Supplementary Information

Text S1. Dechlorinated Tap Water Preparation Method

Dechlorinated tap water was prepared by aeration. Specifically, tap water was placed in an open glass container and continuously aerated for 48 hours using an aquarium air pump (aerator pore size <50 μm). To verify the dechlorination effect, randomly selected water samples were tested with a portable spectrophotometer (DPD spectrophotometric method, Hach, Model: DR1900), confirming that the residual chlorine concentration was below the detection limit (<0.05 mg/L).

Text S2. Calculation Method for Ultraviolet Irradiation Dose

To The ultraviolet irradiation dose (mJ/cm^2) is calculated as the product of the ultraviolet irradiation intensity and the irradiation time, as shown in the formula:

$$D = I \times T$$

Where: I is the ultraviolet irradiation intensity (mW/cm^2)

T is the ultraviolet irradiation time (s)

In this study, the ultraviolet light intensity to which the chironomid larvae were exposed remained constant. Therefore, the irradiation dose was controlled by varying the exposure time of the larvae, so as to investigate its effect on the inactivation of chironomid larvae within the system.

Text S3. Scanning Electron Microscopy Observation Method

(1) SEM Observation Procedure

The chironomid larval tissues obtained in Section 2.2.7 were removed, and the fixative solution was discarded. The samples were rinsed three times with 0.1 M phosphate buffer (PB, pH=7.2), each for 15 min, followed by fixation with 1% osmium tetroxide solution for 1–2 h. The waste osmium solution was carefully removed, and the samples were washed three times with 0.1 M PB (pH=7.2), each for 15 min. Dehydration was performed using a graded ethanol series (30%, 50%, 70%, 80%, 90%, and 95%), with each concentration applied for 15–20 min. The samples were then treated with 100% ethanol twice (20 min each) and stored in fresh 100% ethanol.

The samples were dried using a Quorum K850 critical point dryer with CO_2. Subsequently, they were mounted on stubs with conductive carbon adhesive and sputter-coated with gold for 30 s using a Cresington 108 Auto ion sputter coater. Finally, the samples were observed under a Hitachi SU8010 scanning electron microscope.

(2)Transmission Electron Microscopy Observation Method

The obtained chironomid larval tissues were collected and rinsed three times with 0.1 M phosphate buffer (PB, pH 7.2), 15 min each. The samples were fixed with 1% osmium tetroxide solution for 1-2 h. After careful removal of the waste osmium solution, the samples were washed three times with 0.1 M PB (pH 7.2), 15 min each. Tissue dehydration was performed using a graded ethanol series (30%, 50%, 70%, and 90%), with each concentration applied for 20 min, followed by two treatments with 100% ethanol. The samples were then dehydrated twice with 100% acetone, 20 min each.

Infiltration was conducted using acetone:embedding agent mixtures (1:1) at 37°C for 3 h, followed by acetone:embedding agent (1:3) at 37°C for 4 h, and finally pure embedding agent at 37°C overnight. The embedding agent was poured into embedding molds containing the samples and polymerized at 70°C for 12-48 h. The resulting resin blocks were sectioned to 70-90 nm thickness using an ultramicrotome, and the ultrathin sections were collected on copper grids. The sections were stained with uranyl acetate for 8-15 min and lead citrate for 5-10 min. After drying, the samples were observed using a Hitachi HT7800 transmission electron microscope.

Text S4. Antioxidant System Analysis Methods

(1) Protein Content Measurement

The protein content was determined using the Coomassie brilliant blue method, where the dye's anions bind to protein-NH_3^+ groups, producing a blue color. A 1% tissue homogenate and protein standard solution were mixed with Coomassie brilliant blue reagent and allowed to stand for 10 min. The absorbance was measured at 595 nm using a 10 mm cuvette with a UV spectrophotometer (Pharo 300).

(2) MDA Content Measurement

The MDA content in chironomid larvae tissues was measured using the thiobarbituric acid (TBA) method, where MDA condenses with TBA to form a red product. A 10% tissue homogenate was mixed with reagents and boiled uncovered for 40 min, cooled under running water, and centrifuged at 3500 r/min for 10 min. The supernatant was analyzed at 523 nm using a 10 mm cuvette with a UV spectrophotometer (Pharo 300).

(3) Na^+-K^+ -ATPase Activity Measurement

Na^+-K^+ -ATPase activity was determined colorimetrically based on ATP hydrolysis to ADP and inorganic phosphate (Pi). A 10% tissue homogenate was incubated with reagents at 37°C for exactly 10 min, followed by termination reagent addition. After centrifugation at 4000 r/min for 10 min, the supernatant underwent Pi quantification by reaction with phosphate reagent at 45°C for 20 min. The absorbance was measured at 660 nm using a 10 mm cuvette with a UV spectrophotometer (Pharo 300).

