

Physiological and Transcriptomic Responses of the Freshwater Hydrozoan *Craspedacusta sowerbii* to Antibiotic and Heavy Metal Pollution: Insights into Adaptive Mechanisms and Ecological Risks

[Hailong Yan](#)^{*}, Yu Wang, Yufan He, Jinglong Wang, [Mengyao Wu](#), Shang Shi, Jianing Shi, Jingjing Guo, [Nicola Fohrer](#), [Jianguang Qin](#), [Yuying Li](#)^{*}

Posted Date: 4 December 2025

doi: 10.20944/preprints202512.0485.v1

Keywords: *Craspedacusta sowerbii*; transcriptomic response; water pollution; antibiotic; heavy metal



Preprints.org is a free multidisciplinary platform providing preprint service that is dedicated to making early versions of research outputs permanently available and citable. Preprints posted at Preprints.org appear in Web of Science, Crossref, Google Scholar, Scilit, Europe PMC.

Copyright: This open access article is published under a [Creative Commons CC BY 4.0 license](#), which permit the free download, distribution, and reuse, provided that the author and preprint are cited in any reuse.

Disclaimer/Publisher's Note: The statements, opinions, and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions, or products referred to in the content.

Article

Physiological and Transcriptomic Responses of the Freshwater Hydrozoan *Craspedacusta sowerbii* to Antibiotic and Heavy Metal Pollution: Insights into Adaptive Mechanisms and Ecological Risks

Hailong Yan ^{1,*†}, Yu Wang ^{2†}, Yufan He ¹, Jinglong Wang ¹, Mengyao Wu ¹, Shang Shi ¹, Jianing Shi ¹, Jingjing Guo ¹, Nicola Fohrer ^{1,3}, Jianguang Qin ⁴ and Yuying Li ^{1,*}

- ¹ International Joint Laboratory of Watershed Ecological Security for Water Source Region of Middle Route Project of South-North Water Diversion in Henan Province, College of Water Resource and Modern Agriculture, Nanyang Normal University, Nanyang 473061, China
- ² Henan Academy of Fishery Sciences, Henan Academy of Agricultural Sciences, Zhengzhou 450044, China
- ³ Department of Hydrology and Water Resources Management, Kiel University, 240980 Kiel, Germany
- ⁴ College of Science and Engineering, Flinders University, Adelaide, 5001, SA, Australia
- * Correspondence: nynu hailong@163.com (H.Y.); Tel.: +8613487096520. lyying200508@163.com (Y.L.); Tel.: +8615938462454
- † These authors contributed equally to this work.

Simple Summary

Freshwater ecosystems face escalating threats from pollution caused by human activities, including industrial waste and the improper disposal of pharmaceuticals. These contaminants pose risks not only to large aquatic animals but also to smaller, often overlooked organisms, such as the jellyfish relative *Craspedacusta sowerbii*, commonly known as freshwater jellyfish. In this study, researchers investigated the response of *C. sowerbii* to two prevalent pollutants: the antibiotic sulfamethoxazole, a common component of wastewater, and cadmium, a toxic heavy metal originating from industrial runoff. Through morphological observations and genetic analyses, the team discovered that both pollutants caused significant damage. Exposure to the antibiotic resulted in lethargy and body shrinkage in the jellyfish, while cadmium exposure led to complete loss of movement, physical disintegration, and rapid mortality within 24 hours. The study further revealed that *C. sowerbii* attempted to mitigate these effects by activating specific genes associated with stress resilience and damage repair. However, the pollutants overwhelmed these defense mechanisms, disrupting critical biological processes essential for survival. These findings underscore the vulnerability of even the smallest and least visible organisms to environmental contamination and highlight their potential as indicators of freshwater ecosystem health. By elucidating how *C. sowerbii* reacts to pollution, the study emphasizes the necessity for more effective strategies to safeguard water quality and preserve the biodiversity it sustains. This research serves as a poignant reminder that protecting our waterways requires proactive measures to prevent harmful chemicals and waste from infiltrating natural habitats.

Abstract

Water pollution poses a significant threat to global aquatic ecosystems. However, the adaptive mechanisms of aquatic organisms under such stress remain poorly understood. This study investigates the physiological and transcriptomic responses of the freshwater hydrozoan *Craspedacusta sowerbii* to two prevalent water pollutants: the antibiotic sulfamethoxazole (SMZ, 20 µM) and the heavy metal cadmium sulfate (Cd, 10 µM). Physiological observations revealed that exposure to SMZ reduced motility and induced body shrinkage, while exposure to Cd resulted in a complete loss of motility, physical disintegration, and mortality within 24 hours. Transcriptomic analysis via RNA-seq identified significant alterations in gene expression patterns. SMZ exposure

primarily up-regulated genes associated with oxidative stress, apoptosis, and immune responses, whereas Cd exposure resulted in extensive down-regulation of genes involved in metabolic pathways, cell cycle regulation, and anti-aging processes. Comparative analysis highlighted both shared and distinct pathways affected by the two pollutants, including disrupted cell motility, cytokinesis, and molecular transducer activity. Notably, Cd exposure induced more severe transcriptional suppression, correlating with higher mortality rates. These findings underscore the ecological significance of *C. sowerbii* as a sensitive indicator of water quality and provide molecular insights into its adaptive strategies under pollution stress. Furthermore, the study offers critical implications for understanding the broader impacts of water pollutants on aquatic biodiversity, emphasizing the need for improved pollution control measures. Future research should focus on the long-term multi-pollutant effects, field validation, and cross-species comparisons to enhance ecological conservation strategies.

Keywords: *Craspedacusta sowerbii*; transcriptomic response; water pollution; antibiotic; heavy metal

1. Introduction

Global environmental challenges are intensifying at an alarming rate, with issues such as climate change, air pollution, land degradation, and water pollution intricately intertwined [1,2]. Pollution problems, arising from a confluence of natural and anthropogenic factors, have inflicted unprecedented damage on the current ecological environment, leading to a severe decline in biodiversity [3,4]. Among these multifaceted environmental concerns, water pollution stands out as particularly critical, serving as a significant bottleneck to sustainable development [5]. The indiscriminate release of vast amounts of industrial wastewater, agricultural runoff laden with pollutants, and untreated domestic sewage directly into water bodies has precipitated a notable decrease in water quality [6]. This has led to frequent occurrences of eutrophication, a process characterized by excessive nutrient levels that trigger an overgrowth of algae, depleting oxygen levels and disrupting aquatic ecosystems [7]. Additionally, the substantial influx of pollutants from sewage into aquatic environments not only threatens the safety of drinking water supplies, heightening the risk of disease transmission, but also results in the destruction of vital habitats for aquatic organisms [6,8]. Incidents of aquatic biological poisoning have become increasingly frequent, further destabilizing fragile aquatic ecosystems. This, in turn, triggers a precipitous decline in biodiversity, with numerous rare aquatic species experiencing drastic population reductions and facing the imminent threat of extinction [8-10]. The decline biodiversity undermines critical ecosystem services, including water purification and flood mitigation, while reducing resilience to external stressors, thus perpetuating a vicious cycle of environmental deterioration [6,10].

The presence of emerging contaminants and heavy metals within aquatic ecosystems has escalated into a pressing global ecological crisis [11,12]. Alarming, millions of tons of these substances have been released into water bodies without adequate treatment [13,14]. Both types of pollutants are pervasive in contaminated aquatic systems, and their detrimental effects propagate through ecosystems via food chain transmission, resulting in far-reaching ecological repercussions [15,16]. Antibiotic pollutants, whether naturally occurring or synthetic, are prime examples of emerging contaminants, infiltrating water bodies through medical wastewater, livestock farming practices, and aquaculture discharges [17,18]. Study has shown that tetracycline, an antibiotic produced by Actinomycete, significantly reduces microbial community diversity in aquatic ecosystems [19]. Moreover, antibiotic pollutants exert toxic effects on the liver, kidneys, and reproductive organs of aquatic organisms [20,21]. In severe cases, they can even lead to mortality, thereby threatening aquatic biodiversity and disrupting the delicate balance of the aquatic environment [21]. In contrast, heavy metal pollution induces more persistent ecological disturbances, leading to malformations and extinctions of organisms, and thus exerting a profound impact on biodiversity [22]. Elements such as mercury, cadmium, and lead enter water bodies through mining operations, electroplating processes, and industrial wastewater discharges. Subsequently, they

accumulate in sediments and become increasingly concentrated as they ascend the food chain [22,23]. The toxicity of heavy metals to aquatic organisms manifests as damage to multiple physiological systems, for instance, cadmium toxicity causes severe damage to the liver and bones and can reduce calcium ion uptake in the body [23]. The accumulation of cadmium can induce tissue-specific variations and severely compromises the integrity of tissue structure and function, as well as the antioxidant defense system and the immune system in both fish and freshwater cladocerans [24,25]. Although extensive research has investigated emerging contaminants and heavy metals, key knowledge gaps still exist regarding effectively managing their ecological effects [26].

Craspedacusta sowerbii (Lankester, 1880), a freshwater hydrozoan belonging to the phylum Cnidaria [27-29], is originally from the Yangtze River in China, but has since dispersed globally, colonizing every continent except Antarctica, thereby establishing itself as one of the most successful aquatic colonizers [30-32]. Despite its widespread distribution, *C. sowerbii* has rarely been the subject of recent research, primarily due to the challenges associated with cultivating it in laboratory settings or sourcing it from wild populations [33]. The successful colonization of *C. sowerbii* largely depends on suitable environmental conditions and the availability of biological resources [34,35]. Various factors, including temperature, water quality, food availability, light and osmotic pressure, can independently or collectively influence the life cycle stages of *C. sowerbii* [34,36,37], rendering its presence in freshwater ecosystems a reliable indicator of good water quality [27,33,34]. In certain regional cultures, particularly in China, this species is often metaphorically referred to as the "giant panda in water" to emphasize its rarity and the fragility of its population, while simultaneously urging greater attention to the protection of its habitat from water pollution [27,38]. Research efforts have been directed towards elucidating the response mechanisms of *C. sowerbii* when confronted with stressful environments. Notably, study has revealed that intense light significantly influences the vertical migration patterns of *C. sowerbii*, thereby shaping its position in the water column [37]. Additionally, food scarcity and drought conditions stimulate the formation of a chitin-covered resting stage in *C. sowerbii*, enabling it to endure extreme conditions [28,39]. The survival duration of *C. sowerbii* declines sharply when osmotic pressure exceeds 34 mOsm/L [36]. Through phenotypic plasticity, *C. sowerbii* can adjust its population dynamics in response to varying dietary qualities [40]. Despite the increasing number of reported sightings, our understanding of the genetics, physiology, and ecology of *C. sowerbii* still remains notably limited, highlighted by a significant lack of data regarding its physiological responses to environmental challenges [41-44].

