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Life history traits of low-toxicity alternative bisphenol S on *Daphnia magna* with short breeding cycles: A multigenerational study

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ABSTRACT

Due to relatively lower toxicity, bisphenol S (BPS) has become an alternative to previously used bisphenol A. Nevertheless, the occurrence of BPS and its ecological impact have recently attracted increasing attentions because the toxicology effect of BPS with life cycle or multigenerational exposure on aquatic organisms remains questionable. Herein, *Daphnia magna* (*D. magna*) multigenerational bioassays spanning four generations (F0–F3) and single-generation recovery (F1 and F3) in clean water were used to investigate the ecotoxicology of variable chronic BPS exposure. For both assays, four kinds of life-history traits (i.e., survival, reproduction, growth and ecological behavior) were examined for each generation. After an 18-day exposure under concentration of 200 μg/L, the survival rate of *D. magna* was less than 15 % for the F2 generation, whereas all died for the F3 generation. With continuous exposure of four generations of *D. magna* at environmentally relevant concentrations of BPS (2 μg/L), inhibition of growth and development, prolonged sexual maturity, decreased offspring production and decreased swimming activity were observed for the F3 generation. In particular, it is difficult for *D. magna* to return to its normal level through a single-generation recovery in clean water in terms of reproductive function, ecological behavior and population health. Hence, multi-generational exposure to low concentrations of BPS can have adverse effects on population health of aquatic organisms with short breeding cycles, highlighting the necessity to assess the ecotoxicology of chronic BPS exposure for public health.

1. Introduction

Bisphenol A (BPA) is restricted or prohibited in the United States, Canada, China and other countries due to its genotoxicity, reproductive toxicity, endocrine disrupting effects and neurotoxicity (Liu et al., 2021). Bisphenol S (BPS), as the main substitute of BPA, has been wildly used in the manufacture of food packaging, feeding bottles and thermal paper (Choi and Lee, 2017; Mustieles et al., 2020). According to the data from the European Chemicals Agency, BPS output in Europe has reached 1000–10,000 tons/year (ECHA, 2015). In terms of molecular structure, BPS replaces the methyl group of two phenolic rings in the structure of BPA by sulfone, resulting in longer persistence, accumulation risk and specific toxicity mechanism of BPS (Chen et al., 2016; Usman and Ahmad, 2016). At present, with the increase of the detection frequency and concentration of BPS in environmental media (Liu et al., 2021), its ecological safety is worthy of further study.

BPS has been wildly detected in foodstuffs, paper products, indoor dust, surface water, sediment samples, sludge, sewage, aquatic organisms and human urine (Sun et al., 2017; Vasiljevic and Harner, 2021). In surface water, concentrations of BPS were generally between dozens of ng/L and hundreds of ng/L (Catenza et al., 2021), which is comparable to BPA, but the concentration levels of BPS in sediment samples were

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much lower than those of BPA (Wu et al., 2018). Compared with South Korea, Japan, and India, the pollution level of BPS in aquatic environment of China was at a medium level, and the average concentrations of BPS were between 1.1–135 ng/L in surface water and 0.0073–4.1 ng/g dw in sediment (Liu et al., 2021). Meanwhile, BPS pollution was the most serious in surface water of Indian, reaching to 7200 ng/L (Yamazaki et al., 2015). In China, the study area of BPS pollution is mainly concentrated in the Taihu Lake, Liaohe River and Pearl River basins, where the detection frequency of BPS in water and sediment samples was 100 %, and the highest concentration in water was 1600 ng/L (Jin and Zhu, 2016). Interestingly, the average concentrations of BPS in water and sediment samples of the Taihu Lake increased 20- and 26-fold from 2013 to 2016, respectively (Yan et al., 2017). This results further indicate that BPS has been widely used as an alternative of BPA, and its environmental health and ecological risks require continuous attention.

Toxicological studies on BPS mainly focus on endocrine disruption, immune system disorders, neurotoxicity, and reproductive developmental effects (Mokra et al., 2017; Qiu et al., 2019). The acute toxicity data indicated that BPS belonged to low toxicity chemicals (55 mg/L $\leq$ L(E)C_{50} $\leq$ 361 mg/L), which was significantly lower than BPA (5.7 mg/L $\leq$ L(E)C_{50} $\leq$ 19.6 mg/L) (Liu et al., 2021). Similar to BPA, BPS has estrogenic and anti-androgenic effects, with 32 % and 25 % of toxicity of BPA, respectively (Rochester and Bolden, 2015). BPS can also alter the levels of steroid hormones and thyroid hormones in zebrafish (Roelofs et al., 2015), is 30-fold less toxic to zebrafish embryo hatchability than BPA (Moreman et al., 2017). Although the toxic effects of BPS on reproductive development and neurotoxicity are lower than those of bisphenols such as BPA, BPAF, and BPF (Liu et al., 2021), the current studies mainly focused on the short-term or single-generation toxic effects of BPS on aquatic organisms. Whereas the ecological risk of long-term or multi-generation exposures has not been carried out, which deviates from the actual pollution stress characteristics of BPS in aquatic environment. Given current status of BPS pollution, the generational exposure risk of aquatic organisms to BPS is underestimated, especially for those with short breeding cycles.

Based on earlier knowledge on short-term exposure of vertebrates (e.g., fish and frogs) and acute exposure of aquatic animals to BPS on the reproduction, gene expression and estrogen effects (Liu et al., 2021), a toxicological study on multi-generational exposure of aquatic organism with short breeding cycles to BPS will be carried out. Specifically, *Daphnia magna* is selected as the model organism and an environmental concentration of BPS (2 μg/L) is used as the initial exposure concentration. The objectives of this study are to explore 1) effect of BPS on the survival of *D. magna*, 2) toxicity of BPS to reproduction, growth and other ecological behaviors of *D. magna*, and 3) self-recovery capability of *D. magna* after multi-generational exposure to BPS. The obtained knowledge is expected to shed light on the assessment of ecotoxicology of BPS and BPA substitutes to aquatic organisms with short breeding cycles.

