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## Nematodes in the Ba Lai River, inhabiting sediments rich in hydrogen sulfide and methane, serve as effective bioindicators for environmental monitoring and management

## SUMMARY OF DISSERTATION ON BIOLOGY

**Major: Ecology**

**Code: 9 42 01 20**

*Ha Noi – 2026*

The dissertation is completed at: Graduate University of Science and Technology, Vietnam Academy Science and Technology.

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The dissertation is examined by Examination Board of Graduate University of Science and Technology, Vietnam Academy of Science and Technology at 9:00 AM, February 2nd, 2026.

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# INTRODUCTION

## 1. The urgency

The Mekong Delta in Vietnam plays a major role as a vast "food basket" for the country and for the global food market, exporting mainly rice, fruits, and seafood worldwide [1,2]. However, this delta is facing problems of serious salt intrusion and drought due to reduced freshwater discharge from upstream areas [3]. In order to solve these problems, the construction of dams along the coastal region and estuarine areas has been a priority for the Vietnamese government. In 2002, the first largest dam of the North Ben Tre Irrigation Project in the Mekong delta was built across the mainstream of the Ba Lai estuary [4]. The Ba Lai sluice gate has brought some benefits, such as flood control, freshwater supply, and irrigation, hence favoring the economic development of local communities [5, 6]. Its operation however has also resulted in long-term negative effects on the environmental quality. The erection of the Ba Lai dam is considered one of the reasons for the differences between its intertidal and subtidal nematode assemblages and those of the remaining Mekong estuaries both in terms of densities and species and genera diversity. Furthermore, the dam's construction had an effect on the biochemical components of the estuary, as observed by the increase in total suspended solids, heavy metal concentrations [7, 8], and significant oxygen depletion. The dam's presence may contribute to driving the Ba Lai's ecosystem to a tipping point. Indeed, soon after the dam began to operate, the quantity of microalgae, the concentration of phosphates and nitrates, and the suspended sediments were higher upstream of the dam and lower near the river mouth area [9]. Furthermore, turbidity levels increased since the dam construction due to alluvial silty deposition [10]. Also, sediments discharged from upstream could be trapped above the dam's sluice forming a stagnant area [11]. Combined with continuous siltation input, this has led to a reduction of the water capacity and subsequently the depletion of oxygen in the bottom layers turning the river's function into a shallow "lake–river" and supporting anaerobic processes that produce a large amount of greenhouse gases such as methane with a warming potential far greater than that of carbon dioxide (Ramaswamy et al., 2001), but also hydrogen sulfide, which is toxic for most eukaryotic life forms [12].

In aquatic ecosystems, $H_2S$ is highly toxic to most organisms and strongly affects benthic fauna, particularly free-living nematodes [13]. In contrast, the effects of methane on nematodes remain poorly studied, although $CH_4$ often co-occurs with $H_2S$ in anaerobic sediments [14]. Elevated $CH_4$ and $H_2S$ concentrations can reduce nematode genus richness and abundance [15]. As sensitive bioindicators, nematodes respond rapidly to environmental change and are widely used in ecological monitoring and assessments of anthropogenic impacts on aquatic habitats [16].

Given this context, the study "Nematodes in the Ba Lai River, inhabiting sediments rich in hydrogen sulfide and methane, serve as effective bioindicators for environmental monitoring and management" was conducted to establish a scientific basis for proposing solutions aimed at protecting, sustainably using, restoring, and conserving biodiversity in the Ba Lai River basin.

## 2. Research objectives

- Determine the concentration of hydrogen sulfide ($H_2S$) and methane ($CH_4$) emitted from the sediment in the Ba Lai River, Ben Tre Province.

- Study the free-living nematode community in the sediment under the influence of environmental conditions in the river mouth affected by the dam on the Ba Lai River, Ben Tre Province.

- Investigate the application of nematodes as bioindicators for monitoring and managing the environment of the Ba Lai River.

## 3. Scientific and practical significance of the Dissertation

➢ **Scientific significance:**

The dissertation provides a foundational database on the distribution of free-living nematode genera in the Ba Lai River, serving as a reference for future research in wetland conservation and aquatic environmental management. Additionally, it contributes to refining and advancing the methodological application of nematode communities as a superior bioindicator tool for water quality assessment.

➢ **Practical significance:**

The research highlights the impacts of $CH_4$ and $H_2S$ on nematode communities, elucidating their effects on the aquatic environment and benthic organisms. The findings offer a basis for biodiversity conservation strategies in wetlands and provide scientific support for sustainable

environmental monitoring and management. Moreover, the study underscores the potential of using nematodes as a bio-monitoring tool for assessing environmental quality in the Ba Lai River and similar aquatic systems. The dissertation also clarifies the ecological impacts of dam construction on the environment and benthic communities through nematode assemblages, an essential indicator group. These insights contribute valuable scientific evidence for the planning, operation, and management of dam-reservoir systems, aiming to mitigate negative environmental impacts.

## CHAPTER 1. OVERVIEW

Chapter 1 consists of 29 pages, presenting the current state of dam construction and its recorded impacts on ecosystems worldwide, as well as on the natural ecosystem of the Ba Lai River, Ben Tre. It provides an overview of the formation and emission of hydrogen sulfide ($H_2S$) and methane ($CH_4$) gases, along with their effects on species composition, distribution structure, and biodiversity of free-living nematode communities in dam-affected aquatic sediments. Additionally, it summarizes research on the ecological characteristics, density, and distribution of estuarine nematode communities globally and in Vietnam, with a particular focus on the Mekong estuary. The chapter also describes the study area of the Ba Lai River. The research gap in Vietnam is the absence of studies integrating $H_2S$, $CH_4$, and nematode communities for ecological assessment in hydrologically fragmented estuarine systems.

## CHAPTER 2. MATERIALS AND METHODS

Chapter 2 comprises 11 pages, presenting the study area, the research period, and the methodological approaches employed in the dissertation

### 2.1. Study area and sampling period

Sampling was conducted at 16 locations along the tidal zone of the Ba Lai River (designated G1 to G16), arranged sequentially from the river mouth toward the upstream section. Samples were collected during both the dry and wet seasons within the study years: the dry season (March), when the barrage gates were closed, and the wet season (October), when the gates were opened. In total, four sampling campaigns were carried out: wet seasons of 2019 and 2020, and dry seasons of 2020 and 2021.

**2.2. Analytical methods for hydrogen sulfide (H₂S), methane (CH₄), and sediment environmental parameters in the Ba Lai River**

- Surface sediment samples (0 – 10 cm depth) were collected using core tubes. Environmental parameters (pH, ORP, and salinity) were measured in situ using portable handheld meters. Sediment grain-size composition was analyzed in the laboratory using a RETSCH (Germany) sieve shaker, following the procedure of Zancanaro et al. (2020) [216].

- For the sulfide (H₂S) analysis, the top 2 cm of undisturbed sediments were carefully placed in a 50 mL PE conical bottom tube with thread cap (ISOLAB). The sediment samples were kept on dry ice during sampling and transportation and stored at −18 °C prior to the laboratory analysis. Total free H₂S concentrations were measured following the method of Brown et al. (2011). Briefly, the sediment sample stored in the 50 mL plastic tube was mildly defrosted at 4 °C overnight, and then centrifuged at 3000 rpm for 5 min. The supernatant was decanted and the sediment was mixed well with a stainlesssteel spatula. A 10 mL portion of the homogenized sediment was taken and placed into another 50 mL PE tube containing 10 mL of sulfide antioxidant buffer. The mixture was vortexed, and the concentration of sulfide was quickly measured on a potentiometer (Mettler Toledo) equipped with a sulfide ion selective electrode. The concentrations of sulfide in the samples were calculated based on a sulfide calibration curve prepared in the same manner samples [217].

