



Zooplankton Biodiversity Along a Pollution-Salinity Gradient in Bahr Al-Najaf, Iraq: Implications for Biomonitoring and One Health

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ABSTRACT

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Aquatic ecosystems are increasingly threatened by climate variability, political instability, and anthropogenic pollution that affect biological indicators for early environmental assessment. The purpose of this study was to investigate the diversity and morphometric responses of zooplankton along a pollution-salinity gradient in the Bahr Al-Najaf depression, Iraq, a vulnerable shallow aquatic ecosystem. Sampling was conducted over 18 months (March 2023-August 2024) across three sites representing varying pollution levels. A total of 18 zooplankton taxa were identified in reference areas, declining to 9 taxa in highly impacted zones. Biodiversity indices indicated marked ecological degradation, with Shannon diversity decreasing from 2.45 ± 0.15 to 1.10 ± 0.12 and Simpson diversity declining by 45% (0.82 ± 0.04 to 0.45 ± 0.08). These changes coincided with severe environmental stress, including dissolved oxygen (DO) <4 mg/L, total dissolved solids (TDS) >4000 mg/L, and heavy metal pollution index (HPI) >2.5 . In the reference sites, large filter-feeding cladocerans (*Daphnia* spp., *Diaphanosoma* spp., and *Simocephalus* spp.) dominated the community composition, while in the degraded areas, pollution-tolerant taxa such as *Brachionus angularis*, *Mesocyclops leuckarti*, and *Rotaria neptunia* were dominant. Total abundance increased 2.4-fold, but populations were dominated by tolerant species. Strong sublethal stress responses were revealed by morphometric analyses (*Daphnia* body length decreased by $>90\%$, copepods shrunk by 27-30%), suggesting energy trade-offs involved in osmoregulation and detoxification. Community restructuring was mainly driven by chemical pollution and heavy metals ($p = 0.001$), confirmed by canonical correspondence analysis and ANOVA. In the Indicator Species Analysis (ISA), the indicator species for reference conditions were *Euchlanis dilatata* and *Daphnia magna*, while *Brachionus angularis* and *Mesocyclops leuckarti* were characteristic of degraded habitats. The integration of taxonomic and morphometric metrics is an affordable method for the identification of ecological degradation and supports the use of zooplankton bioindicators in environmental monitoring and One Health models in arid ecosystems.

1. INTRODUCTION

Inland aquatic ecosystems in arid and semi-arid areas are among the most ecologically fragile systems on the planet, experiencing unparalleled stress from the synergistic impacts of anthropogenic activities and climate change [1, 2]. These ecosystems, which include temporary wetlands, saline lakes, and shallow depressions, are home to a wide range of plants and animals and provide important ecosystem services like cleaning water, storing carbon, and making fish more productive [3, 4]. But the water is getting scarcer, the temperatures are rising, and the pollution is getting worse. This has caused a lot of damage to both aquatic food webs and people's jobs [5, 6]. The combined effects of different stressors, such as salinisation, organic enrichment, and heavy metal contamination, cause biological responses that work

together in ways that are often missed when stressors are looked at one at a time [7, 8]. To develop effective monitoring and management strategies for vulnerable dryland regions globally, it is essential to comprehend these intricate interactions.

Zooplankton communities, comprising rotifers (Phylum Rotifera), cladocerans, ostracods (Subphylum Crustacea), and copepods (Subclass Copepoda), are integral to aquatic food webs and function as bioindicators of ecosystem health [9, 10]. Because they have short generation times, populations that change quickly, and known physiological tolerances, they can quickly respond to changes in the environment, which can be an early warning sign of ecological harm. Two main ways to measure how zooplankton react are functional trait analysis and morphometric analysis. These are in addition to regular surveys. Functional traits encompass feeding mechanisms,

reproductive strategies, life-history characteristics, and stress resilience [11, 12]. Morphometric traits assess physical attributes such as body length, lorica, carapace dimensions, and biomass [13]. Functional traits elucidate ecological strategies and community assembly, whereas morphometric traits signify physiological stress and phenotypic alterations. Using both methods together makes it easier to find ecosystem decline before taxonomic loss.

The One Health concept offers a crucial framework for comprehending ecological findings within broader contexts of environmental, animal, and human health [14]. One Health was first created to deal with the rise of zoonotic diseases. It recognises the connections between the health of wildlife, the health of ecosystems, and the health of people. In aquatic ecosystems, environmental degradation proliferates through food webs via bioaccumulation and biomagnification, impacting human populations dependent on fishery resources [15, 16]. Zooplankton are an important link between the environment and higher trophic levels. Changes in the traits, morphology, and community composition of zooplankton are early signs that have direct effects on the health of the food web, fish production, and human exposure to pollutants. This current study uses the One Health concept to see how zooplankton respond to pollution, linking it to ecological degradation, risks to wildlife, and human communities that depend on wetlands.

Inland arid ponds are globally significant yet exhibit knowledge deficiencies concerning zooplankton responses to various stressors, particularly when amalgamating morphometric diagnostics with community ecology and One Health. In Iraq, inland waters are deteriorating due to untreated wastewater, agricultural runoff, solid waste, and salinisation [17, 18]. The majority of studies depend on presence-absence or abundance data rather than sublethal indicators [19, 20]. This decline is shown by Bahr Al-Najaf (31°59' N, 44°18' E), a shallow depression in the middle of Iraq. It used to be a great place for migratory birds and fishing [21, 22], but now it is losing biodiversity because of sewage, waste, sediment contamination, invasive macrophytes, rising temperatures, and droughts [23]. The most commonly used indicators in assessing zooplankton biodiversity in wetland environments are the Shannon-Wiener index and abundance. Relatively low values for the former were recorded due to high salinity, hydrological fluctuations, and environmental stress, leading to the dominance of species tolerant to harsh conditions and a decrease in sensitive species. For example, study [24] in Lake Habbaniyah recorded values ranging from 0.7 to 2.8, while the abundance index recorded values ranging from 1 to 4, indicating that sites affected by environmental factors showed low biodiversity. The study [25] in the Najaf Sea recorded low values for the Shannon-Wiener index, ranging between 0.48 and 2.42, while high values for the flow index were recorded during the spring season, reaching 7.2. As for the homogeneity index, it recorded the highest values during the winter and spring seasons, reaching 0.61. This indicates that the water quality of the Najaf Sea ranged from low to moderate diversity due to high salinity and water pollution.

We hypothesise that changes in pollution and salinity will decrease zooplankton diversity, favour tolerant taxa, and lead to morphometric reductions in crustaceans, thereby impacting ecosystem services and One Health. This study seeks to: (i) delineate zooplankton variation along pollution-salinity gradients; (ii) assess morphometric responses as indicators of

stress; and (iii) elucidate environmental determinants of community structure from a One Health perspective, emphasising impacts on the environment, wildlife, and human health.

