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论文题目: Effect and Mechanism of Ultrasound on Killing *Chironomus* kiiensis' Eggs Clutch

Effects and Mechanism of Ultrasound Treatment on *Chironomus kiiensis'* Eggs Clutch

刘博栋

Abstract:

Chironomids are abundant insects in freshwater ecosystems and spawn in still or slow-moving water. The walls of sedimentation tanks in drinking water treatment plants (DWTP) provide such spawning habitat, which can lead to larval outbreaks in plant effluent. While chironomid larvae are often associated with poor hygiene, effective methods to control outbreaks are needed. Here, we assessed the effect of ultrasound treatment on *Chironomus kiiensis*' eggs. The mortality rate of eggs was examined after ultrasound treatment, and the protein content (heat shock protein 70 and hemoglobin) and enzymatic activities (acetyl cholinesterase, cytochrome P_{450}, and glutathione S-transferases) involved in the ultrasound-induced stress response were analyzed before and after treatment. COMSOL software was also used to examine the characteristics of the ultrasonic field, including frequency, power, and exposure distance and time. Higher egg mortality was observed at lower frequencies. At 28 kHz, 450 W, 15 mm exposure distance, and 75 s exposure time, 72.4% of eggs showed apoptosis after exposure. At higher frequencies (68 kHz), mortality decreased to 50.9%. Exposure time and distance also significantly affected egg mortality. From the geometric models, it could be seen that *C. kiiensis*' eggs sustained much greater acoustic pressure (2379 Pa) with 28 kHz exposure than that with 68 kHz exposure (422 Pa); however, the propagation distance was greater at the higher frequency. The vibration effect of the ultrasonic radiation appeared to be the primary factor in egg mortality. Our results indicate that ultrasonic transducers embedded in the walls of water treatment plants could be effective in killing *Chironomus*' eggs and highlight the potential for ultrasound as an effective treatment for the prevention of *Chironomus* outbreaks in treatment plant effluents.

Keywords: ultrasonic treatment, Chironomidae, egg mortality, COMSOL

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1. Introduction

Chironomids, from the dipteran family Chironomidae (Sun *et al*., 2007), are widely distributed and are often the most abundant insects in freshwater habitats at temperate latitudes (Armitage *et al*., 1995). *Chironomus* species undergo a complete metamorphosis, from egg to larva, pupa, and adult (Khosrovyan *et al*., 2020). After the nuptial flight, adult chironomids spawn egg clutches on substrates with standing or slow-moving water. Thus, the side walls of horizontal sedimentation tanks in drinking water treatment plants (DWTP) are desirable habitat for *Chironomus* egg laying and hatching, leading to a boom in larval chironomids in plant effluent. There have been many serious incidents of *Chironomus* larvae pollution in water treatment systems in Britain and the USA (Bay *et al*., 1993; Alexander *et al*., 1997). Since it began operating in 2003, Nanzhou DWTP (the largest DWTP in south China) has been affected by *Chironomus* eggs and larvae (Supplementary Information Figure S3). Although there are no indications that the larvae pose a threat to public health, their presence is not appreciated because most people associate the presence of *Chironomus* larvae with poor hygiene (Bay *et al*., 1993).

Chemical oxidant reagents such as chlorine, hydrogen peroxide, and chlorine dioxide have been used to kill *Chironomus* eggs (Liu *et al*., 2004). For example, the spraying of hydrogen peroxide (10% v/v) was reported to be an effective method for egg removal (Zhang *et al*., 2001) but only when using high concentrations of oxidants. With chlorine-based reagents, chloric exposure (>2 mg L^{-1}) did not inhibit the hatching rate of *Chironomus* eggs (>95%). While high concentrations of oxidants can be effective for egg removal, any excess residual reagent can contribute to water quality degradation, limiting their application in drinking water processing

systems.

Ultrasound has proven effective in the inactivation of some microorganisms (Joyce *et al*., 2003; Koda *et al*., 2009) and the invasive mussel *Limnoperna fortunei* (Zhou *et al*., 2021). Because it involves sound energy and requires no other chemicals, ultrasound has been applied when bio-contamination is a concern (Wu *et al*., 2012). Except for a study by Lu *et al*. (2001), who reported invalid hatching with ultrasonic doses >10 mJ cm^{-2}, there are few studies, of which we are aware, that have attempted to control *Chironomus* eggs using ultrasound.

In this study, experiments were conducted at a bench-scale to evaluate the effect of ultrasound on *Chironomus kiiensis* eggs. Mortality rate, based on the integrity of the eggs, as well as heat shock protein 70 (HSP70), hemoglobin (Hb), acetyl cholinesterase (AChE), cytochrome P_{450} (P450), and glutathione S-transferases (GSTs) activities before and after ultrasound treatment, were examined to determine the apoptosis process of eggs after ultrasound treatment. Moreover, COMSOL was introduced to calculate the distribution characteristics of the ultrasonic field, including frequency, power, and exposure distance and time. The results presented herein offer insight into the efficiency and mechanism of *Chironomus* egg mortality with ultrasound treatment.

2. Materials and methods

2.1 *Chironomus* egg clutch collection and experimental design

Because *Chironomus* eggs hatch to larvae in only five hours, egg collection and experimentation were performed as soon as possible after laying. *Chironomus* eggs (Supplementary Information Figure S3) were collected from Nanzhou DWTP (ca. 23.05°N, 113.32°E), placed into a flask, and transported to the laboratory at 4°C, between May and July,

2021. Prior to experimentation, the viability of the eggs was examined using a microscope. *Chironomus* eggs with a broken cell wall were considered to be "dead." Egg clutches with >95% viable eggs were retained and placed into a urine cup (20 mL) with a lid. Each cup filled with water contained approximately 150 mg eggs wet weight, defined as one sample.

Experiments were performed in 40 L plastic tanks (Fig. 1) with an ultrasonic generator, ultrasonic transducer, and adjustable bracket (used to vary the power source distance to the samples). The four variables were ultrasonic power, ultrasonic frequency, and exposure distance and time, while the dependent variable was egg mortality (%), based on the integrity of the cell wall. To achieve the most effective parameter ranges, single-factor tests, Box-Behnken design (BBD), and response surface methodology (RSM) were used to optimize parameters for *Chironomus* egg mortality (detailed in Supplementary Information Text S1).