2. Materials and methods

2.1. Experimental reagents

BPS (CAS: 80-09-1, purity > 98 %) was purchased from J&K Scientific Ltd. (Shanghai, China). Ethanol, acetone and methanol (chromatographic purity) were purchased from Merck (Darmstadt, Germany). BPS stock solution of 1 g/L was prepared with ethanol as the solvent and stored at − 20 °C in dark for preparation every two weeks. The exposure solution was obtained by stepwise dilution of 1 g/L BPS, and the solvent concentration in each exposure group did not exceed 0.1 % ethanol (v/v). No immobilization and mortality was observed in solvent control (0.1 % ethanol).

2.2. Test organism

D. magna was provided by the Wuhan Institute of Hydrobiology, Chinese Academy of Sciences. According to OECD TG 211 guidelines (OECD, 2012), *D. magna* was acclimated in laboratory for 14 days before the exposure experiments. Briefly, the acclimation was conducted in the drinking water after 24 h of aeration under 21 ± 1 °C with light/dark cycle of 16–8 h and light intensity of 1000 lux. *D. magna* were fed with *Scenedesmus obliquus* at a feeding density of 3×10^5 cells/mL.

2.3. Chronic experimental settings

In the multi-generation chronic experiment, a total of 4 experimental groups were set up, including blank control (medium), solvent control group (with 0.1 % ethanol in the medium), low concentration exposure group of BPS (2 μg/L, measured concentration of 1.93 ± 0.38 μg/L) and high concentration exposure group of BPS (200 μg/L, with actually measured concentration of 195.2 ± 4.23 μg/L). Each experimental group was performed 20 replicates, which were composed of 50 mL exposure solution and a 6–24 h neonate (F0 generation) respectively. After the F0 generation given birth to the first generation of neonate, the parent and neonate *D. magna* were separated in time (within 12 h), and the isolated neonates of the F1 generation were exposed to the same exposure conditions as the F0 generation, until they were exposed to the F3 generation. Each generation was exposed for 21 days. All experimental beakers were sealed with transparent plastic wrap to reduce water evaporation as much as possible, and several small holes were opened on the plastic wrap to meet oxygen demand of *D. magna*. All experimental groups were placed in an intelligent light incubator with a 16–8 h light/dark cycle and temperature of 21 ± 1 °C. The tested parameters, including intrinsic rate of natural increase (r), time to first brood (TFB), number of first brood per female (NFBF), brood times (BT), total number of offspring (TNO) and number of offspring per brood per female (NOBF) of *D. magna* were recorded every day during 21 days. During the exposure period, the exposure solution was changed and the beaker was cleaned every 2 days. *D. magna* were fed with fresh *Scenedesmus obliquus* (3×10^5 cells/mL) every day. After the 21-day exposure of each experimental group, the body length (from the top of the head to the bottom of the caudal vertebra) (BL), heartbeat (HR), thoracic limb beat (TLB), swimming velocity (V) and accelerated velocity (AV) of *D. magna* were measured under the microscope with a video camera.

2.4. Recovery experiments

The recovery experiment of clean water was performed for the F1 and F3 generation neonate produced by the F0 and F2 generations. The recovery period was 21 days. During the recovery process, the exposure conditions were the same as those of the experimental group of BPS the exposure solution, which was replaced with clean water. The measurement of all indicators during the recovery experiment was exactly the same as that of the chronic experimental group.

2.5. Analysis of physiological and behavior indexes

Refer to Yang et al. (2018), one *D. magna* was placed on a slide containing 50 μL of exposure solution, and cotton wool fibers were used to restrict the movement of the *D. magna*. Microscopic views (> 1 min) of the tested *D. magna* were recorded with a digital camera (EOS 1500D, Canon, Japan). The software Tracker® was used to analyze the HR and TLA at 30 frames per second. Six replicates were determined for each exposure group.

For swimming activity, the exposed *D. magna* was placed in a petri dish with a diameter of 5 cm and allowed to adapt for 5 min. Then a one-minute video was recorded for the swimming activity under light intensity of 1000 lux, and the software Tracker® was used to analyze the swimming trajectory, speed and acceleration of *D. magna* by frame-by-

frame method.

2.6. Data analysis

The intrinsic rate of natural increase (r) is a comprehensive indicator for evaluating the reproductive toxicity of pollutants to the tested organisms (Lu et al., 2017). The r value of *D. magna* was calculated by using Euler equation: $\sum e^{-r_x} l_x m_x = 1$. Where x is the age of *D. magna*, l_x is the survival rate of *D. magna* on the x day and m_x is the number of offspring by each *D. magna* on the x day. The exact value of r was solved by the stepwise approximation method, which is calculated by combining EXP function and SUM function in Excel function library.

The ggplot2 package in R was used for Pearson correlation analysis. Statistical differences among data sets were expressed as mean ± standard deviation (SD). A one-way ANOVA followed by Tukey's post hoc test was used to analyze the significance level between the treatments. Significant differences were expressed as $p < 0.05$ (*) and $p < 0.01$ (**).

3. Results and discussion

3.1. Survival rates

During the exposure period, the significant differences of tested endpoints were not presented in the blank control and solvent control groups. In this study, the solvent control group was used as the reference group for data analysis. In the control group, the mortality rates of *D. magna* during the exposure period were lower than 10 %, except that the mortality rate of *D. magna* F3 generation reached 15 % on the 19 days (Fig. 1A). For Low concentration group (2 μg/L), significant difference of the survival rate was not observed in F0–F3 generations of *D. magna* (Fig. 1B). For high concentration group (200 μg/L), the survival rate of *D. magna* was clearly decreased with the increase of exposure generations. In high concentration group, the survival rate of F0 and F1 generations *D. magna* did not changed significantly (mortality rate < 10 %), decreased to less than 75 % on the day 6 and 15 % on the day 18 of the F2 generation, and was less than 65 % on the day 5 and all died on the day 18 of the F3 generation (Fig. 1C).