In this study, *C. sowerbii* was experimentally exposed to two prevalent water pollutants: the sulfamethoxazole antibiotic (SMZ) and the heavy metal salt cadmium sulfate (Cd). Subsequently, the physiological responses of *C. sowerbii* were observed. Specimens exhibiting distinct physiological symptoms were collected for transcriptome sequencing, to analyze the transcriptional responses of *C. sowerbii* to these water pollutants. The results revealed that both SMZ (20 μ M) and Cd (10 μ M) significantly impacted the motility of *C. sowerbii*. Notably, exposure to Cd for 6 hours resulted in a complete loss of motility, followed by physical disintegration of the organism after 24 hours. The transcriptome data provided profound insights into the molecular mechanisms underlying the transcriptional responses of *C. sowerbii* to these two types of water pollutants. On one hand, a substantial number of genes associated with oxidative stress, pathological signaling pathways, apoptosis, and programmed cell death were up-regulated. Conversely, genes involved in critical metabolic pathways such as the cell cycle, immunity, and anti-aging processes were down-regulated. The primary objective of this study is to leverage omics data to unravel the physiological responses of *C. sowerbii* when confronted with pollution stress. Furthermore, it serves as a clarion call for heightened awareness regarding the decline in biodiversity instigated by water pollutants and underscores the paramount importance of safeguarding aquatic environments and their invaluable organisms.

2. Materials and Methods

2.1. Pharmaceutical Management and Biosafety

SMZ was procured from Sigma-Aldrich (PubChem CID: 329824585). To prepare the stock solution, SMS powder was accurately weighed using an analytical balance, and subsequently added

to a 0.4% sodium hydroxide solution. The mixture was stirred thoroughly until the powder was completely dissolved, yielding a stock solution with a concentration of 30 mg/mL. The solution was then filtered through a 0.22 μ m filter membrane for sterilization purposes, and transferred to a sterile container for future use. Cd was obtained from a domestic reagent supplier. The Cd powder was precisely weighed and dissolved in deionized water to prepare a stock solution with a concentration of 10 mg/mL. This solution was also filtered through a 0.22 μ m filter membrane and transferred to a container for subsequent use. Following the experiments, the culture solutions containing these chemicals were collected and transported to a qualified facility responsible for the proper treatment and disposal of contaminated water.

2.2. Cultivation of *C. sowerbii* and Morphological Observation

Over 450 specimens of *C. sowerbii* were collected from a natural pond [27] and carefully transferred into a 10-liter plastic bucket. These specimens were cultured using water drawn directly from the same pond. Subsequently, they were transported to an indoor laboratory for further experimentation. Upon arrival, the specimens were randomly divided into nine groups, with three serving as control groups (CK). The remaining three groups were exposed to the addition of SMZ, at a final concentration of 20 μ M, while the last three groups were treated with Cd, achieving a final concentration of 10 μ M. Each group, consisting of approximately 50 individuals, was placed in a 3-liter glass aquarium measuring 25 cm in length, 10 cm in width, and 12 cm in height. All the aquariums were then placed inside an incubator, set to maintain a constant temperature of 25°C. Morphological observations of *C. sowerbii* individuals were conducted using a Canon 80D digital camera equipped with a 50 mm macro lens (Canon Ltd., Tokyo, Japan). Both photographs and videos were captured to accurately quantify the frequency of swimming movements exhibited by *C. sowerbii* per minute, a metric that serves as an indicator of their relative activity dynamics. These photographs and videos were edited to enhance contrast and calculate swimming movements, utilizing software such as Adobe Premiere Pro and Adobe Photoshop (Adobe Systems Incorporated, San Jose, CA, USA).

2.3. Sample Preparation for RNA-Seq Analysis

Fifteen individuals of *C. sowerbii* were collected from various groups, including the CK groups, SMZ-treated groups sampled at 2 hours post-inoculation (SMZ_2h), SMZ-treated groups sampled at 24 hours post-inoculation (SMZ_24h); Cd-treated groups sampled at 2 hours post-inoculation (Cd_2h) and Cd-treated groups sampled at 6 hours post-inoculation (Cd_6h). Each individual was carefully picked using a pipette with a diameter of approximately 10 mm, and all 15 *C. sowerbii* individuals were subsequently placed into a 15 mL freezing tube. A rolled-up filter paper was inserted into the freezing tube to absorb any remaining water. The samples were promptly frozen in liquid nitrogen and stored at -80°C until RNA extraction was performed. For each treatment, three replicates were prepared. The total RNA of these samples was extracted using Trizol reagent (Invitrogen, CA, USA), strictly adhering to the manufacturer's instructions. The RNA quantity was then measured using a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, MA, USA), and RNA integrity was assessed using an Agilent 2100 bioanalyzer system along with an RNA Nano 6000 Assay kit (Agilent Technologies, CA, USA). Only qualified RNA was reverse-transcribed into double-stranded cDNA, which was subsequently subjected to sequencing, performing in 2×150-bp paired-end runs utilizing an Illumina NovaSeq X Plus system (Illumina, CA, USA). This entire sequencing process was performed at Novogene Co., Ltd. (Beijing, China).

2.4. Bioinformatics Analysis Pipeline

The raw data, presented in fastq format, initially underwent processing using the fastp software. During this preprocessing stage, clean data were generated by eliminating reads containing adapters, reads with poly-N sequences, and low-quality reads from the raw dataset. All subsequent analyses were conducted based on this high-quality clean data. Transcriptome assembly of the RNA-seq data was carried out using Trinity (v2.15.1) software. Building upon the transcriptome assembly results produced by Trinity, Corset (Nadia M. Davidson, Alicia Oshlack, 2014) was utilized to segregate

transcripts exhibiting differential expression across samples from their original clusters and to form new clusters. Ultimately, each cluster was designated as a Gene. The assembly quality of the Trinity.fasta, unigene.fa, and cluster.fasta files derived from the assembly process was evaluated using the BUSCO software (Benchmarking Universal Single-Copy Orthologs). Gene functional annotation was performed based on databases including Nr (the protein sequence database of NCBI); Nt (the nucleic acid sequence database of NCBI); Pfam (the most comprehensive classification system for protein domain annotations); KOG (euKaryotic Ortholog Groups); Swiss-Prot (a database curating and studying protein sequences by experienced biologists); GO (an internationally standardized classification system for describing gene functions); and KEGG (a database analyzing the metabolic pathways of gene products and compounds within cells, along with their functions). The transcriptome assembled by Trinity served as the reference sequence (Ref). For each sample, the clean reads were aligned to the Ref. During this alignment process, reads with a mapping quality score below 10, successfully mapped unpaired reads, and reads mapped to multiple genomic regions were filtered out. The alignment was performed using the RSEM (v1.3.3) software, incorporating the bowtie2 parameter "mismatch 0" (the default parameter of bowtie2) within RSEM.

2.5. Differential Expression Analysis

Differential expression analysis between two groups was conducted using the DESeq2 R package (version 1.42.0). DESeq2 provides statistical tools for identifying differential expression in digital gene expression data, employing models based on the negative binomial distribution. The P-values obtained were adjusted using the Benjamini-Hochberg method to control the false discovery rate. The criteria for significant differential expression were established as: an adjusted p-value (padj) ≤ 0.05 and an absolute $\log_2$ fold-change ($\log_2\text{FC}$) ≥ 1 . Gene Ontology (GO) enrichment analysis of differentially expressed genes (DEG) was performed using the Goseq R package, which corrects for gene length bias. GO terms with a corrected p-value less than 0.05 were considered significantly enriched among DEG. The Kyoto Encyclopedia of Genes and Genomes (KEGG) is a database resource that facilitates the understanding of the high-level functions and utilities of biological systems, such as cells, organisms, and ecosystems, based on molecular-level information, particularly large-scale molecular datasets generated by genome sequencing and other high-throughput experimental technologies (<http://www.genome.jp/kegg/>). We utilized KOBAS software to statistically assess the enrichment of DEG in KEGG pathways.

3. Results

3.1. The Symptoms and Features of *C. sowerbii* Under Water Pollution

As previously reported, tentacle kinematics and the frequency of swimming movements per minute serve as key indicators for assessing the relative activity dynamics of *C. sowerbii* [45]. In this study, specimens of *C. sowerbii* were cultivated indoors under various pollutant treatments for 24 h, and their relative activity dynamics were meticulously recorded. In the CK groups, the individuals exhibited continuous vertical swimming, remaining suspended in the water column. The frequency of swimming movements remained stable throughout the observation period, with no discernible differences in individual morphology (Table 1). The tentacle activity of *C. sowerbii* in this treatment was relatively high, with tentacles remaining spread out and positioned above the body, regardless of whether observed from the anterior or apical view (Figure 1A-B). In the SMZ-treated groups, the initial frequency of swimming movements remained relatively stable (Table 1), with no obvious differences in individual morphology observed within the first 12 h (Figure 1C). However, over time, about 24 h post SMZ treatment, the swimming movement frequency in this group declined to approximately 54% of that in the CK groups (Table 1). After 24 h of cultivation, notable abnormal behaviors and delayed morphological responses were evident in individual specimens of the SMZ-treated groups. Some individuals began to sink to the bottom of the water and exhibited signs of body shrinkage (Figure 1D). In the Cd-treated groups, no obvious differences in individual morphology were observed within the first 1 h post treatment (Table 1). However, the swimming movement frequency of *C. sowerbii* began to decline after just 2 h of Cd treatment (Table 1), and the movement ceased entirely by the 12 h mark (Table 1). The body of *C. sowerbii* started to shrink within just 2 h,

and this shrinkage became increasingly pronounced as time progressed (Figure 1E-F). Eventually, by the 6 h mark, nearly every *C. sowerbii* individual in the Cd-treated groups had curled into a compact mass, with all movement ceasing (Figure 1F). Subsequently, the bodies disintegrated into fragments after 24 hours of Cd treatment (Data not shown).