2. MATERIALS AND METHODS

2.1 Study area and sampling design

Bahr Al-Najaf is a small, shallow basin with a surface area of about 24 square kilometers and an average depth of 1.5 to 3.0 meters. It is in central Iraq, 10 km southwest of Najaf City (31°59' N, 44°18' E). The basin gets its water from seasonal rain, drainage from nearby date palm farms, and mostly untreated municipal wastewater and runoff from solid waste from Old Najaf City. The system is very likely to become salty and polluted because it loses a lot of water (about 2,000 mm per year) and doesn't get much new water [1, 3].

Three sampling zones were created to capture the spatial gradient in environmental stress. These zones were chosen based on (i) visible signs of pollution (like water clarity, smell, and floating trash); (ii) how close the pollution sources are (like sewage outfalls and solid waste dumping sites); (iii) how easy it is to get to and how safe it is; and (iv) how well the depression covers the area (Figure 1):

- Zone 1 (Reference/Low Impact): Northern peripheral areas (31°59'45" N, 44°17'30" E) with very little direct sewage input, not a lot of people around, fairly stable water quality, and submerged macrophyte beds mostly made up of *Potamogeton* spp.
- Zone 2 (Moderate Impact): Central transitional areas (31°59'20" N, 44°18'15" E) that get wastewater discharge on occasion, mixed agricultural and urban runoff, and moderate turbidity with patchy invasive macrophyte (*Eichhornia crassipes*) coverage.
- Zone 3 (High Impact): Southern areas (31°58'50" N, 44°18'50" E) next to direct sewage inflows and active solid waste dumping sites. These areas are always hypoxic, have high turbidity, a bad smell, salinity stratification, and dense organic pollution.

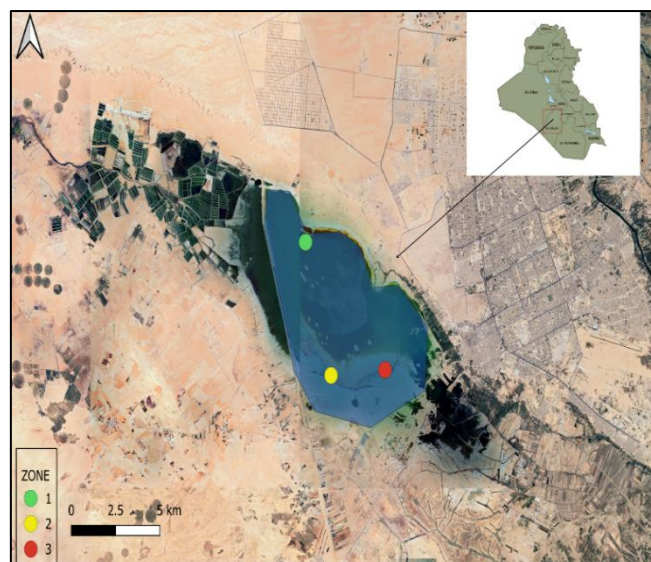


Figure 1. Geographic location of Bahr Al-Najaf

2.2 Sampling protocol and temporal design

The sampling took place over 18 months (March 2023 to August 2024) to characterize major seasonal trends in water levels while also taking into account the limitations that are common in dry wetland systems. There were six sampling campaigns: March, April, May, June, September, and October 2023; and March, April, May, June, July, and August 2024. These months were chosen because (i) they are hydrologically stable, avoiding times of complete drying out (July-August) or very cold temperatures (December-February) that make it hard for living things to move around; (ii) they are when Mesopotamian wetlands have the most diverse zooplankton [26, 27]; and (iii) they are when flooding makes it hard to get around. To cut down on daily changes in physicochemical parameters and zooplankton vertical migration, all sampling was done between 08:00 and 10:00 hours local time.

Within-zone microhabitat heterogeneity was obtained by establishing three (replicate) sampling points approximately 100 m apart within each zone with similar environmental conditions. Within each zone, the sampling points were selected to have similar water depth, substrate type, hydrological connectivity, and macrophyte coverage to reduce local habitat variability. Before each sampling campaign, sites were visually inspected for consistency in physicochemical characteristics and absence of major habitat disturbances. To reduce the temporal and diel variability, all samples were taken between 08:00 and 10:00 h. All samples were collected under comparable weather conditions and using identical sampling procedures and equipment in all zones. During each sampling campaign, replicate points were located with a GPS and revisited to ensure that spatial consistency was maintained throughout the study period. Temporal replicates ($n = 12$ sampling events per zone) were pooled for statistical analysis after confirming no significant temporal heterogeneity among sampling periods (PERMANOVA, $p > 0.05$), thereby reducing pseudo-replication while maintaining spatial replication (3 zones \times 3 replicate points = 9 spatial replicates per sampling event).

The study design was capable of capturing seasonal and spatial variability over an 18-month period, but the logistical scope of the present investigation would not have allowed for continuous high-frequency monitoring. Therefore, short-term pollution pulses and rapid hydrological variations may not have been fully resolved. However, repeated standardised sampling over several seasons provided a robust representation of long-term ecological trends within Bahr Al-Najaf.

2.3 Environmental analysis

In situ physicochemical parameters were measured at 0.5 m depth using a calibrated multiparameter probe (YSI ProPlus, USA), including water temperature ($^{\circ}\text{C}$), pH, dissolved oxygen (DO, mg/L), electrical conductivity (EC, $\mu\text{S}/\text{cm}$), salinity (ppt), and total dissolved solids (TDS, mg/L). The YSI probe was calibrated daily prior to field measurements using manufacturer-recommended standard solutions, and calibration accuracy was verified after every sampling campaign. Detection limits for probe-based measurements were as follows: DO = 0.01 mg/L, EC = 1 $\mu\text{S}/\text{cm}$, salinity = 0.1 ppt, and TDS = 1 mg/L.

Water samples (2 L) were collected from the surface layer (0-0.3 m depth) for laboratory analyses of nutrients and

organic pollution indicators. Nitrate (NO_3^-), nitrite (NO_2^-), ammonium (NH_4^+), and phosphate (PO_4^{3-}) concentrations were determined spectrophotometrically according to APHA (2017) standard methods (4500- NO_3^- , 4500- NO_2^- , 4500- NH_3 , and 4500-P). Biochemical oxygen demand (BOD_5 , mg/L) was determined using the 5-day incubation method (APHA, 2017; Method 5210B), while chemical oxygen demand (COD, mg/L) was measured using the closed-reflux colorimetric method (APHA, 2017; Method 5220D). Total organic carbon (TOC, mg/L) was analyzed by high-temperature combustion using a Shimadzu TOC-VCPH analyzer. Method detection limits (MDLs) for nutrient analyses were: $\text{NO}_3^- = 0.01$ mg/L, $\text{NO}_2^- = 0.001$ mg/L, $\text{NH}_4^+ = 0.01$ mg/L, $\text{PO}_4^{3-} = 0.005$ mg/L, $\text{BOD}_5 = 0.5$ mg/L, COD = 1 mg/L, and TOC = 0.1 mg/L.