Previous study showed that after exposure to 10 mg/L BPS for 21 days, no adverse effects on the survival of single generation *D. magna* was observed (Park et al., 2019). In our study, the exposure concentrations of BPS (2 and 200 μg/L) were much lower than that of 48 h-LC_{50} (55 mg/L) for *D. magna* (Moreman et al., 2017), but the survival rates of *D. magna* were clearly inhibited by BPS with the increase of exposure generations and exposure concentrations. These data indicated that low toxic compound BPS can have distinct negative effects on aquatic organisms through a short-term of multi-generational exposure, especially for organisms with short breeding cycles.

3.2. Reproduction performance

In this study, the solvent control group was still selected for data comparison analysis. Survival rates of F2 and F3 generation *D. magna* exposed to high concentration group of BPS (200 μg/L) exceeds 20 %, reproductive and developmental toxicity data will not be discussed for the high exposure group in the following content.

Reproductive functions are important parameters to characterize the population health of *D. magna*, in which the TFB and the NOBF characterize the speed of maturation and population fecundity of *D. magna* (Blewett et al., 2017). As shown in Fig. 2, both low and high exposure groups of BPS had no obvious interference effect on the six reproductive indicators of the F0 generation. When exposed to the F1 generation, 200 μg/L BPS significantly delayed the sexual maturation time (Fig. 2A), clearly decreased the BT, the TNO and the NOBF (Fig. 2C–E), and eventually induced a markedly reduction in reproductive ability (Fig. 2F). For F1 generation exposed to low concentration group (2 μg/L), BPS had no significant effect on all reproductive indexes of *D. magna*. However, when exposed to F2 generation, the biotoxic effects of 2 μg/L of BPS began to appear, the TNO was significantly inhibited after 21 days exposure (Fig. 2D). Compared with the F2 generation, the inhibitory effect of 2 μg/L BPS on the reproductive ability of *D. magna* F3 generation was more obvious. Low concentration BPS (2 μg/L) significantly prolonged the sexual maturation time, and clearly reduced the BT and the TNO and the r of *D. magna* F3 generation. The NFBF in all exposure groups and NOBF in low concentration group were not obviously changed (Figs. 2B and 2E).

After comparing the effects of high and low concentration groups on reproduction for the F0-F3 generations, no obvious toxicological effect of BPS was found on the reproduction for the F0 generation of *D. magna*. However, the high concentration group of BPS exhibited obvious reproductive inhibitory effect for the offspring of F1 generation, and caused the deaths of the large number of the offspring of F2 generation (Fig. 1C). At environmental concentration of BPS (2 μg/L), the inhibition effects of reproduction in *D. magna* only begins in the F2 generation, and the population reproduction capacity (r) of *D. magna* was significantly inhibited by BPS from the F3 generation. The above data suggest that low-toxicity pollutants can produce high reproductive toxicity to aquatic organisms with short breeding cycles through multi-generations of exposure, which is easy to underestimate if they are only exposed through single-generation or short-term toxicological experiments.

Under the stress of environmental concentration pollutants, aquatic organisms will develop a certain tolerance to pollutants, and this tolerance will last for 1–2 generations (Zhou et al., 2016). With the prolongation of exposure time, aquatic organisms with short breeding cycles are most susceptible to the double pressure of direct exposure toxicity and genotoxicity of pollutions (Guan and Wang, 2006; Xie et al., 2019). For example, the reproduction rate of *D. magna* exposed to 100 μg/L of tetracycline decreased generation by generation, the reproduction rate of the F0 generation decreased by 16.6 %, while that

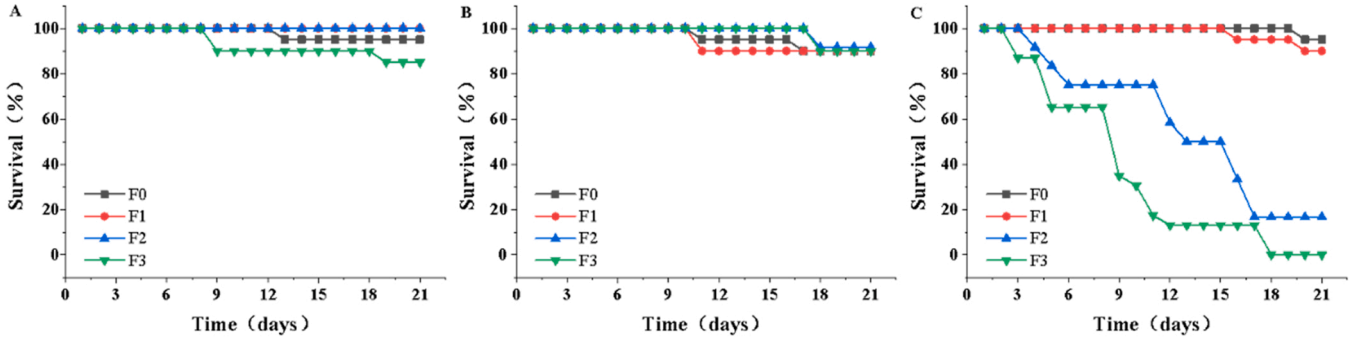

Fig. 1. Survival rates of F0-F3 generation *D. magna* exposed to BPS for 21 days. A, B and C, indicates the solvent control group, BPS low concentration group (2 μg/L) and BPS high concentration group (200 μg/L), respectively.