Table 1. Number of swimming movement per minute, which indicates the relative activity dynamics of *C. sowerbii*, during the cultivation time period under various treatments.

Group	1 h	2 h	6 h	12 h	24 h
CK	42.3 ± 8.1	43.9 ± 5.6	45.5 ± 6.0	43.1 ± 7.2	43.5 ± 6.4
SMZ	43.9 ± 10.8	41.7 ± 13.8	40.4 ± 12.3	41.8 ± 7.5	23.4 ± 8.7
Cd	42.7 ± 6.9	20.1 ± 8.3	4.3 ± 5.0	0	0

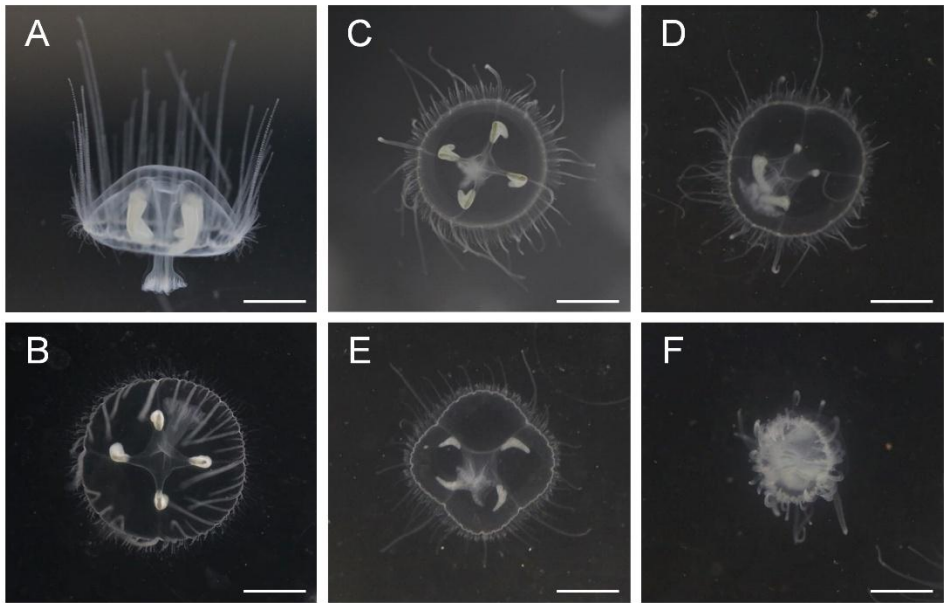


Figure 1. Visual representation of typical *C. sowerbii* during the cultivation period under various treatment conditions. Anterior (A) and apical view (B) of the specimens in the CK groups. Apical view of the SMZ_2h groups (C), SMZ_24h groups (D), Cd_2h groups (E), and Cd_6h groups (F). It should be noted that *C. sowerbii* individuals remain suspended in the water in A-C, whereas they sink to the bottom of the water and exhibit body shrinkage in D-F. Bars = 0.5 cm.

3.2. Transcriptomic Variations of *C. sowerbii* Under Water Pollution

The SMZ_2h and Cd_2h groups represent the initial stages when *C. sowerbii* begins to show abnormal living characteristics in response to water pollutants, and the SMZ_24h and Cd_6h groups mark the later stages of its response to these pollutants. Specimens from these groups were collected to analyze the transcriptomic variations of *C. sowerbii* under different types of water pollution, with the CK groups serving as the control. A total of 60,966, 51,097, 61,999, 51,448, and 58,308 genes were identified through RNA-seq analysis in the CK groups, SMZ_2h groups, SMZ_24h groups, Cd_2h groups, and Cd_6h groups, respectively (Figure 2A, E). Under SMZ pollution conditions, a total of 43,046, 49,493, and 43,203 genes were co-expressed in the comparisons of SMZ_2h vs CK, SMZ_24h vs CK, and SMZ_24h vs SMZ_2h groups, respectively (Figure 2A). Among these, 2,408 and 752 genes exhibited differential expression in the SMZ_2h vs CK and SMZ_24h vs CK groups, respectively (Figure 2B, File S1). Specifically, 682 and 497 genes were up-regulated (Figure 2C), while 1,726 and 255 genes were down-regulated (Figure 2D) in these comparisons. Notably, a total of 39,910 genes were co-expressed across the CK, SMZ_2h, and

SMZ_24h groups (Figure 2A). Of these, 105 genes were differentially expressed (Figure 2B), including 55 up-regulated (Figure 2C) and 37 down-regulated genes (Figure 2D).

Upon exposure to Cd pollution, co-expression analysis indicated that 43,603, 46,643, and 42,900 genes were commonly expressed in the pairwise comparisons of Cd_2h vs CK, Cd_6h vs CK, and Cd_6h vs Cd_2h groups, respectively (Figure 2E). Among the analyzed genes, 2,307 and 3,975 genes exhibited differential expression patterns in the Cd_2h vs CK and Cd_6h vs CK groups, respectively (Figure 2F, File S1). Specifically, 466 and 1,609 genes were up-regulated (Figure 2G), while 1,841 and 2,366 genes were down-regulated (Figure 2H) in these comparisons. Notably, a core set of 39,512 genes was consistently co-expressed across the CK, Cd_2h, and Cd_6h groups (Figure 2E). Of these, 1,202 genes displayed differential expression (Figure 2F), with 96 up-regulated (Figure 2G) and 1,096 down-regulated genes (Figure 2H).

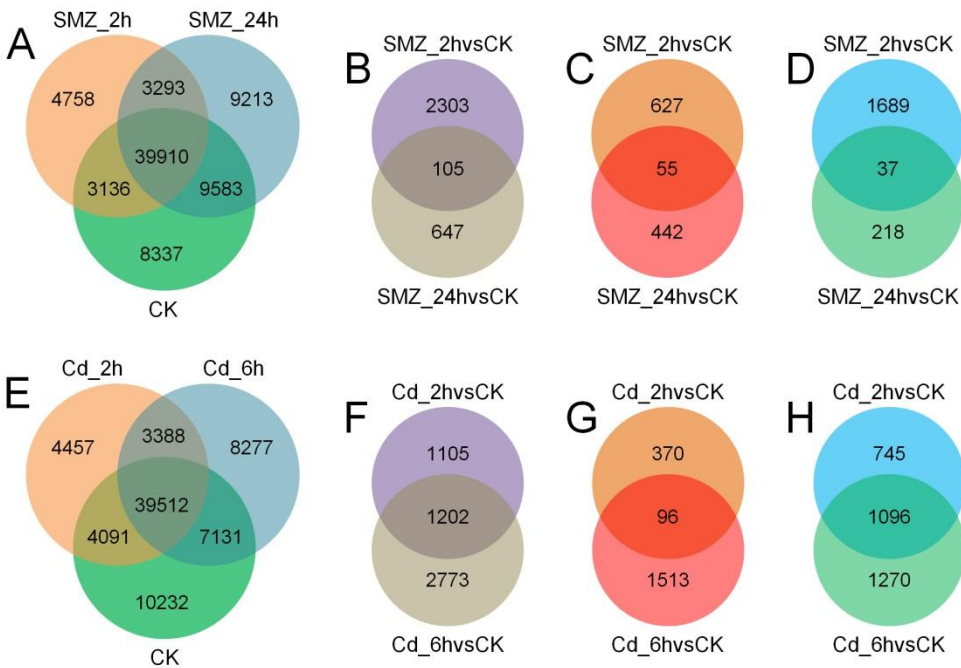


Figure 2. Overview of the transcriptomic variations of *C. sowerbii* in response to water pollution. SMZ-treated groups with co-expression (A), differential expression (B), up-regulation (C), and down-regulation (D) patterns. Cd-treated groups with co-expression (E), differential expression (F), up-regulation (G), and down-regulation (H) patterns.

3.3. Metabolic Pathway Alterations in *C. sowerbii* in Response to Water Pollution

The DEG are likely of critical importance, playing pivotal roles in the metabolic processes that enable *C. sowerbii* to respond effectively to water pollution. Consequently, GO and KEGG analyses were performed on these genes (File S1), aiming to thoroughly investigate the potential functions associated with the top 20 most notably altered terms. However, significant alterations in GO and KEGG terms were observed only in the comparison groups of SMZ_2h vs CK and Cd_6h vs CK (File S2).

Under GO analysis, the metabolic pathways of the SMZ_2h groups exhibited notable changes compared to the CK groups. Specifically, pathways related to cytokinesis (involving 14 genes) and cell motility (also involving 14 genes) showed significant alteration (Figure 3A). Among these, pathways associated with structural molecule activity (involving 30 genes) demonstrated a marked up-regulation (Figure 3B). Conversely, pathways related to cell motility (12 genes), cilium (10 genes), and cytokinesis (9 genes) displayed significant down-regulation (Figure 3C). In the comparison between the Cd_6h and CK groups, although no metabolic pathways exhibited significant overall alteration (Figure 3D) or significant down-regulation (Figure 3F), pathways associated with molecular transducer activity (encompassing 43 genes) demonstrated significant up-regulation (Figure 3F). It is particularly noteworthy that, in addition to the markedly altered metabolic

pathways, pathways related to signaling, defense response, programmed cell death, and wound healing were among the most significantly up-regulated. In contrast, pathways associated with molecular activity, cell cycle progression, cell motility, and immune system processes were among the most notably down-regulated in *C. sowerbii* when exposed to water pollutants (Figure 3). The activation or suppression of these pathways may indicate the cellular and molecular damage inflicted by water pollutants, as well as the organism's efforts to mitigate these toxic effects through various defense and repair mechanisms.