Surface sediment samples (0-5 cm depth) were collected using a stainless-steel Ekman grab sampler (225 cm^2), air-dried at ambient temperature (25 ± 3 $^{\circ}\text{C}$) for 72 h, homogenized, and sieved through a 63 μm nylon mesh. Subsamples (0.5 g dry weight) were digested using an acid mixture of HCl: HNO_3 (3:1, v/v) following USEPA Method 3050B. Concentrations of lead (Pb), cadmium (Cd), copper (Cu), zinc (Zn), nickel (Ni), and chromium (Cr) were quantified using inductively coupled plasma optical emission spectrometry (ICP-OES; PerkinElmer Optima 8300). ICP-OES calibration was performed at the beginning of each analytical run and verified after every 10 samples using multi-element standard solutions to minimize instrumental drift. Instrumental limits of detection (LOD, mg/kg dry weight) were: Pb = 0.01, Cd = 0.002, Cu = 0.01, Zn = 0.02, Ni = 0.01, and Cr = 0.01.

Quality assurance and quality control (QA/QC) procedures included calibration using certified multi-element standards ($R^2 > 0.995$ for all calibration curves), procedural blanks ($n = 2$ per batch), duplicate analyses ($n = 3$ per batch; relative percent difference $< 10\%$), and certified reference materials (NIST SRM 1643e; recoveries ranged from 90-110%). MDLs were calculated as three times the standard deviation of blank measurements, and all measured concentrations exceeded their respective MDLs. Prior to statistical analyses, datasets were screened for potential outliers and analytical anomalies. Values exceeding ± 3 standard deviations were rechecked against laboratory records, calibration performance, and field observations. Samples associated with instrument instability, contamination, or QA/QC failure were re-measured or excluded, whereas environmentally plausible extreme values were retained to preserve natural ecological variability.

The heavy metal pollution index (HPI) was calculated following [28] to integrate multiple metal contaminants into a single quantitative metric:

$$HPI = \frac{\sum(Q_i \times W_i)}{\sum W_i}$$

where, $Q_i = C_i/S_i$ represents the sub-index for metal (i), C_i is the measured metal concentration (mg/kg), S_i is the corresponding sediment quality guideline value based on the Canadian Sediment Quality Guidelines (CCME, 2001), and $W_i = 1/S_i$ is the unit weight. The sediment guideline values used were: Pb = 35 mg/kg, Cd = 0.6 mg/kg, Cu = 35.7 mg/kg, Zn = 123 mg/kg, Ni = 18 mg/kg, and Cr = 37.3 mg/kg. HPI values were classified as follows: < 1.0 = low pollution, 1.0-2.5 = moderate pollution, 2.5-5.0 = high pollution, and > 5.0 = severe pollution.

2.4 Zooplankton collection and processing

We used a 30-cm diameter conical plankton net with a 64–200 μm mesh to collect zooplankton. The net was suitable for catching small rotifers and juvenile cladocerans. A calibrated mechanical flowmeter (General Oceanics, Model 2030R) was attached to the net opening to measure the amount of water filtered (mean volume per tow: $2,450 \pm 380$ L). Tows were done at a speed of about 1 m/s over 100 m long, parallel to the coast. To stop predation and degradation, samples were quickly preserved in 4% buffered formalin (final concentration). We used an Olympus CX43 compound microscope (100–400 \times magnification) in the lab to count and concentrate the samples. The number of zooplankton was given in individuals per cubic meter (ind. m^{-3}).

For morphometric analysis, 30 adult specimens per dominant taxon (determined by the presence of eggs, embryonic development, or fully differentiated morphological characteristics) were randomly selected from each zone and season. Using an Olympus DP27 digital camera with a calibrated scale bar, we took pictures of the specimens. We used ImageJ software (version 1.53k) [29] to measure the following: (i) for rotifers: lorica length and width; (ii) for cladocerans: body length (not including the spine), carapace length, and antennae length; and (iii) for copepods: prosome length and total body length. All measurements adhered to established protocols [30, 31].

2.5 Species identification and taxonomic analysis

We employed established identification keys to find the lowest possible taxonomic level for zooplankton taxa: Rotifera according to studies [32, 33]; Cladocera according to studies [30, 34]; Copepoda according to research [31, 35]. The genus *Chydorus* was given special attention because it has a lot of phenotypic plasticity and hidden diversity. Diagnostic features like valve sculpture, rostrum morphology, postabdominal claw structure, and setation patterns were checked in at least 10 individuals per zone to make sure they were correct [30].

Functional traits were ascribed to each identified taxon according to the Freshwater Ecology.info database [36] and regional literature [37], as shown in Figure 2.

The included traits were: (i) feeding mode (filter-feeder, predator, detritivore, or mixed); (ii) body size class (small: $<200 \mu\text{m}$, medium: $200\text{--}500 \mu\text{m}$, large: $>500 \mu\text{m}$); (iii) reproductive strategy (mictic/amictic ratio for rotifers; parthenogenetic/sexual for cladocerans); (iv) salinity tolerance (stenohaline, mesohaline, euryhaline); and (v) organic pollution tolerance (sensitive, tolerant, highly tolerant) following research [3, 38].