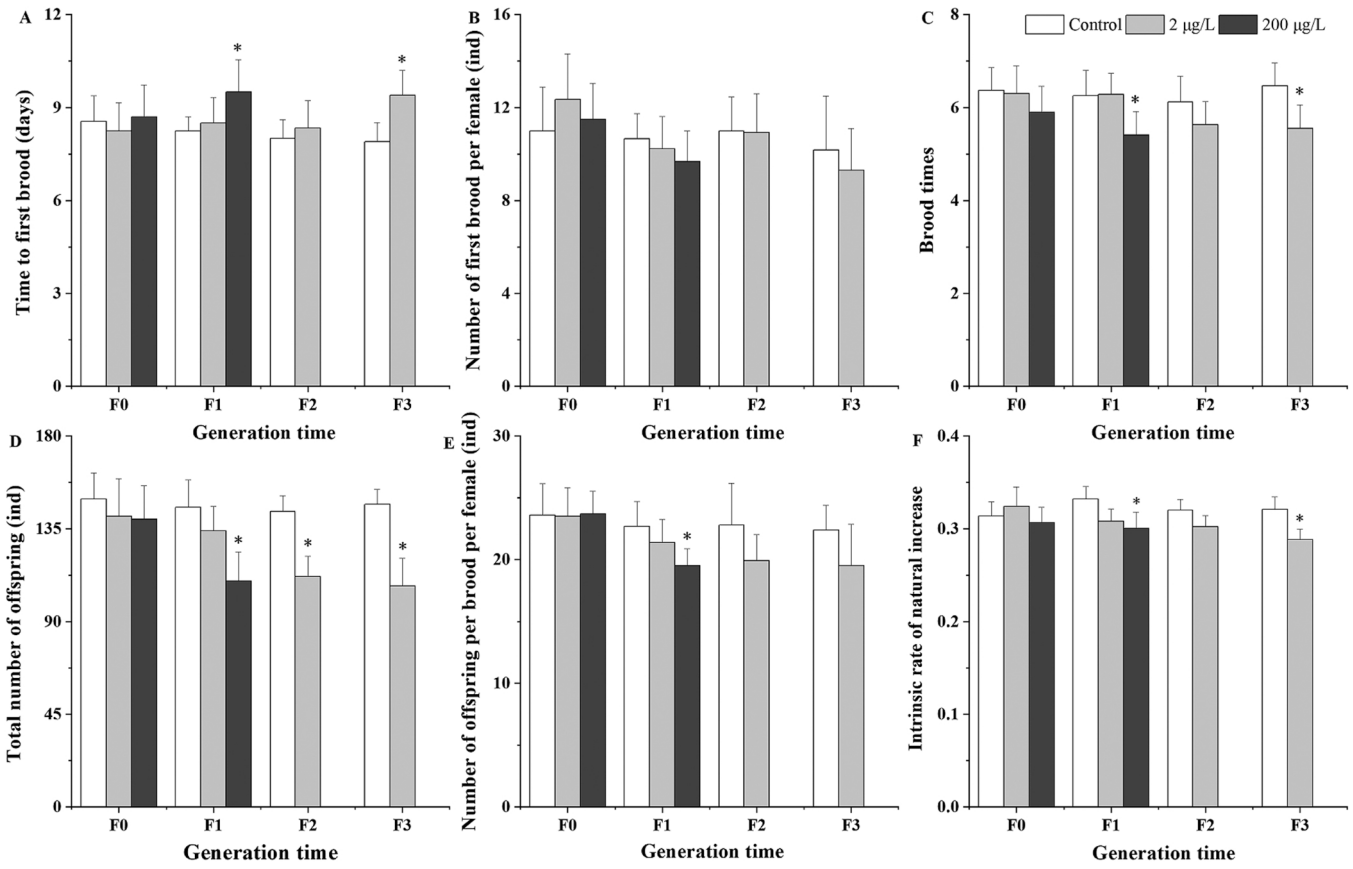

Fig. 2. Reproductive parameters of F0-F3 generation *D. magna* exposed to BPS for 21 days. (A) Time to first brood (TFB), (B) number of first brood per female (NFBF), (C) brood times (BT), (D) total number of offspring (TNO), (E) number of offspring per brood per female (NOBF) and (F) Intrinsic rate of natural increase (r). Data are expressed as the mean ± SD. * expresses significant difference ($P < 0.05$).

of the F3 generation decreased by more than 60 % (Kim et al., 2012). For single generation exposure, the reduction of spawning and sperm counts in zebrafish exposed to BPS of 10 μg/L did not occur until 75 days (Naderi et al., 2014). At low environmental concentration levels, multiple generations of continuous exposure of aquatic organisms may enhance the reproductive toxicity of bisphenols (Sohoni et al., 2001).

The r is a comprehensive indicator that characterizes the health of the population based on parameters such as species fecundity, maturity time, and survival rate (Sancho et al., 2018). With the increase of exposure generations, the population health of *D. magna* continued to decline, especially the significant decrease of r in F3 generation exposed to 2 μg/L of BPS and in F1 generation exposed to 200 μg/L of BPS (Fig. 2F). Correlation analysis showed that the r of F0-F3 generation exposed to environmental concentration was significantly negatively correlated with the time to first brood (TFB) ($R^2 = 0.70$, $P < 0.05$), and was significant positive correlated with the TNO and NOBF, respectively ($R^2 = 0.83$, $P < 0.05$ and $R^2 = 0.91$, $P < 0.05$) (Fig. 3). The results indicated that the reduction of population health caused by the delay of sexual maturity and the decrease of NOBF under BPS stress. Long-term exposure of *Moina macrocopa* to BPS could lead to its significant reproductive retardation (Park et al., 2019). Previous studies have found that BPS can alter the level of estradiol in plasma and the normal development of oocytes by affecting the feedback regulatory loop of the hypothalamic-pituitary-gonadal axis (HPG) in *Caenorhabditis elegans* (Campen et al., 2018; Xiao et al., 2019), resulting in delayed hatching and reduced hatchability in zebrafish (Ji et al., 2013). BPS can also disrupt the endocrine system by regulating the hormone signaling pathway-related genes of *Diaphanosoma celebensis*, thereby delaying the emergence time of the first offspring (Cho et al., 2022). Long-term exposure of *D. magna* to BPA and its metabolites can cause insufficient