(4) SOD Activity Measurement

SOD activity in chironomid larvae tissues was determined using the WST-1 method. A 5% tissue homogenate was added to a 96-well plate, followed by sequential addition of enzyme working solution and substrate application solution. The mixture was incubated at 37°C for 20 min, and the absorbance was measured at 450 nm using a microplate reader.

(5) CAT Activity Measurement

CAT activity was measured using the ammonium molybdate method, where catalase-mediated H_2O_2 decomposition was rapidly terminated by ammonium molybdate, with residual H_2O_2 forming a colored complex. A 10% tissue homogenate was mixed with reagents for a specified reaction time, followed by termination with ammonium molybdate. The absorbance was measured at 405 nm using a 5 mm cuvette with a UV spectrophotometer (Pharo 300).

(6) T-AOC Measurement

Total antioxidant capacity (T-AOC) was assessed by the ABTS method, where antioxidant substances inhibit the formation of oxidized ABTS products. A 10% tissue homogenate was added to a 96-well plate with reagents, reacted at room temperature for 6 min, and measured at 405 nm using a microplate reader. T-AOC values were calculated by comparison with a standard curve.

All assay kits used in this study were purchased from Nanjing Jiancheng Bioengineering Institute. The data are expressed as relative levels compared to blank controls (a).

$$a = \frac{\text{Experimental group}}{\text{Control group}} \times 100\%$$

Results represent the mean ± SEM of three independent experiments. Statistical analyses were performed using SPSS software (version XX). One-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) post hoc test was employed to determine intergroup differences. The significance level was set at 0.05. Significant differences are indicated by lowercase letter superscripts, where groups sharing the same letter are not significantly different ($p \geqslant 0.05$),

while different letters denote statistically significant differences ($p < 0.05$).

Text S5. Economic Cost Analysis for UV/ClO_2 Inactivation of Chironomid Larvae

This study employed the EE/O method, proposed by the International Union of Pure and Applied Chemistry (IUPAC), to conduct a cost-benefit assessment of the system. The EE/O (kWh/m³ • order) is defined as the electrical energy (kWh) required to reduce the target pollutant by one order of magnitude in one cubic meter of contaminated water, with detailed calculations provided by Equations 4.5 to 4.8.

$$EE/O_{total} = EE/O_{UV} + EE/O_{oxidant} \quad (S1)$$

$$EE/O_{UV} = \frac{P \times t \times 1000}{V \times 60 \times n} \quad (S2)$$

$$EE/O_{oxidant} = Eq_{oxidant} \times \frac{[Oxidant] \times 1000}{n} \quad (S3)$$

$$Eq_{oxidant} = \frac{Price \times Mass}{10^6 \times Purity \times EC}(kWh \bullet mol^{-1}) \quad (S4)$$

Where:

P is the output power of the UV lamp (kW);

t is the ultraviolet irradiation time (min);

V is the volume of the solution in the reactor (L);

n is the inactivation logarithm, i.e., $\log(N_0/N)$, where N_0 and N are the initial and post-treatment numbers of Chironomid larvae, respectively;

$Eq_{oxidant}$ is the equivalent electrical energy per mole of oxidant (kWh/mol);

[Oxidant] is the concentration of the oxidant (mol/L);

Price represents the average price of the oxidant (CNY/ton);

Mass denotes the molar mass of the oxidant (g/mol);

Purity refers to the purity of the oxidant (%);

EC represents the average electricity cost (CNY/kWh).

Text S6. Reactive Species Generation Experiments

(1) Chemicals, and analytical methods.

The probe compounds (i.e. NB, and BA) were quantified by the high performance liquid chromatography with a UV detector (Thermo U3000) and a Syncronis C18 column (4.6 × 250 mm, 5 μm) at 265 nm, and 227 nm, respectively. The mobile phase consisted of 0.1% acetic acid in ultrapure water (A) and acetonitrile (B) at a flow rate of 1.0 mL min-1, and the gradient elution was 40% A and 60% B.

(2) Experimental method.

Nitrobenzene and benzoic acid were selected as characteristic probe molecules for hydroxyl radicals (·OH). NB reacts rapidly with HO• at a second-order rate constant ($K_{HO^{\bullet},NB}$) of 3.9×10^9 $M^{-1}s^{-1}$. BA reacts rapidly with HO• and Cl• at a second-order rate constant of 5.9×10^9 $M^{-1}s^{-1}$ ($K_{HO\bullet,BA}$) and 1.8×10^{10} $M^{-1}s^{-1}$ ($K_{Cl\bullet,BA}$), respectively [1].

$$K_{obs-NB} = K_{HO\bullet,NB} \times [HO\bullet]ss \quad (S5)$$

$$K_{obs-BA} = K_{HO\bullet,BA} \times [HO\bullet]ss + K_{Cl\bullet,BA} \times [Cl\bullet]ss \quad (S6)$$

where and refer to the observed first-order rate constants of NB, and BA during the co-exposure

of ClO_2 and UV radiation, which are obtained from Fig. S9.

