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Review

Ecological Health Assessment of the Mekong River and Its Tributaries: Challenges and Future Perspective – A Review

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Abstract

The Mekong River Basin (MRB) and its tributaries play an important role in the biodiversity, livelihoods, and ecological services for millions of people in Southeast Asia. In recent decades, economic development, expanded urbanization, hydropower establishment, and climate change have significantly impacted river health. This review highlights the current knowledge of the Ecological River Health Assessment (ERHA) using bioindicators, including algae, nematodes, zooplankton, macroinvertebrates, and fish, to assess water quality and ecosystem health of the MRB. We identify key challenges and propose future directions for the sustainable management of the MRB. The results highlighted that the ecological condition of the MRB varied from poor to good levels with a decreasing trend downstream. Each biological group offers distinct advantages in ERHA within the MRB. Microalgae and zooplankton are effective indicators for short-term variations, while

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nematodes and macroinvertebrates are reliable indicators of sediment conditions. Macroinvertebrates are best for long-term indicators, and fish reflect broader ecosystem health. Biomonitoring methodologies have advanced to incorporate multimetric indices (MMIs) and bioindicators, but challenges remain in ensuring data consistency, taxonomic accuracy, and methodological standardization. Future research should aim to standardize sampling techniques, establish consistent reference materials, and improve data processing methods, which will improve the results for the Mekong River system.

Keywords: bioindicators, biotic indices, ecosystem health, multimetric indices, water quality

Introduction

The ecological health of river systems depends on ascertaining their status, especially with the increasing human impact and climate change pressure on the environment. Through the response of aquatic organisms to environmental stressors, ascertaining ecological river health provides a scientific foundation for the quantification of water quality, biodiversity, and ecosystem function [1, 2]. Over the past century, innovation in biomonitoring has changed Ecological River Health Assessment (ERHA) from the simple recording of species presence/absence to using complex multimetric approaches combining biological, chemical, and physical variables [2, 3]. These advances have greatly enhanced our ability to detect pollution effects, monitor long-term ecological trends, and inform conservation efforts.

The use of biological communities as indicators of water pollution was first proposed in the United States in 1887, marking the beginning of biomonitoring [4]. Kolkwitz and Marsson (1908) later introduced the Saprobic System, classifying aquatic organisms by their tolerance of organic pollution [5]. In the 1960s, Hynes emphasized the importance of benthic macroinvertebrates in assessing stream conditions, and by the middle of the 20th century, important biological indicators like fish, macroinvertebrates, and algae were essential to river health assessments [3, 6]. During this time, popular diversity indices that measure species richness and evenness were also developed, such as the Shannon-Wiener Index and the Simpson Index. Specialized tools such as the Saprobic Index and the Biological Monitoring Working Party (BMWP) score were also introduced to evaluate organic pollution using aquatic invertebrates [7, 8].

The development of the Index of Biotic Integrity (IBI) by Karr in 1981 marked a shift toward multimetric biomonitoring, using fish to assess ecosystem health [9, 10]. In the 1980s and 1990s, the integration of geographic information systems (GIS) enabled broader-scale ecosystem assessments [11]. Biological monitoring became central to water resource management through legislation such as the U.S. Clean Water Act (1972) and the EU Water Framework Directive (2000) [12]. Emerging technological advances in the past decades have transformed biomonitoring, enabling data collection in real-time and monitoring of ecosystems remotely. River health assessment is more convenient with the help of technologies such as drones, satellites, and artificial intelligence (AI), along with conventional field surveys [13]. International activities like the United Nations Decade on Ecosystem Restoration (2021-2030) and Sustainable Development Goals (SDGs 6 and 15) have reiterated the pressing need for sustainable management of water resources, thereby facilitating the increased use of ecosystem-based methods [14].