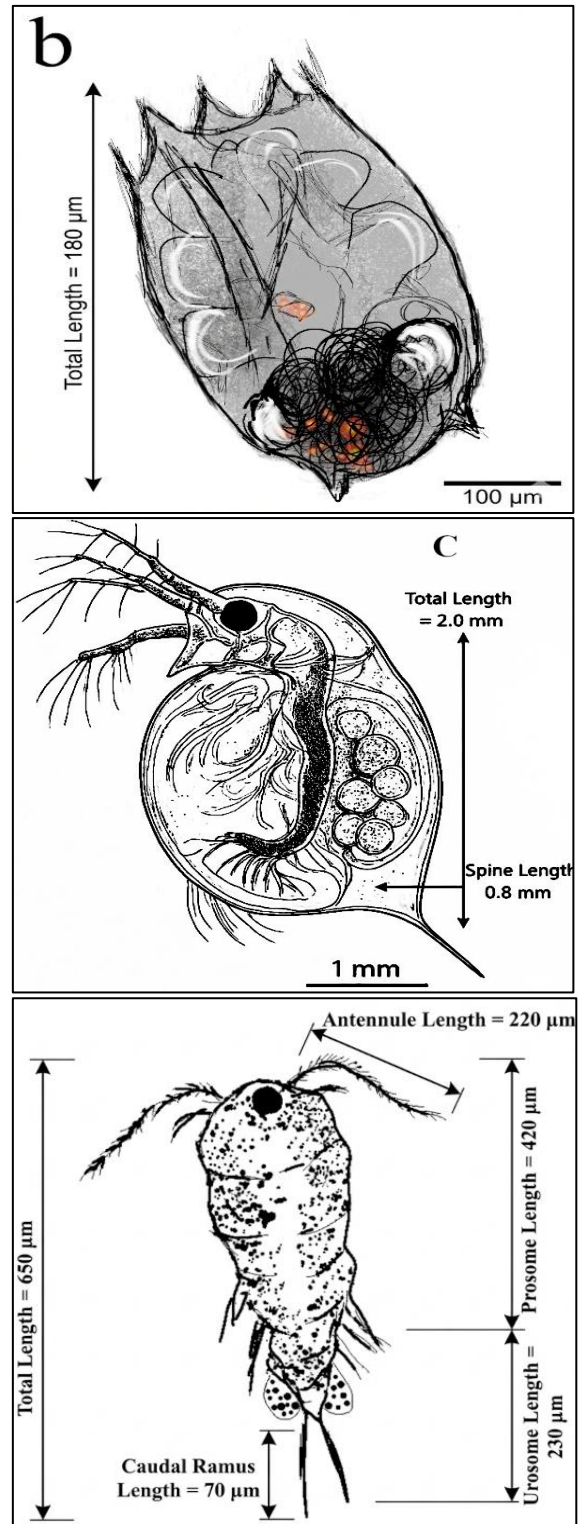
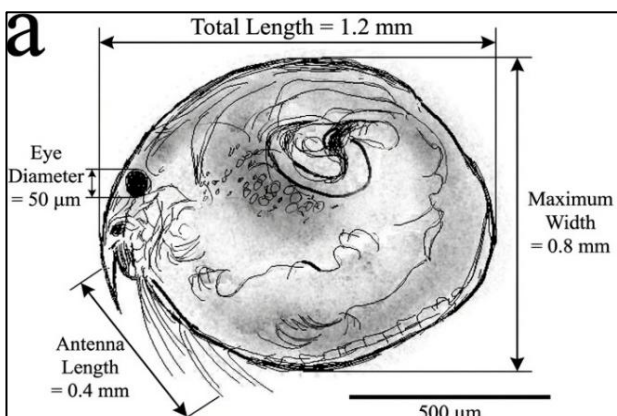


Figure 2. Scientific schematic of representative zooplankton taxa based on taxonomic identification criteria

2.6 Statistical analysis

Biodiversity was quantified using: (i) species richness (S , total number of taxa); (ii) Shannon-Wiener diversity index ($H' = -\sum p_i \times \ln(p_i)$); and (iii) Simpson's index of diversity ($1-D$, where $D = \sum p_i^2$), calculated as $1 - \sum p_i^2$, where p_i = proportional abundance of species i [39]. This formulation (also known as the Gini-Simpson index) ranges from 0 (no diversity, single species dominance) to 1 (infinite diversity, all species equally abundant). This approach was selected to ensure an intuitive interpretation, in which higher values indicate greater

diversity, consistent with the Shannon index.

To lessen the effect of species with high abundance and double zeros, zooplankton abundance data were Hellinger-transformed ($y' = \sqrt{y/\text{row sum}}$) [40]. Detrended correspondence analysis (DCA) was used to compare linear redundancy analysis (RDA) and unimodal canonical correspondence analysis (CCA) ordination methods. A gradient length of 3.2 standard deviations on Axis 1 showed unimodal responses, which supported the choice of CCA. We used forward selection with 999 Monte Carlo permutations ($p < 0.05$) to choose the environmental variables (DO, TDS, BOD, COD, TOC, HPI) for CCA. We looked at variance inflation factors (VIF) to get rid of collinear variables (VIF threshold = 10). Permutation tests (999 iterations) were used to find out how important the first canonical axis and the overall analysis were.

Differences in community composition between zones were tested using permutational multivariate analysis of variance (PERMANOVA; 999 permutations), based on Bray-Curtis dissimilarity across all taxa identified. We conducted an Indicator Species Analysis (ISA) to identify taxa significantly associated with individual zones (indicator value > 0.25 , $p < 0.05$). All statistical analyses were conducted in R version 4.2.2 [41]. Community ecology and multivariate analyses were performed using the vegan package [42], indicator species analyses were conducted using indicspecies, and graphical outputs were generated using ggplot2.

Previous taxonomic studies identified the need for detailed morphological differences to definitively identify Chydorus species, such as areas with cryptic diversity and poorly documented taxa [30].

3. RESULTS

3.1 Environmental gradients across zones

A clear spatial gradient in water quality was observed across the three study zones (Table 1 and Figure 3). Overall, physicochemical conditions deteriorated progressively from Zone 1 (reference) to Zone 3 (high impact), reflecting increasing environmental stress. Temperature showed no significant variation among zones ($p = 0.12$), remaining relatively stable between 24.2 ± 2.1 °C and 26.5 ± 2.6 °C. In contrast, pH declined significantly ($p = 0.02$), from slightly alkaline conditions in Zone 1 (7.8 ± 0.3) to lower values in Zone 3 (7.1 ± 0.5). DO decreased markedly along the gradient, from 7.6 ± 0.5 mg/L in Zone 1 to 3.1 ± 0.9 mg/L in Zone 3 ($p < 0.001$), indicating hypoxic conditions in the most impacted sites. Concurrently, EC, salinity, and TDS increased significantly ($p < 0.001$), with EC rising from $2,400 \pm 380$ $\mu\text{S/cm}$ to $8,900 \pm 1,200$ $\mu\text{S/cm}$, salinity from 1.2 ± 0.2 ppt to 4.8 ± 0.7 ppt, and TDS from $1,200 \pm 190$ mg/L to $4,800 \pm 650$ mg/L. Nutrient concentrations also increased significantly across zones ($p < 0.001$). Nitrate (NO_3^-) increased from 0.8 ± 0.2 mg/L in Zone 1 to 2.9 ± 0.8 mg/L in Zone 3, while ammonium (NH_4^+) rose from 0.3 ± 0.1 mg/L to 2.4 ± 0.7 mg/L. Similarly, phosphate (PO_4^{3-}) concentrations rose substantially from 0.05 ± 0.02 mg/L to 0.38 ± 0.11 mg/L. Indicators of organic pollution followed the same pattern. BOD₅ increased significantly from 2.0 ± 0.6 mg/L in Zone 1 to 8.5 ± 2.3 mg/L in Zone 3, while COD rose from 12.5 ± 3.1 mg/L to 68.0 ± 10.5 mg/L ($p < 0.001$). TOC also increased markedly from 3.2 ± 0.8 mg/L to 18.4 ± 4.2 mg/L. Last but not least, the HPI

showed an increase in sediment contamination ($p < 0.001$), with values rising from 0.9 ± 0.2 in Zone 1 to 4.5 ± 1.1 in Zone 3, indicating severe pollution levels in the most impacted sites, as shown in Figure 4.