Under standardized conditions ($[ClO_2]_0$ = 5.0 mg/L, [NB]0 = [BA]0 = 0.8 μM, pH = 7.0, I=0.0597 mW/cm²), three parallel experimental groups were set up: "UV only" irradiation group, "ClO_2 only" oxidation group, and "UV/ClO_2" combined treatment group, along with groups at different pH levels (pH = 6.0, 7.0, 8.0).

After initiating the reaction, samples were taken at predetermined time intervals (0, 3, 7.5, 15, 22.5, 30, 45, 60 minutes) and immediately quenched by adding a termination agent TBA (tert-butanol). The residual concentration of the probe substances at each time point was analytically determined. The change in the natural logarithm ratio of the probe concentration, $\ln(C/C_0)$ (where C is the concentration at time t, and C_0 is the initial concentration), over time was used as the key indicator for evaluating the free radical generation potential of the different systems (Fig. S9).

Table S1. Experimental instruments

Name	Model	Manufacturer
Scanning Electron Microscope	Hitachi SU8010	Hitachi (China) Co., Ltd.
Critical Point Dryer	Quorun k850	Micronano Technologies Co., Ltd.
Ion Sputter Coater	Cresstington 108 Auto	Cresstington Ltd. (UK)
Transmission Electron Microscope	Hitachi HT7800	Hitachi (China) Co., Ltd.
Ultramicrotome	Leica UC7	Leica Microsystems (Germany)
Diamond Knife	Diatome Ultra 45°	Diatome Ltd. (Switzerland)
Centrifuge	TG16-WS	Hunan Xiangyi Lab Instrument Development Co., Ltd.
Tissue Grinder	BK-HTG80	Bkmam Biotech Co., Ltd.
Portable Spectrophotometer	DR1900	Hach Company (USA)
UV Spectrophotometer	Pharo 300	Merck (Germany)
Microplate Reader	Synergy LX	BioTek Instruments (USA)
Ultrapure Water System	ULUP-II-100	Sichuan UP Ultra-Pure Technology Co., Ltd.
Incubator	MGC-150H	Shanghai Zuole Instrument Co., Ltd.
Ultrasonic Cleaner	LC-UC-100	Shanghai Lichen Bangxi Instrument Co., Ltd.
Analytical Balance (0.1 mg)	AL 204	Mettler Toledo
Oil Bath Magnetic Stirrer	DF-101S	Zhengzhou Ketao Lab Equipment Co., Ltd.

Name	Model	Manufacturer
pH Meter	Hach Pocket pro+	Hach Company (USA)
Turbidity Meter	WZB-172	Shanghai Leici Instrument Co., Ltd.
Constant Temperature Water Bath	AB-2	Shanghai Zhulan Instrument Technology Co., Ltd.

Table S2. Orthogonal test results and range analysis

Test No.	A Temperature	B COD_{Mn}	C pH	D Blank	Survival Rate (%)
1	1	1	1	1	74
2	1	2	2	2	88
3	1	3	3	3	64
4	2	1	2	3	75
5	2	2	3	1	74
6	2	3	1	2	57
7	3	1	3	2	55
8	3	2	1	3	51
9	3	3	2	1	47
		Range Analysis			
K1	226	204	182	195	
K2	206	213	210	200	
K3	153	168	193	190	
k1	75.33	68	60.67	65	
k2	68.67	71	70	66.67	
k3	51	56	64.33	63.33	
R	24.33	15	9.33	3.33	

Table S3. Synergistic effects of UV and different concentrations of ClO_2 treatment alone and in combination on the inactivation of chironomid larvae

Inactivation Method	Inactivation Count at 8 h（log）	S_n	k_{max}（h^{-1}）	R^2	S_k
UV alone	0.10	-	0.012	0.996	-
1 mg/L ClO_2	0.12	0.836<1	0.008	0.838	1.480>1
UV-1 mg/L ClO_2	0.18		0.029	0.946	

3 mg/L ClO_2	0.20	1.671>1	0.039	0.982	1.493>1
UV-3 mg/L ClO_2	0.49		0.077	0.976	
5 mg/L ClO_2	0.47	1.698>1	0.098	0.990	1.506>1
UV-5 mg/L ClO_2	1.00		0.165	0.984	
7 mg/L ClO_2	0.71	1.483>1	0.123	0.993	1.573>1
UV-7 mg/L ClO_2	1.20		0.213	0.986	

Table S4. Inactivation number and synergy of combined system inactivating chironomid larvaeunder different UV irradiation durations

Inactivation Method	Inactivation Count at 8 h（log）	S_n
UV alone	0.10	-
ClO_2 alone	0.47	-
2 h irradiation	0.57	1.006>1.0
4 h irradiation	0.71	1.256>1.0
6 h irradiation	0.85	1.510>1.0
8 h irradiation	1.00	1.698>1.0