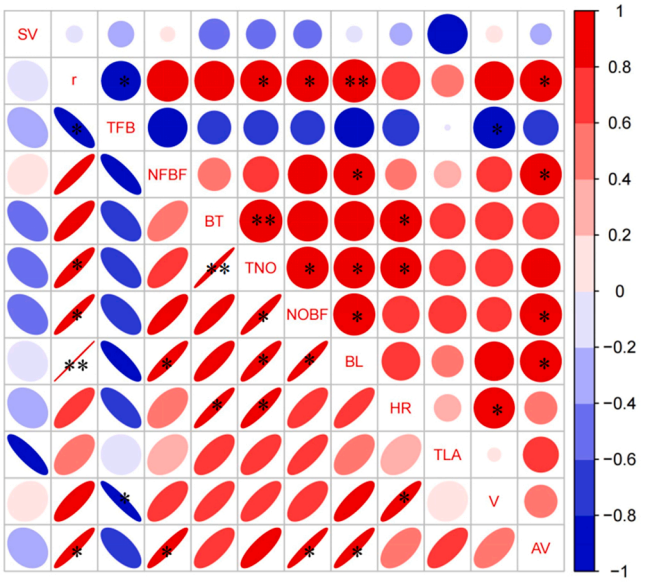

Fig. 3. The values of Pearson's correlation coefficient between tested parameters, including survival rate (SV), r, TFB, NFBF, BT, TNO, NOBF, BL, HR, TLA, V and AV. Significant correlations ($P < 0.05$) are indicated by *, and significant correlations ($P < 0.01$) are indicated by * *.

reproductive energy supply and inhibit its reproductive function, which is more severe in the multi-generation exposure of aquatic organisms (Li et al., 2018). In our study, the reproduction time of *D. magna* from F0 to

F3 generation was about 21 days, which was similar to the exposure time of single generation, however, the toxicological effects of multiple generations exposed to environmental relevant concentration of BPS were much higher than those of single generations of exposure on the same days.

3.3. Growth, development and other ecological behaviors

The characteristics of *D. magna* are rapid development and short growth cycle, and the development process is susceptible to environmental stress. High concentration group of BPS significantly inhibited the BL, HR, TLA, V and AV of *D. magna* F0 and F1 generations, and the maximum inhibition rates reached 21 %, 18 %, 16 %, 65 % and 64 %, respectively (Fig. 4). Low concentration of BPS significantly inhibited the HR and swimming activity of *D. magna* F0–F3 (Figs. 4B, 4D and 4E) and the BL of *D. magna* F1–F3 (Fig. 4A), but had no obvious interference effect on the thoracic limb beating frequency of *D. magna* during the whole exposure period (Fig. 4C).

The HR and the TLA have been frequently used to reflect the stress of *D. magna* exposed to bisphenols, which are closely related to respiration, feeding and metabolic functions (Lovern et al., 2007; Liu et al., 2020). The significant inhibitory effect of HR in *D. magna* was observed in the F0 generation at 2 μg/L, and the inhibition rate of BPS on the HR increased continuously with the increase of exposure generations, and the maximum inhibition rate was up to 22 % (Fig. 4B). However, the TLA only had a significant inhibitory effect at high concentration BPS exposure, no significant change was found in the low concentration group (Fig. 4C). Heartbeat is an indispensability energy source for the activities of the thoracic limbs. Thoracic limbs beating is not only the main way of feeding, but also completes the exchange of air between the *D. magna* and the external environment (Liu et al., 2020). In view of the important roles of heartbeat and thoracic limb beating, we speculated that the dual inhibition of two indicators the main cause of the death of F2 generation *D. magna* exposed to high concentration of BPS. The low mortality rate of F0–F3 generations *D. magna* exposed to the environmentally relevant concentration of BPS is probably profit from the normal TLA, which ensured the normal energy acquisition path of *D. magna* (Lari et al., 2017).

The BL is an essential indicator of the individual health of *D. magna*, small changes can sensitively characterize the population stability of the species. The BL of *D. magna* exposed to 2 μg/L of BPS for 21 days had no obvious change in the F0 generation, but which were significantly reduced in the BL of the offspring (F1–F3 generations) (Fig. 4A). Previous results were also found in BPA toxicity studies, where long-term generational exposure to 1140 μg/L of BPA could significantly reduce the body size of offspring *D. magna*, but had no effect on the parental generation (Chen et al., 2021). Similarly, the inhibition rate of 53.2 μg/L pentachlorophenol on the BL of *D. magna* increased from 6.8 % in F0 generation to 21.9 % in F2 generation (Chen et al., 2014). BPS can significantly reduce the feeding rate of *D. magna* by inhibiting thoracic limb beating, and cause growth inhibition and developmental retardation (Liu et al., 2019). The inhibitory effect of phenolic compounds on the content of thyroid hormones (T3 and T4) may be the main cause of growth retardation and BL reduction of *D. magna* (Chen et al., 2021; Qiu et al., 2018; Zhang et al., 2018).