Table 1. Physicochemical parameters across sampling zones

Parameter	Zone 1	Zone 2	Zone 3	F-Value	P-Value
Temperature (°C)	24.2 ± 2.1^a	25.8 ± 2.4^a	26.5 ± 2.6^a	2.34	0.12
pH	7.8 ± 0.3^a	7.5 ± 0.4^{ab}	7.1 ± 0.5^b	4.56	0.02
DO (mg/L)	7.6 ± 0.5^a	6.9 ± 0.7^a	3.1 ± 0.9^b	45.2	<0.001***
EC ($\mu\text{S/cm}$)	$2,400 \pm 380^a$	$4,200 \pm 650^b$	$8,900 \pm 1,200^c$	89.7	<0.001***
Salinity (ppt)	1.2 ± 0.2^a	2.1 ± 0.3^b	4.8 ± 0.7^c	92.3	<0.001***
TDS (mg/L)	$1,200 \pm 190^a$	$2,100 \pm 325^b$	$4,800 \pm 650^c$	91.5	<0.001***
NO_3^- (mg/L)	0.8 ± 0.2^a	1.4 ± 0.4^b	2.9 ± 0.8^c	18.9	<0.001***
NH_4^+ (mg/L)	0.3 ± 0.1^a	0.9 ± 0.3^b	2.4 ± 0.7^c	24.6	<0.001***
PO_4^{3-} (mg/L)	0.05 ± 0.02^a	0.12 ± 0.04^b	0.38 ± 0.11^c	31.2	<0.001***
BOD ₅ (mg/L)	2.0 ± 0.6^a	3.8 ± 1.0^b	8.5 ± 2.3^c	28.4	<0.001***
COD (mg/L)	12.5 ± 3.1^a	24.0 ± 5.2^b	68.0 ± 10.5^c	56.8	<0.001***
TOC (mg/L)	3.2 ± 0.8^a	6.1 ± 1.5^b	18.4 ± 4.2^c	42.3	<0.001***
HPI	0.9 ± 0.2^a	1.6 ± 0.4^b	4.5 ± 1.1^c	67.4	<0.001*

Values mean \pm standard deviation ($n = 18$). The letters (a, b, c) indicate significant differences between zones (Tukey's HSD, $p < 0.05$). HPI = heavy metal pollution index; *** $p < 0.001$.

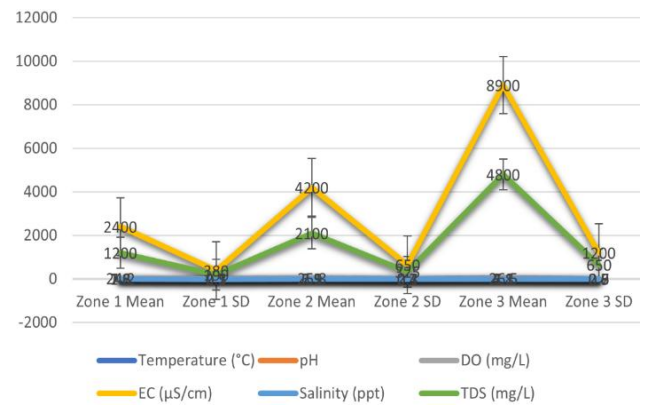


Figure 3. Spatial variation of physical parameters across study zones

Mean (\pm SD) values for temperature, pH, DO, EC, salinity, and TDS in the three study zones, demonstrating the environmental gradient from reference to high-impact conditions.

Mean (\pm SD) nutrient concentrations (NO_3^- , NH_4^+ , PO_4^{3-}) and pollution indicators (BOD₅, COD, TOC, HPI) were measured across zones. Results indicate a significant increase in contamination toward the high-impact zone.

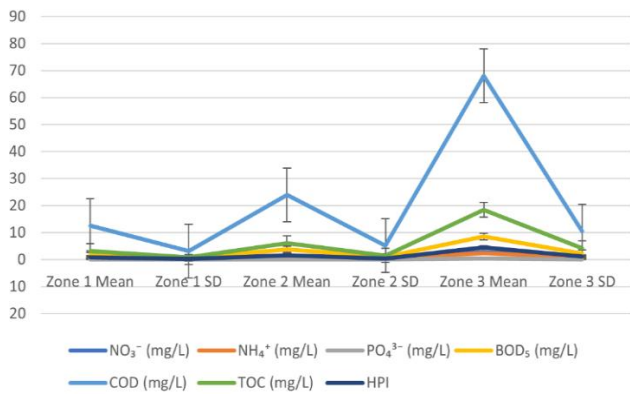


Figure 4. Distribution of chemical and pollution indicators across study zones

3.2 Zooplankton community composition and diversity

Samples were collected from multiple water zones using plankton nets with mesh sizes ranging from 64 to 200 μm . Specimens were systematically identified and enumerated under a light microscope according to standard taxonomic keys. Eighteen zooplankton taxa were identified, comprising nine Rotifera, eight Cladocera, and one Copepoda (see Table 2 and Figure 5). Biodiversity indices demonstrated a pronounced degradation trend along the pollution gradient (Table 3). Species richness (S) declined significantly from 18 taxa in Zone 1 to 9 taxa in Zone 3 ($F = 67.4$, $p < 0.001$). Simpson's index of diversity (1-D) decreased from 0.82 ± 0.04 in Zone 1 to 0.45 ± 0.08 in Zone 3, indicating a transition from high, even diversity to strong dominance by a limited number of tolerant taxa. This 45% reduction in Simpson diversity was accompanied by a corresponding decline in Shannon diversity (H' : 2.45 to 1.10) and evenness (J' : 0.85 to 0.50). The Simpson index value of 0.45 in Zone 3 indicates reduced diversity with dominance by a few stress-tolerant taxa species, with *Mesocyclops* spp. and *Brachionus angularis* collectively comprising 39% of total abundance, compared to only 12% dominance by the top species in Zone 1. The rare and low-abundance taxa were mainly found in Zones 1 and 2, while Zone 3, which was highly impacted, was dominated by a few pollution-tolerant taxa. The reduction in diversity indices was mainly due to the loss of several stenohaline and sensitive rotifers and cladocerans. The retention of rare taxa in the dataset helped maintain ecological heterogeneity and increase sensitivity to community-level environmental change.



