be further studied by analyzing acetylcholine activity and dopamine concentration. Furthermore, compared to previous conventional analysis techniques, the use of molecular technologies to detect target genes and regulatory pathways not only improves the sensitivity of the assay (Iqbal et al. 2000) but also helps to understand the intrinsic causes of the effects of electrolysis on aquatic animals. Neural-related genes, such as *syn2a*, *mbp*, *nestin*, and *AChE*, which are sensitive molecular targets to stressors, were analyzed in this study (Garbarino et al. 2014; Brösamle and Halpern 2002; Chen et al. 2010, 2012; Behra et al. 2002). Zebrafish have been widely used as model animals due to advantages such as small size, strong fertility, short development cycle, transparency, ease of observation, and clear genetic background (Hill et al. 2005). In addition, zebrafish are vertebrates with conservative nervous system structures and complex behavioral patterns (Pullaguri et al. 2020) and have been increasingly used in behavioral neuroscience and behavioral research (Wang et al. 2020a).

In this study, we used a series of biological research methods, such as analyses of behavioral activity, enzyme activity, dopamine concentration, and gene differential expression, to explore the effects of electrolysis on the neurodevelopment of zebrafish in its early development stage. Thus, the purposes of this study were first to explore the effect of prolonged electrolysis with low current density on zebrafish development and their neural system, and second to determine the no-observed-effect electrolytic reaction conditions in order to provide a reference for comprehensive and systematic evaluation of the biological safety of electrolysis applied in aquatic ecosystems.

Materials and methods

Zebrafish and experimental design

All experimental zebrafish (wild-type AB strain) were initially obtained from the China Zebrafish Resource Center (Wuhan, China). Afterwards, zebrafish were domesticated in a recirculating culture system (ESEN, Beijing, China) over 30 days. They were kept in fish cultured water (temperature 27 ± 1 ℃, pH 7.2–7.6, dissolved oxygen 7.6–7.8 mg/L, electrical conductivity 518–520 μS/cm), and the photoperiod was a cycle of 14 h light and 10 h dark. The lights were turned on at 8:00 am daily. During the culture period, all zebrafish were fed twice per day with newly hatched brine shrimp (*Artemia Sinica*) containing a high protein content. The breeding zebrafish were all approximately 2–3 months old with a body length of 3.8 ± 0.3 cm and a bodyweight of 0.5 ± 0.1 g, and their fertilized embryos were all collected from the natural spawning paired breeding zebrafish in the morning once the lights were turned on at 8:00 am. The embryos were washed several times and incubated in fish cultured water. Before electrolysis exposure, all embryos were observed under a stereoscopic microscope (Leica, Solms, Germany) to select normal embryos. Embryos that were coagulated, lacked a heartbeat, failed to develop somatic cells, or had unseparated tails were identified as dead embryos and discarded. The normal embryos were

stored in a petri dish with a diameter of 60 mm until the experiment.

The testing apparatus is shown in Fig. 1. At 8 h postfertilization (hpf), approximately 100 normal embryos were randomly assigned to the testing apparatus containing 500 mL fish cultured water, and a $Ti/IrO_2/RuO_2$ mesh was inserted vertically as the anode and a Ti mesh as the cathode with an electrode spacing of 22 cm. The effective working area of the electrode was 45.45 cm^2. The electrodes leaned on the walls of the plastic container and were connected by wiring alligator clips to a Zhaoxin Model KXN-3020D DC regulated power supply (Zhaoxin Electronic Instruments and Equipment Co., Ltd., Shenzhen, China) with a voltage range from 0 to 30 V and amperage from 0 to 5 A. The DC regulated power provided a series of current densities by adjusting the voltage. The total electrolysis time was 112 h as sampling zebrafish developed from embryos (8 hpf) to larvae (120 hpf).

In the zebrafish fertilization period and larval period (96 hpf), dead embryos were identified in real time and calculated at the 96th hour according to the Organization for Economic Co-operation and Development (OECD) 236 zebrafish embryo acute toxicity test method (OECD 2013). The electrolysis used in many studies is defined in terms of the required current rather than voltage to affect aquatic organisms (Jung-Schroers et al. 2020; Lambooij et al. 2008). To obtain current densities corresponding to different mortality rates, several different current density groups were set, and the dose–effect curve of mortality of the larval period (96 hpf) was fitted by using a variable slope model (GraphPad Prism 7, San Diego, CA, USA). No electrolysis reactions were added in the control groups. The electrolysis groups and control groups all contained 3 replicates.

Morphological measurement

After electrolysis exposure, zebrafish embryos and larvae were all examined under a stereoscopic microscope (Leica, Solms, Germany) to screen morphology, and abnormalities were recorded for each electrolysis exposure of different current densities corresponding to different mortality rates (10%, 20%, and 50%). Ten zebrafish embryos were randomly selected from each testing apparatus ($n = 3$) to measure the autonomous movement (autonomous movement/30 s) of the embryo at 24 hpf and the heart rate (heart rate/15 s) of the embryo at 48 hpf under a stereoscopic microscope (Leica, Solms, Germany).

Autonomous movement and neurobehavioral analysis

The autonomous movement of zebrafish embryos was measured at 24 hpf (Jia et al. 2020). Normally developed 110 hpf zebrafish larvae in the electrolysis groups and control groups were transferred into 96-well plates. Each well contained one larva and 200 µL fish cultured water, 36 larvae for each group. The plate was incubated at 28 ℃ in a constant temperature light incubator for 10 h. Then, the behavior of zebrafish larvae (120 hpf) was analyzed using a zebrafish behavior analyzer (Viewpoint Life Sciences, Inc., France) (Chen et al. 2012). The system setting parameters included three light–dark periods, 10 min darkness followed by 10 min light for each cycle. After recording their movement distance (mm) and movement speed (mm/min), the data were analyzed using R language.