- For methane (CH₄) analysis, about 10 mL of the 2 cm top of undisturbed sediment was placed into a 40 mL thread bottle containing 5 mL of 0.1 N NaOH to terminate further bacterial activity. The vial was immediately capped with a Teflon-lined silicone septum and kept cool with dry ice. Once returned to the laboratory, samples were stored at -18°C until analysis. Methane in sediment samples was analyzed following the method of Leloup et al. (2007). In summary, methane in the headspace was determined by gas chromatography equipped with an Alumina Sulfate PLOT (30 m×0.53 mm × 10 μm, Supelco, USA), a split/splitless injector, and a flame ionization detector. The injector was operated at splitless mode, and temperature of 35 °C. The linear velocity of nitrogen as carrier gas was set at 90 cm/s and the detector was operated at 250 °C, air flow rate of 400 mL/min, and hydrogen flow rate of 40 mL/min. Quantification of methane was carried out using a

calibration curve made by injecting various volumes of standard methane gas 15 ppmv (Agilent) with five replicated injections of every sample and standard [218].

- Gas data were processed as mean ± standard deviation using Microsoft Excel 2019. Shapiro – Wilk and Levene tests were used to assess statistical assumptions; non-normal data were log- or square-root–transformed. When assumptions were met, two-way ANOVA tested the effects of season, site, and their interaction; otherwise, two-factor PERMANOVA was applied. Tukey HSD or pairwise comparisons were conducted when significant differences occurred ($p < 0.05$). Pearson correlations evaluated relationships between $CH_4$, $H_2S$, and environmental variables, and regression models were developed based on high $R^2$ and $p < 0.05$. Analyses were performed using Statgraphics Centurion 18 and PRIMER v.6 + PERMANOVA.

## 2.3. Methods for studying free-living nematode communities in Ba Lai River sediments

For analysis of the nematode, communities' triplicate samples were collected at the same 16 intertidal stations at both riverbanks by means of 10 cm2 transparent plastic cores that were pushed into the sediment to at least 10 cm depth. Samples were preserved in 7% neutralized formaldehyde (pre-heated to 60 – 70 °C) before extracting from the sediment by washing the sample over a 1-mm sieve and keeping the fraction retained on a 38-µm sieve. The extracted samples were then separated and collected by flotation technique using Ludox-TM50 (specific gravity of 1.18) and stained with 1% solution of Rose Bengal (Vincx, 1996). All nematode individuals in each sample were counted under a stereomicroscope. About 200 nematodes per sample (if the sample consists of less than 200, all nematodes in that sample) were randomly picked out and processed for making permanent slides for taxonomic identification (De Grisse, 1969). The pictorial key on free-living marine nematodes part III (Warwick et al., 1998), the identification manual for freshwater nematode genera (Zullini, 2021), the free-living nematodes in Vietnam (Nguyen Vu Thanh, 2007), the handbook on freshwater nematodes: ecology and taxonomy (Eyualem-Abbe et al., 2006), and the NEMYS database (Bezerra et al., 2020) were referred for the identification. The nematodes were classified into four feeding categories, based on the structure of the buccal cavity according to Wieser (1953): (1A) selective deposit-

feeders, (1B) non-selective depositfeeders, (2A) epistratum feeders, and (2B) predators or omnivores (Jensen, 1987)

Nematode data were presented by the averages and standard deviations of triplicate samples per station per transect (16 station). The indices of nematode communities, such as density (N), genera richness (S), Shannon–Wiener diversityH' (Shannon & Weaver, 1949), Hill indices N1 and N2 (Hill, 1973), feeding type (1A, 1B, 2A, 2B) (Jensen, 1987; Wieser, 1953), trophic diversity index–TD (Heip et al., 1988), and the Maturity index–MI (Bongers,1990; Bongers et al., 1991), were tested for significant difference

Nematode density and diversity data were tested for normality (Shapiro–Wilk) and homogeneity of variance (Levene). When assumptions were satisfied, two-way ANOVA and Tukey HSD were applied; otherwise, data were transformed or analyzed using PERMANOVA. Multivariate analyses, including CLUSTER with SIMPROF and MDS, were used to assess differences and distributional patterns of nematode communities. Spearman correlations were performed to examine relationships among nematode metrics, environmental variables, and $H_2S$ and $CH_4$ concentrations. All analyses were conducted using STATISTICA 7.0, Excel 2019 with the XLSTAT-R engine, PRIMER 6.0, and Statgraphics Centurion 18.

## 2.4. Ecological health assessment method for the Ba Lai River

Benthic ecological health was assessed by classifying nematodes according to their c-p (1–5) values, which indicate ecological tolerance and recovery capacity. The Maturity Index (MI) was calculated following Bongers (1991) to evaluate the degree of environmental disturbance. Biodiversity was quantified using the Shannon–Weiner index (H'), while the Index of Trophic Diversity (ITD) reflected changes in nematode feeding functional groups. Threshold values for MI, H', and ITD were interpreted according to the environmental quality categories proposed by Moreno et al. (2011) (Table 2.2). When the indicators yielded inconsistent results, environmental quality was determined using the mean ranking approach described by Chen et al. (2018) (Fig. 2.8).

Table 2.2. Environmental quality classification thresholds based on nematode community characteristics, as proposed by Moreno et al. (2011) [186].

| The ecological quality status | c-p | Maturity Index (MI) | Shannon – Weiner Index (H') | Index of Trophic Diversity (ITD) |
|---|---|---|---|---|
| Hight | c-p 2 ≤ 50% c-p 4 > 10% | MI > 2.8 | H' > 4.5 | 0 < ITD ≤ 0.25 |
| Good | c-p 2 ≥ 50% c-p 4 > 10% | 2,8 ≤ MI < 2,6 | 3,5 < H' < 4,5 | 0.25 < ITD ≤ 0.4 |
| Moderate | c-p 2 ≥ 50% 3 < c-p 4 < 10% | 2,6 ≤ MI < 2,4 | 2,5 < H' < 3.5 | 0.4 < ITD ≤ 0.6 |
| Poor | c-p 2 > 60% c-p 4 < 3% | 2,4 ≤ MI < 2,2 | 1 < H' ≤ 2.5 | 0.6 < ITD ≤ 0.8 |
| Bad | c-p 2 > 80% | MI ≤ 2,2 | 0 < H' ≤ 1 | 0.8 < ITD ≤ 1 |
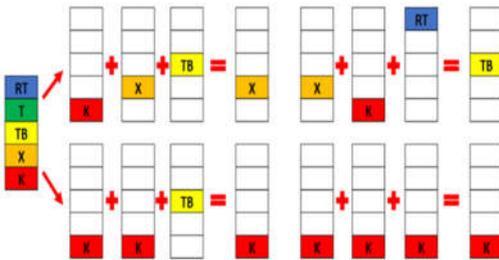


***Fig. 2.8.*** *Principles for assessing integrated environmental quality based on multiple indicators (Adapted from Chen et al., 2018)* [235]