This global evolution of biomonitoring techniques has profound implications for regional river systems, particularly in biodiversity hotspots like the Mekong River Basin (MRB). Spanning six countries, including China, Myanmar, Laos, Thailand, Cambodia, and Vietnam, the MRB is one of the most biologically diverse and economically critical river systems in the world. Managed by the Mekong River Commission (MRC), the basin covers approximately 795,000 km² and supports over 65 million people. With an estimated 850 to 1,148 documented fish species, the MRB ranks as one of the most species-rich freshwater ecosystems globally [15, 16]. The global conservation relevance of the river is highlighted by iconic species, including the giant Mekong catfish (*Pangasianodon gigas*), giant freshwater stingray (*Urogymnus polylepis*), and Irrawaddy dolphin (*Orcaella brevirostris*). The Mekong also supports the largest inland fishery in the world, therefore producing about 15-20% of all inland fish [17]. Though ecologically and financially significant, the MRB is under increasing pressure that endangers its long-term health and viability. Hydropower development, including 11 mainstream dams and over 400 tributary dams, has changed flow patterns, obstructed fish migration, and disturbed sediment transport, therefore degrading habitats [16, 18, 19]. Urbanization and intensive agriculture have further polluted the river through pesticide runoff, overuse of fertilizers, and industrial discharge. Moreover, climate change compounds these pressures through rising temperatures, shifting rainfall patterns, sedimentation, and increased natural disasters [20].

Although many studies have examined the Mekong's ecological health, research remains fragmented and often focuses on specific biological groups [16, 19, 20]. This has led to inconsistent results and difficulty identifying reliable bioindicators. Moreover, every study focuses on a particular section of the river restricted to one country using different techniques and categories for different goals to draw their findings; thus, information on the ERHA of the whole MRB is sparse [18, 21, 22]. A complete study of river health assessment in the MRB is absolutely necessary to understand the general condition and suggest efficient management and conservation plans for the MRB. To address this gap, this paper aims to: (i) offer a thorough examination of ecological river health assessments employing important aquatic bioindicators including algae, nematodes, zooplankton, macroinvertebrates, and fish; (ii) assess present techniques, sampling strategies, and diversity indices; (iii) highlight issues with data standardization and cross-border cooperation; and (iv) suggest future paths for improving ERHA by means of advanced technologies and ecosystem-based approaches. Relevant peer-reviewed articles and scholarly books from 1999-2025 were identified via systematic searches in Google Scholar, Scopus, and Web of Science using keywords such as: "ecological monitoring", "biomonitoring", "environmental biomonitoring" "river ecological health assessment", "river ecological status evaluation", "biological water quality monitoring", "bioindicators-based monitoring" "aquatic biological surveillance", "biological integrity monitoring", "river bioassessment protocol", "river ecological condition reporting" "river health monitoring framework" and "biomonitoring methods for the Lower Mekong River and its tributaries". This review is based on 109 documents, including 88 peer-reviewed papers, 7 books, and 14 reports, all focused on biomonitoring using major aquatic groups as biological indicators. It examines key aspects such as sampling methods, taxonomic richness, abundance, bio-indices, and multimetric analysis. Spatial and temporal distribution analyses are also considered essential to understand when and where aquatic group-based biomonitoring has been conducted, thereby helping to identify and address existing research gaps.

Results and Discussion

Ecological River Health Assessment Using Different Aquatic Groups

Aquatic organisms are key bioindicators in ERHA, reflecting water quality and ecological health through their biological and functional roles [23]. River health is shaped by interacting factors such as biodiversity, water quality, flow regimes, and the integrity of riparian zones and physical habitats. These elements collectively influence ecosystem structure, processes, and resilience. Disturbance in any component can lead to ecological degradation. In the MRB, commonly assessed groups include algae, nematodes, zooplankton, macroinvertebrates, and fish (Fig. 1), all essential to ecosystem function [24]. Macroinvertebrates, microalgae, and fish are preferred due to their sensitivity to pollution and ease of sampling [7, 25]. These organisms support nutrient cycling, energy flow, and water quality monitoring [26]. Standard indices (e.g., BMWP, Saprobic Index), alongside multimetric and multivariate approaches, provide comprehensive assessments of biodiversity, pollution, and habitat conditions. ERHA also helps evaluate pollution control and restoration outcomes, guiding sustainable river management [27].

Microalgae

Microalgae communities, including phytoplankton, periphyton, and diatoms, are valuable bioindicators for the ERHA [22, 28]. As primary producers, they respond sensitively to water quality, flow regimes, and nutrient levels, making them effective for tracking ecological changes [29, 30] (Fig. 1). Their occurrence in various aquatic settings, such as water columns, riverbeds, and submerged surfaces, enables them to mirror both localized and broader ecological conditions [22, 31].