Based on these optimized parameters, the mortality (%) of *Chironomus* eggs with exposure time was determined using fixed ultrasonic frequencies (28, 40, and 68 kHz) at a fixed ultrasonic power of 450 W and an exposure distance of 5.0 mm. Three replicate tests were conducted in independent tanks, while other samples were immersed in water without ultrasound treatment as a control. After treatment, the *Chironomus* eggs in the urine cup were left to stand for 10 min, the supernate was removed, and then the egg clutch was assessed for mortality (%), stress protein content, and antioxidant enzyme activities. Mortality (%) was determined in accordance with the criteria described in Section 2.1.

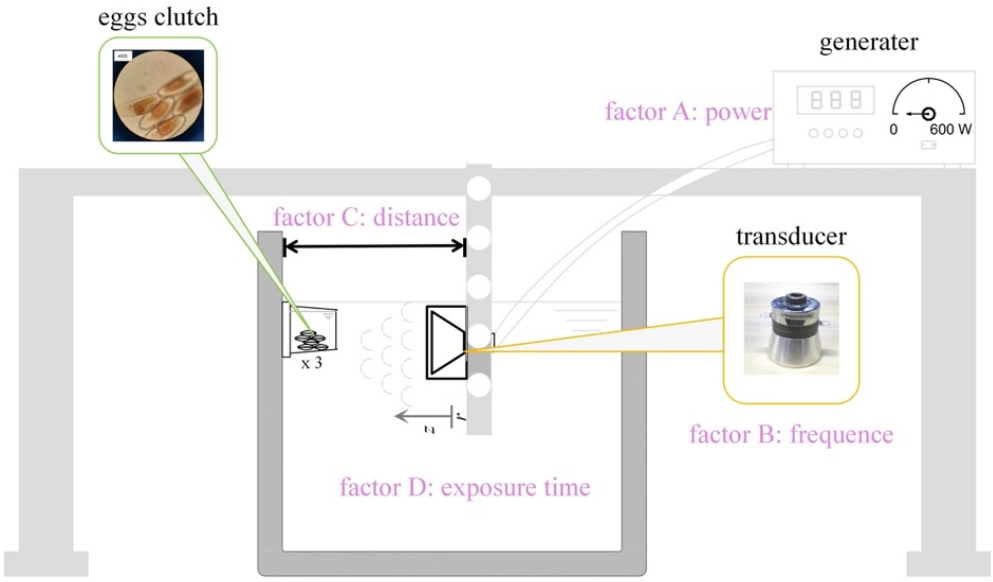


Fig. 1. Experimental equipment.

2.2 Assay of stress protein content and antioxidant enzyme activities

All reagents were purchased from Merck KGaA (Darmstadt, Germany), except when specified otherwise. Before further analyses, samples were homogenized in T-PER lysis buffer (adding 1 mL buffer to 100 mg tissue) freshly supplemented with 1% Halt Protease Inhibitor Cocktail (Thermo Fisher Scientific, Waltham, MA, USA) using a Potter-Elvehjem tissue homogenizer. Homogenates were centrifuged at 3,000 ×*g* for 10 min, and supernatants were used for further measurements.

To detect HSP70, Hb, AChE, P450, and GSTs, insect ELISA kits were used (Mybiosource, San Diego, CA, USA; ML230641, ML360344, ML56074, ML360374, and ML69034, respectively). First, 50 µL standard solution or sample solution (10 µL combined with 40 µL PBS) was measured into the wells, while blanks remained empty. Next, 100 µL horseradish peroxidase (HRP)-labeled streptavidin was added to the standard and sample wells. After 60 min incubation at 37°C and five washing steps (described in the

manufacturer's protocol), 50 μL Substrate A and 50 μL Substrate B solutions were added, followed by 15 min incubation away from light, at 37°C. The reaction was stopped by adding 50 μL Stop Solution, and absorbance values were measured at 450 nm.

2.3 COMSOL Multiphysics emulation

To illustrate the sound field distribution for ultrasonic frequency at 28, 40, and 68 kHz, COMSOL Multiphysics (Version 5.4, COMSOL, Inc., Stockholm, Sweden) software was used to simulate this process. Sound pressure intensity and pressure were used to characterize the sound field distribution.

2.4 Data analysis

The experimental design and data analysis were performed using Minitab (Pennsylvania State University, PA, USA) and Design Expert software (Stat-Ease Inc., version 10). To illustrate the sound field distribution during the experiments, COMSOL Multiphysics (version 5.3a) software was used to simulate the acoustic distribution. Based on the results for HSP70, Hb, AChE, P450, and GSTs before and after ultrasonic exposure, the increment (%) was calculated as follows:

Increment (%) = (value at t exposure time/initial value -1) × 100

Origin 10.0 was applied to gather the increments for stress protein content and antioxidant enzyme activities, while the differences were evaluated by one-way ANOVA (SPSS® software, IBM., version 21.0) to assess whether stress protein content and antioxidant enzyme activities at exposure time t were significantly different (at the $p < 0.05$ level) from the initial values.

3. Results and discussion

3.1 Effect of ultrasound on *Chironomus* eggs

Prior to experiments, *Chironomus* eggs were collected for species identification. Using multiple sequence alignment integrated with 911-HCO2198 primers (Sharley *et al*., 2004), identification as *C. kiiensis* was confirmed (Supplementary Information Figure S4). For the experimental results, no post-assay recovery of "dead" eggs was apparent. Mortality in the control treatments was below 1.0%. Under conditions of 40 kHz and 450 W, mortality presented a negative correlation with exposure distance (1–20 mm), while exposure time (3–150 s) showed a positive correlation. For ultrasonic frequency, at 28 kHz with 450 W, 15 mm exposure distance and 75 s exposure time, 72.4% of eggs showed apoptosis after exposure. As the frequency approached 68 kHz under the same conditions, mortality decreased to 50.9%. Based on data in Supplementary Information Text S1, a Pareto chart (Fig. 2) was generated to illustrate significant factors affecting the mortality of *C. kiiensis* eggs. Data were variable at $\alpha = 0.05$, indicating that exposure time following ultrasonic frequency and exposure distance significantly influenced egg mortality, while the effect of ultrasonic power was unremarkable.