Table S5. Synergistic effects of different pretreatment methods on chironomid larvae inactivation

Inactivation Methods	Inactivation Count at 8 h（log）	S_n	k_{max} (h^{-1})	R^2	S_k
UV alone	0.10	—	0.012	0.996	—
		$[ClO_2]_0$=5mg/L			
ClO_2 alone	0.47	—	0.097	0.990	—
UV/ ClO_2	1.00	1.698>1	0.165	0.984	1.505>1
UV pre-irradiation	1.35	2.386>1	0.185	0.991	3.567>1
ClO_2 pre-irradiation	1.03	1.817>1	0.143	0.995	1.306>1
		$[ClO_2]_0$=7mg/L			
ClO_2 alone	0.71	—	0.127	0.990	—
UV/ ClO_2	1.20	1.483>1	0.213	0.986	1.533>1
UV pre-irradiation	1.76	2.179>1	0.248	0.974	1.784>1
ClO_2 pre-irradiation	1.46	1.807>1	0.230	0.995	1.659>1

Table S6. Synergism of different UV pre-irradiation time to inactivate chironomid larvae

Inactivation Method	Inactivation Count at	S_n	k_{max} (h^-	R^2	S_k

	8 h（log）		[1])		
UV alone	0.10	-	0.012	0.996	-
ClO_2 alone	0.47	-	0.097	0.990	-
Direct UV+ClO_2	1.00	1.698	0.165	0.984	1.506
Pre-oxidation (30 min)	1.01	1.732	0.168	0.979	1.534
Pre-oxidation (60 min)	1.03	1.817	0.143	0.995	1.306
Pre-oxidation (90 min)	1.13	1.991	0.179	0.903	1.636

Table S7. Synergism of different UV pre-irradiation time to inactivate chironomid larvae

Inactivation Method	Inactivation Count at 8 h（log）	S_n	k_{max} (h^{-1})	R^2	S_k
UV alone	0.10	-	0.012	0.996	-
ClO_2 alone	0.47	-	0.098	0.990	-
Direct UV+ClO_2	1.00	1.698	0.165	0.984	1.506
Pre-irradiation (30 min)	1.05	1.854	0.175	0.990	1.594
Pre-irradiation (60 min)	1.35	2.386	0.185	0.993	1.689
Pre-irradiation (90 min)	1.55	2.738	0.184	0.982	1.678
Pre-irradiation (120 min)	2.07	3.647	0.199	0.972	1.817