## CHAPTER 3. RESULTS AND DISCUSSION

### 3.1. Environmental characteristics of sediment in the Ba Lai River

#### *3.1.1. Hydrogen sulfide*

$H_2S$ concentrations in the Ba Lai River displayed a consistent spatial pattern in both seasons, with elevated levels at the river mouth, dam-foot area, and upstream section, and the lowest values in the mid-river zone. Wet-season concentrations were higher outside the dam (0.19–1.03 mM) than inside (0.10–0.77 mM), and a similar trend occurred in the dry season. The
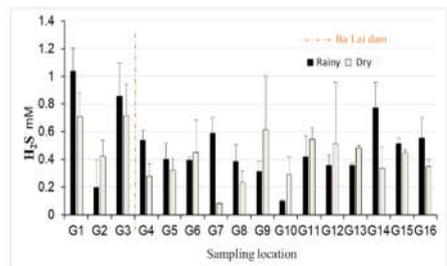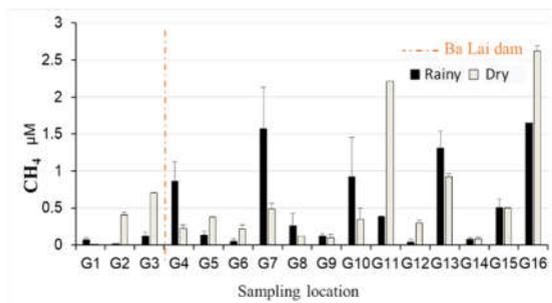


*Fig. 3.1. Hydrogen sulfide (± SD) concentrations at survey sites in the Ba Lai River during rainy and dry seasons.*

mid-river sites (G7 – G8) consistently exhibited the lowest $H_2S$ levels (0.08 – 0.31 mM). No significant seasonal difference was detected (p = 0.14), whereas spatial variation among sites was highly significant (p < 0.001), with G1 and G3 showing the highest concentrations.

### 3.1.2. Methane

During the wet season, $CH_4$ concentrations were generally low across most sites (0.01–0.51 µM), except for G4, G7, G10, G13, and G16, which exhibited unusually high values (up to 1.64 µM). In the dry season, $CH_4$ concentrations increased significantly compared to the

**Figure 3.4.** *Methane (± SD) concentrations at survey sites in the Ba Lai River during rainy and dry seasons.*

wet season (p = 0.02). Sites G13 and G16 maintained high $CH_4$ levels (0.91 and 2.62 µM, respectively), whereas G4, G7, and G10 showed reduced concentrations. In contrast, $CH_4$ increased markedly at G2, G3, G11, and G16 during the dry season, with G11 and G16 reaching exceptionally high values (2.21 and 2.62 µM) (Fig. 3.4). Tukey HSD post-hoc analysis indicated that G16 had significantly higher $CH_4$ concentrations than all other sites, and sites G7, G11, and G13 also showed significantly elevated levels relative to the remaining locations (Fig. 3.5b; Appendix 2).

### 3.1.3. Other Environmental Parameters

#### 3.1.3.1. pH

During the wet season, pH ranged from 6.78 ± 0.03 to 7.28 ± 0.008. Sites G2 and G8 showed pH > 7 (7.28 ± 0.008 and 7.26 ± 0.04, respectively), whereas G12 and G13 exhibited the lowest values (6.84 ± 0.05 and 6.78 ± 0.03). Most other sites were close to neutral. Overall, pH was significantly higher in the wet season than in the dry season (p < 0.001). In the dry season, elevated pH persisted at sites outside the dam, particularly G2 (7.20 ± 0.04). In contrast, sites near the dam foot (G4–G6) had low pH (6.93 ± 0.09; 6.78 ± 0.03; 6.81 ± 0.05), and similarly low values occurred upstream at G12, G13, and G14 (6.77 ± 0.01; 6.69 ± 0.01; 6.76 ± 0.05).

### 3.1.3.2. Salinity

Except for site G1, where salinity was similar between the wet and dry seasons (wet: $19.47 \pm 0.87$ g/L; dry: $18.07 \pm 0.48$ g/L), all other sites showed significantly lower salinity during the wet season ($p < 0.001$). As in other Mekong estuaries, freshwater discharge during the wet season (June – December) reduced salinity in the estuarine zone. Salinity in the Ba Lai River exhibited a clear spatial pattern, with lower values inside the dam and higher values outside ($p < 0.001$). During the wet season, salinity outside the Ba Lai dam ranged from $7.52 \pm 0.16$ to $19.47 \pm 0.97$ g/L, compared to $0.26 \pm 0.0005$ to $1.54 \pm 0.31$ g/L inside the dam. In the dry season, salinity increased from $15.36 \pm 0.01$ to $19.43 \pm 0.47$ g/L outside the estuary, and from $1.61 \pm 0.11$ to $4.41 \pm 0.20$ g/L inside the dam.

### 3.1.3.3. Oxidation - Reduction Potential (ORP)

ORP values were negative across all sites in both seasons, indicating reducing conditions associated with organic-rich sediments. During the wet season, ORP ranged from $-339.95$ mV (G4) to $-170.50$ mV (G12), while in the dry season values varied from $-266.85$ mV (G13) to $-117.10$ mV (G2). Several sites (e.g., G2, G6, G12) showed particularly low ORP in the dry season. Overall, ORP increased slightly in the wet season ($p = 0.045$), likely due to rainwater inputs enhancing ionization and redox reactions within the sediment, thereby influencing gas formation and sediment geochemistry.

### 3.1.3.4. Sediment Composition

Across both seasons, silt overwhelmingly dominated the sediment composition at all sites, reflecting the fine-grained characteristics of the Ba Lai River. Silt content decreased slightly from the wet to the dry season ($p = 0.001$), while sand and clay fractions increased (p-sand $= 0.002$; p-clay $= 0.01$). During the wet season, silt reached up to 94.15% (G7), whereas in the dry season it was lower, with a maximum of 91.03% (G9). Sand and clay contents were generally low in the wet season but increased markedly in the dry season, particularly at sites G14 and G16 (sand) and G5 and G7 (clay). These seasonal shifts indicate sediment redistribution driven by changes in flow regime, water extraction activities, and upstream material inputs.

### 3.1.4. Relationships between $CH_4$, $H_2S$, and environmental parameters

In the wet season, gas concentrations were correlated only with salinity: $H_2S$ showed a positive correlation ($p = 0.027$, $r = 0.391$), whereas $CH_4$ was

negatively correlated with salinity (p = 0.018, r = −0.417). In the dry season, salinity again correlated positively with $H_2S$ (p = 0.046, r = 0.355), but no relationship with $CH_4$ was detected. $H_2S$ was also negatively correlated with clay content (p = 0.008, r = −0.460). Conversely, $CH_4$ in the dry season correlated positively with sand content (p = 0.011, r = 0.441) and negatively with silt content (p = 0.033, r = −0.377). When data from both seasons were combined, $H_2S$ remained positively correlated with salinity (p = 0.041, r = 0.257) and negatively correlated with clay (p = 0.017, r = −0.298), while $CH_4$ showed a negative correlation with salinity (p = 0.044, r = −0.252)

## 3.2. Characteristics of Free-living nematode communities by season

### 3.2.1. Species Composition

Across the two sampling seasons, a total of 190 genera, 67 families, 11 orders, and 2 classes were recorded. The community was overwhelmingly dominated by Chromadorea (90.54%), with Enoplea accounting for only 9.46%. Monhysterida was the dominant order (60.65%), followed by Araeolaimida (14.15%), Chromadorida (8.27%), and Enoplida (7.17%). The families Xyalidae and Monhysteridae played major roles in the assemblage, contributing 37.10% and 12.88%, respectively, followed by Axonolaimidae (9.70%), Linhomoeidae (9.52%), Chromadoridae (5.47%), and Comesomatidae (4.11%). The dominance of Xyalidae was largely attributed to two genera *Daptonema* and *Theristus*. Several other genera, including *Parodontophora, Terschellingia, and Monhystera*, were also relatively abundant.