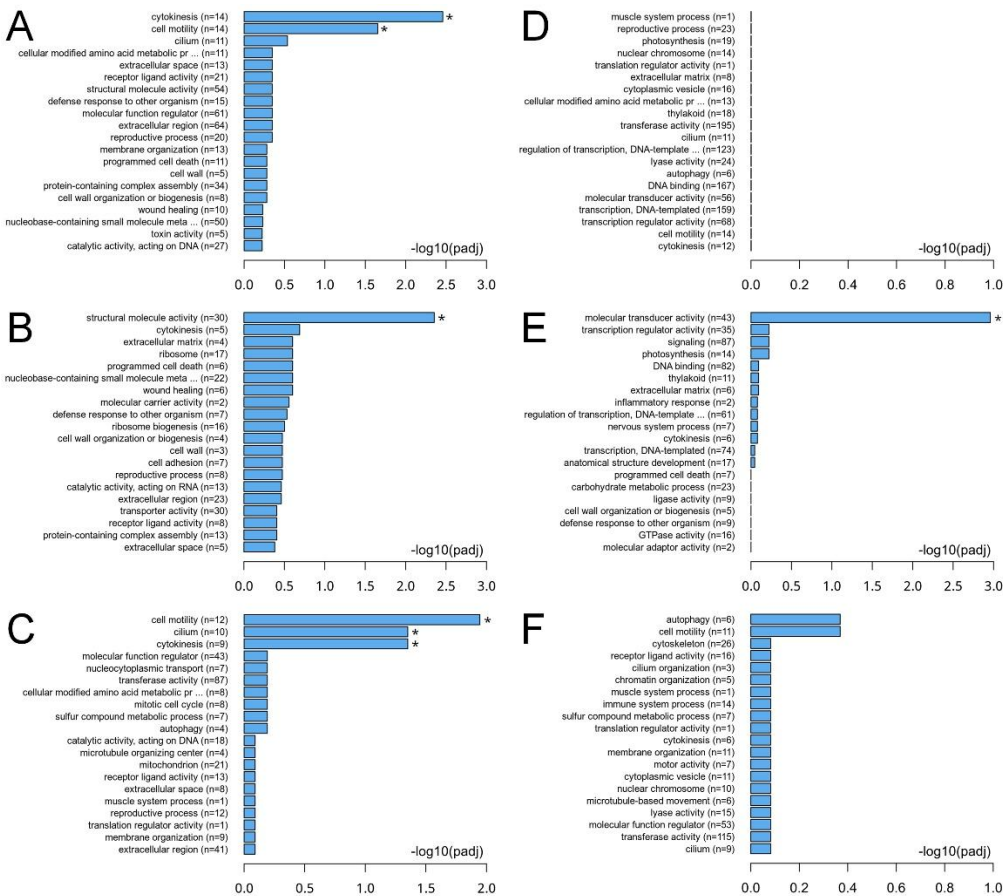


Figure 3. GO analysis of DEG in *C. sowerbii* across different comparison groups. The top 20 GO terms for the overall (A), up- (B) and down- (C) regulated metabolic pathways in the SMZ_2h vs CK groups. The top 20 GO terms for the overall (D), up- (E) and down- (F) regulated metabolic pathways in the Cd_6h vs CK groups.

Under KEGG analysis, distinct patterns of pathway alterations emerged in the comparison groups of *C. sowerbii*. Results indicated that in the SMZ_2h vs CK groups, pathways associated with cellular senescence, encompassing 14 genes, demonstrated significant changes (Figure 4A). Among these top regulated pathways, the phototransduction pathway (involving 5 genes), the oxytocin signaling pathway (with 12 genes), and the vascular smooth muscle contraction pathway (comprising 13 genes) exhibited notable up-regulation (Figure 4B). Conversely, pathways related to the cell cycle (9 genes), progesterone-mediated oocyte maturation (7 genes), and cellular senescence (9 genes) showed significant down-regulation (Figure 4C). When comparing the Cd_6h and CK groups, pathways linked to fatty acid degradation (7 genes), cellular senescence (14 genes), and neuroactive ligand-receptor interaction (20 genes) displayed significant alteration (Figure 4D). Notably, the neuroactive ligand-receptor interaction pathway, involving 18 genes, showed marked up-regulation (Figure 4E). In contrast, pathways related to the FoxO signaling pathway (7 genes), the PPAR signaling pathway (5 genes), cell cycle (9 genes), progesterone-mediated oocyte maturation (8 genes), fatty acid degradation (7 genes), and cellular senescence (12 genes) all exhibited significant down-regulation (Figure 4F). Other metabolic pathways that underwent the most significant changes under KEGG analysis included signaling and cell cycle (Figure 4). These results provide fundamental

insights into the molecular responses of *C. sowerbii* to different environmental stressors, highlighting the complex regulation of various biological processes under water pollution conditions.

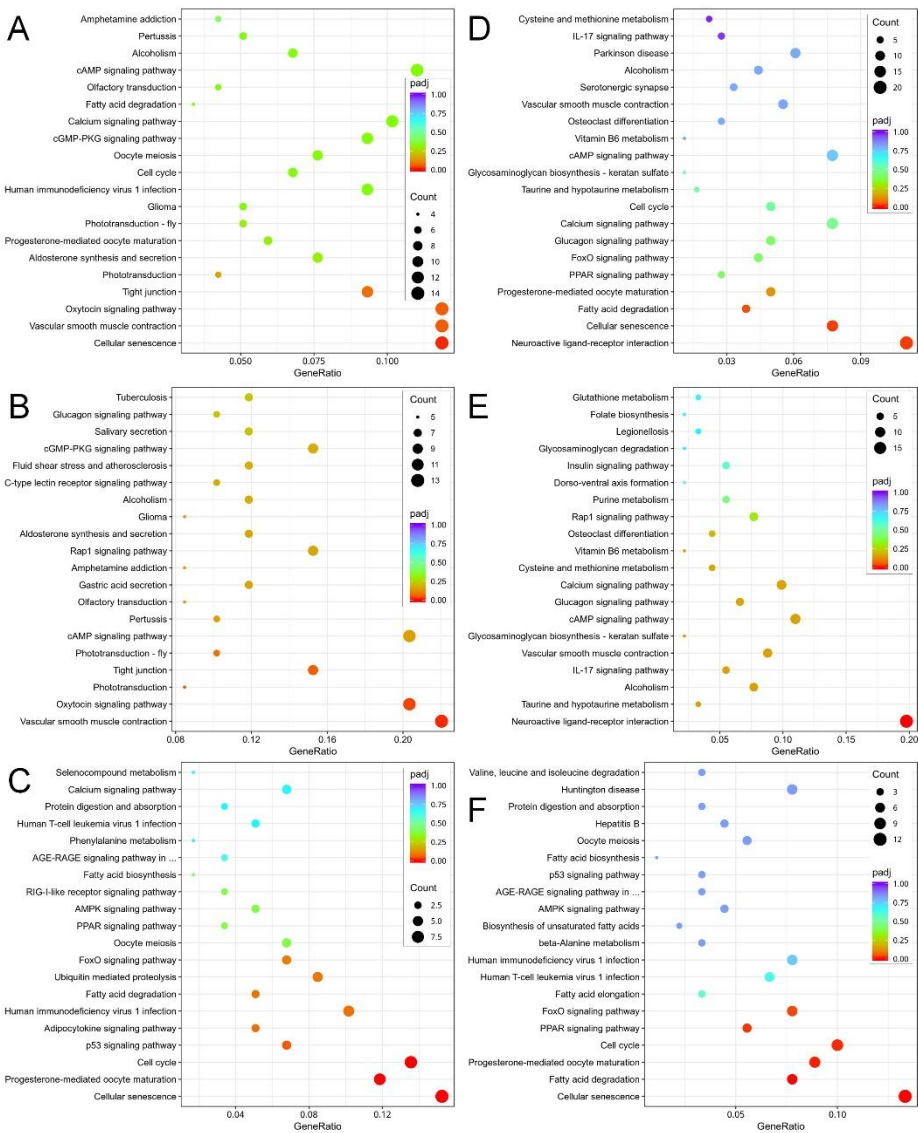


Figure 4. KEGG functional enrichment analysis of DEG in *C. sowerbii* across diverse comparison groups. The top 20 KEGG terms for the overall (A), up- (B) and down- (C) altered metabolic pathways in the SMZ_2h vs CK groups. The top 20 KEGG terms for the overall (D), up- (E) and down- (F) altered metabolic pathways in the Cd_6h vs CK groups.

3.4. Alterations in Gene Expression Profiles of *C. sowerbii* in Response to Water Pollution

Based on the observed symptoms and the alterations in the metabolic pathways of *C. sowerbii* under water pollution conditions, particular attention has been directed towards examining the gene expression patterns within key metabolic pathways under KEGG enrichments (File S1), which encompass cell cycle, cellular senescence, apoptosis, oxidative phosphorylation, FoxO signaling pathway, and the Ras-Rap1 signaling.

A total of ten apoptosis-related genes were identified, with four demonstrating up-regulation in the SMZ_2h vs CK comparison. In the Cd_6h vs CK groups, two genes were up-regulated while four were down-regulated. Notably, the comparisons of SMZ_24h vs CK and Cd_2h vs CK exhibited only limited expression alterations (Figure 5). Additionally, 14 cell cycle-related genes were identified, all of which consistently demonstrated down-regulation across treatment groups (Figure 5). Among the 21 cellular senescence-associated genes, the majority exhibited down-regulated expression patterns (Figure 5). Furthermore, nine genes involved in oxidative phosphorylation were identified, with most showing up-regulation. Specifically, Cluster-10902.35885 and Cluster-10902.19054 exhibited

particularly striking log2FC values of 7.87 and 4.94 in the SMZ_2h vs CK and Cd_6h vs CK groups, respectively (Figure 5). In the FoxO signaling pathway, ten DEG were identified, with the exception of one gene showing a log2FC of 1.20 in the Cd_6h vs CK group, all others exhibited down-regulation (Figure 5). Lastly, in the Ras-Rap1 signaling pathway, 24 genes were identified, the majority of which displayed up-regulated expression patterns (Figure 5).