AChE activity and dopamine concentration in zebrafish larvae

The AChE activity and dopamine concentration were determined by using enzyme-linked immunosorbent assay (ELISA) kits (Jiangsu Meimian Industrial Co. Ltd., Yancheng, China) according to the manufacturer's instructions. Briefly, zebrafish larvae (120 hpf) in the electrolysis groups and control groups (50 larvae in each group, and all with 3 replicates) were added to normal saline homogenate,

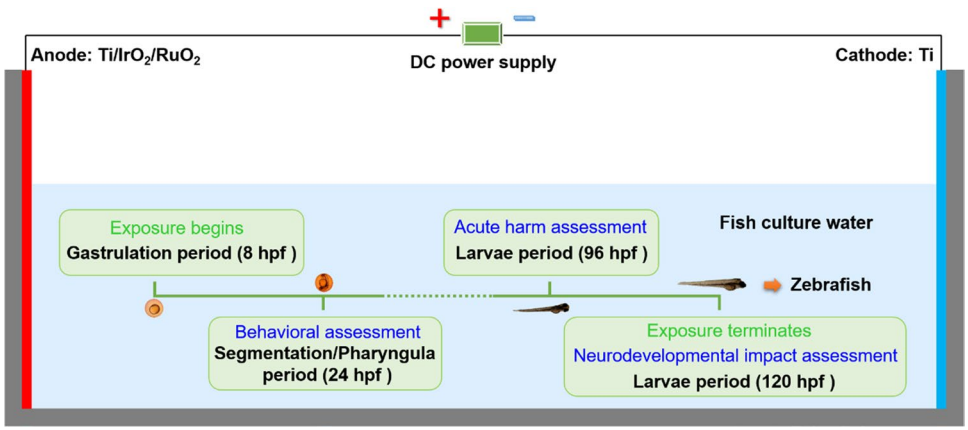

Fig. 1 Schematic diagram of the experimental design testing apparatus and details of the procedure of the short-term exposure procedure

and, operating on ice, a 10% homogenate was prepared (Chen et al. 2012). Then, the reaction reagent was added according to the instructions, and the absorbance was measured at 412 nm using a spectrophotometer to calculate the AChE activity. The AChE activity of zebrafish larvae was calculated as the amount of enzyme that catalyzes the production of 1 nmol 5-mercapto-nitrobenzoic acid (TNB) per milligram of protein per minute. Similarly, the absorbance was measured at 450 nm using a spectrophotometer to calculate the dopamine concentration.

RNA extraction of zebrafish larvae and real-time fluorescence quantitative PCR analysis of genes involved in neural development

The total RNA of homogeneous zebrafish larvae (120 hpf) in the electrolysis group or control group was extracted using RNAiso Plus reagent (TaKaRa Bio Inc., Japan) following the protocol described by the manufacturer. The PrimeScript RT reagent kit with gDNA Eraser (TaKaRa Bio Inc., Japan) was used to reverse transcribe cDNAs for subsequent fluorescence quantitative analysis. Fluorescence quantitative PCR assays were performed using the CFX Connect Real-Time System (Bio-Rad, USA). The reaction procedure for real-time fluorescence quantitative PCR was as follows: heating at 95 ℃ for 10 min and then repeating at 95 ℃ for 5 s and 60 ℃ for 18 s for 40 times. Each sample was calibrated using *β-actin* as an internal reference. Primers of target genes were designed using Primer Premier 5 (Premier Biosoft, San Francisco, USA) and then sent to Sangon Biotech, Shanghai, China, for synthesis, and the specificity and amplification efficiency of each primer were verified in the pre experiment. The primer information used in this experiment is shown in Table 1. The relative mRNA expression level calculation was conducted using the relative expression software (Bio-Rad) based on the $2^{-\Delta\Delta Ct}$ method.

Statistical analysis

All data were processed using SPSS 16.0 (SPSS, Chicago, IL, USA) and GraphPad Prism 7 (GraphPad Software, San Diego, CA, USA). Differences between the electrolysis groups and control groups were assessed using one-way analysis of variance (ANOVA) and Tukey's test. All data are expressed as the mean ± standard error. A p value of less than 0.05 indicated that the differences were statistically significant.

Results

Early development of zebrafish embryos

The fatality of zebrafish embryos was tested under different electric current densities of electrolysis. Based on a dose–effect curve of current density (Fig. 2A), the 10% lethal electric current density was 0.86 A/m^2, the 20% lethal electric current density was 0.89 A/m^2, and the 50% lethal electric current density was 0.94 A/m^2. These three lethal electric current densities were all set as the electrolysis groups.

The effects of electrolysis on the autonomous movement, heart rate, and malformation rate were measured (Fig. 2B–C). There was a slight decrease in autonomous movement of zebrafish embryos (24 hpf) in the electrolysis groups compared with the control groups (Fig. 2B); however, no significant differences were observed. The heart rate of zebrafish embryos (48 hpf) gradually increased to 43.67 ± 0.62 (heart rate/15 s) when the current density increased to 0.94 A/m^2. In particular, the heart rates of zebrafish embryos were significantly higher in the electrolysis groups of 0.89 A/m^2 and 0.94 A/m^2 than those in the control groups ($p < 0.05$, Fig. 2C). In addition, the deformity of zebrafish larvae (96 hpf) was further observed under a stereoscopic microscope, and the teratological effect of zebrafish larvae (96 hpf) in the electrolysis groups is shown in Fig. 2E. The main deformities of zebrafish embryos and larvae were pericardial and yolk cysts, while tail deformities were also occasionally observed. In the morphological observation, we found that the deformity rates increased from 9.17 ± 1.60 to 17.50 ± 2.85% with the current densities from 0 to 0.94 A/m^2, which was significantly different when the electric current density was greater than 0.89 A/m^2 ($p < 0.05$, Fig. 2D).