### 3.2.2. Community Density

Nematode densities varied markedly by season and site. During the wet season, densities ranged from $76 \pm 7$ ind./10 $cm^2$ at G6 to $2379 \pm 63$ ind./10 $cm^2$ at G3. High densities were also observed at the estuarine sites G1 and G2 ($430 \pm 215$ and $911 \pm 303$ ind./10 $cm^2$), whereas sites within the Ba Lai impoundment (e.g., G9, G14, G16) showed low values (< 100 ind./10 $cm^2$). Overall, densities tended to be higher outside the dam, with the dam-foot area showing relatively elevated values, though no consistent spatial pattern was observed across all sites.

In the dry season, densities increased substantially, ranging from $173 \pm 21$ ind./10 $cm^2$ at G15 to $1642 \pm 1215$ ind./10 $cm^2$ at G2. High densities were recorded at G1, G3, G5, G7, and G8 (793–1946 ind./10 $cm^2$), with G16 and

G6 also showing elevated values. A general increase from the estuary toward the dam was evident, with mid-river sites (G5–G8) showing particularly high densities, whereas most upstream sites remained low except for G16.

Two-way ANOVA and Tukey HSD indicated significantly higher densities in the dry season than in the wet season ($p < 0.001$) and significant spatial differences among sites ($p < 0.001$), with G2, G3, G5, G7, and G8 displaying distinctly higher densities than other locations.

### 3.2.3. Biodiversity of biological communities

*3.2.3.1. Genus richness index (S)*

Genus richness (S) varied by season and site. In the wet season, S was highest at G2 ($32.00 \pm 3.00$) and other sites outside the dam (G1: $31.00 \pm 2.65$; G3: $25.67 \pm 3.79$), while low values occurred at G12 ($14.33 \pm 4.04$) and several impounded sites (G5, G6, G14, G16). During the dry season, richness remained high outside the dam (G1–G3: 26–29) and at upstream sites (G10–G15: 27.33–38.00), with the lowest value at G16 ($12.00 \pm 1.73$).

Two-way ANOVA showed significant effects of season ($p < 0.001$), site ($p < 0.001$), and their interaction ($p < 0.001$). Tukey HSD indicated higher richness in the dry season and consistently low richness at G16 compared to other sites.

*3.2.3.2. Margalef diversity index (d)*

The Margalef index (d) varied across seasons and sites. In the wet season, values ranged from $2.46 \pm 0.16$ (G5) to $5.53 \pm 0.77$ (G9). A decreasing trend was observed from the estuary toward the Ba Lai dam, with $d = 5.02 \pm 0.28$, $4.59 \pm 0.61$, and $3.18 \pm 0.39$ at G1, G2, and G3, respectively. Low diversity occurred at G4 and G12 ($2.88 \pm 0.37$; $2.85 \pm 0.63$), while mid-river sites (G7–G11) showed higher values ($3.66 \pm 0.60$–$5.53 \pm 0.77$). Upstream sites (G14–G16) had moderate values ($3.66 \pm 0.94$–$4.60 \pm 0.56$). Overall, wet-season diversity was generally high except near the dam foot.

In the dry season, d ranged from $1.64 \pm 0.26$ (G16) to $6.94 \pm 1.50$ (G11). The three estuarine sites (G1–G3) showed similar diversity (3.73–3.92). Mid-river sites (G5–G9) had lower values ($2.69 \pm 0.27$–$3.34 \pm 0.35$), whereas upstream sites (G10–G15) recorded higher diversity ($5.13 \pm 0.61$–$6.94 \pm 1.50$). Thus, dry-season diversity was highest outside the dam and in the upstream section.

Two-way ANOVA showed no significant seasonal effect (p = 0.053), but diversity differed significantly among sites (p < 0.001) with a significant season × site interaction (p < 0.001). Tukey HSD identified G16 as having significantly lower d compared to all other sites.

### 3.2.3.3. Shannon–Wiener diversity index (H' log$_2$)

In the wet season, Shannon–Weiner diversity (H') was low at G4, G5, G6, G12, and G13 (2.25–2.73), while higher values were observed at other sites, particularly outside the Ba Lai dam (G1–G3: 3.11–4.12). In the dry season, H' ranged from 2.19 ± 0.09 (G16) to 4.47 ± 0.22 (G10). Estuarine sites (G1–G3) maintained high diversity (3.6–3.9), whereas sites such as G7–G9 and G16 showed low values (2.2–2.6).

Two-way ANOVA revealed significant seasonal (p < 0.001), spatial (p < 0.001), and season × site interaction effects (p < 0.001). Tukey HSD identified higher H' in the dry season and consistently low values at G16 compared to all other sites.

### 3.2.3.4. Pielou's evenness Index (J')

In the wet season, Pielou's evenness (J') ranged from 0.55 ± 0.16 (G6) to 0.83 ± 0.06 (G14). Estuarine sites (G1, G2) showed relatively high evenness (~0.82), whereas several impounded sites (G4, G5, G13) had lower values (0.55–0.66). In the dry season, J' ranged from 0.56 ± 0.14 (G7) to 0.84 (G10, G11, G15). Site G1 also showed high evenness (0.84 ± 0.04), while the upstream site G16 had the lowest value (0.61 ± 0.02).

Two-way ANOVA showed no significant seasonal effect (p = 0.063), but significant spatial variation (p < 0.001) and a significant season x site interaction (p < 0.001). Tukey HSD indicated that G6 and G7 had significantly lower J' than sites such as G1, G11, and G14.

### 3.2.3.5. Hill's diversity indices (N$_1$, N$_2$, N$\infty$)

In the wet season, Hill indices (N$_1$, N$_2$, N$\infty$) were highest at sites outside the Ba Lai dam, with N$_1$ ranging from 11.83 ± 1.90 to 17.69 ± 3.95, N$_2$ from 7.45 ± 0.50 to 12.14 ± 4.08, and N$\infty$ from 3.77 ± 0.58 to 5.73 ± 1.35. Lower values occurred at dam-foot sites (G4–G6) and at G12–G13 (N$_1$: 5.27 ± 2.83 – 6.72 ± 1.27), while mid-river and upstream sites showed intermediate to high diversity (N$_1$: 8.73 ± 1.76 – 12.80 ± 1.25).

During the dry season, Hill diversity increased at estuarine and upstream sites (N$_1$: 12.48 ± 2.27 – 22.34 ± 3.19), with a pronounced rise at the dam-

foot section (N$_1$: 18.52 ± 3.68). In contrast, sites G5–G9 and G16 showed lower values.