For ERHA purposes, microalgae can be divided into two main groups: benthic diatoms and phytoplankton. Sampling methods vary by target group: periphyton and benthic diatoms are typically collected using a 10 cm^2 plastic sheet and brushed with a toothbrush, while phytoplankton are sampled using plankton nets (10-30 μm), membrane filtration, or surface tows [32-35]. These methodological differences influence species richness estimates and may affect the comparability of results across studies.

The number of phytoplankton species in the Mekong River ranges widely from 42 to 422 [34-39], varying by sample site and tributaries (Table S1). Chlorophyta are dominant in slower-moving freshwater sections, while Cyanobacteria are prevalent in eutrophic waters, such as Ubol Ratana Dam [40]. Bacillariophyta are dominant organisms in marine and estuarine environments. Phytoplankton density varies significantly based on sample location, frequency, and taxonomic classification, with reported ranges from 13 to 1,140,000 individuals/L [36]. According to Nguyen et al. (2023), phytoplankton densities in the My Thanh River (Mekong Delta) fluctuate between 30,000 and 83,000 individuals/L, with an increase observed during the rainy season and a decrease during the dry season in upstream areas [41]. The middle and lower reaches exhibited elevated diatom cell counts, whereas the upper reaches showed a preference for euglenoids due to salinity and nutrient gradients. The primary source of this variance was predominantly the research groups, rather than factors such as geography, habitat, or water quality. Benthic diatom richness ranges from 79 [42] to 307 [43], with Achnanthales, Cymbellales, and Naviculales being most common [44]. Densities span from 660 to more than 30,000 cells/cm^2, depending on substrate stability and sediment loads [35]. Periphyton thrives in clearer, stable-bottom environments, but is reduced by turbidity and scouring flows [32]. The communities of planktonic and benthic algae serve as indicators of the Mekong's dynamic hydrology, influenced by the annual flood pulse

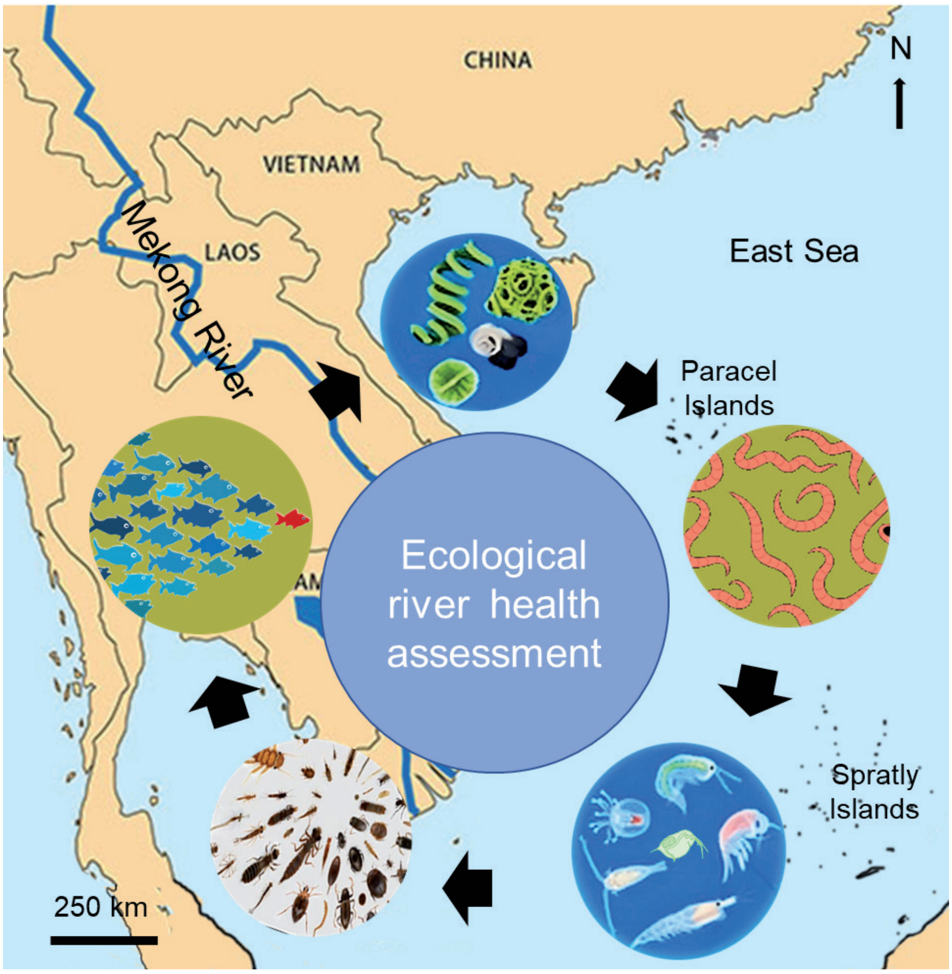

Fig. 1. The main groups of aquatic organisms used to assess the ecological health of rivers in the Mekong River Basin.

and local factors such as pollution and flow constraints, which can either encourage or inhibit blooms.