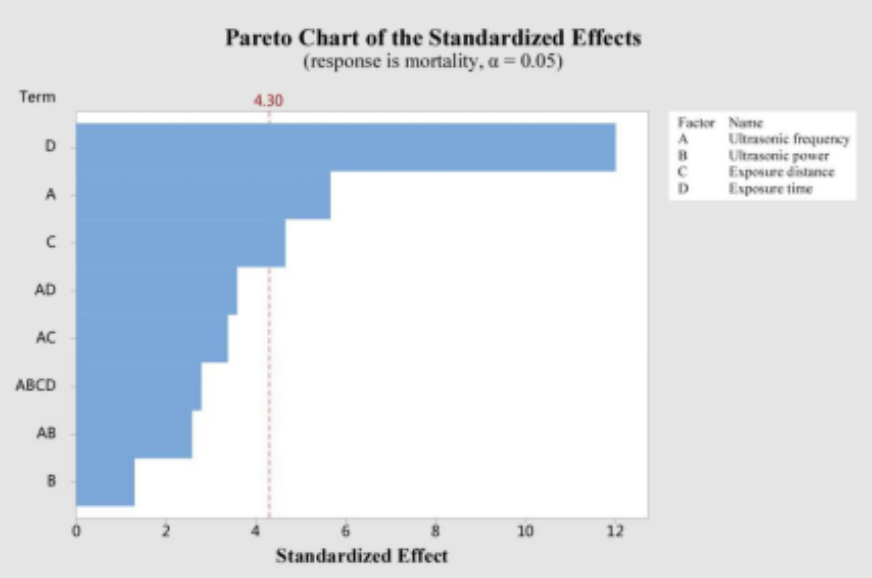


Fig. 2. Pareto plot for (a) ultrasonic frequency, (b) ultrasonic power, (c) exposure distance, and (d) exposure time, used to kill Chironomidae eggs.

Considering the practical application of this method in DWTPs, an ultrasonic transducer embedded in a wall could inhibit *C. kiiensis* breeding (conceptual sketch shown in Fig. 3). Mortality within an exposure distance of 5 mm was the main content. The effect of ultrasonic frequency on mortality was obvious, especially at frequencies below 68 kHz. For example, at 68 kHz and 450 W, 55% of eggs survived after exposure for 61 ± 17 s, while at 28 kHz, 82% mortality of *C. kiiensis* eggs required 56 ± 13 s. To kill all eggs within 120 s at 5 mm exposure distance, ultrasonic frequency should be set to 28 kHz at 450 W.

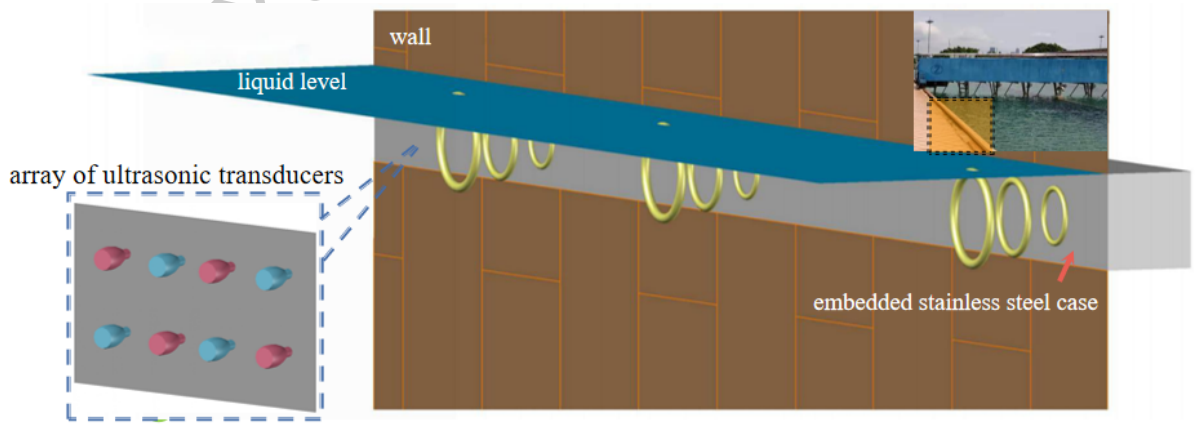
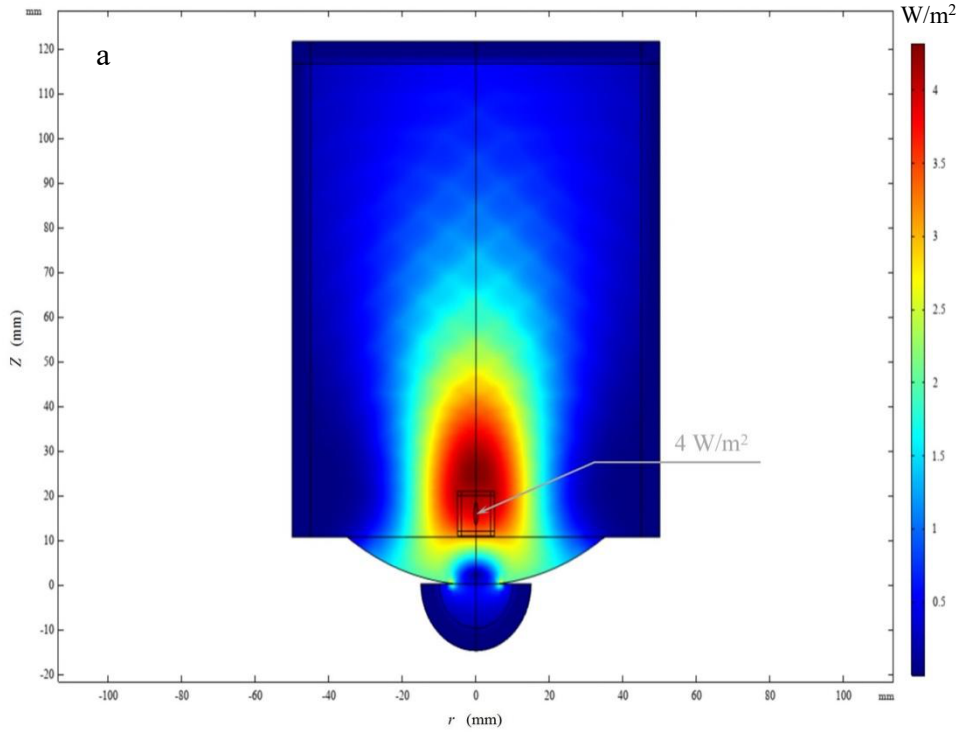
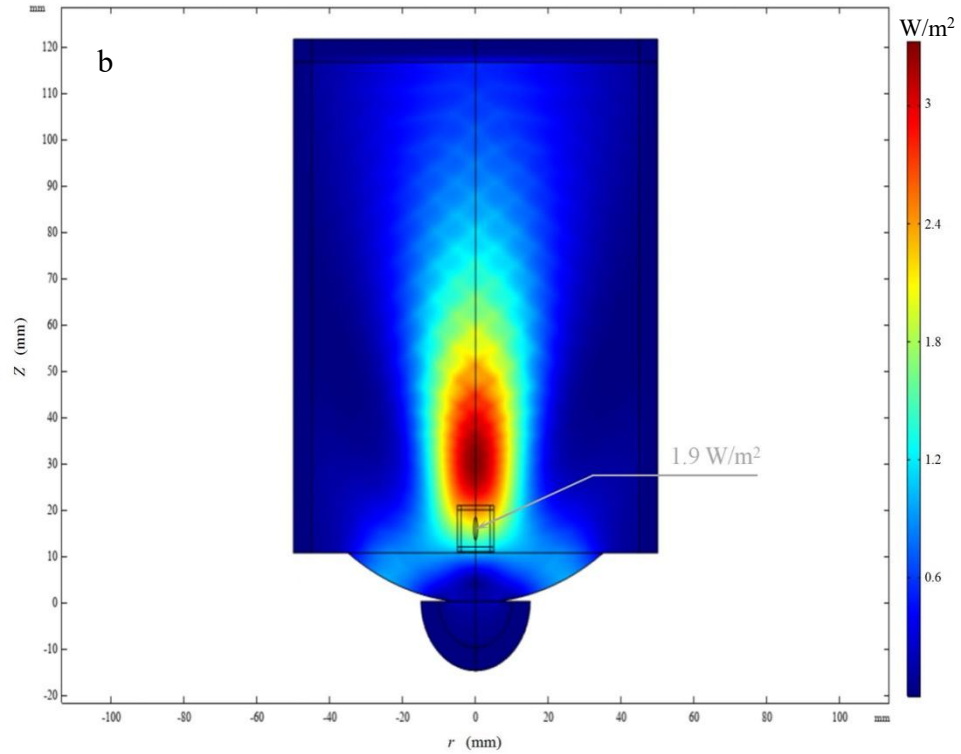