Two-way ANOVA revealed significant effects of season, site, and their interaction (p < 0.001), with consistently higher diversity in the dry season and markedly lower values at G16 compared to other sites.
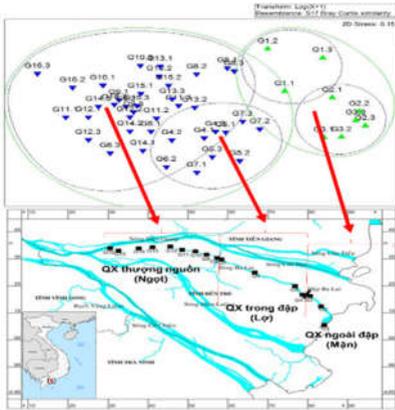
Overall, nematode communities in the Ba Lai River displayed relatively high density and diversity compared with several global and regional studies for example, coastal Northumberland (UK) [260], the Mediterranean Sea [261], the Elbe, Oder, and Rhine rivers (Germany) [262], and the Taro and Ticino rivers (Italy) [263] (Appendix 7). Within the Ba Lai system, nematode communities in the intertidal zone (this study; Ngo et al., 2016 [7]; Tran et al., 2022 [173]; Nguyen et al., 2022 [170]) generally exhibited higher density and diversity than those in the subtidal zone (Table 3.2).

### 3.2.4. Distribution Structure
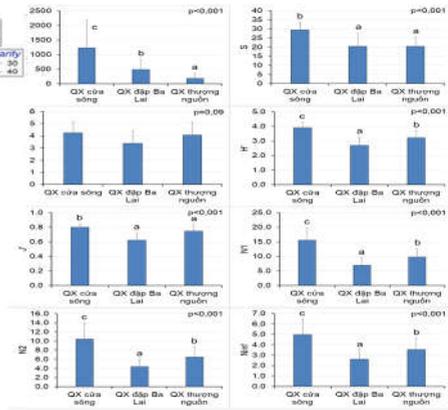
#### 3.2.4.1. Wet Season

Multivariate MDS analysis of wet-season nematode assemblages showed that at 30% similarity, the Ba Lai River community separated into two groups: sites outside the dam (G1 – G3) and sites inside the dam (G4 – G16). At a higher similarity threshold of 40%, the assemblage further separated into three groups: (1) outside the dam (G1 – G3), which included two subgroups the estuary (G1) and the near-dam outer sites (G2 – G3); (2) inner-dam sites (G4 – G7); and (3) upstream sites (G8–G16) (Fig. 3.36).

PERMANOVA indicated significant differences in community structure among the three nematode assemblages in the Ba Lai River (p = 0.001), reflecting distinct ecological characteristics under differing environmental conditions. The outside-dam assemblage exhibited the highest density (1240 ± 953 ind./10 cm²) and diversity, whereas the dam-foot (492 ± 356 ind./10 cm²) and upstream assemblages (188 ± 169 ind./10 cm²) had lower densities. Diversity indices (S, H′, J′, Hill numbers) all showed markedly higher values outside the dam. For example, H′ reached 3.92 ± 0.37 outside the dam, compared to 2.70 ± 0.57 and 3.22 ± 0.44 in the dam-foot and upstream assemblages, respectively. Although upstream density was lower, its diversity exceeded that of the dam-foot region.

**Fig. 3.36.** *Metric Multidimensional Scaling (MDS) analysis of the average distribution structure of the nematode community in the Wet season.*

**Fig 3.37.** *Ecological characteristics of the three nematode assemblages in the Ba Lai River during the Wet season*

Two-way ANOVA confirmed significant differences among assemblages for density and most diversity indices (except Margalef's d). Tukey HSD showed that density, H′, and Hill numbers were significantly higher outside the dam compared with the other two assemblages, while S and J′ did not differ significantly between the upstream and dam-foot groups (Fig. 3.37).

SIMPER analysis (Table 3.3) showed similar within-group similarity across assemblages (49.90 – 50.74%). Genera such as *Daptonema, Parodontophora, Terschellingia, and Theristus* contributed strongly to similarity in all three groups due to their wide ecological tolerance. Marine-associated genera (*Leptolaimoides, Linhystera, Paracomesoma, Spilophorella, Trissonchulus*) characterized the outside-dam assemblage, whereas freshwater genera (*Adoncholaimus, Eumonhystera, Monhystrella*) dominated inside the Ba Lai dam.
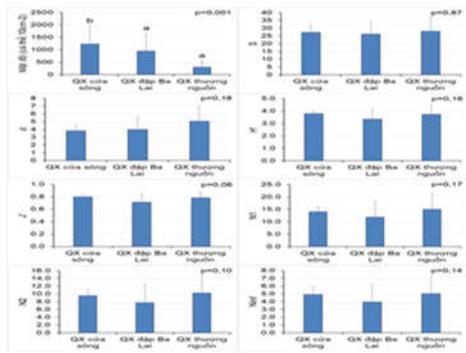
*3.2.4.2. Dry Season*

MDS analysis in the dry season showed that at 30% similarity, nematode assemblages separated into two groups: outside the dam (G1–G3) and inside the dam (G4 – G16). At 40% similarity, three assemblages were distinguished: outside dam (G1–G3), inner-dam (G4–G10), and upstream (G11–G16). Notably, the inner-dam assemblage extended farther upstream

to G10 in the dry season beyond the An Hoa confluence whereas during the wet season it was limited to G4–G7 (Fig. 3.38).

PERMANOVA confirmed significant differences among the three assemblages (Pseudo-F = 10.95; p = 0.001), indicating distinct community structures corresponding to different environmental conditions. Overall, the outside-dam assemblage exhibited the highest density ($1236 \pm 735$ ind./10 cm$^2$), followed by the inner-dam ($953 \pm 704$ ind./10 cm$^2$) and upstream groups ($307 \pm 244$ ind./10 cm$^2$) (p < 0.001).
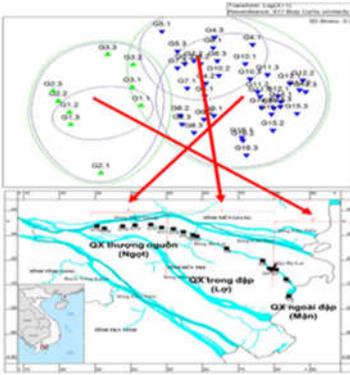


Fig. 3.38. Metric Multidimensional Scaling (MDS) analysis of the average distribution structure of the nematode community in the dry season.

Fig. 3.39. Ecological characteristics of the three nematode assemblages in the Ba Lai River during the dry season

Tukey HSD indicated no significant difference in density between the inner-dam and upstream assemblages, and diversity indices did not differ significantly among the three groups (p > 0.05) (Fig. 3.39).

In the dry season, the spatial extent of the Ba Lai dam assemblage expanded; however, diversity indices did not differ significantly among assemblages, while species composition showed clear differentiation. This indicates that species composition responds more sensitively to environmental variation than diversity indices, reflecting localized environmental changes and conditions across the Ba Lai River.

SIMPER analysis for the dry season (Table 3.7) showed low within-group similarity in the outside-dam and inner-dam assemblages (43.83% and

45.11%), while upstream assemblages exhibited higher similarity (51.67%). Numerous genera with broad ecological tolerance were present across at least two habitat types, including *Daptonema, Dichromadora, Eumonhystera, Linhystera, Monhystera, Mononchulus, Parodontophora, Sphaerolaimus, Sphaerotheristus, Terschellingia, Thalassomonhystera, Theristus,* and *Tobrilus*.