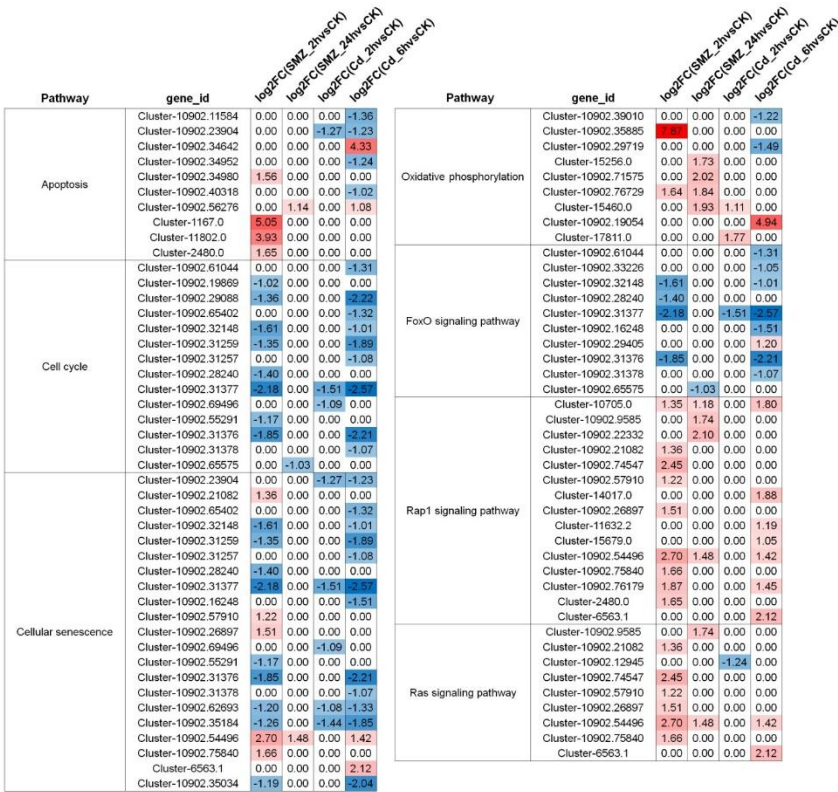


Figure 4. Impact of water pollutants on gene expression patterns in *C. sowerbii*. Differential gene expression was visualized using log2FC values. Genes exhibiting significant up-regulation were highlighted in red, while down-regulated genes were marked in blue across experimental groups. A log2FC value of 0 indicated no differential expression of the gene.

4. Discussion

4.1. Similarities and Differences of *C. sowerbii* Under Different Water Pollution Treatments

When confronted with environmental pollutants, certain organisms can gradually acclimate to their surroundings by modulating their physiological metabolism, while others are unable to effectively counteract the physiological toxicity induced by these pollutants, ultimately leading to individual mortality [24,46]. Among the most frequently abused pollutants pervasive in various aquatic environments are the antibiotic SMZ and the heavy metal Cd [11,47]. Studies have demonstrated that the extensive use of SMZ has contaminated aquatic ecosystems, resulting in oxidative damage and immune system malfunctions in the livers and gills of organisms, for example, exposure to SMZ at concentrations ranging from 1 to 10 mg/L has been shown to affect the heart rate and swimming behavior of zebrafish [48,49]. The toxicity of Cd to aquatic animals is lethal, exposure of zebrafish larvae to Cd at concentrations of 3–8 mg/L significantly elevated the mortality rate, accompanied by a notable increase in the expression of heat shock proteins [50,51]. In this study, the freshwater hydrozoan *C. sowerbii* was exposed to SMZ and Cd at concentrations of 20 μM (approximately 5 mg/L) and 10 μM (approximately 2.5 mg/L), respectively. Morphological observations revealed abnormal behavioral alterations in *C. sowerbii* following treatment with SMZ or Cd for varying durations. These changes primarily manifested as a decrease in swimming activity (Table 1) and symptoms such as body shrinkage (Figure 1). In severe cases, such as after 24 hours of

Cd treatment, individuals of *C. sowerbii* succumbed and their bodies disintegrated, indicating that these water pollutants were detrimental to the growth and survival of *C. sowerbii*.

The physiological toxicities of these two water pollutants on *C. sowerbii* exhibit both similarities and differences. Phenotypically, both pollutants affect the movement of *C. sowerbii*. However, while individuals treated with SMZ show reduced motility, those subjected to Cd exposure experience not only decline in swimming ability, but also the threats of death and disintegration (Figure 1). Furthermore, RNA-seq analysis indicates that when *C. sowerbii* is exposed to SMZ pollution at later stage, the metabolic response primarily involves the up-regulation of DEG (Figure 2). In contrast, when confronted with Cd pollution, a greater number of DEG exhibit down-regulation patterns (Figure 2). Moreover, the performance of *C. sowerbii* varies at different time points following exposure to different pollutants. Two hours after SMZ treatment, there are 2,408 DEG, whereas after 24 hours of SMZ exposure, the number of DEG decreases to only 752 (Figure 2). This suggests that *C. sowerbii* rapidly initiates gene transcriptional regulatory responses when exposed to SMZ pollution, thereby conferring a certain degree of resistance to SMZ contamination. This observation is also supported by the absence of lethal outcomes in *C. sowerbii* at the phenotypic level (Figure 1). However, the scenario markedly differs when *C. sowerbii* is exposed to Cd pollution. Firstly, two hours after Cd exposure, there are 2,307 DEGs, a number that increases to 3,975 six hours post-exposure (Figure 2). This suggests that, at the transcriptional level, gene regulation in *C. sowerbii* becomes increasingly intense. Secondly, following Cd treatment, the DEG of *C. sowerbii* predominantly exhibit a down-regulation pattern (Figure 2), indicating that many metabolic pathways are inhibited and normal ones are inevitably disrupted. This might explain why *C. sowerbii* ceases movement and begins to deteriorate after Cd treatment.

Transcriptomic data provides a robust approach to characterizing metabolic alterations in organisms in response to pollution stress, thereby elucidating the hazards that pollutants pose to living organisms [52,53]. In this study, the most significant metabolic regulation in *C. sowerbii* was observed in the SMZ_2h vs CK and Cd_6h vs CK treatment groups (Figure 3-5). Consequently, a detailed analysis of the transcriptomic data from these two treatment groups was performed. The results indicated that a total of 10,383 genes were co-expressed in both the SMZ_2h vs CK and Cd_6h vs CK groups, with 1,005 genes possessing a KEGG orthology identity (KO_id) (File S1, Figure 6A). Among all identified genes, 1,270 DEG were shared between the two groups, encompassing 128 KO_id (File S1). Of these 1,270 DEG, 116 exhibited an up-regulation pattern (with 20 KO_id), while 1,152 displayed a down-regulation pattern (with 108 KO_id) (Figure 6A). The up- and down-regulated genes were subsequently enriched based on their KO_id (Figure 6B-C). The results demonstrated that the top 12 most up-regulated KEGG orthologies were enriched in pathways related to cancer, neurodegeneration, Wnt signaling, Rap1 signaling, calcium signaling, vascular smooth muscle contraction, proteoglycans in cancer, neuroactive ligand signaling, cAMP signaling, glucagon signaling, melanogenesis and alcoholism (Figure 6B). Although these pathways span a wide array of biological domains, including cancer biology, neurodegeneration, metabolism, endocrine signaling, and behavioral processes, they converge on shared mechanisms that regulate cell fate determination [54,55]. Moreover, these factors contribute to the disruption of homeostasis and exhibit responsiveness to extracellular stimuli, collectively indicating that *C. sowerbii* is undergoing pathological conditions. In contrast, the top 12 most significantly down-regulated KEGG orthologies were enriched in pathways related to cellular senescence, neurodegeneration, cell cycle, progesterone-mediated oocyte maturation, fatty acid metabolism, shigellosis, fatty acid degradation, human immunodeficiency virus 1 infection, calcium signaling, oocyte meiosis, motor proteins, and ubiquitin mediated proteolysis (Figure 6C). These pathways predominantly represent interconnected networks that regulate cell-cycle progression, stress response, and survival [56-58]. The combined up- and down-regulation of these pathways suggests that *C. sowerbii* is exhibiting a pathological state when exposed to SMZ or Cd contamination.

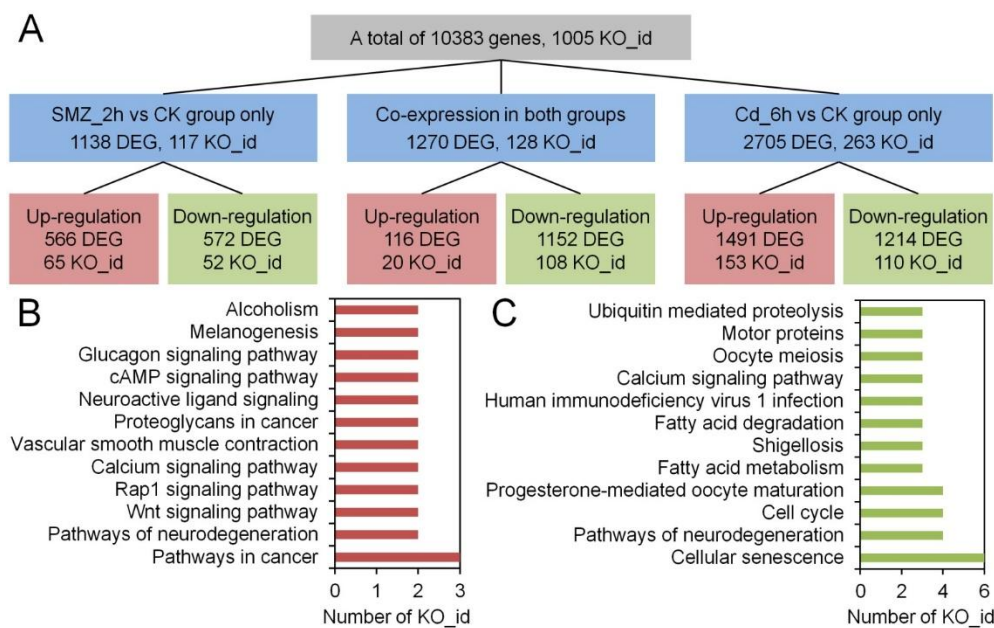


Figure 6. Overview of the transcriptomic variations of *C. sowerbii* in response to water pollutants. The composition of DEG and their corresponding KO_id across various comparison groups (A), along with the distribution patterns of KO_id exhibiting up-regulated (B) and down-regulated (C) expression patterns among the DEG that were commonly identified in both the SMZ_2h vs CK groups and the Cd_6h vs CK groups.