Changes in swimming behavior of *D. magna* are a comprehensive manifestation of their physiological, sensory, neural and muscular system regulation, which are usually characterized by V, AV and trajectory (Parolini et al., 2018). The results showed that the significant inhibition of swimming activity by environmentally related concentrations of BPS became more serious with the increase of exposure concentration and exposure generation. The inhibition rates of V and AV of *D. magna*

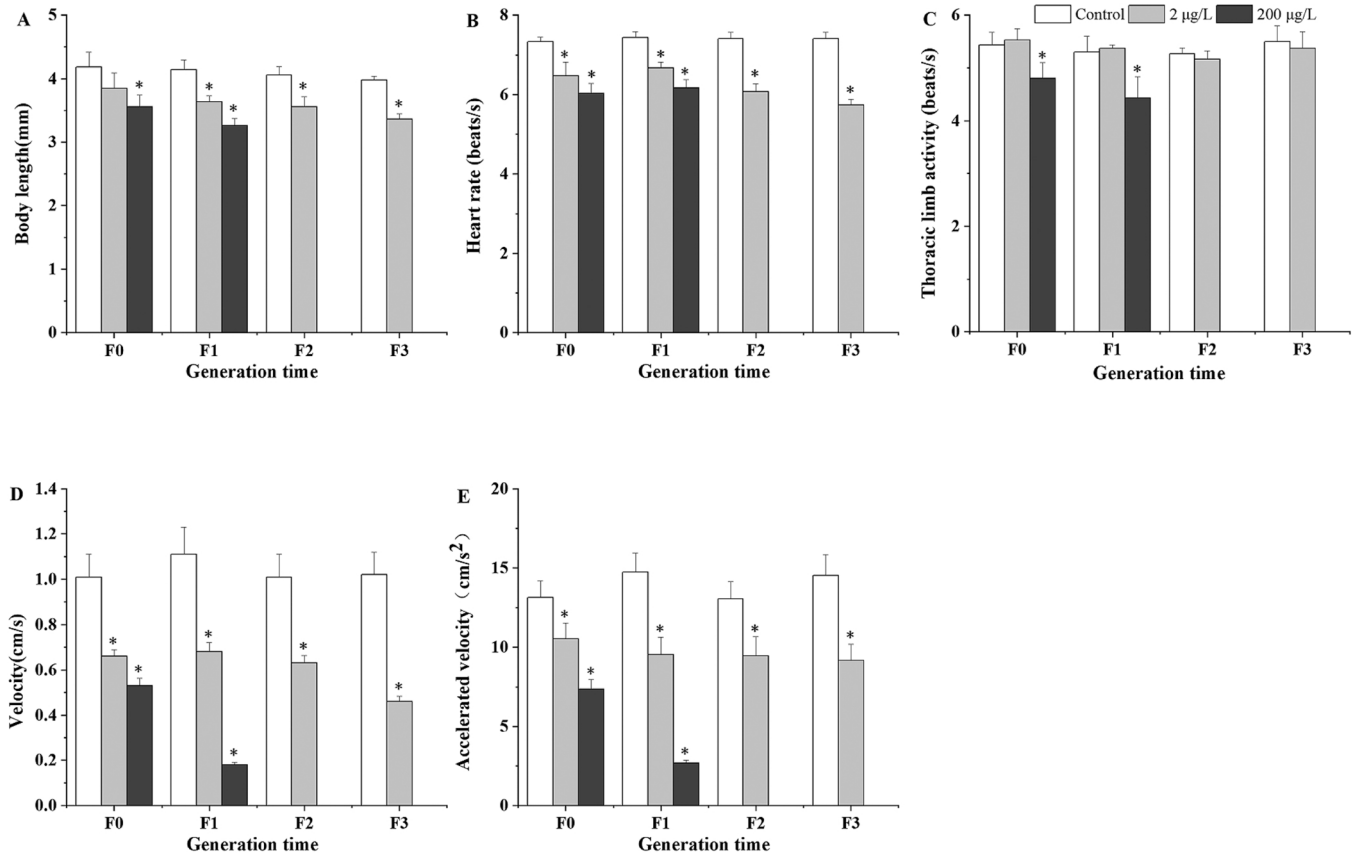

Fig. 4. Changes in (A) Body length (BL), (B) heart rate (HR), (C) thoracic limb activity (TLA), (D) velocity (V), and (E) accelerated velocity (AV) of F0–F3 generation *D. magna* exposed to BPS for 21 days. Data are expressed as the mean ± SD. * expresses significant difference ($P < 0.05$).

exposed to 2 μg/L BPS increased from 35 % (F0) to 55 % (F3) and 13 % (F0) to 37 % (F3), respectively (Figs. 4D and 4E), and the swimming trajectories of *D. magna* exposed to 2 μg/L BPS decreased obviously in F3 generation (Fig. 5). Previous study found that BPS can disturb the acetylcholinesterase activity and the expression of genes related to nervous system differentiation in *D. magna* (Liu et al., 2019), cause damage of the central nervous system and brain cells of zebrafish (Gyimah et al., 2021), and eventually lead to decreased swimming activity. The reduction of swimming activity will weaken the predatory ability of *D. magna* to some extent, increase its risk of predation, and threaten the stability of the biological population (Bownik, 2017).

The correlation analysis between reproduction and growth indicators is shown in Fig. 3. For reproductive indicators, TNO was negatively correlated with TFB and significantly positively correlated with BT. As a comprehensive index to characterize the toxic effects of pollutants on the fertility of *D. magna* population, r is not only correlated with reproductive indicators, but also positively correlated with BL. The BL change of *D. magna* is also a defense mechanism of organisms under environmental stress. Hence, even subtle changes in BL may affect the population stability of *D. magna* (Imhof et al., 2017). HR was significantly positively correlated with most reproductive indicators, especially the TNO. Furthermore, the behavioral indicators of V and AV were also significantly correlated with most reproductive growth indicators. Previous studies have shown that inhibition of swimming behavior may have a negative impact on filtration activity and food uptake of *D. magna*, which can lead to a decrease in growth and reproduction (Parolini et al., 2018). The above results also confirmed that involving multiple generations of chronic exposure allows a more effectively assessment of the eventual ecological hazard of BPS to aquatic receptors at different exposure periods.