The outside-dam assemblage was characterized by typical marine genera such as *Aegialoalaimus, Anoplostoma, Eleutherolaimus, Halalaimus, Haliplectus, Hopperia, Onyx, Paracomesoma, Paracyatholaimus, Pierrickia, Pseudolella, Sabatieria, Spilophorella,* and *Trissonchulus*. In contrast, dominant genera in the inner-dam and upstream assemblages were mainly euryhaline or freshwater-tolerant forms, reflecting their adaptation to low-salinity environments.

### 3.2.5. Colonizer-Persister Index (c-p) and Maturity Index (MI) of the Nematode Community

During the wet season, c-p 1&2 nematodes dominated most sites, particularly at the Ba Lai dam-foot (G4: 94.01%; G5: 92.46%) and outside the dam (G2: 68.35%; G3: 88.00%). Mid-river sites such as G8 and G10 showed much lower proportions (40.86% and 51.44%). A similar pattern occurred in the dry season, although proportions decreased outside the dam (e.g., G2: 43.67%). Two-way ANOVA showed significant spatial variation (p = 0.009) but no seasonal effect (p = 0.96), with G3, G4, and G16 consistently exhibiting lower proportions of c-p 1&2 (Fig. 3.41; Appendix 8).

The c-p 3 group was generally low in the wet season (4.33% – 12.78%), except at G8 (29.96%), and extremely low at the dam-foot (G4 – G6: 0.79% – 1.88%). In the dry season, c-p 3 proportions increased significantly (p < 0.001), especially at G2 (50.23%) and G3 (34.77%). The dam-foot also showed notable increases (G4 – G6: 12.44% – 17.88%), whereas upstream sites remained relatively stable (4% – 13.15%).

The c-p 4&5 group showed higher proportions in the wet season particularly at G6 (21.68%) and G10 (36.46%) but decreased markedly in the dry season. There was a strong seasonal effect (p < 0.001) but no spatial effect (p = 0.28). This pattern contrasts with c-p 1&2, which were higher in the dry season.

Maturity Index (MI) values in the wet season were low at the estuary and dam-foot (G1 – G5: 2.1 – 2.2), but higher upstream (G12 – G16: 2.4 – 2.9). In the dry season, MI remained low at mid-river sites (G6 – G9: 2.1 – 2.3) and some dam-foot locations, but higher upstream (G10 – G15: 2.4 – 2.6). ANOVA showed significant seasonal ($p = 0.03$) and spatial effects ($p = 0.02$), with the wet season having higher MI overall, and G1, G2, and G5 exhibiting significantly lower MI than other sites.

### 3.2.6. Nematode Feeding type and Trophic Diversity Index (ITD)

The proportion of 1A bacterivores was markedly higher outside the dam in both seasons. During the wet season, G1 – G3 ranged from 31.35% to 36.67%, while inner-dam sites varied widely (4.34% at G6 to 36.14% at G10). In the dry season, G2 – G3 maintained high proportions (27.06%–28.64%), and several inner-dam sites (G4, G5, G13) also showed elevated values (25.50% – 31.69%). Two-way ANOVA indicated significant effects of season ($p = 0.03$) and site ($p < 0.001$).

Feeding type 1B bacterivores (large-mouthed) dominated inner-dam assemblages in both seasons (43.05% – 93.78%), while lower proportions occurred outside the dam (20.80% – 46.08%). Site differences were significant ($p = 0.01$), but no seasonal effect was detected ($p = 0.18$).

Feeding type 2A fungivores were more abundant outside the dam (wet: 22.38% – 41.73%; dry: 25.93% – 34.87%), with several inner-dam sites (G7, G8, G15) also showing relatively high values in the dry season (23.95% – 33.48%). Differences occurred among sites ($p = 0.002$), but not between seasons ($p = 0.15$).

Feeding type 2B carnivores/predators were concentrated in mid-river sites in both seasons (wet: 14.38% – 27.16% at G6 – G12), and remained low outside the dam (<4%). Seasonal ($p = 0.15$) and spatial ($p = 0.79$) differences were not significant.

The trophic diversity index (ITD) was lower in the wet season, particularly at G1 – G3 (~0.38) and G7 – G10 (0.33 – 0.45), while higher values occurred at the dam-foot (G4–G6) and upstream sites (0.47 – 0.69). In the dry season, ITD increased across most inner-dam sites (0.43 – 0.89), whereas outside-dam sites remained low (0.40–0.42). ANOVA showed significant seasonal ($p = 0.04$) and spatial effects ($p < 0.001$), with higher

ITD in the dry season. Sites G6, G3, and G4 exhibited significantly lower ITD than other locations.

### 3.3. Relationships between Nematode community characteristics and Sediment environmental parameters in the Ba Lai River

#### 3.3.1. Correlations between Nematode Community attributes and Sediment conditions

##### 3.3.1.1. Wet Season

DistLM showed that the first two dbRDA axes explained 70.27% of the variation in nematode community structure (dbRDA1: 55.05%; dbRDA2: 15.22%). Salinity (p = 0.001), pH (p = 0.002), silt (%) (p = 0.021), and $CH_4$ (p = 0.042) were the key variables influencing community patterns, in the order: salinity > pH > Silt > $CH_4$ (Fig. 3.57). Salinity and pH structured the outside-dam assemblages, whereas $CH_4$ and Silt (%) were more influential within the Ba Lai impoundment.

In addition, nematode community attributes were negatively correlated with $H_2S$ and clay content, but positively correlated with $CH_4$, pH, salinity, ORP, sand, and silt. $H_2S$ exerted a stronger influence than $CH_4$ and had a more pervasive effect on community structure. Among sediment fractions, silt and clay were more influential than sand. Overall, pH, salinity, and ORP were the key environmental drivers of nematode density and diversity, with pH and salinity showing particularly strong positive correlations.
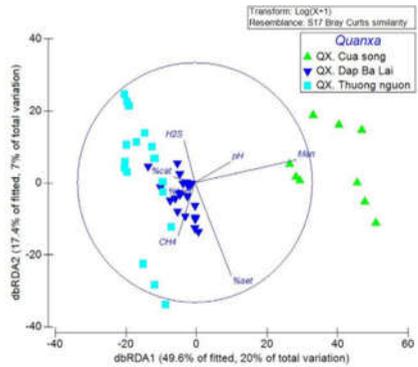
##### 3.3.1.2. Dry Season

DistLM analysis showed that the first two dbRDA axes explained 67.06% of the variation in dry-season nematode community structure (dbRDA1: 49.63%; dbRDA2: 17.43%). Significant environmental predictors included salinity (p = 0.001), clay (%) (p = 0.001), $CH_4$ (p = 0.001), and pH (p = 0.033), with contributions ranked as: salinity > clay (%) > $CH_4$ > pH (Fig. 3.58). Salinity and pH primarily structured estuarine assemblages, whereas clay (%) and $CH_4$ influenced the inner-dam and upstream communities.