Table 1 Primers for significantly expressed genes in the neural development of zebrafish embryos

Gene	Forward primer sequence (5′—3′)	Reverse primer sequence (5′–3′)
β-actin	TGCTGTTTTCCCCTCCATTG	TCCCATGCCAACCATCACT
Syn2a	GTGACCATGCCAGCATTTC	TGGTTCTCCACTTTTTTCACCTT
mbp	AATCAGCAGGTTCTTCGGAGGAGA	AAGAAATGCACGACAGGGTTGACG
nestin	ATGCTGGAGAAACATGCCATGCAG	AGGGTGTTTACTTGGGCCTGA
AChE	CCAAAAGAATAGAGATGCCATGGACG	TGTGATGTTAAGCAGACGAGGCAGG

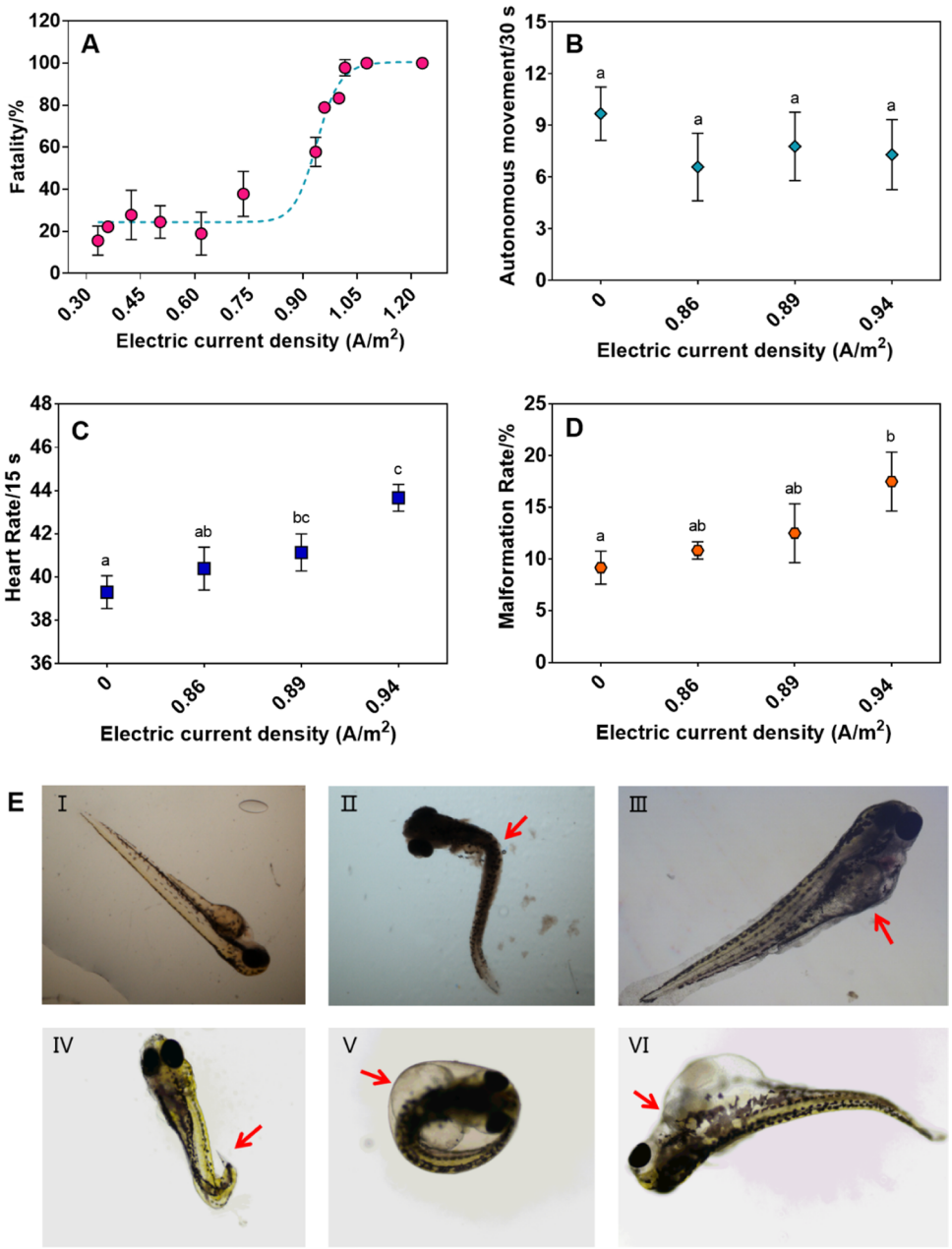

Fig. 2 Effects of electrolysis on the development of zebrafish embryos. **A** Half lethality electric current density (A/m^2) at 96 hpf. **B** Autonomous movement at 24 hpf. **C** Heart rate at 48 hpf. **D** Malformation rate at 96 hpf. **E** Typical malformation schematic diagram, which occurred when the current density exceeded 0.86 A/m.^{2} (I, normal; II, spinal curvature; III, yolk sac anomaly; IV, tail malformation; V, egg congeal; VI, pericardium edema (red arrow point to malformation). Bars show the mean ± *SE* ($n = 30$). Different letters indicate statistically significant differences ($p < 0.05$)