Figure 5. Microscopic images of different zooplankton species (A) *Daphnia* spp., (B) *Brachionus angularis*, (C) *Filinia* spp., (D) *Keratella* spp., (E) Nauplius-larva, (F) Egg (immature stage), (G) *Chydorus piger*, (H) *Halicyclops*. Scale bar = 50 μm .

Table 2. Zooplankton taxa list with diagnostic measurements, morphometric ranges, and relative abundance (%) across zones

Taxon	Feeding Mode	Size Class	Salinity Tolerance	Pollution Tolerance	Zone 1 (%)	Zone 2 (%)	Zone 3 (%)
<i>Brachionus angularis</i>	Filter-feeder	Small	Euryhaline	Highly tolerant	7.0	6.5	15.0
<i>Brachionus calyciflorus</i>	Filter-feeder	Small	Euryhaline	Tolerant	5.5	4.0	8.0
<i>Filinia longiseta</i>	Filter-feeder	Small	Stenohaline	Sensitive	4.0	3.0	0.0
<i>Keratella cochlearis</i>	Filter-feeder	Small	Stenohaline	Sensitive	4.0	2.0	0.0
Rotifera <i>Keratella tropica</i>	Filter-feeder	Small	Mesohaline	Tolerant	3.5	2.5	0.0
<i>Lecane nana</i>	Detritivore	Small	Euryhaline	Tolerant	9.0	10.0	6.0
<i>Lecane lunaris</i>	Detritivore	Small	Euryhaline	Tolerant	6.0	7.0	7.0
<i>Rotaria neptunia</i>	Detritivore	Medium	Euryhaline	Highly tolerant	8.0	8.0	15.0
<i>Trichotria tetractys</i>	Filter-feeder	Medium	Stenohaline	Sensitive	4.0	5.0	0.0
<i>Euchlanis dilatata</i>	Filter-feeder	Large	Stenohaline	Sensitive	12.0	15.0	8.0
<i>Cephalodella gibba</i>	Predator	Small	Stenohaline	Sensitive	3.0	4.0	0.0

	<i>Daphnia magna</i>	Filter-feeder	Large	Stenohaline	Sensitive	5.0	3.0	0.0
	<i>Daphnia lumholtzi</i>	Filter-feeder	Large	Mesohaline	Tolerant	3.5	2.0	0.0
	<i>Simocephalus vetulus</i>	Filter-feeder	Large	Stenohaline	Sensitive	4.0	2.0	0.0
	<i>Diaphanosoma excisum</i>	Filter-feeder	Large	Stenohaline	Sensitive	2.0	1.0	0.0
Cladocera	<i>Ceriodaphnia reticulata</i>	Filter-feeder	Medium	Mesohaline	Tolerant	3.0	2.0	0.0
	<i>Moina micrura</i>	Filter-feeder	Medium	Euryhaline	Tolerant	4.0	3.0	0.0
	<i>Bosmina longirostris</i>	Filter-feeder	Small	Euryhaline	Tolerant	6.0	5.0	10.0
	<i>Chydorus sphaericus</i>	Filter-feeder	Small	Euryhaline	Highly tolerant	2.0	2.0	8.0
	<i>Chydorus piger</i>	Filter-feeder	Small	Euryhaline	Highly tolerant	1.0	0.0	2.0
Copepoda	<i>Alona rectangularis</i>	Filter-feeder	Small	Euryhaline	Tolerant	2.0	2.0	5.0
	<i>Mesocyclops leuckarti</i>	Predator	Medium	Euryhaline	Highly tolerant	2.5	6.0	14.0
	<i>Mesocyclops hyalinus</i>	Predator	Medium	Euryhaline	Highly tolerant	1.5	4.5	10.0

Size classes: Small < 200 µm, Medium 200–500 µm, Large > 500 µm.

Table 3. Diversity indices and community structure

Zone	Species Richness (S)	Shannon H'	Simpson (1-D)	Evenness (J')	Total Abundance (ind. m ⁻³)	Dominant Taxon (% of Total)
Zone 1 (Reference)	18 ± 1.2 ^a	2.45 ± 0.15 ^a	0.82 ± 0.04 ^a	0.85 ± 0.05 ^a	485 ± 68 ^a	<i>Euchlanis dilatata</i> (12.0%)
Zone 2 (Moderate)	16 ± 1.5 ^a	2.30 ± 0.18 ^a	0.79 ± 0.06 ^a	0.83 ± 0.06 ^a	720 ± 95 ^b	<i>Euchlanis dilatata</i> (15.0%)
Zone 3 (High Impact)	9 ± 0.8 ^b	1.10 ± 0.12 ^b	0.45 ± 0.08 ^b	0.50 ± 0.07 ^b	1,150 ± 142 ^c	<i>Mesocyclops</i> spp. (24.0%)

Values are expressed as mean ± standard deviation. Different superscript letters (a–c) indicate significant differences among zones (p < 0.05). Simpson's index (1-D, index of diversity) is provided, where higher values reflect greater diversity of the community and lower values reflect lower diversity and greater dominance of stress-tolerant taxa.

Table 4. Morphometric responses of dominant crustacean taxa

Taxon	Measurement	Zone 1	Zone 2	Zone 3	Reduction %	ANOVA F	P-Value
Cladocera							
<i>Daphnia magna</i>	Body length (µm)	3,850 ± 245 ^a	2,100 ± 198 ^b	Absent	45.5%	78.4	<0.001***
	Carapace length (µm)	1,420 ± 89 ^a	780 ± 65 ^b	—	45.1%	82.1	<0.001***
<i>Simocephalus vetulus</i>	Body length (µm)	2,100 ± 156 ^a	1,150 ± 98 ^b	Absent	45.2%	65.7	<0.001***
<i>Diaphanosoma excisum</i>	Body length (µm)	1,680 ± 124 ^a	890 ± 76 ^b	Absent	47.0%	71.2	<0.001***
<i>Ceriodaphnia reticulata</i>	Body length (µm)	920 ± 68 ^a	510 ± 45 ^b	Absent	44.6%	58.9	<0.001***
<i>Moina micrura</i>	Body length (µm)	780 ± 58 ^a	420 ± 38 ^b	Absent	46.2%	62.3	<0.001***
<i>Bosmina longirostris</i>	Body length (µm)	450 ± 34 ^a	380 ± 29 ^{ab}	290 ± 22 ^b	35.6% (Z1→Z3)	12.4	<0.001***
<i>Chydorus sphaericus</i>	Carapace length (µm)	320 ± 24 ^a	280 ± 21 ^a	240 ± 18 ^b	25.0%	8.9	0.001**
Copepoda							
<i>Mesocyclops leuckarti</i>	Prosome length (µm)	680 ± 52 ^a	580 ± 45 ^b	490 ± 38 ^c	27.9%	15.6	<0.001***
	Total length (µm)	1,120 ± 84 ^a	950 ± 72 ^b	780 ± 61 ^c	30.4%	18.2	<0.001***

Values are mean ± standard deviation. Different superscript letters (a–c) indicate significant differences among zones (p < 0.05). "Absent" indicates the absence of the species in the respective zone.