Fig. 3. Conceptual sketch for an ultrasonic transducer in a DWTP horizontal sedimentation tank

3.2 Acoustic field mapping

Acoustic pressure and pressure intensity calculated using COMSOL were applied to characterize the sound field distribution at different frequencies (28, 40, and 68 kHz). Figure 4 shows that ultrasound irradiated the water in a cylindrical volume with a diameter of 50 mm and a height of 120 mm. The simulated acoustic pressure intensity distribution in an r–*Z* plane (vertical) is shown, where red indicates a high acoustic pressure intensity. The transducer interface was located at $Z = 10$ mm, while *C. kiiensis*' egg clutches were fixed ≤5 mm from the transducer interface. At 28 kHz (Fig. 4a), the high acoustic pressure intensity region covered 9–45 mm on the *Z* axis, while the same region at 68 kHz (Fig. 4c) was 25–63 mm on the *Z* axis, with a narrower pattern than that at 28 kHz. During the experiments, *C. kiiensis* eggs were right in the high acoustic pressure intensity region for 28 kHz under an average pressure intensity of 4.0 W m^{-2} (Fig. 4a). At 68 kHz, the eggs were outside the high acoustic pressure intensity region, with a lower average pressure intensity of 0.9 W m^{-2} (Fig. 4c), indicating that higher mortality was achieved at the lower frequency. The 2D half geometric model (Fig. 5) for acoustic pressure distribution supported this finding, depicting that *C. kiiensis*' eggs sustained much greater acoustic pressure (2379 Pa) with 28 kHz exposure in comparison with that during 68 kHz exposure (422 Pa). However, at $Z = 800$ mm, which was far from the transducer interface, higher acoustic pressure (27 Pa) was observed at 68 kHz exposure than that at 28 kHz, indicating an increase in propagation distance with ultrasonic frequency.


a
W/m²
4
3.5
3
2.5
2
1.5
1
0.5
4 W/m²
Z (mm)
r (mm)
mm


b
W/m²
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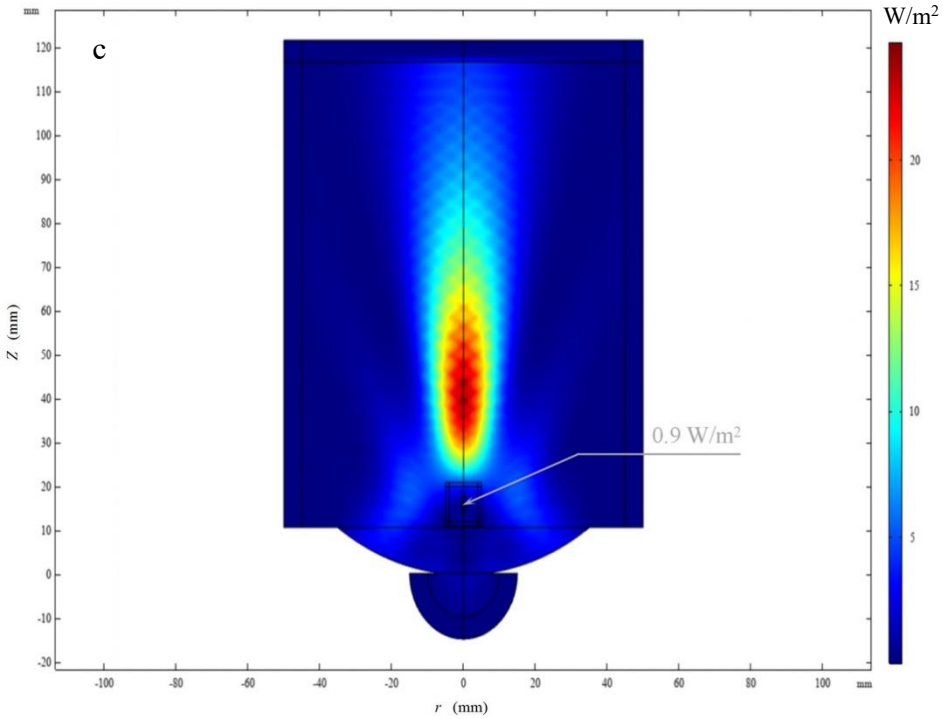


Fig. 4. Simulated sound pressure intensity (W m^{-2}) distribution during ultrasonic treatment with frequencies of (a) 28 kHz, (b) 40kHz, and (c) 68 kHz.