Motor behavior activity of zebrafish larvae

Neurological-related motor changes are readily monitored in zebrafish larvae and are highly associated with motor neuron development. Here, the effect of electrolysis on the neural behavior of zebrafish larvae (120 hpf) was analyzed to explore the no-observed-effect current density of electrolysis on the nervous system of zebrafish. In this study, it was clear that zebrafish larvae (120 hpf) had significantly reduced movement speed in light (30.34 mm/min of average activity), and excessive activity in darkness (56.85 mm/min of average activity) in the electrolysis groups and control groups ($p < 0.05$, Fig. 3). There was no significant difference in movement speed among the electrolysis groups and control groups under light stimulation ($p < 0.05$). However, compared with the control group (65.48 ± 23.69 mm/min), the movement speeds of zebrafish larvae were significantly reduced in a dark environment in 0.89 A/m^2 for the electric current density electrolysis group (48.08 ± 22.73 mm/min) and 0.94 A/m^2 group (44.47 ± 20.75 mm/min; $p < 0.05$). Thus, the no-observed-effect current density for neural behavior of zebrafish larvae should not be greater than 0.86 A/m^2.

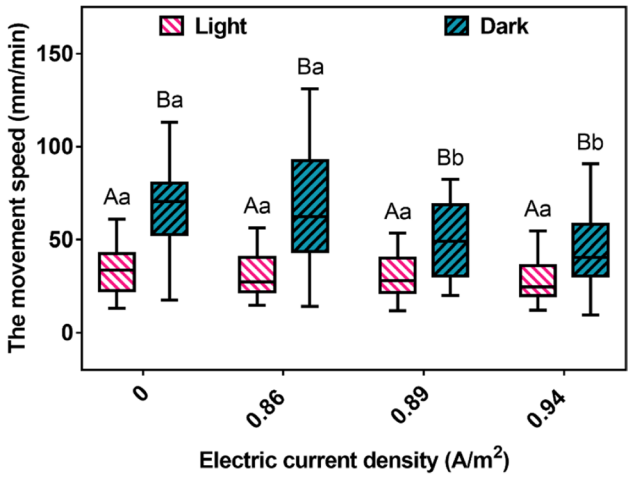

Fig. 3 The movement speed of zebrafish larvae (120 hpf) under different electric current densities with a strong light stimulus and in the dark. Bars show the mean ± *SE* ($n = 36$). Different letters indicate statistically significant differences: the uppercase letters show the comparison of the same electric current density in light and dark environments, and the lowercase letters show the comparison of different electric current densities in the same environment ($p < 0.05$)

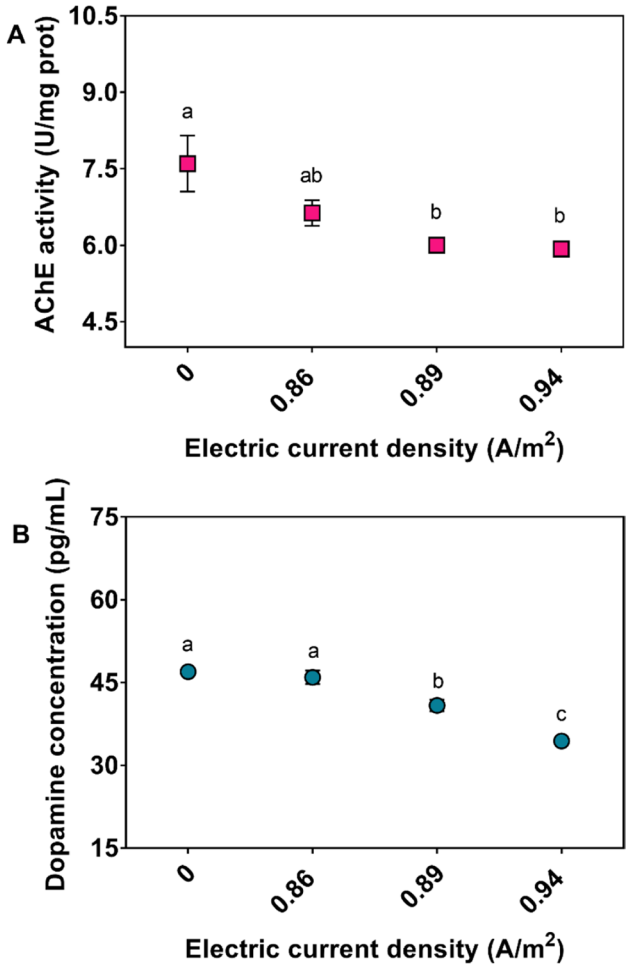

Fig. 4 AChE activity (**A**) and dopamine concentration (**B**) of zebrafish larvae (120 hpf) under different electric current densities. Bars show the mean ± *SE* ($n = 3$). Different letters indicate statistically significant differences ($p < 0.05$)

AChE activity and dopamine concentration in zebrafish larvae

As described above, zebrafish larval motor behavior activity decreased at the excessive current density. Therefore, we next assessed the AChE activity and dopamine concentration to further examine the change in behavior. The AChE activity of zebrafish larvae (120 hpf) gradually decreased from 7.60 ± 0.55 to 6.00 ± 0.01 U/mg prot with an electric current density from 0 to 0.89 A/m^2 in the electrolysis groups (Fig. 4A). Compared to the control group, the AChE activity decreased significantly when the electric current density was greater than 0.89 A/m^2 ($p < 0.05$).

The dopamine concentration gradually decreased with increasing electric current density in the electrolysis groups (Fig. 4B). Compared to the control group (46.96 ± 0.85 pg/mL), the dopamine concentration was significantly lower in the 0.89 A/m^2 electrolysis group (40.86 ± 1.05 pg/mL; $p < 0.05$). Therefore, the no-observed-effect current density was also less than 0.86 A/m^2.