Fig. S1. UV inactivation device

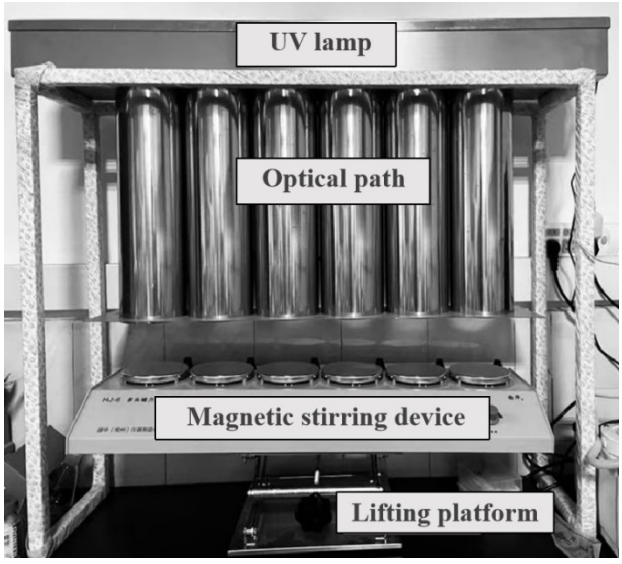


Fig. S2. Cultivation Device for Chironomid Larvae

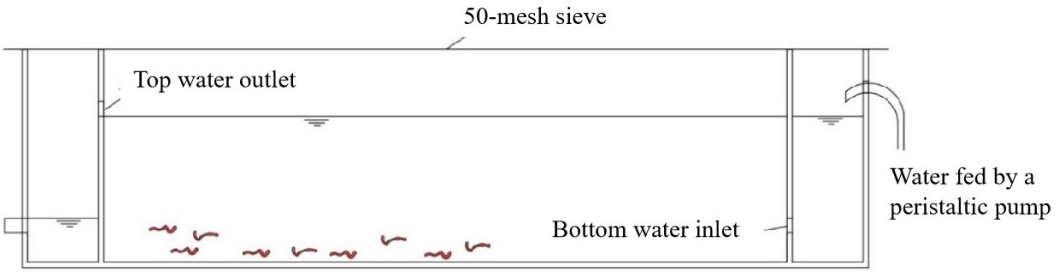


Fig. S3. Effect of Turbidity on the Survival of Chironomid Larvae

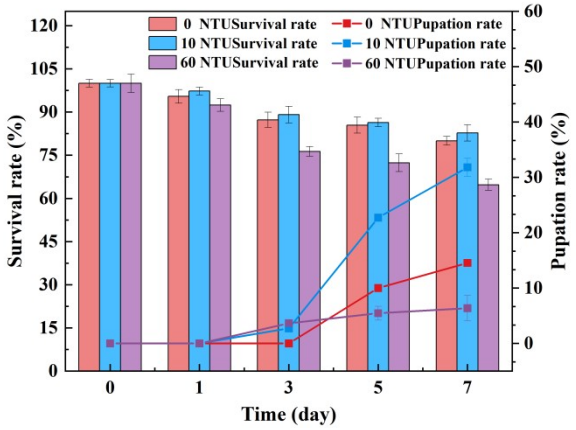


Fig. S4. Maximum inactivation rate constant (k_{max}) and synergistic factor (S_k) of UV and different concentrations of ClO_2 alone or in combination for inactivating Chironomid Larvae

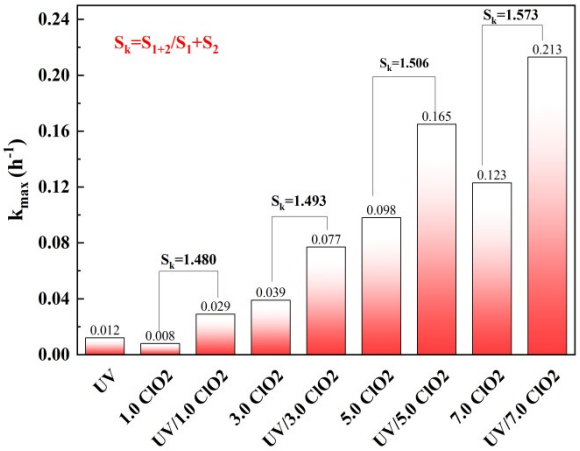


Fig. S5. The synergistic factor S_k of UV irradiation duration on the inactivation of chironomid larvae in a combined system

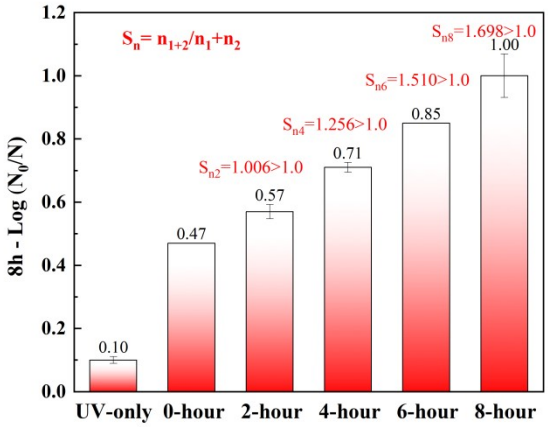


Fig. S6. Effect of pH on the inactivation of chironomid larvae by ClO_2 alone (a) and the UV/ClO_2 combined system (b)

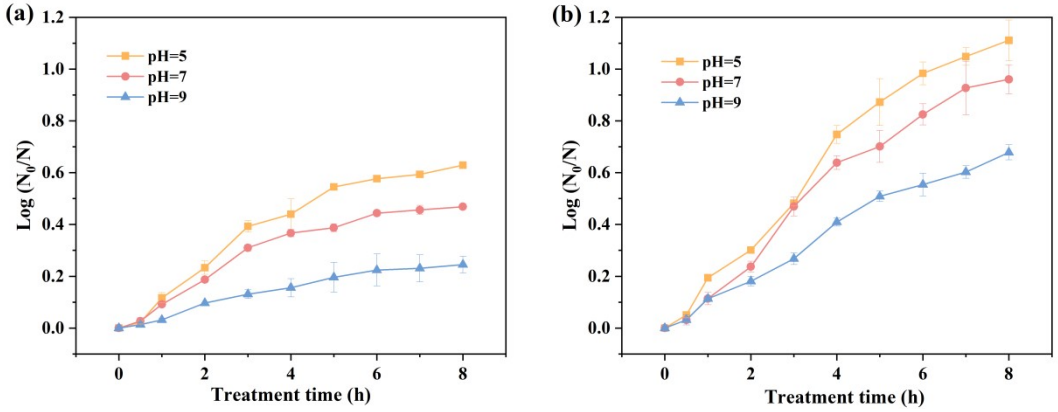


Fig. S7. (a)Effect of HA on the inactivation of chironomid larvae by ClO_2 alone and (b) the UV/ClO_2 combined system.