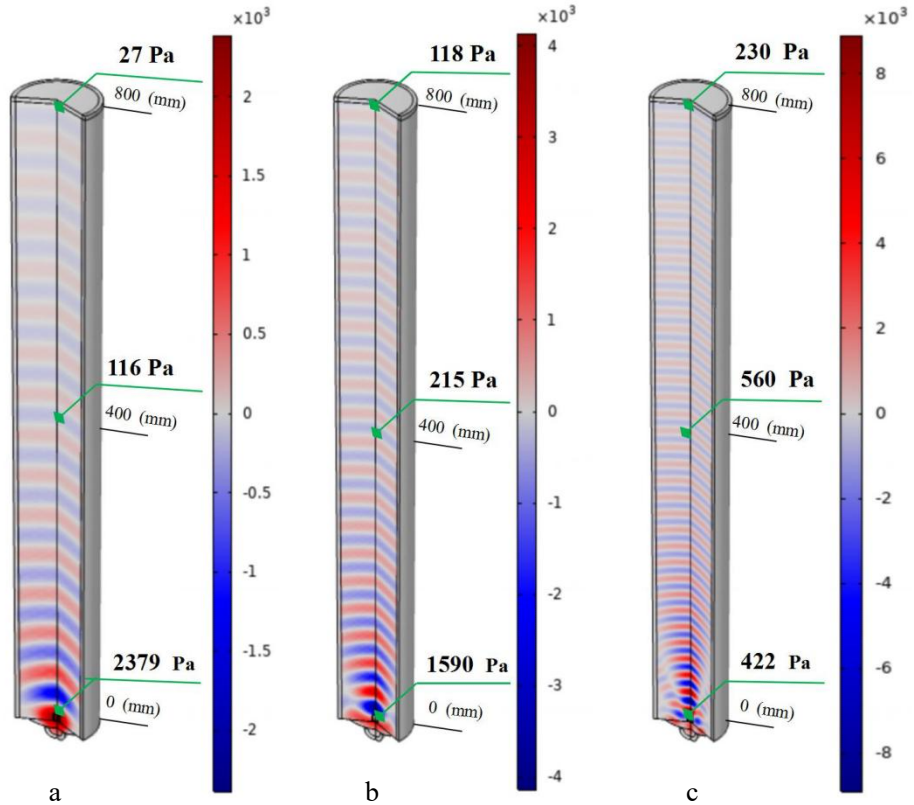


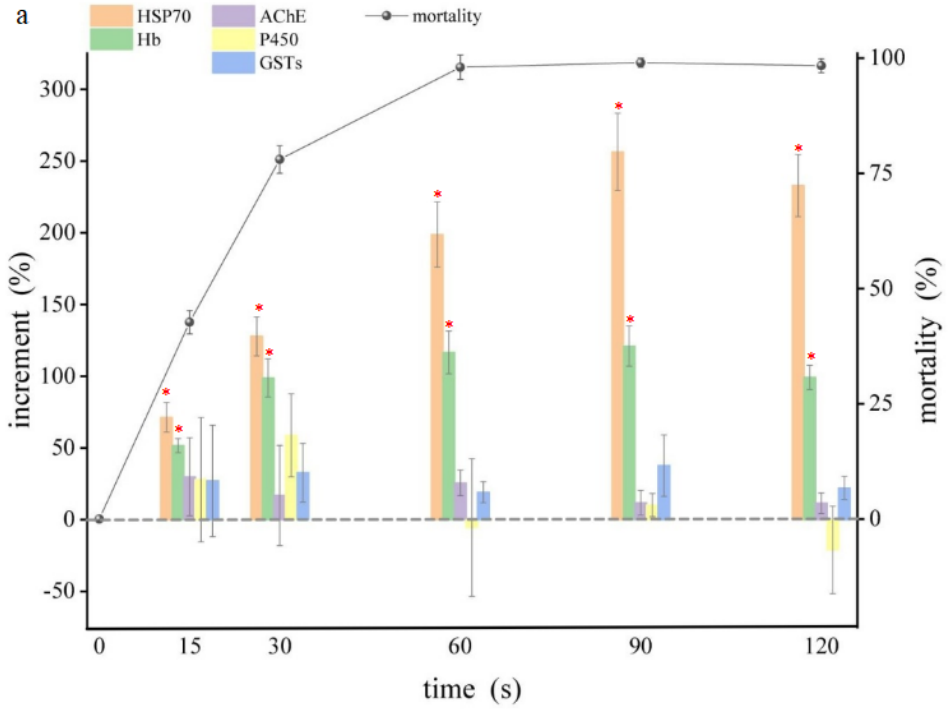
Fig. 5. Simulation of acoustic pressure (Pa) distribution in r–*Z* planes with frequencies of (a) 28 kHz, (b) 40 kHz, and (c) 68 kHz.

3.3 Mechanism of Chironomidae egg mortality with ultrasound

Ultrasonic radiation enhances heat and mass transfer processes and can be qualitatively illustrated as follows: (1) a vibration effect, which introduces a rapidly alternating compression series together with expansion cycles (Cárcel *et al*., 2012; Yao *et al*., 2015); (2) a heating effect, which can increase the temperature (Yao *et al*., 2016); (3) a cavitation effect, which can absorb sound energy and collapse to release energy in a very short time, and generate high temperatures of 1900–5200 K and high pressure over 50 MPa in a very small space around the target (Qiu *et al*., 2018). The emergence of a cavitation effect can be attributed to location-based maximum pressure greater than 0.2 MPa (Caupin *et al*., 2006), where water absorbs the energy produced by the cavitation of bubbles to generate $OH^{\bullet}$, $H^{\bullet}$ radicals (Zhang *et al*., 2019). In Figure 5, a high acoustic pressure region can be seen near the transducer interface, with average pressures of 2379, 1590, and 422 Pa for 28, 40, and 68 kHz, respectively. All these values were far below the cavitation threshold of 0.2 MPa, indicating that the cavitation effect played a minor role in our experiments. Meanwhile, according to the simulation via COMSOL and practical measurements of temperature rise, increases of only 0.1–0.2°C were recorded in the water column around the eggs after ultrasonic exposure (Supplementary Information Fig. S5). With the exclusion of the heating effect, the vibration effect was assumed to be the pivotal function for killing *C. kiiensis*' eggs.