3.4. The self-recovery ability of D. magna after BPS exposure

In the actual aquatic environment, pollution sources are generally discharged discretely, and aquatic organisms are in an alternating state of pollution stress and health recovery. To understand the health recovery of *D. magna*, we performed clean water recovery experiments on F1 and F3 generation offspring. Compared with F0 exposure group, the survival rate of *D. magna* exposed to 2 μg/L of BPS in F1 recovery group increased by 3.3 % (from 90 % to 93.3 %), while the survival rate of F3 generation recovery group was 5.5 % lower than that of F2 generation exposure group (Fig. 6A). In the high concentration exposure group (200 μg/L), the survival rate of *D. magna* decreased significantly after three generations of BPS exposure (F0–F2 generations), and increased more than three times after one generation of recovery (F3-recovery), but did not return to the normal level (Fig. 6B).

Studies have shown that reproductive parameters are more sensitive than survival parameters under exposure to sublethal concentrations (Villarroel et al., 2003; Luo et al., 2008). All the reproductive indexes of *D. magna* exposed 2 μg/L of BPS at the F0 generation were not obviously changed, however the r value of *D. magna* showed a significant reduction in the F1 recovery group of 2 μg/L BPS (Fig. 7A). With the increase of exposure concentration, the impacts of BPS on the offspring of *D. magna* increased, the BT, TNO and NOBF showed a continuous decreasing trend in the F1 recovery group of 200 μg/L BPS (Fig. 7B). With the increase of exposure generation (F0–F2), the reproductive recovery ability of *D. magna* exposed to 2 μg/L BPS was similar to that of 200 μg/L BPS of F0 generation (Fig. 7C). The recovery rates of the BT, TNO and NOBF for F3 generation exposed to 2 μg/L BPS ranged from 82 % to 88 %, compared with 83–93 % of recovery rates for F0-offspring exposed to 200 μg/L BPS. These results indicated that environmental relevant concentration of BPS by three generations exposure could produce reproductive damage similar to high concentration (200 μg/L) of BPS by single generation exposure, and it is difficult to return to normal level. Previous study also found that when 10 μg/L triethyl butylphosphate was exposed to two successive generations for 21 days, the reproductive function of *D. magna* of the F0-F1 generation could not be improved after being recovered by the F2 generation with clean water (Giraudo et al., 2017).

In the growth and development indicators of *D. magna*, the BL and HR always returned to normal level after F0 and/or F0–F2 generations of BPS exposure (Fig. 7A–C). As a non-sensitive index, the TLA was not obviously changed in the F0-F3 generations of 2 μg/L BPS exposure, however, the inhibitory effect of TLA was not effectively recovered in the F1-200 μg/L recovery group (Fig. 7B). Swimming activity was significantly inhibited by BPS at all exposure groups of BPS. The inhibitory effect of swimming activity did not recover clearly at the F1-2 μg/L recovery and F3-2 μg/L recovery groups, and the swimming ability was further weakened in the F1-200 μg/L recovery group (Fig. 7B). Long-term exposure of *D. magna* to BPS may lead to genetic and/or epigenetic changes across generations and maintain symptoms during development (Chen et al., 2018).

The above results also indicate that the health damage of *D. magna* caused by longer exposure time and/or higher exposure concentration is difficult to recover. Previous study found that the health recovery of organisms caused by pollutants is related to the recovery time (Gonçalves et al., 2018). For example, after two generations of exposure to tris (2-butoxyethyl) phosphate, the reproductive function of *D. magna* did not improve effectively through only one generation of clean water recovery (Giraudo et al., 2017). However, after a 60-day exposure to tris (1, 3-dichloro-2-propyl) phosphate, the BL and gene expression of *Tetrahymena thermophila* basically returned to the level of the control

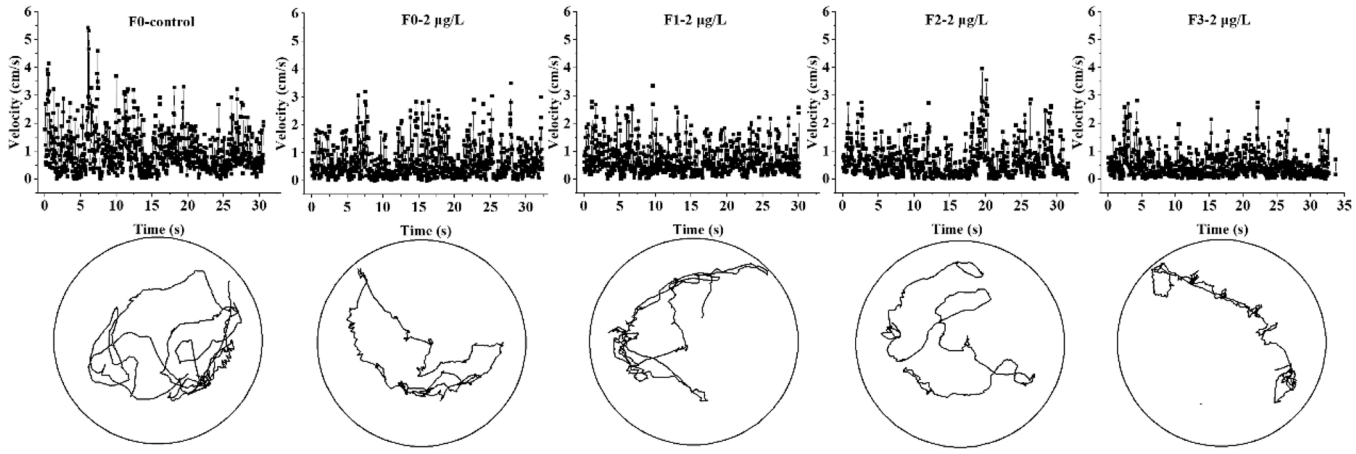

Fig. 5. The velocities and trajectories of the movement of F0-F3 generations *D. magna* exposed to 2 μg/L of BPS. A–E indicates F0-control, F0–2 μg/L, F1–2 μg/L, F2–2 μg/L and F3–2 μg/L, respectively.