3.3 Morphometric responses to stress

Morphometric analyses indicated clear evidence of chronic sublethal stress, as crustacean body size decreased significantly along the pollution-salinity gradient (Table 4). Average body-length reductions among cladoceran taxa ranged between 25% and 47%, with several taxa disappearing

entirely in Zone 3. Copepods in polluted zones were also significantly smaller (Figure 6). The reported percentages refer to the taxa's relative contributions across pooled samples rather than to absolute abundances. Rare species with very low abundance values were kept in the dataset to preserve ecological variability and to avoid underestimating patterns of biodiversity.

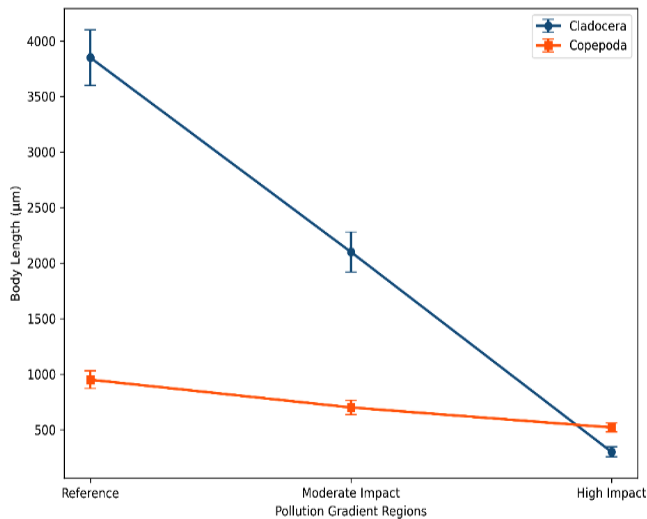


Figure 6. Bar chart showing the decline in mean body length for major groups (Cladocera, Copepoda) across the three zones, with error bars

3.4 Multivariate drivers of community structure

The CCA ordination (Figure 7) revealed strong correlations between species and environmental variables. The first two canonical axes accounted for 58% of the total variance in species data (Axis 1: 76.6%, eigenvalue = 0.42; Axis 2: 16.9%, eigenvalue = 0.22). A Monte Carlo permutation test with 999 iterations confirmed the significance of both the first axis and the overall analysis ($p = 0.001$). The biplot distinctly separated the zones based on environmental factors. Zone 3 samples clustered together and were strongly associated with high TDS (salinity), BOD/COD/TOC (organic pollution), and HPI (heavy metals). In contrast, Zone 1 was linked to higher DO levels and included sensitive species such as *Daphnia* spp. and *Euchlanis dilatata*. PERMANOVA confirmed significant differences in community composition among the zones ($F = 4.12$, $p = 0.001$). ISA identified *E. dilatata* and *Daphnia* spp. as indicators of reference conditions (Zone 1), while *B. angularis* and *Mesocyclops* spp. were markers of heavily impacted areas (Zone 3).

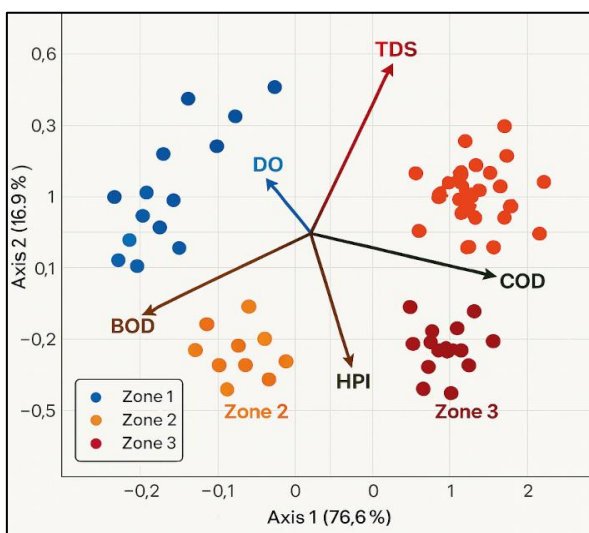


Figure 7. Canonical correspondence analysis (CCA) biplot illustrating the relationship between zooplankton assemblages and key environmental variables

4. DISCUSSION

4.1 Community restructuring under multi-stressor gradients

This study demonstrates that zooplankton communities in Bahr Al-Najaf are structured by interacting stressors, including salinity, organic enrichment, and metal contamination. The most prominent pattern is the decoupling of abundance and diversity: although total zooplankton density increased in the most impacted zone, both taxonomic and functional diversity declined significantly. This indicates a transition from diverse assemblages dominated by large-bodied cladocerans to simplified communities composed primarily of small, stress-tolerant taxa.

Elevated salinity (approximately 4.8 ppt) likely imposed substantial osmoregulatory costs on freshwater crustaceans, reducing fitness and excluding sensitive taxa such as *Daphnia*, *Diaphanosoma*, and *Simocephalus*. Simultaneously, increased metal bioavailability under saline conditions and high organic loading from sewage inputs likely intensified physiological stress. These combined pressures function as ecological filters, favoring opportunistic, r-selected species such as *Brachionus*, *Rotaria*, and *Mesocyclops*, which can exploit detrital and microbial pathways. Comparable patterns of community simplification and dominance by tolerant taxa have been widely documented in eutrophic and polluted wetlands, supporting the conclusion that multiple stressors operate synergistically rather than independently.

Observed shifts in zooplankton community composition are likely a product of both independent effects of salinity and pollution gradients and the synergistic interactions of multiple environmental stressors. Higher salinity could increase the bioavailability and physiological toxicity of heavy metals and organic pollutants, resulting in higher stress for stenohaline and pollution-sensitive taxa. These interacting stressors may explain the observed drastic decline in cladoceran diversity and concurrent dominance of stress-tolerant rotifers and cyclopoid copepods in Zone 3. Similar synergistic effects among salinity, heavy metal contamination and eutrophication have been reported in degraded inland wetlands and hypersaline transitional ecosystems, where combined environmental pressures drive biotic homogenisation and loss of sensitive zooplankton assemblages.

4.2 Body size reduction as an early ecological indicator

A pronounced reduction in cladoceran body size was observed along the pollution gradient, with decreases exceeding 50% in highly impacted areas. This trend reflects sublethal stress responses, in which energy is diverted from growth and reproduction to maintenance processes such as osmoregulation and detoxification. Additionally, metal exposure and organic pollution further disrupt moulting and metabolic processes, thereby reinforcing size suppression.