Neural-related gene expression levels in zebrafish larvae

To investigate the molecular pathways that interfere with the nervous system development of zebrafish larvae caused by electrolysis, we used real-time fluorescence quantitative PCR technology to analyze the expression of genes related to the nervous system. The expression of key genes related to the development of the nervous system (*syn2a*, *mbp*, *nestin*, *AChE*) in zebrafish larvae (120 hpf) decreased with increasing electric current density (Fig. 5). No significant changes in the gene expression of *syn2a*, *mbp*, or *nestin* were observed when the current density was less than 0.86 A/m^2, while a significant downregulation of *AChE* gene expression was observed ($p < 0.05$). In addition, compared with the control group (0 A/m^2), the mRNA expressions of *syn2a* (*fold change* 0.69 ± 0.12), *mbp* (*fold change* 0.70 ± 0.07), *nestin* (*fold change* 0.45 ± 0.06), and *AChE* (*fold change* 0.55 ± 0.05) were significantly downregulated when the current density was greater than 0.89 A/m^2 ($p < 0.05$, Fig. 5). Consequently, the no-observed-effect current density of the expression of neural-related genes in zebrafish larvae should be less than 0.86 A/m^2.

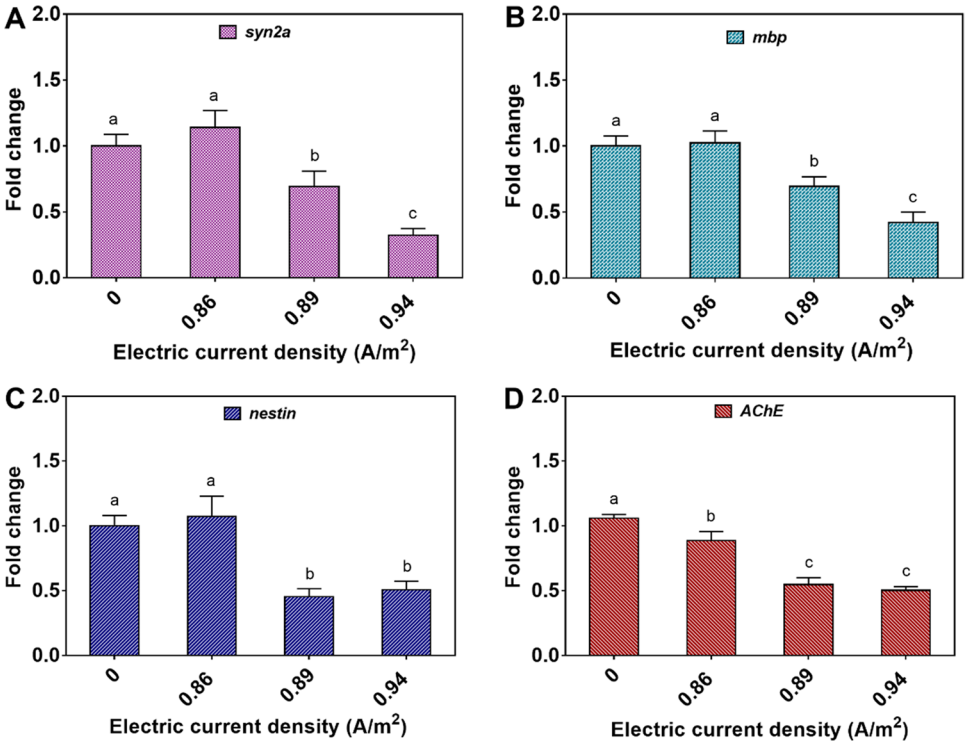

Fig. 5 The mRNA expression levels of the *syn2a* gene (**A**), *mbp* gene (**B**), *nestin* gene (**C**), and *AChE* gene (**D**) related to nervous system development in zebrafish larvae (120 hfp) under different electric current densities. Bars show the mean ± *SE* ($n = 6$). Different letters indicate statistically significant differences ($p < 0.05$)

Discussion

Although fish are not in direct contact with the electrodes when the electrolysis devices are applied to the river, the effect of the electric field near the electrodes on the fish cannot be ignored. Work from Dwyer et al. (1993) showed that buried embryos can be killed by electroshock. Bohl et al. (2010) demonstrated that the embryos were protected from the electric fields if a reasonable minimum distance (e.g., 0.5 m) was maintained between electrodes and embryos. Furthermore, the nervous system is the most complex and important signal transmission system in animals and plays an important role in controlling almost every aspect of animal physiology (Lin et al. 2020). Due to its low resistance, the nervous system is one of the most sensitive systems to electric current (Kandeel et al. 2017). Therefore, studies exploring nervous system damage and the no-observed-effect current density of electrolysis on the nervous system in zebrafish are necessary for the promotion of the application of electrolysis technology.

In this study, there was no significant difference in the autonomous movement of zebrafish embryos at 24 hpf between the electrolysis groups and the control groups; however, the zebrafish larvae at 120 hpf in the electrolysis groups exhibited a negative behavioral response when the electric current density was greater than 0.89 A/m^2. These results are consistent with previous studies that showed a decrease in swim capacities by exposing warmwater stream fish to electric fields (Gatz and Linder 2008). Thomas et al. (2019) found that fish are likely to be paralyzed or injured (spinal injuries from sudden muscular contractions) from electrical currents. This is consistent with our findings of increased malformation rates in zebrafish. Motor behavior activity is the sum of activities controlled by the nervous system in response to external stimuli (Ping and Zhla 2020; Pullaguri et al. 2021). Injuries are more extreme with longer electroshock durations (Schreer et al. 2004). This suggests that prolonged electrolysis with a low current density can cause greater damage to the zebrafish nervous system.