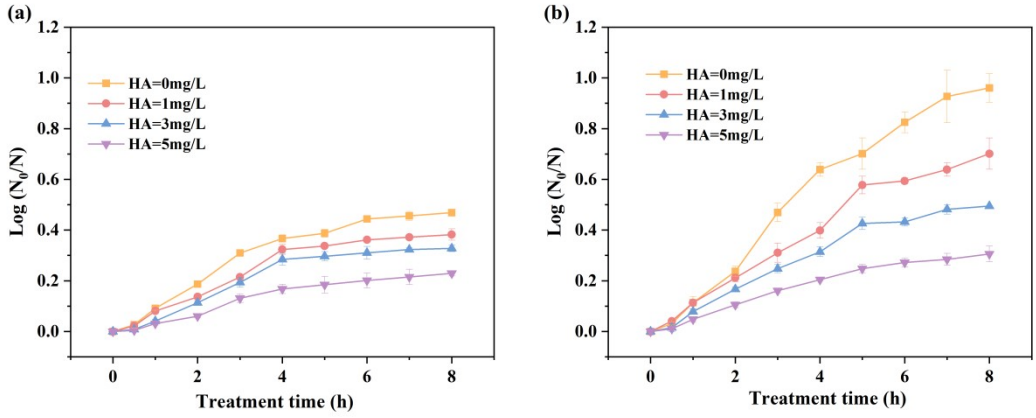


Fig. S8. (a) Effect of different ClO_2 pre-oxidation times on the inactivation efficiency of the combined system; (b) Effect of different UV pre-irradiation times on the inactivation efficiency of the combined system.

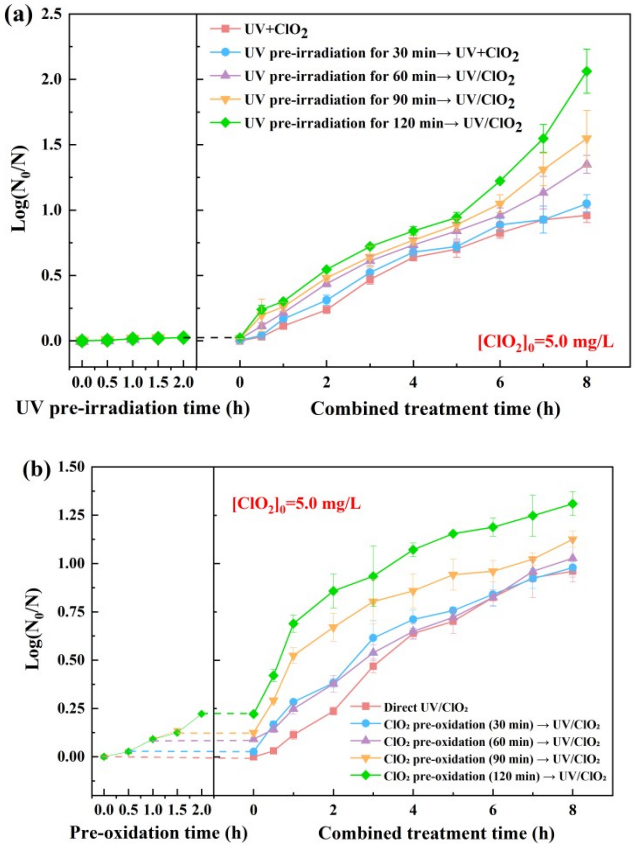


Fig. S9. The degradation of (a) NB, and (b) BA at pH = 6.0–8.0, by ClO2 oxidation alone, UV radiation, and the UVC + ClO_2 . (Conditions: $[ClO_2]_0$ = 5.0mg/L, $[NB]_0$ = $[BA]_0$ = 0.8 μM, I=0.0597 mW/cm²)

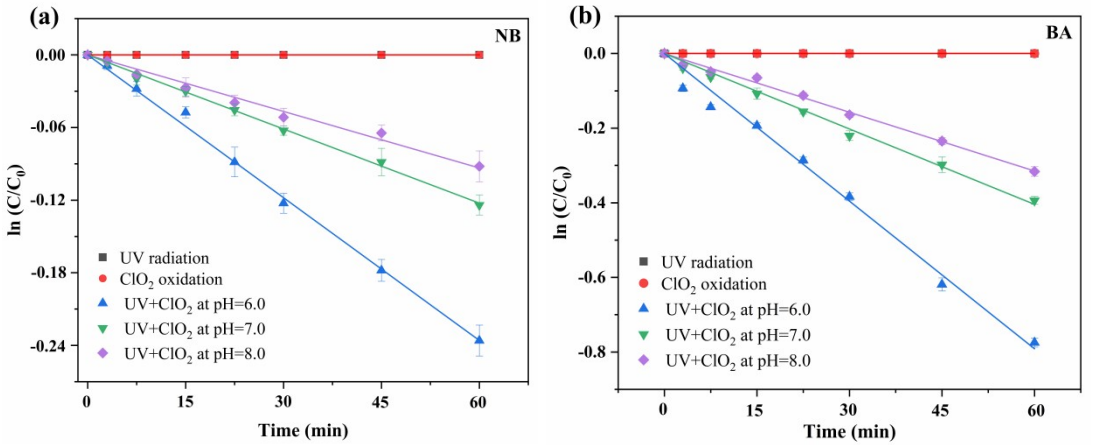


Fig. S10. Ultrastructural observation of chironomid larval cuticle under different treatments:(a) Control group SEM image (×1.0k);(b) UV/ClO_2-treated group SEM image (×1.0k);(c) Control group TEM image (×2.0k);(d) UV/ClO_2-treated group TEM image (×2.0k).