On the other hand, notable differences were observed in the gene expression profiles of *C. sowerbii* under different water pollution treatments. Among the 10,383 identified genes, 1,138 DEG were uniquely present in the SMZ_2h vs CK groups, encompassing 117 KO_id. Of these 1,138 DEG, 566 exhibited an up-regulation pattern (with 65 KO_id), while 572 displayed a down-regulation pattern (with 52 KO_id) (File S1, Figure 6A). Additionally, another 2,705 DEG were uniquely identified in the Cd_6h vs CK groups, encompassing 263 KO_id. Among these 2,705 DEG, 1,491 showed an up-regulation pattern (with 153 KO_id), whereas 1,214 exhibited a down-regulation pattern (with 110 KO_id) (Figure 6A). All these up- and down-regulated genes were subsequently enriched based on their KO_id (Figure 7). Notably, pathways in cAMP signaling (up-regulation) and cellular senescence (down-regulation) were enriched not only in the shared gene portions between the SMZ_2h vs CK and Cd_6h vs CK groups, but also in gene portions unique to either the SMZ_2h vs CK groups or the Cd_6h vs CK groups (Figure 7). Apart from these two pathways, the remaining pathways exhibited distinct enrichment patterns (Figure 7). This suggests that when subjected to different water pollution treatments, gene expression at the transcriptome level in *C. sowerbii* displays both similarities and differences. These similarities give rise to analogous physiological responses in *C. sowerbii* under the two separate pollution scenarios. Conversely, the differences provide an explanation for why SMZ treatment merely impacts the motility of *C. sowerbii*, while Cd treatment causes their mortality due to its toxic effects.

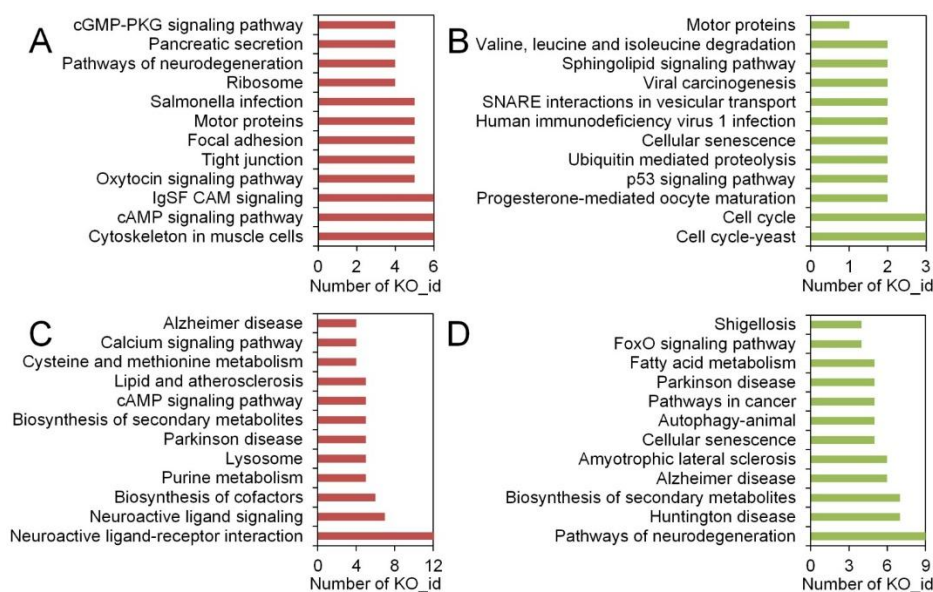


Figure 7. The distribution patterns of KO_id uniquely presented in different groups. Up- (A) and down- (B) enriched patterns in the SMZ_2h vs CK groups only. Up- (C) and down- (D) enriched patterns in the Cd_6h vs CK groups only.

4.2. Adaptation and Evolution of Aquatic Lives Under the Stress of Water Pollution

While the proliferation of *C. sowerbii* depends on high-quality aquatic habitats [27,33,34], the prevalence of water pollution poses significant changes for this species in avoiding contamination. Currently, *C. sowerbii* is globally distributed and displays a tendency to expand its range further due to climate change [30,31,59]. The success of its colonization can be attributes to multiple factors. Research has demonstrated that activities such as fish stocking, bird migrations, and the importation of ornamental aquatic plants and pets, facilitate the efficient spread of *C. sowerbii*, and significantly increasing opportunities for transmission [32,60,61]. Furthermore, *C. sowerbii* employs evolutionary strategies to thrive in adverse conditions, including adopting stress-resistant forms or developing chitinous resting stages to endure harsh environmental circumstances [39,62]. Despite these adaptations, the impact of water pollutants on *C. sowerbii*'s survival remains a critical concern. Firstly, pollutants such as SMZ and Cd can directly disrupt its behavior and gene expression, imposing toxic effects on individuals. Secondly, these contaminants can also indirectly hinder reproduction by altering aquatic ecosystem dynamics, such as disrupting algal, zooplankton, or microbial communities, thereby depleting nutritional resources for *C. sowerbii* [20,22,27]. These together indicate that not only *C. sowerbii* but also other aquatic organisms face increasing threats from pollution. By utilizing *C. sowerbii* as a model organism and SMZ/Cd as representative pollutants, this study investigates the toxicological effects of common environmental contaminants on aquatic lives, and aims to raise awareness about the ecological consequences of pollution, encourage proactive environmental stewardship, and underscore the urgency of protecting biodiversity.

This study also provides foundational insights into the physiological and molecular adaptive mechanisms of *C. sowerbii* when exposed to water pollutants, specifically, the antibiotic SMZ and the heavy metal Cd. These findings not only enhance our understanding of *C. sowerbii*'s evolutionary strategies but also offer critical references for other aquatic organisms facing similar pollution challenges. On one hand, the species' reliance on gene expression regulation to mitigate pollution stress establishes a predictive framework for interpreting molecular responses in other aquatic taxa exposed to analogous contaminants. On the other hand, the observed physiological and behavioral changes, such as reduced motility, body shrinkage, and disintegration underscore the shared vulnerability of aquatic organisms facing extreme environmental stress. This emphasizes the necessity of prioritizing pollution-sensitive keystone species in ecosystem conservation, as their decline could trigger the destruction of ecological chain stability, destabilizing entire aquatic systems.

Despite the advancements made in elucidating the adaptive responses of *C. sowerbii* to water pollution, several limitations remain. First, the research primarily focused on the short-term effects of two isolated pollutants (SMZ and Cd), leaving the long-term consequences of chronic, multi-pollutant exposure unresolved. Future studies should investigate the synergistic interactions among pollutants and their cumulative impacts on the physiological and ecological functions of *C. sowerbii*. Second, while transcriptomic analyses were predominant in this work, the limited integration of proteomic and metabolomic approaches restricts holistic insights into the molecular regulatory networks. Multi-omics strategies should be prioritized to unravel the species' adaptive mechanisms more comprehensively. Finally, laboratory-based experiments may not fully replicate natural conditions, potentially skewing the results. Strengthening field monitoring alongside controlled studies will enhance ecological relevance, providing more robust scientific foundations for conservation and environmental management.

5. Conclusions

This study systematically investigates the pathological and transcriptional responses of the freshwater hydrozoan *C. sowerbii* to two prevalent water pollutants: the antibiotic SMZ and the heavy metal Cd. Through an integrated approach that combines physiological observations with transcriptomic analyses, several critical insights into the species' vulnerability, adaptive strategies, and ecological implications are revealed.

1, Both SMZ and Cd exert significant detrimental effects on *C. sowerbii*. Exposure to SMZ results in reduced motility and body shrinkage, indicating a stress response that compromises the organism's ability to maintain normal physiological functions. In contrast, Cd exposure induces more severe outcomes, including complete loss of motility, physical disintegration, and rapid mortality within 24 hours.

2, Transcriptomic analysis provides profound insights into the molecular mechanisms underlying *C. sowerbii*'s responses to these pollutants. SMZ exposure primarily up-regulates genes associated with oxidative stress, apoptosis, and immune responses, suggesting that the organism attempts to counteract the toxic effects of the antibiotic through enhanced stress resilience and damage repair mechanisms. Conversely, Cd exposure results in extensive down-regulation of genes involved in metabolic pathways, cell cycle regulation, and anti-aging processes, indicating a more severe transcriptional suppression that correlates with higher mortality rates.

3, The study highlights the ecological significance of *C. sowerbii* as a sensitive indicator of water quality. The observed physiological and transcriptional responses to water pollution underscore the potential of this species to serve as an early warning system for detecting environmental contamination. By elucidating the molecular mechanisms underlying *C. sowerbii*'s adaptive strategies under pollution stress, this research provides critical references for understanding the broader impacts of water pollutants on aquatic biodiversity.

4, Future studies should explore the synergistic interactions among pollutants and their cumulative impacts on *C. sowerbii*'s physiological and ecological functions. Multi-omics strategies should be prioritized to unravel the species' adaptive mechanisms more comprehensively. Strengthening field monitoring alongside controlled studies will enhance ecological relevance and provide more robust scientific foundations for conservation and environmental management.

In conclusion, this study provides fundamental insights into the physiological and molecular adaptive mechanisms employed by *C. sowerbii* when confronted with water pollutants. These insights not only enhance our comprehension of the species' evolutionary strategies but also offer crucial references for other aquatic organisms facing similar pollution threats. By shedding light on the ecological repercussions of pollution, this research fosters a proactive approach to environmental stewardship and emphasizes the pressing need to safeguard biodiversity within freshwater ecosystems. It reinforces the critical imperative to protect aquatic environments and their rich biodiversity, while simultaneously laying a scientific groundwork for future investigations and informing evidence-based conservation policies.

Supplementary Materials: File S1. Raw Data of the DEG. File S2. GO and KEGG functional enrichment.

Author Contributions: Conceptualization, H.Y., Y.W., and Y.L.; data curation, H.Y., Y.W., Y.H., J.W., M.W., and S.S.; formal analysis, H.Y. and Y.W.; funding acquisition, H.Y., Y.L. and Y.W.; visualization, H.Y., J.S. and J.G.; supervision, Y.L., J.Q. and N.F.; writing—original draft preparation, H.Y., and Y.W.; writing—review and editing, H.Y., Y.L. and N.F.. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Science and Technology Innovation Leading Talent Program of Henan Province (Grant No. 254000510014), the Overseas Expertise Center for Discipline Innovation (D23015), the Nanyang Normal University Doctoral Special Project (2025ZX002), the Key Research Program of Higher Education in Henan Province (25B180001), the Nanyang Municipal Science and Technology Plan Project (24JCQY021), the Cultivation Project of the National Natural Science Foundation of China at Nanyang Normal University (2026PY024), the Basic Research Business Fund Project of Henan Province (2025ZC121) , and the Youth Science Foundation of Henan (232300420183).