Most research to date has focused on the survival, growth, and injury of fish exposed to electricity (Roach 1999; Schreck et al. 1976). The physiological stress response and potential injuries that may result from exposing fish to electric fields are only partially understood (Snyder 2003). Previous studies have shown reduced growth in centrarchids after repeated exposure to electroshocking (Wahl et al. 2007). However, these studies did not explore the molecular mechanisms underlying these effects. Therefore, in this study, we examined the expression of several genes important in the development of the central nervous system (CNS) to characterize the effect of electrolysis on zebrafish. To our knowledge, this study is the first to explore the potential mechanisms of nerve damage from electrolysis to zebrafish at the molecular level. AChE is essential during the neuronal development of zebrafish embryos (Behra et al. 2002). A decreased acetylcholine concentration can cause muscle contraction and low activity of behavioral responses (Chen et al. 2012). In our study, the mRNA expression of the cholinergic

system-related gene (*AChE*), which can affect acetylcholine-mediated neurotransmission, decreased significantly when the electric current density was greater than 0.89 A/m^2. Similar results were found for AChE activity, which was significantly reduced. Thus, the cholinergic system was damaged during electrolysis exposure. Electrolysis harms the nervous system development of zebrafish larvae, which may occur through cholinergic system dysfunction. Additionally, we investigated the expression of dopamine, which is a neurotransmitter with an important role in the modulation of neurotoxic effects such as movement regulation, motivation, and the development of dopaminergic neurons (Jia et al. 2020; Wang et al. 2015). In this study, we observed a significant decrease in dopamine concentration when the current density exceeded 0.89 A/m^2. A reduced dopamine concentration may contribute to decreased larval locomotion.

Furthermore, changes in dopamine have also been implicated in functional abnormalities of the CNS (Wang et al. 2015). Here, we analyzed the mRNA expression levels of several important CNS-related genes (*syn2a*, *mbp*, *nestin*) to investigate the effect of electrolysis on the zebrafish nervous system. Gene expression analysis was based on the phenotypic motor behaviors of zebrafish to explore potential molecular pathways. The expression of the *syn2a*, *mbp*, and *nestin* genes in zebrafish larvae (120 hpf) was significantly inhibited and exhibited a dose–response effect to the current density when the current density was more than 20% of the lethal current density (0.89 A/m^2). As a result, we hypothesized that the low expression of important CNS-related genes may lead to the interruption of neural development, which could cause damage to the nervous system of zebrafish. Previous studies have shown that the cytoskeleton of the nervous system is more sensitive to certain poisons, including *mbp*, which is required for myelin formation during the development of the nervous system (Brösamle and Halpern 2002). Consequently, the downregulation of *mbp* expression may affect the structure and function of brain tissue. *Syn2a* is a biomarker of synapse formation in mammals and plays an important role in synapse generation and neurotransmitter release (Kao et al. 1998). *Syn2a* is not only related to the nervous system but may also be related to the embryonic development of zebrafish (Garbarino et al. 2014). Downregulation of *Syn2a* expression can affect synapse generation and neuronal differentiation. Gene expression levels of both *mbp* and *Syn2a* were downregulated in our experiments, and the results were consistent with Wang et al. (2015), who found that organic flame retardants can downregulate the expression of genes related to the nervous system. In addition, nestin is a selective neural progenitor cell intermediate filament involved in cytoskeletal functions (Lendahl et al. 1990). The expression of the *nestin* gene was inhibited by electrolysis when the current density was greater than 0.89 A/m^2, which suggests that the motor neurons were developmentally deficient. Consequently, our results suggest that electrolysis led to altered expression of CNS-related genes, which supports that the electrolysis treatment has a neurotoxic effect on zebrafish when the current density of electrolysis is greater than 0.89 A/m^2.

Thus, the current density of electrolysis must be controlled if electrolysis is used to remove the nitrogen and phosphorus pollutants. Gao et al. (2018) combined electrolysis with biochar to enhance the removal rates of nitrate (49.54%) and P (74.25%) under a current density of 0.02 mA/cm^2 and an electrolysis time of 24 h. The current density (0.02 mA/cm^2) was lower than the no-observed-effect current density from our results, which means that the apparatus was relatively safe. However, as previously mentioned, the current density used by Yan et al. (2020) in the river water treatment was 0.37 mA/cm^2, which may hurt the neural development of fish. Moreover, the current densities adopted by other studies may be even higher (Wang et al. 2020b). Therefore, it is necessary to study the no-observed-effect distance in the future.

Conclusion

The application of electrolysis technology in aquatic environment treatment is increasing, but little is known about the impact of electrolysis with lower current densities for longer periods of time on aquatic animals. Here, we examined the prolonged effects of electrolysis with low current density on the development of the zebrafish nervous system. Electrolysis induced neurodevelopmental damage to early-stage zebrafish when the electric current density was greater than 0.89 A/m^2, and the damage was dose dependent. In addition, the behavioral activity, AChE activity, dopamine concentration, and the regulation of neural-related genes (*syn2a*, *mbp*, *nestin*, and *AChE*) of zebrafish larvae were reduced when the electric current density was greater than 0.86 A/m^2. To summarize, our data provide new insights into the prolonged effects of electrolysis on the neural development of zebrafish, particularly that the no-observed-effect electric current density is 0.86 A/m^2 for nervous system development in zebrafish. Thus, the electric current density should be controlled in the use of electrolysis technology in aquatic pollutant treatment.

Author contribution Chaoqun Zheng: conceptualization, methodology, investigation, formal analysis, writing original draft. Yan Gao: funding acquisition, conceptualization, methodology, formal analysis. Jinling Zhu: conceptualization, methodology, formal analysis. Lin Gan: conceptualization, methodology. Mengmeng Wang: conceptualization, methodology. Wen Zhang: conceptualization, methodology. Shunqing Yang: conceptualization, methodology. Liuyan Yang: funding acquisition, conceptualization, project administration, writing—reviewing and editing.