Size reduction was observed prior to local extinction, particularly in moderately impacted zones, highlighting its potential as an early-warning indicator. Shifts toward smaller body sizes have significant ecological consequences, including reduced grazing efficiency, altered nutrient cycling, and diminished energy transfer to higher trophic levels. The transition from large-bodied ($>500 \mu\text{m}$) to small-bodied ($<300 \mu\text{m}$) communities thus represents both taxonomic loss and functional degradation. These results support the integration

of morphometric traits into biomonitoring frameworks. In comparison to behavioural indicators, body size offers a robust, quantifiable, and cost-effective proxy for chronic environmental stress, especially in arid wetland systems where monitoring resources are limited. The reduction in body size and simplification of community structure observed may be an adaptive ecological response to environmental stress. High salinity and pollution stress might divert a large part of the metabolic energy for osmotic pressure regulation and physiological functions other than for growth and reproduction. Such strategies of energy allocation can favour smaller, stress-tolerant organisms, while sensitive species gradually decline in the community. Therefore, the community structure becomes less complex and increasingly dominated by organisms that can survive unstable environmental conditions. These mechanisms could account for the observed decreasing of diversity and simplification of structure in the studied sites.

4.3 Ecological and One Health implications of stress-tolerant assemblages

The predominance of stress-tolerant taxa in degraded zones indicates advanced ecosystem disturbance and carries significant ecological and health implications. Rotifers, including *Brachionus* and *bdelloids* (Rotaria), along with cyclopoid copepods (*Mesocyclops*), are established indicators of eutrophic, hypoxic, and organically enriched environments. The increased abundance of these taxa suggests a transition toward microbial-based food webs, which diminishes trophic efficiency and compromises ecosystem stability.

Ecologically, the decline of large, lipid-rich cladocerans limits food resources for fish larvae and other consumers, which may hinder recruitment and reduce biodiversity. Concurrently, stress-tolerant taxa are frequently linked to elevated pathogen loads and contaminant accumulation, thereby increasing risks to water quality and food safety. These trends underscore the function of zooplankton as sensitive indicators of environmental change, with implications for ecosystem, animal, and human health.

4.4 From ecological signals to monitoring and management

The strong associations between zooplankton community structure and environmental gradients confirm their effectiveness as cost-efficient bioindicators. Shifts in community composition, reductions in organism size, and changes in dominance patterns reliably correspond to critical thresholds of ecosystem stress, such as low DO, high salinity, and elevated metal concentrations. These findings support the use of zooplankton metrics in early-warning monitoring systems. Integrating taxonomic, quantitative, and trait-based indicators yields a more ecologically relevant assessment than reliance on physicochemical parameters alone. In settings with limited resources, these approaches provide practical tools for detecting ecosystem degradation, informing management strategies, and promoting sustainable wetland utilization.

4.5 Comparison with literature

Shannon diversity values obtained in Bahr Al-Najaf were comparable to those reported from other stressed saline wetlands in the Middle East. Diversity values in the relatively less impacted Zone 1 ($H' = 2.45$) were similar to those reported

from moderately productive Iraqi marsh ecosystems, while the significantly reduced diversity in Zone 3 ($H' = 1.10$) was comparable to conditions reported from heavily polluted hypersaline wetlands and eutrophic lagoons in arid regions of Iran and Egypt. These results support the interpretation that increased salinity and organic pollution strongly simplify the zooplankton community structure in transitional desert wetlands.

5. STUDY LIMITATIONS

Several limitations should be acknowledged in the present study. First, greenhouse gas emissions such as methane (CH_4) were not quantified, limiting assessment of broader biogeochemical consequences associated with eutrophication and organic loading. Second, the study design was observational and correlative; therefore, causal relationships between specific environmental stressors and zooplankton responses could not be experimentally validated. Third, sampling was restricted to three zones within a single basin, which may limit broader regional extrapolation of the findings. In addition, although detailed morphological identification was conducted using standard taxonomic keys, the reliance on morphology-based taxonomy may have underestimated cryptic diversity, particularly among rotifers, copepods, and morphologically similar taxa such as *Chydorus* spp. without molecular confirmation, the possibility of cryptic species misidentification cannot be completely excluded. While the 18-month dataset successfully captured major seasonal variability, longer-term monitoring would be necessary to assess interannual ecological dynamics in this climatically variable wetland system. Future studies integrating high-frequency environmental monitoring, experimental mesocosm approaches, greenhouse gas measurements, DNA barcoding, and other molecular techniques would substantially strengthen ecological interpretation and taxonomic resolution.

6. CONCLUSIONS

This study demonstrates that zooplankton communities in Bahr Al-Najaf are highly sensitive to combined salinity and pollution gradients, showing predictable changes in diversity, size structure, and functional composition. The shift toward low-diversity, small-bodied, stress-tolerant assemblages signals a significant loss of ecological function and indicates ecosystem degradation. By integrating abundance, diversity, and trait-based indicators, this research advances biomonitoring for arid wetlands and establishes zooplankton as effective indicators of environmental change. These results have direct implications for ecosystem management, biodiversity conservation, and the protection of ecosystem services in vulnerable freshwater systems. The results highlight the importance of long-term environmental monitoring programs and mitigating actions to prevent the slow deterioration of wetland ecosystems. Routine monitoring of physical, chemical and biological indicators can improve environmental evaluation and assist sustainable management decisions. In addition, wetland conservation methods and minimizing the inflow of untreated pollutants can be advocated to save the ecological community structure, ecological balance, and biodiversity of the impacted ecosystem.

7. RECOMMENDATIONS

Arid wetlands are fragile ecosystems highly susceptible to climate change and human activities. Their protection requires integrated management, including the following measures:

- Protecting water resources through regular monitoring of water quality, regulating dams and water diversions, and working to reduce water depletion from rivers that feed wetlands.
- Reducing water pollution through environmental awareness of the importance of water, preventing the discharge of sewage and industrial waste, reducing the use of agricultural fertilizers and pesticides in areas near wetlands, establishing treatment plants, and enforcing laws that prevent encroachment, dredging, and draining.
- Involving local communities in the management of protected areas and expanding the areas covered by legal protection.
- Combating climate change involves planting native, drought- and salinity-resistant plants and afforestation around wetlands to reduce desertification, as well as adopting modern irrigation techniques to avoid and minimize water waste.

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APPENDIX

Figure A1 presents the three sampling sites in Bahr Al-Najaf. The photographs highlight the differences in pollution intensity and environmental conditions among the three study zones.



Figure A1. Representative field images of polluted and reference sites in Bahr Al-Najaf