Institutional Review Board Statement: The animal study protocol was approved by the Institutional Review Board of Nanyang Normal University (protocol code 2024NYNU-WZ-1, 02/09/2024).

Informed Consent Statement: Not applicable.

Data Availability Statement: The data are contained within the article.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. van Vliet, M.T.H.; Thorslund, J.; Strokal, M.; Hofstra, N.; Flörke, M.; Ehalt Macedo, H.; Nkwasa, A.; Tang, T.; Kaushal, S.S.; Kumar, R.; et al. Global river water quality under climate change and hydroclimatic extremes. *Nature Reviews Earth & Environment* **2023**, *4*, 687-702, doi:10.1038/s43017-023-00472-3.
2. Manca, F.; Benedetti-Cecchi, L.; Bradshaw, C.J.A.; Cabeza, M.; Gustafsson, C.; Norkko, A.M.; Roslin, T.V.; Thomas, D.N.; White, L.; Strona, G. Projected loss of brown macroalgae and seagrasses with global environmental change. *Nature Communications* **2024**, *15*, 5344, doi:10.1038/s41467-024-48273-6.
3. Kaijser, W.; Musiol, M.; Schneider, A.R.; Prati, S.; Brauer, V.S.; Bayer, R.; Birk, S.; Brauns, M.; Dunne, L.; Enss, J.; et al. Meta-analysis-derived estimates of stressor–response associations for riverine organism groups. *Nature Ecology & Evolution* **2025**, doi:10.1038/s41559-025-02884-4.
4. Xie, J.; Wang, T.; Zhang, P.; Zhang, H.; Wang, H.; Wang, K.; Zhang, M.; Xu, J. Effects of multiple stressors on freshwater food webs: Evidence from a mesocosm experiment. *Environmental Pollution* **2024**, *348*, 123819, doi:https://doi.org/10.1016/j.envpol.2024.123819.
5. Jiang, X.; Kirsten, K.L.; Qadeer, A. Contaminants in the Water Environment: Significance from the Perspective of the Global Environment and Health. *Water* **2025**, *17*, 1257.
6. Yu, K.; Mohapatra, S.; Chen, Y.; Jiang, P.; Tong, X. Interactive Effects of Climate Change and Contaminants in Aquatic Ecosystems on Environmental-Human Health. *Current Pollution Reports* **2025**, *11*, 46, doi:10.1007/s40726-025-00379-1.
7. Ho, J.C.; Michalak, A.M.; Pahlevan, N. Widespread global increase in intense lake phytoplankton blooms since the 1980s. *Nature* **2019**, *574*, 667-670, doi:10.1038/s41586-019-1648-7.
8. Rajak, P.; Ganguly, A.; Nanda, S.; Mandi, M.; Ghanty, S.; Das, K.; Biswas, G.; Sarkar, S. 14 - Toxic contaminants and their impacts on aquatic ecology and habitats. In *Spatial Modeling of Environmental Pollution and Ecological Risk*, Shit, P.K., Datta, D.K., Bera, B., Islam, A., Adhikary, P.P., Eds.; Woodhead Publishing: 2024; pp. 255-273.
9. Madesh, S.; Gopi, S.; Sau, A.; Rajagopal, R.; Namasivayam, S.K.R.; Arockiaraj, J. Chemical contaminants and environmental stressors induced teratogenic effect in aquatic ecosystem – A comprehensive review. *Toxicology Reports* **2024**, *13*, 101819, doi:https://doi.org/10.1016/j.toxrep.2024.101819.
10. Jan, S.; Mishra, A.K.; Bhat, M.A.; Bhat, M.A.; Jan, A.T. Pollutants in aquatic system: a frontier perspective of emerging threat and strategies to solve the crisis for safe drinking water. *Environmental Science and Pollution Research* **2023**, *30*, 113242-113279, doi:10.1007/s11356-023-30302-4.
11. Arambawatta-Lekamge, S.H.; Pathiratne, A.; Rathnayake, I.V.N. Sensitivity of freshwater organisms to cadmium and copper at tropical temperature exposures: Derivation of tropical freshwater ecotoxicity

- thresholds using species sensitivity distribution analysis. *Ecotoxicology and Environmental Safety* **2021**, 211, 111891, doi:https://doi.org/10.1016/j.ecoenv.2021.111891.
12. Wang, F.; Xiang, L.; Sze-Yin Leung, K.; Elsner, M.; Zhang, Y.; Guo, Y.; Pan, B.; Sun, H.; An, T.; Ying, G.; et al. Emerging contaminants: A One Health perspective. *The Innovation* **2024**, 5, 100612, doi:https://doi.org/10.1016/j.xinn.2024.100612.
 13. Bashir, I.; Lone, F.A.; Bhat, R.A.; Mir, S.A.; Dar, Z.A.; Dar, S.A. Concerns and Threats of Contamination on Aquatic Ecosystems. In *Bioremediation and Biotechnology: Sustainable Approaches to Pollution Degradation*, Hakeem, K.R., Bhat, R.A., Qadri, H., Eds.; Springer International Publishing: Cham, 2020; pp. 1-26.
 14. Schar, D.; Klein, E.Y.; Laxminarayan, R.; Gilbert, M.; Van Boeckel, T.P. Global trends in antimicrobial use in aquaculture. *Scientific Reports* **2020**, 10, 21878, doi:10.1038/s41598-020-78849-3.
 15. Singh, B.K.; Paul, S.; Das, I.; Singha, E.R.; Giri, A. Global Warming and Emerging Contaminants: Impacts on Aquatic Organisms and Their Responses. *International Journal of Environmental Research* **2025**, 19, 186, doi:10.1007/s41742-025-00859-8.
 16. Grenni, P.; Ancona, V.; Barra Caracciolo, A. Ecological effects of antibiotics on natural ecosystems: A review. *Microchemical Journal* **2018**, 136, 25-39, doi:https://doi.org/10.1016/j.microc.2017.02.006.
 17. Xiong, J.-Q.; Govindwar, S.; Kurade, M.B.; Paeng, K.-J.; Roh, H.-S.; Khan, M.A.; Jeon, B.-H. Toxicity of sulfamethazine and sulfamethoxazole and their removal by a green microalga, *Scenedesmus obliquus*. *Chemosphere* **2019**, 218, 551-558, doi:https://doi.org/10.1016/j.chemosphere.2018.11.146.
 18. Zhang, H.; Quan, H.; Song, S.; Sun, L.; Lu, H. Comprehensive assessment of toxicity and environmental risk associated with sulfamethoxazole biodegradation in sulfur-mediated biological wastewater treatment. *Water Research* **2023**, 246, 120753, doi:https://doi.org/10.1016/j.watres.2023.120753.
 19. Peng, P.; Yan, X.; Zhou, X.; Chen, L.; Li, X.; Miao, Y.; Zhao, F. Enhancing degradation of antibiotic-combined pollutants by a hybrid system containing advanced oxidation and microbial treatment, a review. *Journal of Hazardous Materials* **2024**, 480, 136300, doi:https://doi.org/10.1016/j.jhazmat.2024.136300.
 20. Studziński, W.; Gackowska, A.; Kudlek, E.; Przybyłek, M. Environmental and toxicological aspects of sulfamethoxazole photodegradation in the presence of oxidizing agents. *Environmental Science and Pollution Research* **2025**, 32, 4733-4753, doi:10.1007/s11356-025-36000-7.
 21. Bojarski, B.; Kot, B.; Witeska, M. Antibacterials in Aquatic Environment and Their Toxicity to Fish. *Pharmaceuticals* **2020**, 13, 189.
 22. Galasso, F.; Frank, A.B.; Foster, W.J. Heavy metal toxicity and its role as a major driver of past biodiversity crises. *Communications Earth & Environment* **2025**, 6, 780, doi:10.1038/s43247-025-02781-5.
 23. Haider, F.U.; Liqun, C.; Coulter, J.A.; Cheema, S.A.; Wu, J.; Zhang, R.; Wenjun, M.; Farooq, M. Cadmium toxicity in plants: Impacts and remediation strategies. *Ecotoxicology and Environmental Safety* **2021**, 211, 111887, doi:https://doi.org/10.1016/j.ecoenv.2020.111887.
 24. Liu, Y.; Chen, Q.; Li, Y.; Bi, L.; Jin, L.; Peng, R. Toxic Effects of Cadmium on Fish. *Toxics* **2022**, 10, 622, doi:10.3390/toxics10100622.
 25. Samarakoon, T.; Fujino, T.; Hagimori, M.; Saito, R. Cadmium uptake and oxidative-stress-induced DNA alterations in the freshwater cladoceran *Moina macrocopa* (Straus 1820) following consecutive short-term exposure assessments. *Limnology* **2023**, 24, 9-23, doi:10.1007/s10201-022-00702-5.
 26. El-Sharkawy, M.; Alotaibi, M.O.; Li, J.; Du, D.; Mahmoud, E. Heavy Metal Pollution in Coastal Environments: Ecological Implications and Management Strategies: A Review. *Sustainability* **2025**, 17, 701.
 27. Yan, H.; Wang, Y.; Wu, M.; Li, Y.; Wang, W.; Zhang, D.; Guo, J.; Fohrer, N.; Li, B.L. Feeding Behavior and Ecological Significance of *Craspedacusta sowerbii* in a Freshwater Reservoir: Insights from Prey Composition and Trophic Interactions. *Biology* **2025**, 14, 665, doi:10.3390/biology14060665.
 28. Jankowski, T. The freshwater medusae of the world – a taxonomic and systematic literature study with some remarks on other inland water jellyfish. *Hydrobiologia* **2001**, 462, 91-113, doi:10.1023/A:1013126015171.
 29. Acker, T.S.; Muscat, A.M. The Ecology of *Craspedacusta sowerbii* Lankester, a Freshwater Hydrozoan. *The American Midland Naturalist* **1976**, 95, 323-336, doi:10.2307/2424397.
 30. Luskow, F.; Väinölä, R.; Lehtiniemi, M.; von Numers, M.; Pakhomov, E.A. Evidence for non-indigenous freshwater jellyfish *Craspedacusta sowerbii* spreading in Finland. *Hydrobiologia* **2025**, doi:10.1007/s10750-025-05908-z.