Funding This work was supported by grants from National Special Program of Water Environment (2017ZX07204002), National Natural Science Foundation of China (51908277), the scientific research project of Ecological Environment Department of Jiangsu Province (2020019), the Water Resources Science and Technology Project of Water Resources Department of Jiangsu Province (2019006), and Jiangsu Provincial Natural Science Foundation of China (BK20190320).

Data availability All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

References

Behra M, Cousin X, Bertrand C, Vonesch JL, Biellmann D, Chatonnet A, Strähle U (2002) Acetylcholinesterase is required for neuronal and muscular development in the zebrafish embryo. Nature Neuroence 5:111–118. https://doi.org/10.1038/nn788

Bohl R, Henry T, Strange R (2010) Electroshock-induced mortality in freshwater fish embryos increases with embryo diameter: a model based on results from 10 species. J Fish Biol 76:975–986. https://doi.org/10.1111/j.1095-8649.2010.02552.x

Brösamle C, Halpern ME (2002) Characterization of myelination in the developing zebrafish. Glia 39:47–57. https://doi.org/10.1002/glia.10088

Chen HL, Yuh CH, Wu KK (2010) Nestin is essential for zebrafish brain and eye development through control of progenitor cell apoptosis. PLoS ONE 5:e9318. https://doi.org/10.1371/journal.pone.0009318

Chen L, Yu K, Huang C, Yu L, Zhu B, Lam PKS, Lam JCW, Zhou B (2012) Prenatal transfer of polybrominated diphenyl ethers (PBDEs) results in developmental neurotoxicity in zebrafish larvae. Environ Sci Technol 46:9727–9734. https://doi.org/10.1021/es302119g

Dannehl D (2018) Effects of electricity on plant responses. Sci Hortic 234:382–392. https://doi.org/10.1016/j.scienta.2018.02.007

Dwyer W, Fredenberg W, Erdahl D (1993) Influence of electroshock and mechanical shock on survival of trout eggs. N Am J Fish Manage 13:839–843. https://doi.org/10.1577/1548-8675(1993)013%3c0839:ioeams%3e2.3.co;2

Gao Y, Zhang W, Gao B, Jia W, Miao A, Xiao L, Yang L (2018) Highly efficient removal of nitrogen and phosphorus in an electrolysis-integrated horizontal subsurface-flow constructed wetland amended with biochar. Water Res 139:301–310. https://doi.org/10.1016/j.watres.2018.04.007

Garbarino G, Costa S, Pestarino M, Candiani S (2014) Differential expression of synapsin genes during early zebrafish development. Neuroscience 280:351–367. https://doi.org/10.1016/j.neuroscience.2014.09.015

Gatz A, Linder R (2008) Effects of repeated electroshocking on condition, growth, and movement of selected warmwater stream fishes. N Am J Fish Manage 28:792–798. https://doi.org/10.1577/M07-031.1

Hill AJ, Teraoka H, Heideman W, Peterson RE (2005) Zebrafish as a model vertebrate for investigating chemical toxicity. Toxicol Sci 86:6–19. https://doi.org/10.1093/toxsci/kfi110

Iqbal SS, Mayo MW, Bruno JG, Bronk BV, Batt CA, Chambers JP (2000) A review of molecular recognition technologies for detection of biological threat agents. Biosens Bioelectron 15:549–578. https://doi.org/10.1016/S0956-5663(00)00108-1

Jia M, Teng M, Tian S, Yan J, Meng Z, Yan S, Li R, Zhou Z, Zhu W (2020) Developmental toxicity and neurotoxicity of penconazole enantiomers exposure on zebrafish (*Danio rerio*). Environ Pollut 267:115450. https://doi.org/10.1016/j.envpol.2020.115450

Jung-Schroers V, Hildebrandt U, Retter K, Esser K, Hellmann J, Kleingeld DW, Rohn K, Steinhagen D (2020) Is humane slaughtering of rainbow trout achieved in conventional production chains in Germany? Results of a pilot field and laboratory study. BMC Vet Res 16:197. https://doi.org/10.1186/s12917-020-02412-5

Kandeel S, Elhosary NM, El-Noor MMA, Balaha M (2017) Electric injury-induced Purkinje cell apoptosis in rat cerebellum: Histological and Immunohistochemical study. J Chem Neuroanat 81:87–96. https://doi.org/10.1016/j.jchemneu.2017.02.010

Kao HT, Porton B, Czernik AJ, Feng J, Yiu G, Häring M, Benfenati F, Greengard P (1998) A third member of the synapsin gene family. P Natl Acad Sci 95:4667–4672. https://doi.org/10.1073/pnas.95.8.4667

Lambooij E, Gerritzen MA, Reimert H, Burggraaf D, van de Vis JW (2008) A humane protocol for electro-stunning and killing of Nile tilapia in fresh water. Aquaculture 275:88–95. https://doi.org/10.1016/j.aquaculture.2008.01.026

Lee S, Oh M (2021) Electric stimulation promotes growth, mineral uptake, and antioxidant accumulation in kale (*Brassica oleracea var. acephala*). Bioelectrochemistry 138:107727. https://doi.org/10.1016/j.bioelechem.2020.107727

Lendahl U, Zimmerman LB, Mckay R (1990) CNS stem cells express a new class of intermediate filament protein. Cell 60:585–595. https://doi.org/10.1016/0092-8674(90)90662-X

Li J, Ren Y, Lai L, Lai B (2018) Electrolysis assisted persulfate with annular iron sheet as anode for the enhanced degradation of 2, 4-dinitrophenol in aqueous solution. J Hazard Mater 344:778–787