During the dry season, nematode attributes were correlated with $H_2S$, $CH_4$, pH, salinity, and clay content. Salinity and pH remained positively correlated with nematode density but showed no relationship with diversity indices. $CH_4$ displayed a negative correlation with diversity and a stronger influence than in the wet season; it correlated positively with nematode density. In contrast, $H_2S$ showed positive correlations with H′ and J′. Clay

(%) maintained a broad negative correlation with most community attributes, except density, similar to wet-season patterns (Table 3.14).
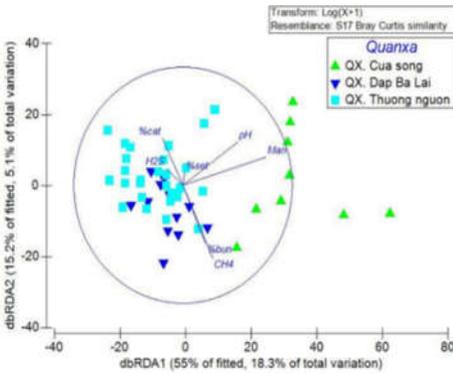


**Fig. 3.57.** DistLM analysis describing the relationship between environmental conditions and nematode community structure in the wet season

**Fig. 3.58.** DistLM analysis describing the relationship between environmental conditions and nematode community structure in the dry season

### 3.3.2. Influence of sediment environmental parameters on nematode community characteristics

#### 3.3.2.1. Effects of Hydrogen sulfide and Methane

The results show that environmental factors such as $H_2S$ and $CH_4$ strongly influence nematode community characteristics, particularly genus diversity, mainly within the Ba Lai impoundment (including the dam-foot and upstream assemblages). Both gases exert negative effects on nematode biodiversity; however, $CH_4$ was positively correlated with nematode density during the dry season, possibly due to the presence of chemosynthetic microorganisms thriving under anaerobic, $CH_4$ and $H_2S$ rich conditions.

#### 3.3.2.2. Effects of pH

Nematode communities developed best under neutral to alkaline conditions (pH > 7) and declined markedly under acidic conditions (pH < 6.5). The restricted water circulation caused by the Ba Lai dam led to the accumulation of organic-rich sediments and the release of toxic gases such as $CH_4$ and $H_2S$, particularly near the dam area (G3 – G6).

#### 3.3.2.3. Effects of Salinity and Sediment Composition

Salinity and sediment composition also strongly influenced nematode communities. Nematode density and diversity tended to decrease from downstream to upstream as salinity decreased. Moreover, silt-rich sediments (larger particles, better aeration, higher organic content) supported higher biodiversity than clay-rich sediments, which are finer, more anaerobic, and retain toxic compounds, thereby limiting species development. Overall, the results indicate that nematode communities in the Ba Lai River are simultaneously shaped by multiple environmental factors, with $CH_4$, $H_2S$, pH, salinity, and sediment composition being the dominant drivers of community structure, density, and diversity.

## 3.4. Application of Nematode communities in environmental monitoring of the Ba Lai River

### 3.4.1. Nematode genera sensitive or resistant to Hydrogen sulfide and Methane.

During the wet season, 34 genera showed significant interactions with $H_2S$ and $CH_4$. Of these, 10 genera were resistant to $H_2S$ (positively correlated with $H_2S$), 21 were resistant to $CH_4$ (positively correlated with $CH_4$), one genus (*Pseudolella*) was sensitive to $H_2S$ (negative correlation), and two genera (Halichoanolaimus and Halipletus) were sensitive to $CH_4$ (negative correlation) (Table 3.13). Overall, resistant genera outnumbered sensitive ones, reflecting the high adaptability of nematodes. The greater number of genera resistant to $CH_4$ compared with $H_2S$ also suggests that $H_2S$ may exert a stronger toxic effect.

In the dry season, the number of genera interacting with gases decreased to 10. No genera were sensitive to $H_2S$ or $CH_4$. Six genera (*Aphanonchus, Heterocephalobus, Paramonhystera, Parascolaimus, Stephanolaimus, and Tobrilus*) were resistant to $H_2S$, while four genera (*Axonolaimus, Eumonhystera, Monhystera, and Theristus*) showed resistance to $CH_4$ (Table 3.14).

### 3.4.2. Correlations between Biological indices and Sediment environmental parameters

During the wet season, H′, MI, %c-p 3, and %c-p 4&5 were positively correlated with pH and ORP, whereas %c-p 1&2 showed negative correlations. The c-p 4&5 group was the only category sensitive to $CH_4$ (r = – 0.311; p = 0.033). ITD was negatively correlated with pH and ORP (Table

3.15), indicating that ITD, MI, H′, and higher c-p groups reflect benthic habitat quality in a similar increasing trend. The trophic group 1B increased with $H_2S$ and $CH_4$, consistent with organic-rich sediments that promote bacterial growth, its primary food source.

During the dry season, MI and H′ were negatively correlated with clay content (Table 3.16). Salinity decreased %c-p 1&2 but increased %c-p 3, while %c-p 4&5 correlated negatively with salinity, suggesting that c-p indices were less stable than in the wet season. In contrast, ITD was more sensitive, showing a clear negative correlation with salinity and more accurately reflecting changes in benthic habitat quality.

### 3.4.3. Overall assessment of Benthic ecological quality in the Ba Lai River based on Nematode communities

During the wet season, the outside-dam area exhibited the best benthic environmental quality, characterized by high H′, low ITD, and relatively high proportions of c-p 4&5. In contrast, the inner-dam and upstream areas showed only moderate quality (Table 3.17).

Table 3.17. Current ecological quality of the benthic environment in the Ba Lai River during the wet season, assessed using the Shannon–Wiener diversity index (H'), %c-p, the Nematode Maturity Index (MI), and the Nematode Trophic Diversity Index (ITD)

| Area (Site) | % c-p | | Nematode Maturity Index (MI) | | Shannon–Wiener Diversity Index (H') | | Nematode Trophic Diversity Index (ITD) | | Benthic Ecological Environmental Quality | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Value | Classification | Value | Classification | Value | Classification | Value | Classification | Average Score | Classification |
| Downstream of the dam | c-p 1&2: 71,03 c-p 3: 9,15 c-p 4&5: 19,81 | Good | 2,46 | Moderate | 3,91 | Good | 0,39 | Good | 2 | Good |
| Upstream of the dam | c-p 1&2: 74,11 c-p 3: 7,83 c-p 4&5: 18,06 | Good | 2,43 | Moderate | 2,70 | Moderate | 0,51 | Moderate | 3 | Moderate |
| Headwaters | c-p 1&2: 73,48 c-p 3: 8,25 c-p 4&5: 18,27 | Good | 2,44 | Moderate | 3,23 | Moderate | 0,44 | Moderate | 3 | Moderate |

In the dry season, nematode diversity increased; however, the rise of c-p 1&2 and the decline of c-p 4&5 reduced the Maturity Index (MI), while the trophic diversity index (ITD) increased. As a result, all three regions were classified as having only moderate benthic environmental quality. (Table 3.18).