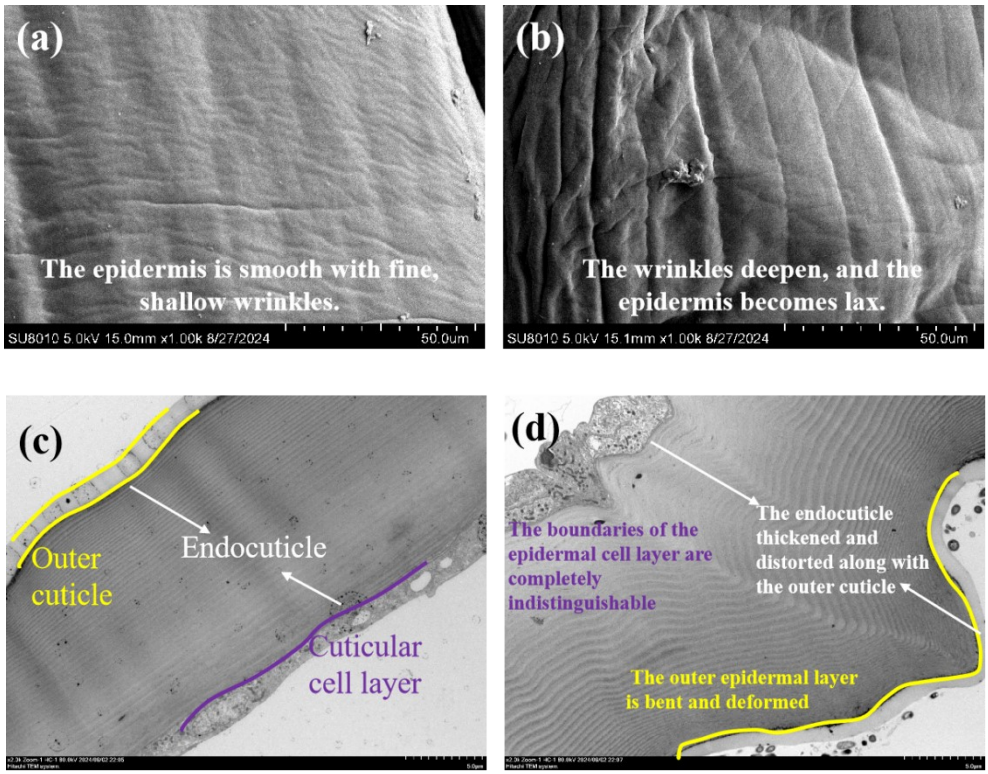


Fig. S11. Ultrastructural observation of chironomid larvae under different treatments:(a) Control group myofibril TEM image (×20.0k);(b) UV/ClO2-treated group myofibril TEM image (×20.0k);(c) Control group mitochondria TEM image (×20.0k);(d)UV/ClO2-treated group mitochondria TEM

image (×20.0k);(e) Control group autophagosome TEM image (×5.0k);(f) UV/ClO2-treated group 4h autophagosome TEM image (×5.0k).