Li Y, Li CX, Lin W, Wang SS, Zhang WX, Jiang YM, Zhang Y, Zhang H, Han YH (2021) Full evaluation of assimilatory and dissimilatory nitrate reduction in a new denitrifying bacterium *Leclercia adecarboxylata* strain AS3-1: Characterization and functional gene analysis. Environ Technol Inno 23:101731. https://doi.org/10.1016/j.eti.2021.101731

Lin YC, Wu CY, Hu CH, Pai TW, Chen YR, Wang WD (2020) Integrated hypoxia signaling and oxidative stress in developmental neurotoxicity of benzo[a]pyrene in zebrafish embryos. Antioxidants 9:731. https://doi.org/10.3390/antiox9080731

Miller M, Bie J, Sharkh S, Kemp P (2021) Behavioural response of downstream migrating European eel (*Anguilla anguilla*) to electric fields under static and flowing water conditions. Ecol Eng 172:106397. https://doi.org/10.1016/j.ecoleng.2021.106397

OECD (2013) Guidelines for the testing of chemicals. Section 2: effects on biotic systems test No. 236: fish embryo acute toxicity (FET) test. Organization for Economic Cooperation and Development (OECD), Paris. https://doi.org/10.1787/9789264203709-en

Payne JF, Mathieu A, Melvin W, Fancey LL (1996) Acetylcholinesterase, an old biomarker with a new future? Field trials in association with two urban rivers and a paper mill in Newfoundland. Mar Pollut Bull 32:225–231. https://doi.org/10.1016/0025-326X(95)00112-Z

Ping LA, Zhla B (2020) Environmental co-exposure to TBT and Cd caused neurotoxicity and thyroid endocrine disruption in zebrafish, a three-generation study in a simulated environment. Environ Pollut 259:113868. https://doi.org/10.1016/j.envpol.2019.113868

Pullaguri N, Grover P, Abhishek S, Rajakumara E, Bhargava Y, Bhargava A (2021) Triclosan affects motor function in zebrafish larva

by inhibiting *ache* and *syn2a* genes. Chemosphere 266:128930. https://doi.org/10.1016/j.chemosphere.2020.128930

Pullaguri N, Nema S, Bhargava Y, Bhargava A (2020) Triclosan alters adult zebrafish behavior and targets acetylcholinesterase activity and expression. Environ Toxicol Phar 75:103311. https://doi.org/10.1016/j.etap.2019.103311

Roach S (1999) Influence of Electrofishing on the Mortality of Arctic Grayling Eggs. N Am J Fish Manage 19:923–929. https://doi.org/10.1577/1548-8675(1999)019%3c0923:IOEOTO%3e2.0.CO;2

Robinson KR, Cormie P (2008) Electric field effects on human spinal injury: Is there a basis in the *in vitro* studies? Dev Neurobiol 68:274–280. https://doi.org/10.1002/dneu.20570

Schreck C, Whaley R, Bass M, Maughan O, Solazzi M (1976) Physiological responses of rainbow trout (*Salmo gairdneri*) to electroshock. J Fish Board Canada 33:76–84. https://doi.org/10.1139/f76-009

Schreer J, Cooke S, Connors K (2004) Electrofishing-induced cardiac disturbance and injury in rainbow trout. J Fish Biol 64:996–1014. https://doi.org/10.1111/j.1095-8649.2004.00364.x

Smith VH, Schindler DW (2009) Eutrophication science: where do we go from here? Trends Ecol Evol 24:201–207. https://doi.org/10.1016/j.tree.2008.11.009

Snyder D (2003) Invited overview: conclusions from a review of electrofishing and its harmful effects on fish. Rev Fish Biol Fisher 13:445–453. https://doi.org/10.1007/s11160-004-1095-9

Stewart C (2014) Retardation of reproduction in the red shiner due to electroshock. N Am J Fish Manage 34:463–470. https://doi.org/10.1080/02755947.2014.882454

Thomas P, Gulland F, Reeves R, Kreb D, Ding W, Smith B, Malik M, Ryan G, Phay S (2019) Electrofishing as a potential threat to freshwater cetaceans. Endang Species Res 39:207–220. https://doi.org/10.3354/esr00962

Wahl D, Einfalt L, Callahan S (2007) Effects of electroshock on bluegill feeding and susceptibility to predation. N Am J Fish Manage 27:1208–1213. https://doi.org/10.1577/M06-138.1

Wang H, Meng Z, Liu F, Zhou L, Su M, Meng Y, Zhang S, Liao X, Cao Z, Lu H (2020a) Characterization of boscalid-induced oxidative stress and neurodevelopmental toxicity in zebrafish embryos. Chemosphere 238:124753. https://doi.org/10.1016/j.chemosphere.2019.124753

Wang H, Wang J, Bo G, Wu S, Luo L (2020b) Degradation of pollutants in polluted river water using Ti/IrO_2-Ta_2O_5 coating electrode and evaluation of electrode characteristics. J Clean Prod 273:123019. https://doi.org/10.1016/j.jclepro.2020.123019

Wang Q, Lai NL, Wang X, Guo Y, Lam PK, Lam JC, Zhou B (2015) Bioconcentration and transfer of the organophorous flame retardant 1, 3-dichloro-2-propyl phosphate causes thyroid endocrine disruption and developmental neurotoxicity in zebrafish larvae. Environ Sci Technol 49:5123–5132. https://doi.org/10.1021/acs.est.5b00558

Yan C, Wang M, Ma T, Yang S, Kong M, Shen J, Yang L, Gao Y (2020) Study on the experimental performance by electrolysis-integrated ecological floating bed for nitrogen and phosphorus removal in eutrophic water. Sci Rep 10:7619. https://doi.org/10.1038/s41598-020-64499-y

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.