Table 3.18. Current ecological quality of the benthic environment in the Ba Lai River during the dry season, assessed using the Shannon–Wiener diversity index (H'), %c-p, the Nematode Maturity Index (MI), and the Nematode Trophic Diversity Index (ITD)

| Area (Site) | % c-p | | Nematode Maturity Index (MI) | | Shannon–Wiener Diversity Index (H') | | Nematode Trophic Diversity Index (ITD) | | Benthic Ecological Environmental Quality | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Value | Classification | Value | Classification | Value | Classification | Value | Classification | Value | Classification |
| Downstream of the dam | c-p 1&2: 75,73 c-p 3: 18,05 c-p 4&5: 6,22 | Moderate | 2,30 | Bad | 3,80 | Good | 0,49 | Moderate | 3 | Moderate |
| Upstream of the dam | c-p 1&2: 66,21 c-p 3: 21,76 c-p 4&5: 12,02 | Good | 2,42 | Moderate | 3,36 | Moderate | 0,60 | Moderate | 3 | Moderate |
| Headwaters | c-p 1&2: 80,00 c-p 3: 10,73 c-p 4&5: 9,26 | Moderate | 2,28 | Bad | 3,75 | Good | 0,45 | Moderate | 3 | Moderate |

The indices H′, MI, %c-p groups, and ITD proved highly effective for assessing the benthic ecological quality of the Ba Lai River, with ITD showing greater sensitivity during the dry season. Overall, benthic quality was classified as good during the wet season but declined to moderate in the dry season. These findings highlight the need for continued management and monitoring of benthic environmental conditions in the Ba Lai River.

## 3.5. Application of Nematode Communities for Environmental Management

The findings indicate that nematode communities are an effective biological tool for assessing and tracking benthic environmental quality in reservoirs and impounded systems affected by barrages. The indices H′, MI, c-p groups, and ITD responded sensitively to seasonal changes and hydrological alterations.

A monitoring program should be conducted at least twice per year (wet and dry seasons), covering three ecological zones (outside dam, inner-dam, upstream), and integrated with key physico-chemical parameters to better interpret ecological dynamics.

Benthic quality can be evaluated using a "traffic-light" alert system (good–warning–degraded), with thresholds adapted to each water body. When degradation is detected, priority management actions include reducing organic and nutrient inputs, sediment management, improving water circulation through dam operation, and applying nature-based measures such as vegetated buffers or constructed wetlands.

# CONCLUSION AND RECOMMENDATIONS

## Conclusion

This study quantified $H_2S$ and $CH_4$ emissions in the sediments of the Ba Lai River. Although both gases originate from anaerobic decomposition of organic matter, their concentrations varied across seasons and sampling locations. $H_2S$ showed no seasonal differences, whereas $CH_4$ emissions were significantly higher in the dry season. $H_2S$ tended to be higher outside the dam, while $CH_4$ peaked at several inner-dam sites, largely influenced by salinity and sediment composition.

The Ba Lai nematode community exhibited high structural diversity compared with previous studies in Vietnam and abroad, comprising 190 genera, 67 families, 11 orders, and 2 classes (Chromadorea and Enoplea). Nematode density and diversity varied significantly by season and location, with higher values in the dry season. Distribution patterns highlighted a clear ecological discontinuity created by the barrage, separating communities into outside-dam and inner-dam assemblages, the latter consisting of two subgroups (dam-foot and upstream). Diversity differed among these assemblages, especially in the wet season, whereas structural differences were consistently strong across both seasons. This indicates that species composition responds more sensitively to environmental change than diversity indices.

Additional community attributes including c-p structure, Maturity Index (MI), trophic groups, and the ITD index also varied temporally and spatially. Wet-season values of MI and ITD were generally higher than those in the dry season. Using H′, MI, c-p, and ITD as bioindicators, benthic environmental quality was classified as good in the wet season but moderate in the dry season. Integrating multiple nematode-based indices is recommended for a more robust ecological assessment.

Environmental variables including $H_2S$, $CH_4$, pH, salinity, and sediment composition significantly influenced nematode communities. $H_2S$ and $CH_4$ primarily affected inner-dam assemblages and reduced biodiversity while having limited effects on density. These findings highlight the potential of nematode communities as bioindicators for $H_2S$ and $CH_4$ emissions

associated with stagnant conditions in barrage- and reservoir-impacted systems.

## Recommendations

Traditional diversity indices alone cannot capture shifts in species composition under environmental stress. Future research should incorporate taxonomic-level diversity metrics, such as Taxonomic Distinctness ($\Delta+$) and Variation in Taxonomic Distinctness ($\Lambda+$), and evaluate their sensitivity relative to traditional indices (Shannon–Wiener H′, Margalef d, Pielou's J′).

To enhance the application of nematode communities in sediment quality assessment, future studies should examine their relationships with a broader suite of environmental variables, including nutrients (TN, TP, OM, Chl, TOC, $NH_4^+$, $NO_3^-$, $NO_2^-$, $PO_4^{3-}$) and heavy metals. This will allow a more comprehensive understanding of how nematode communities respond to complex environmental gradients and pollution pressures.

The underlying mechanisms driving compositional differences among isolated assemblages remain unclear. One hypothesis is that variations in food sources and trophic network structure play a key role. Stable isotope analysis may provide valuable evidence for elucidating trophic dynamics and explaining species-level differences among separated nematode communities.

## NEW CONTRIBUTIONS OF THE DISSERTATION

1. The concentrations of $CH_4$ and $H_2S$ gases along the entire length of the Ba Lai River were, for the first time, comprehensively studied in both spatial and temporal dimensions within the only estuary in the Mekong Delta system that is blocked by a dam on its main channel.

2. The study identified and recorded the levels of influence of $CH_4$ and $H_2S$ gases on the characteristics of free-living nematode communities - including their abundance, distribution density, and diversity properties - through bioindices, indicator group levels, dominant groups, and other intrinsic traits of the free-living nematode communities in the Ba Lai River sediment.

3. The environmental quality of the Ba Lai River was assessed using nematode communities as bioindicators. Moreover, the research provides scientific data and methodological insights related to the application of this animal group as indicators for water environment monitoring and management.

# LIST OF THE PUBLICATIONS RELATED TO THE DISSERTATION

1. Quang N.X., Yen N.T.M., Thai T.T., Yen N.T.H., Van Dong N., **Hoai P.N**., Lins L., Vanreusel A., Veettil B.K., Hiep N.D., Bang H.Q., Quan N.H., Prozorova L., 2022, Impact of a dam construction on the intertidal environment and free-living nematodes in the Ba Lai, Mekong Estuaries, Vietnam, *Environmental Monitoring and Assessment*, 194(Suppl 2):770, pp. 1-23.

2. **Pham Ngoc Hoai\*,** Tran Thanh Thai, Nguyen Thi My Yen, Phan Thi Thanh Huyen, Ngo Xuân Quang, 2022, Seasonal distribution of free - living nematode communities in Ba Lai River, Ben Tre province, *TNU Journal of Science and Technology* 227(05), pp.3-11.

3. **Pham Ngoc Hoai\*,** Tran Thanh Thai, Nguyen Thi My Yen, Nguyen Thi Hai Yen , Phan Thi Thanh Huyen, Ngo Xuân Quang,  2021, Influence of seasonal factors on ecological health assessment of sediment habitat in the Ba Lai River using free living nematodes, *TNU Journal of Science and Technology*, 226(10), pp.170-177.

4. Tran Thanh Thai, Nguyen Thi My Yen, **Pham Ngoc Hoai**, Nguyen Van Dong, Nguyen Lu Nguyet Hang, Ngo Xuan Quang[*], 2023, Greenhouse gases concentrations influence on vertical distribution of nematode communities in the Ba Lai river, Vietnam, *Academia Journal of Biology - Vietnam Academy of Science and Technology*, 45(2), pp.105–122.