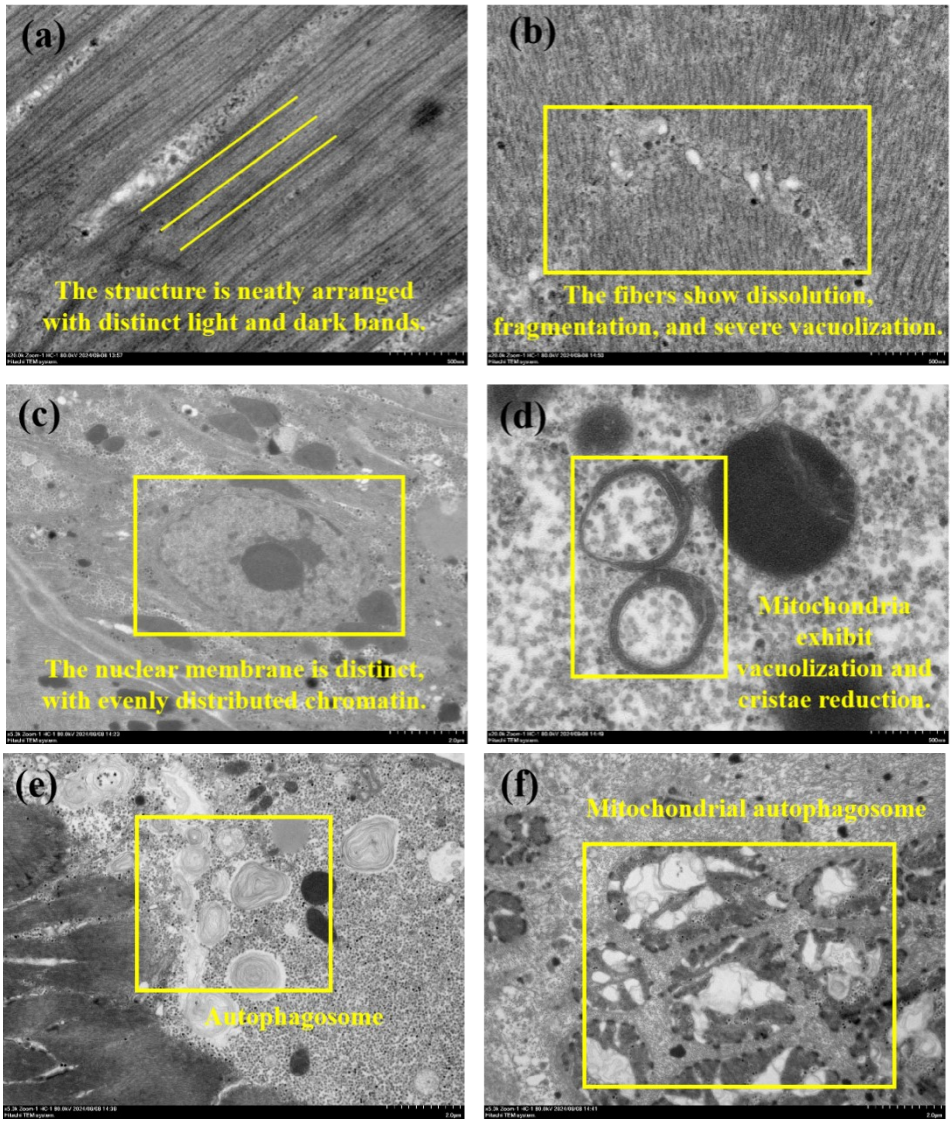
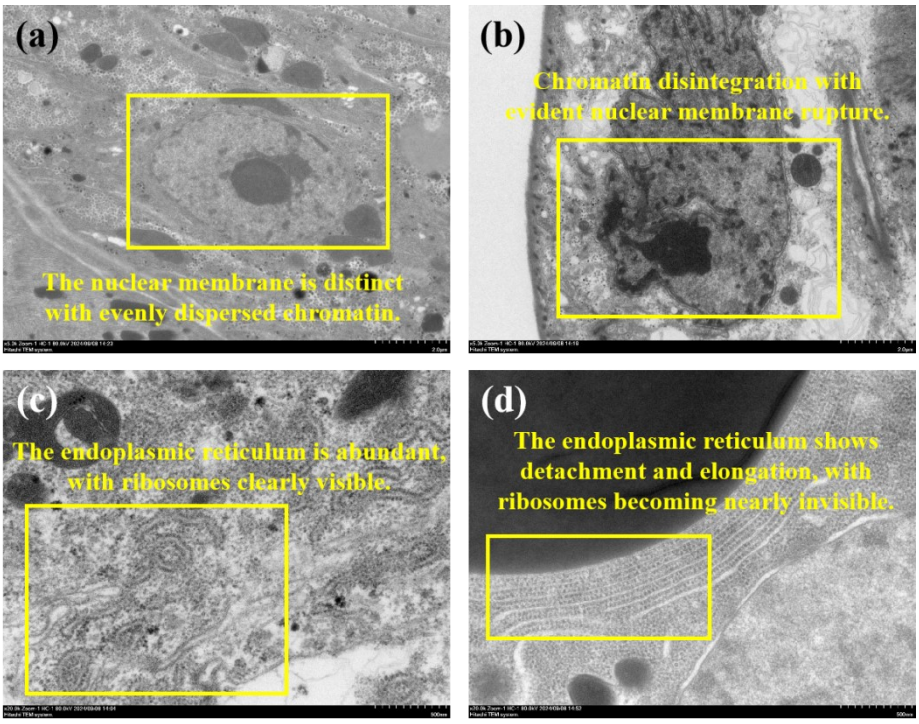


Fig. S12. Ultrastructural observation of somatic cells in chironomid larvae underdifferent treatments:(a) Control group nucleus TEM image (×5.0k);(b)UV/ClO_2-treated group nucleus TEM image (×5.0k);(c) Control group endoplasmic reticulum TEM image (×2.0k);(d) UV/ClO_2-treated groupendoplasmic reticulum TEM image (×2.0k).


(a)
The nuclear membrane is distinct with evenly dispersed chromatin.
(b)
Chromatin disintegration with evident nuclear membrane rupture.
(c)
The endoplasmic reticulum is abundant, with ribosomes clearly visible.
(d)
The endoplasmic reticulum shows detachment and elongation, with ribosomes becoming nearly invisible.