To determine the apoptosis process of *C. kiiensis*' eggs, the protein content (HSP70 and Hb) and enzymatic activities (GSTs, P450, and AChE) involved in the ultrasound-induced stress response were analyzed (Fig. 6). The HSP70 content was 82 ± 6 pg mL^{-1} prior to ultrasound exposure (Supplementary Information Table S4) but upregulated 74.6 ± 3% (Fig.

6a) and 58.8 ± 11% (Fig. 6c), respectively, at frequencies of 28 and 68 kHz, under conditions of 15 s exposure time, 450 W, and 5 mm exposure distance. The HSP70 content of eggs exhibited the most sensitive and amplified response to ultrasonic exposure, especially in the aforementioned high acoustic pressure intensity region at 28 kHz. After 60 s exposure time at a frequency of 28 kHz, almost all the eggs exhibited apoptosis (Fig. 6a), while a peak in HSP70 (253.1 ± 17% increase) was recorded at 90 s.



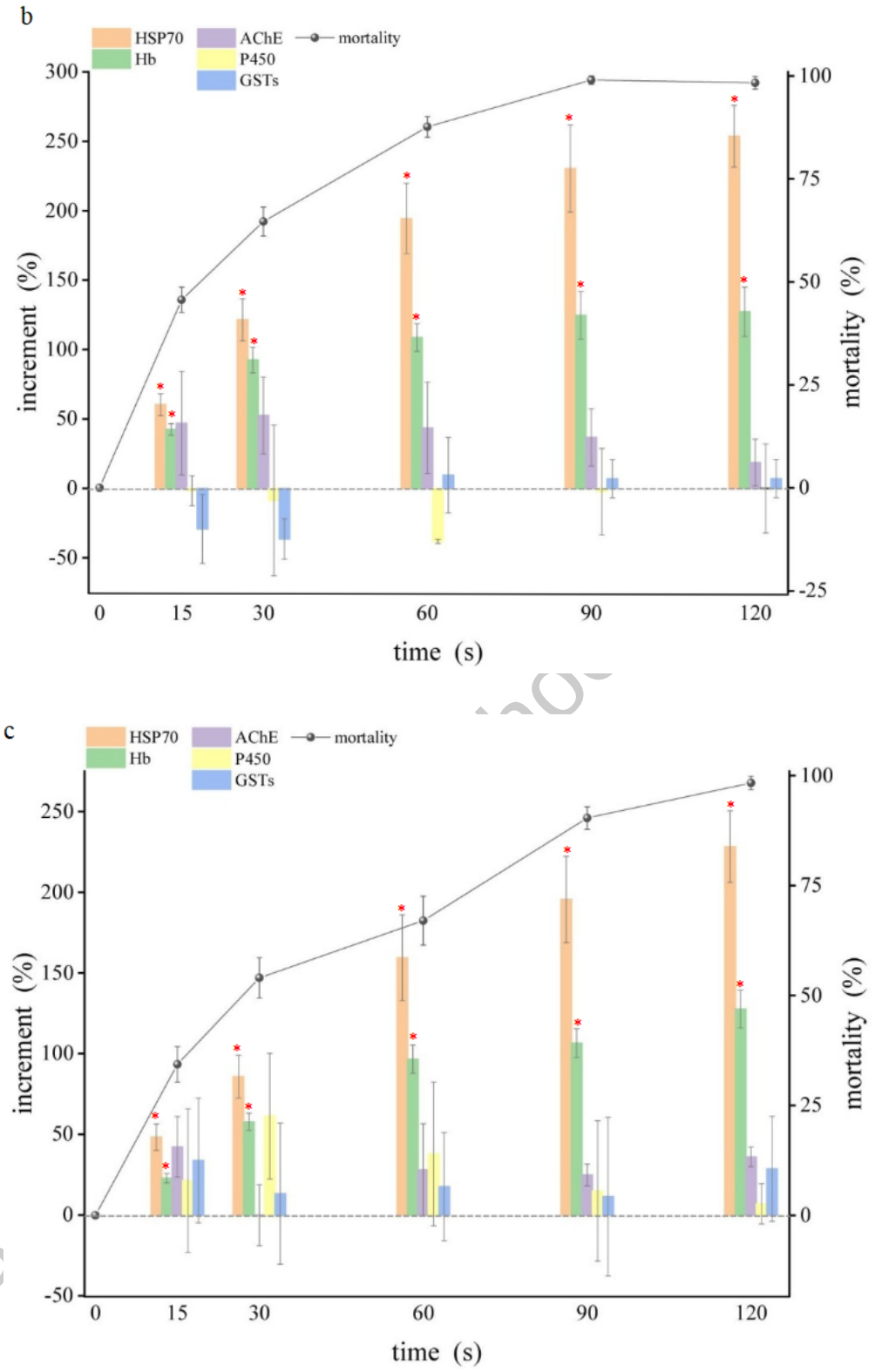


Fig. 6. Mortality (%) and increment (%) of heat shock protein 70 (HSP70), hemoglobin (Hb), glutathione S-transferases (GSTs), cytochrome P_{450} (P450), and acetyl cholinesterase (AChE) from *Chironomus kiiensis*' eggs at frequencies of (a) 28 kHz, (b) 40 kHz, and (c) 68 kHz ultrasonic exposure.

HSP70 is a set of chaperone proteins involved in ensuring the correct folding and

unfolding of proteins, which can be used as a biomarker for the stress response (Wang *et al*., 2021; Carrasco-Navarro *et al*., 2021). HSP70 content is rapidly regulated by changes in physical and chemical conditions (Manfrin *et al*., 2018). Another well-studied biomarker, *Chironomus* Hb, possesses features associated with the transportation and storage of oxygen, such as a high affinity for oxygen, extracellular localization, and a high degree of polymorphism (Osmulski *et al*., 1986). In Figure 6, the Hb content presents a similar pattern to that of HSP70, with a much higher concentration (Supplementary Information Table S4) but lower increase with exposure time (Fig. 6). The upregulation of Hb content suggests that *C. kiiensis*' eggs have more efficient uptake (binding to Hb; Bohr effect) and release of oxygen to cells (Root effect) (Manfrin *et al*., 2018), which may be an attempt to resist apoptosis.