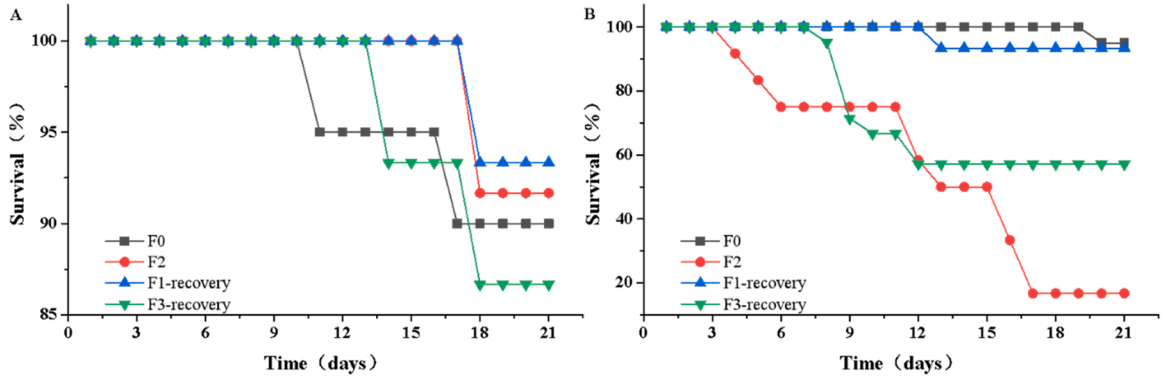

Fig. 6. Survival rates of *D. magna* after clean water recovery. A and B indicates BPS low concentration group (2 μg/L) and BPS high concentration group (200 μg/L), respectively.

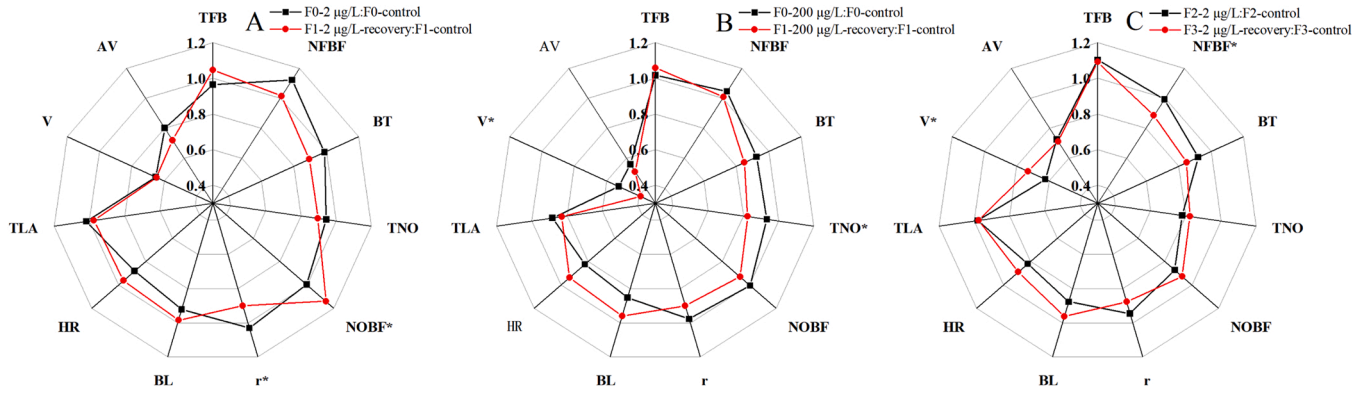

Fig. 7. Changes in reproduction, growth and behavior of *D. magna* after clean water recovery. The tested parameters, including r, TFB, NFBF, BT, TNO, NOBF, BL, HR, TLA, V and AV. Black indicates the ratio of each index between the exposure group and the corresponding control group, while red indicates the ratio between the recovery group and the corresponding control group. * expresses significant difference between the black and red groups ($P < 0.05$).

group through 60 days of clean water recovery (Li et al., 2016). The reproductive and growth indicators of *D. magna* exposed to cadmium (Cd) for 6 generations were basically restored to the control level after 2 generations of recovery (Wang et al., 2018). However, if the recovered *D. magna* is again exposed to the Cd solution, its reproductive and growth indicators will rapidly decrease to the damage situation of continuous exposure for six generations (Guan and Wang, 2006). In the aquatic environment, low toxic substances BPS are frequently discharged, and the occurrence level keeps increasing (Liu et al., 2021). The ecological risks of such BPS based on the short-term exposure of toxicological data could be underestimated for aquatic organisms. Our results suggested that a bioassay involving four generations is sufficient for tolerance of *D. magna* with short breeding cycles to low toxic BPS.

4. Conclusions

This study has demonstrated that multi-generational exposure of *D. magna* to BPS resulted in abnormal development, reproductive derangement and disturbance of ecological behaviors. The multi-generational bioassays after four consecutive 21-day chronic trials indicated that a single 21-day of BPS exposure at both high and low exposure concentrations did not cause fitness defects. However, with the increase of the number of exposure generations, the negative effect on the growth and reproduction of *D. magna* gradually appeared, including increased mortality, inhibition of growth and development, prolonged sexual maturity, decreased offspring production growth and decreased swimming activity, resulting in the death of all the F3 generation exposed to 200 μg/L BPS. Moreover, it is difficult for *D. magna* to recover its reproductive function and ecological behavior to normal levels after three generations of exposure to BPS at environmentally relevant concentration of 2 μg/L. Given a remarkably increasing use of BPS in industries, future work is necessary to unravel the mechanism for the toxicity of BPS on the growth and reproduction of plankton.

CRediT authorship contribution statement

Yixuan Zhang and Jianchao Liu: Writing – original draft, Methodology, Investigation. **Chenyang Jing and Guanghua Lu:** Writing – review & editing, Data curation. **Runren Jiang and Xiqiang Zheng:** Investigation, Software. **Chao He and Wenliang Ji:** Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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