31. Gießler, S.; Strauss, T.; Schachtl, K.; Jankowski, T.; Klotz, R.; Stibor, H. Trophic Positions of Polyp and Medusa Stages of the Freshwater Jellyfish *Craspedacusta sowerbii* Based on Stable Isotope Analysis. *Biology* **2023**, *12*, 814.
32. Marchessaux, G.; Luskow, F.; Sarà, G.; Pakhomov, E.A. Predicting the current and future global distribution of the invasive freshwater hydrozoan *Craspedacusta sowerbii*. *Scientific Reports* **2021**, *11*, 23099, doi:10.1038/s41598-021-02525-3.
33. Marchessaux, G.; Bejean, M. From frustules to medusae: A new culture system for the study of the invasive hydrozoan *Craspedacusta sowerbii* in the laboratory. *Invertebrate Biology* **2020**, *139*, e12308, doi:https://doi.org/10.1111/ivb.12308.
34. Folino-Rorem, N.C.; Reid, M.; Peard, T. Culturing the freshwater hydromedusa, *Craspedacusta sowerbii* under controlled laboratory conditions. *Invertebrate Reproduction & Development* **2016**, *60*, 17-27, doi:10.1080/07924259.2015.1114040.
35. Winata, K.; Zhu, J.A.; Hanselman, K.M.; Zerbe, E.; Langguth, J.; Folino-Rorem, N.; Cartwright, P. Life Cycle Transitions in the Freshwater Jellyfish *Craspedacusta sowerbii*. *Biology* **2024**, *13*, 1069.
36. Zhang, Y.W.; Pan, X.F.; Wang, X.A.; Jiang, W.S.; Liu, Q.; Yang, J.X. Effects of osmotic pressure, temperature and stocking density on survival and sexual reproduction of *Craspedacusta sowerbii*. *Dongwuxue Yanjiu* **2016**, *37*, 90-95, doi:10.13918/j.issn.2095-8137.2016.2.90.
37. Luskow, F.; Polgári, B.; Stibor, H.; Schachtl, K.; Abonyi, A. Light increases surface occurrence of the freshwater jellyfish *Craspedacusta sowerbii* via positive phototaxis. *Hydrobiologia* **2025**, doi:10.1007/s10750-025-05955-6.
38. Luk, C.Y.L. The Chinese Freshwater Jellyfish Unbound: Evolution, Nomenclature, and Bioinvasion of *Craspedacusta sowerbii*, 1880–1941. *Historical Studies in the Natural Sciences* **2024**, *54*, 493-520, doi:10.1525/hsns.2024.54.4.493.
39. Gasith, A.; Gafny, S.; Hershkovitz, Y.; Goldstein, H.; Galil, B. The invasive freshwater medusa *Craspedacusta sowerbii* Lankester, 1880 (Hydrozoa: Olindiidae) in Israel. *Aquatic Invasions* **2011**, *6*, S147-S152, doi:10.3391/ai.2011.6.S1.033.
40. Caputo, L.; Fuentes, R.; Woelfl, S.; Castañeda, L.E.; Cárdenas, L. Phenotypic plasticity of clonal populations of the freshwater jellyfish *Craspedacusta sowerbii* (Lankester, 1880) in Southern Hemisphere lakes (Chile) and the potential role of the zooplankton diet. *Austral Ecology* **2021**, *46*, 1192-1197, doi:https://doi.org/10.1111/aec.13087.
41. Luskow, F.; Boersma, M.; López-González, P.J.; Pakhomov, E.A. Genetic variability, biomass parameters, elemental composition and energy content of the non-indigenous hydromedusa *Craspedacusta sowerbii* in North America. *Journal of Plankton Research* **2022**, *45*, 82-98, doi:10.1093/plankt/fbac064.
42. Luskow, F.; López-González, P.J.; Pakhomov, E.A. Freshwater jellyfish in northern temperate lakes: *Craspedacusta sowerbii* in British Columbia, Canada. *Aquatic Biology* **2021**, *30*, 69-84.
43. Marchessaux, G.; Bejean, M. Growth and ingestion rates of the freshwater jellyfish *Craspedacusta sowerbii*. *Journal of Plankton Research* **2020**, *42*, 783-786, doi:10.1093/plankt/fbaa047.
44. Sreeram, M.P.; Prasad, R.; Sreekumar, K.M.; Raju, A.K.; Augustina, T.A.X.; Luskow, F.; Saravanan, R. Post-flooding blooms of the non-indigenous freshwater jellyfish *Craspedacusta sowerbii* Lankester, 1880 in Kollam District of Kerala, India. *Journal of Plankton Research* **2024**, *00*, 1-4, doi:10.1093/plankt/fbae069.
45. Lucas, K.; Colin, S.P.; Costello, J.H.; Katija, K.; Klos, E. Fluid Interactions That Enable Stealth Predation by the Upstream-Foraging Hydromedusa *Craspedacusta sowerbyi*. *The Biological Bulletin* **2013**, *225*, 60-70, doi:10.1086/BBLv225n1p60.
46. Xu, D.; Xie, Y.; Li, J. Toxic effects and molecular mechanisms of sulfamethoxazole on *Scenedesmus obliquus*. *Ecotoxicology and Environmental Safety* **2022**, *232*, 113258, doi:https://doi.org/10.1016/j.ecoenv.2022.113258.
47. Diogo, B.S.; Rodrigues, S.; Golovko, O.; Antunes, S.C. From bacteria to fish: ecotoxicological insights into sulfamethoxazole and trimethoprim. *Environmental Science and Pollution Research* **2024**, *31*, 52233-52252, doi:10.1007/s11356-024-34659-y.

48. Zhou, J.; Yun, X.; Wang, J.; Li, Q.; Wang, Y.; Zhang, W.; Fan, Z. Biological toxicity of sulfamethoxazole in aquatic ecosystem on adult zebrafish (*Danio rerio*). *Scientific Reports* **2024**, *14*, 9401, doi:10.1038/s41598-024-59971-y.
49. Lin, T.; Yu, S.; Chen, Y.; Chen, W. Integrated biomarker responses in zebrafish exposed to sulfonamides. *Environmental Toxicology and Pharmacology* **2014**, *38*, 444-452, doi:https://doi.org/10.1016/j.etap.2014.07.020.
50. Roy, D.; Mitra, A.; Sen, B.M.; Homechaudhuri, S. Biochemical Responses in Zebra Fish (*Danio rerio*) on Acute Cadmium Exposure Under Temperature Variations. *Proceedings of the Zoological Society* **2024**, *77*, 164-172, doi:10.1007/s12595-024-00518-4.
51. Yuan, W.; Liang, Y.; Xia, X.; Xie, Y.; Lan, S.; Li, X. Protection of *Danio rerio* from cadmium (Cd²⁺) toxicity using biological iron sulfide composites. *Ecotoxicology and Environmental Safety* **2018**, *161*, 231-236, doi:https://doi.org/10.1016/j.ecoenv.2018.05.085.
52. Gao, C.-H.; Cao, H.; Ju, F.; Xiao, K.-Q.; Cai, P.; Wu, Y.; Huang, Q. Emergent transcriptional adaption facilitates convergent succession within a synthetic community. *ISME Communications* **2021**, *1*, 46, doi:10.1038/s43705-021-00049-5.
53. Wang, H.; Xu, Y.; Zhang, Z.; Zhang, G.; Tan, C.; Ye, L. Development and application of transcriptomics technologies in plant science. *Crop Design* **2024**, *3*, 100057, doi:https://doi.org/10.1016/j.crope.2024.100057.
54. Jing, L.; Wang, H.; Xia, S.; Shao, Q. Wnt/Ca(2+) signaling: Dichotomous roles in regulating tumor progress (Review). *Oncol Lett* **2025**, *30*, 399, doi:10.3892/ol.2025.15145.
55. Sanchez-Collado, J.; Lopez, J.J.; Jardin, I.; Salido, G.M.; Rosado, J.A. Cross-Talk Between the Adenylyl Cyclase/cAMP Pathway and Ca²⁺ Homeostasis. In *Reviews of Physiology, Biochemistry and Pharmacology*, Pedersen, S.H.F., Ed.; Springer International Publishing: Cham, 2021; pp. 73-116.
56. Schmidt, M.F.; Gan, Z.Y.; Komander, D.; Dewson, G. Ubiquitin signalling in neurodegeneration: mechanisms and therapeutic opportunities. *Cell Death & Differentiation* **2021**, *28*, 570-590, doi:10.1038/s41418-020-00706-7.
57. Dantuma, N.P.; Bott, L.C. The ubiquitin-proteasome system in neurodegenerative diseases: precipitating factor, yet part of the solution. *Front Mol Neurosci* **2014**, *7*, 70, doi:10.3389/fnmol.2014.00070.
58. Ajoolabady, A.; Pratico, D.; Bahijri, S.; Tuomilehto, J.; Uversky, V.N.; Ren, J. Hallmarks of cellular senescence: biology, mechanisms, regulations. *Experimental & Molecular Medicine* **2025**, *57*, 1482-1491, doi:10.1038/s12276-025-01480-7.
59. Minchin, D.; Caffrey, J.M.; Haberman, D.; Germaine, D.; Walsh, C.; Boelens, R.; Doyle, T.K. First observations of the freshwater jellyfish *Craspedacusta sowerbii* Lankester, 1880 in Ireland coincides with unusually high water temperatures. *Bioinvasions Records* **2016**, *5*, 67-74, doi: http://dx.doi.org/10.3391/bir.2016.5.2.02.
60. Schifani, E.; Viviano, A.; Viviano, R.; Naselli-Flores, L.; Marrone, F. Different lineages of freshwater jellyfishes (Cnidaria, Olindiidae, Craspedacusta) invading Europe: another piece of the puzzle from Sicily, Italy. *Limnology* **2019**, *20*, 143-151, doi:10.1007/s10201-018-0560-4.
61. Seçer, B. New locality records of invasive freshwater jellyfish *Craspedacusta sowerbii* (Lankester, 1880) in Türkiye. *Limnology and Freshwater Biology* **2025**, *2025*, 298-301.
62. Moore, J.P.; Green, H.C.; Stewart, D.J.; Luskow, F.; Wilder, M.L. Invasive freshwater jellyfish (*Craspedacusta cf. sowerbii*) in the Hudson River basin, NYS: comparisons of detection methods. *Hydrobiologia* **2025**, doi:10.1007/s10750-025-05944-9.

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.