AChe activity was observed to be in sync with mortality before apoptosis (Fig. 6). In the case of insects, AChE is a glycosylated dimer attached to the membrane through a glycolipid anchor (Chaabihi *et al*., 1994). Because AChE is an important enzyme in the nervous system, its inhibition would follow egg apoptosis (Supplementary Information Figure S6). For the other two detoxified enzymes, P450 is reportedly the most important enzyme in the functionalization of the detoxification system and is responsible for initiation of the degradation and elimination of endogenous and exogenous oxidative compounds. GSTs are the most important enzymes in conjugation of the detoxification system, available for conjugating reduced glutathione to the electrophilic centers of oxidative compounds (Martínez-Paz *et al*., 2018). Neither of these detoxified enzymes showed statistically significant changes after ultrasonic exposure (Fig. 6). These results support our aforementioned

assumption that ultrasound-induced cavitation related to $OH^{\bullet}$, $H^{\bullet}$ oxidative radicals plays a minor role in *C. kiiensis* egg mortality.

3.4 Implications

To control *Chironomus* eggs and reduce *Chironomus* biomass, ultrasound radiation, mainly its vibration effect, represents a practical and environmentally friendly solution to controlling the incidence of *Chironomus* larvae in DWTP effluent. We noted that the high acoustic pressure intensity region at 28 kHz only reached 9–45 mm vertically, indicating a significant decrease in *Chironomus* egg mortality with an increase in exposure distance. On account of the characteristics of *Chironomus* breeding (i.e., located on the side walls of horizontal sedimentation tanks in DWTP), an ultrasonic transducer embedded in the wall (Fig. 3) would be effective in killing *Chironomus*' eggs. According to the sound distribution mapping by COMSOL (Figs. 4 and 5), the high acoustic pressure intensity region could be extended with upregulation in ultrasonic frequency. Thus, we think that an array of ultrasonic transducers in a criss-cross pattern, comprising both low and high frequencies, would be effective for killing both *Chironomus* eggs and larvae. Besides the vibration effect, we propose that the cavitation effect would become more active during practical operation of an ultrasonic array in DWTP. Because there is a difference in flow rate in horizontal sedimentation tanks, this deviation could result in a vortex, which would produce abundant bubbles (Uchiyama *et al*., 2016). These bubbles are divided into two categories: large and small. The effect of the large bubbles is to scatter the ultrasonic wave emitted by the ultrasonic transducer, making the sound field distribution more uniform, and ensuring full use of the ultrasonic energy. For the small bubbles, their collapse would generate $OH^{\bullet}$, $H^{\bullet}$ radicals (Zhang *et al*., 2019), reducing

the threshold for cavitation.

4. Conclusion

In this study, the mortality of *C. kiiensis* egg by ultrasonic radiation was investigated systematically. The optimal mortality of *C. kiiensis* egg can reach 100% within 120 s with 5 mm exposure distance, ultrasonic frequency of 28 kHz at 450 W. This research set up an ultrasonic process to control *Chironomus* eggs and reduce *Chironomus* biomass in DWTP. And it was found that the effect of ultrasonic radiation to kill *Chironomus* eggs would be more effective and environmentally friendly method than those chemical methods. Meanwhile, COMSOL software was used to examine the characteristics of the ultrasonic field, finding that higher egg mortality were obtained at lower frequencies. What's more, the protein content and enzymatic activities involved in the ultrasound-induced stress response were also studied. The boost of protein content (heat shock protein 70 and hemoglobin) were significant, while unremarkable on enzymatic activities (AChE, P450, and GSTs), indicating ultrasound-induced $OH^{\bullet}$, $H^{\bullet}$ oxidative radicals plays a minor role on killing *C. kiiensis* egg.

From the point of view on economy and operation, the most practical protocol is that, array of high and low frequency ultrasonic transducers embed in the walls of DWTP could be effective on controlling *Chironomus* eggs and reducing *Chironomus* biomass, contributing to the prevention of *Chironomus* outbreaks.

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致谢

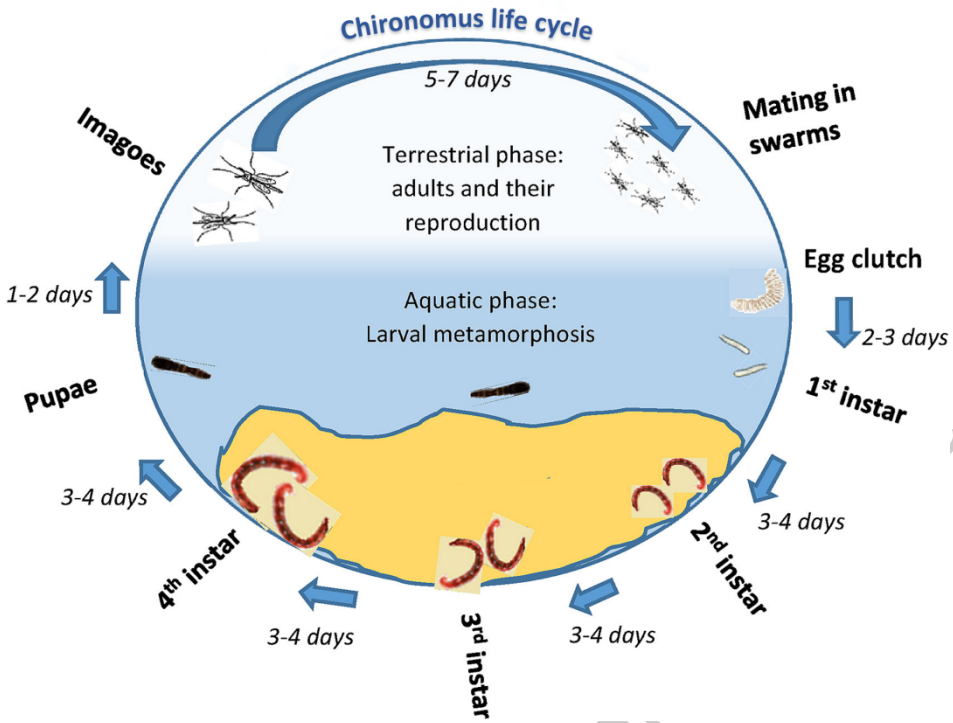
1、论文的选题来源、研究背景

1.1 选题来源：广州市自来水公司合作项目

1.2 研究背景

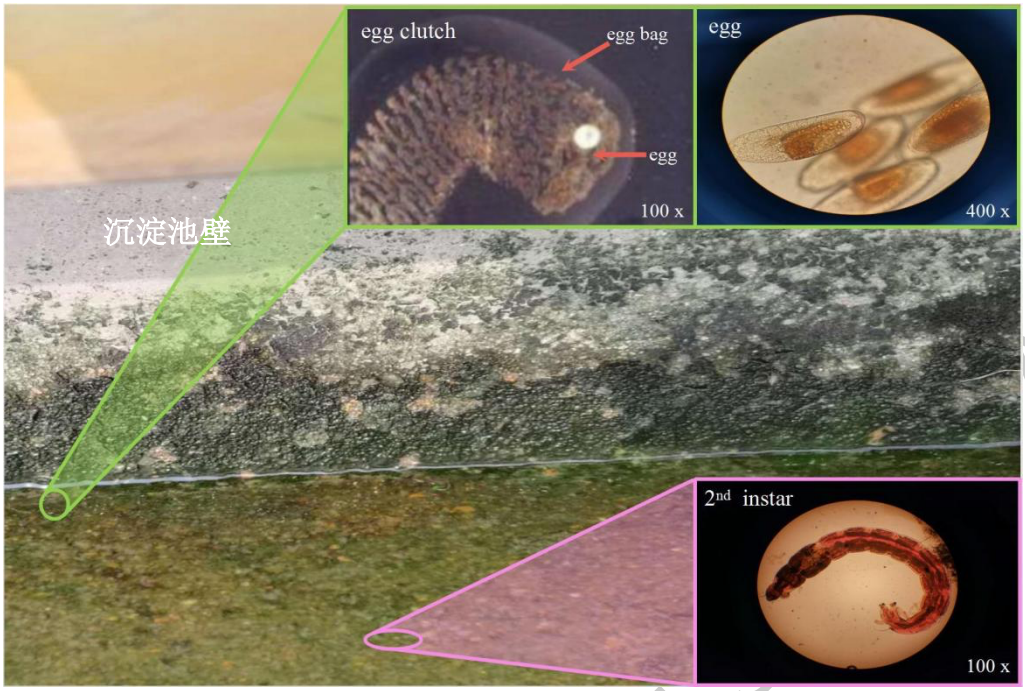
摇蚊是双翅目（Diptera）、长角亚目（*Nematocera*）、摇蚊科（*Chironomidae*）昆虫的统称。全球已知约有400属[1]，种类繁多，分布广泛，是淡水水域中的重要种群之一。摇蚊生命周期分为4个阶段：卵（egg clutch）、幼虫（instar）、蛹（pupae）和成虫（imagoes）（附图 1）。**摇蚊极易在供水系统孳生**，据报道国内的广州市、深圳市、成都市和宁波市等多地的供水系统沉淀池、砂滤池和清水池经常出现大量的摇蚊幼虫[1]。国外供水系统亦存在摇蚊污染问题，20世纪70年代，英国的艾塞克斯城供水厂的蓄水池和管网端用水爆发摇蚊幼虫污染[2]；20世纪80年代，美国塔科马市的4个蓄水池中出现大量摇蚊幼虫，并经管网系统迁移到管网末端，在相同时期，美国印第安那州洛厄尔市城市供水系统亦发生了大规模的摇蚊污染[3]。供水系统摇蚊污染对供水水质的影响主要表现为：

- 幼虫大量出现在沉淀池出水、滤后水、甚至出厂水和管网端用水，影响水质感官指标；
- 大量死亡的摇蚊幼虫严重影响供水流程水质的生物和化学稳定性。



附图 1　　摇蚊的主要发育周期

王珊[4]等认为，大部分的摇蚊幼虫能生存在 pH 值为 6 - 8 的水环境中，供水全流程均是摇蚊幼虫理想的栖息环境。其中，**供水系统沉淀池由于水流速度缓慢，最适宜摇蚊产卵、幼虫初孵和筑巢**。广州市自来水公司下辖水厂——广州市南洲水厂自 2003 年建成以来，其沉淀池壁面水位线经常附着大量摇蚊卵束（见附图 2）。经长期调查，申请人发现南方地区多个供水系统均存在严重的摇蚊污染问题，其中**沉淀池是摇蚊孳生的主要源头，原因在于成虫夜间婚飞并密集地返回沉淀池壁面水位线附近进行产卵，导致卵束大量孳生在沉淀池壁，被卵袋包裹的卵粒在一定的温度条件下孵化为幼虫**。卵束和幼虫随沉淀池出水迁移到滤后水和出厂水，严重影响供水水质的感官和生物化学稳定性指标，迫切需要寻找有效的控制或清除措施，防治供水系统的摇蚊污染，确保提供优质的生活饮用水。



附图 2　广州市南洲水厂沉淀池摇蚊卵束及其幼虫

目前，供水系统摇蚊污染主要通过投加双氧水、液氯、氯胺等化学药剂进行控制[5]。除了化学投加，广州市南洲水厂曾尝试人工刷刮、盐酸洗刷和明火灼烤池壁等手段控制孳生源头，减少摇蚊卵束在池壁的附着密度，从而降低摇蚊污染爆发的频次。然而，持续防治效果较差，存在影响原水水质且成本高昂等缺点，未能从源头长期有效降低供水系统的摇蚊种群生物总量。

由于超声波的机械作用和空化作用会影响生物细胞结构、干扰生物细胞物质代谢及抗氧化酶系统的正常运行[6-8]，导致生物代谢异常，甚至致死。项目组认为，**超声波可直接、有效、并快速灭活摇蚊卵束**。与当前其他防治手段相比，超声波辐射具有对原水水质影响小，可操作性强，持久性好，安装简便等优点，**可从摇蚊孳生源头减少其生物总量**。为了解决供水流程摇蚊污染影响供水水质这个难题，项目组与广州市南洲水厂合作开展超声波灭活摇蚊卵束的静态试验。本项目探索超声波有效灭活摇蚊卵束的参数，成果可为防治供水系统摇蚊污染提供有效的应用方案。

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2、每一个队员在论文撰写中承担的工作以及贡献;

本组成员只有组员刘博栋一人。

3、指导老师与学生的关系，在论文写作过程中所起的作用，及指导是否有偿;

杨晓安为华南师范大学附中校内导师，为学生无偿提供理论指导

荣宏伟为广州大学土木工程学院教授，作为校外导师为学生无偿提供选题参考、理论教学、实验指导和论文撰写指导。

4、他人协助完成的研究成果。

广州市南洲水厂协助采集摇蚊卵与幼虫样本。