To characterize microalgae communities and ecological health, several indices such as the Shannon-Wiener Diversity Index (H'), the Pilou Evenness Index (J'), the Average Tolerance Score Per Taxon (ATSPT), and the Trophic Benthic Diatom Index (TBDI) have been used. In the MRB, H' index ranges from 1.20 to 3.57, and tends to decrease downstream, reflecting variations in species evenness and community structure [33, 36, 42, 45]. The J' index was reported between 0.30 and 0.85, which indicates the degree of uniformity in species distribution [42]. Besides, Moonsin et al. (2013) applied diatom indices for assessments of the Mekong River and found that most sites still fall into mesotrophic (moderate productivity) categories, but some locations influenced by human activities register toward eutrophic conditions. For instance, a biomonitoring study in Thailand noted that while several sampling sites showed moderate water quality, a few sites near urban or agricultural inputs were characterized as "moderate to polluted" (mesotrophic to eutrophic) based on their benthic diatom communities [43]. Moreover, the ATSPT, used to assess human impact, ranges from 30 to 51, and increases downstream direction, indicating greater impacts [32, 46, 47]. Lastly, the TBDI varied from 2.3 to 4.8 in Thailand's main rivers, providing insight into nutrient levels and water quality [34]. Studies have shown that the ecological condition of the river varies from poor to good status and tends to decrease downstream, where human activities have a greater impact.

Numerous studies have explored the relationships between phytoplankton, benthic diatoms, and water quality parameters, providing valuable insights into ecosystem health. Phytoplankton communities are shaped by factors like pH, EC, DO, BOD_5, and nitrate [43], while diatom patterns are influenced by pH, EC, DO, TP, Mg^{2+}, and SiO_2 [44]. Nguyen and Huynh (2020) used cluster and ordination analyses to reveal spatial-temporal shifts in phytoplankton [36]. However, most studies are site-specific and limited in geographic scope, lacking basin-wide consistency. Methodological discrepancies across countries hinder regional integration and long-term comparability. Despite challenges, microalgae remain reliable indicators for classifying ecological status in the MRB (e.g., good, moderate, or poor) [22, 28]. However, seasonal variability, inconsistent sampling, and data gaps limit their broader applicability. To strengthen basin-wide

ERHA, future efforts should prioritize standardized protocols, shared reference materials, and harmonized data analysis methods.

Microalgae-based assessments reveal variable ecological conditions across the MRB, with growing concern over degradation in the lower basin. In Cambodia, some sites still meet ecological thresholds (e.g., ATSPT<40), and H' and J' values suggest moderate health in less disturbed zones [47]. However, ecological conditions tend to deteriorate in southern Lao PDR, Thailand, and Vietnam, where ATSPT threshold values are frequently exceeded, indicating increased pollution and disturbance. Total benthic diatom abundance has declined by ~50%, pointing to rapid ecosystem deterioration. Many sites are now classified as mesotrophic to eutrophic, particularly where urban runoff and agricultural inputs are high. To improve ecological assessment accuracy and support sustainable water management, integrating microalgal data with standardized sampling and robust analysis is essential.

Nematodes

Nematodes are microscopic, thread-like multicellular organisms found in nearly all environments [48], with about 25% living freely in marine sediments, where they can reach densities of up to 12,000,000 individuals/m^2 [49]. They dominate benthic communities, contributing 90-95% of abundance and 50-90% of biomass [50]. Currently, 4,000-5,000 marine species are known [51], but estimates suggest up to one million species globally [52], making nematodes one of the most diverse animal phyla. In aquatic environments, nematodes play essential roles in organic matter decomposition, nutrient cycling, sediment oxygenation, and energy transfer within food webs [53-55]. Their small size, high abundance, sediment-dwelling lifestyle, and sensitivity to environmental changes make nematodes effective bioindicators [56-58]. Their short life cycles and low mobility allow rapid detection of pollution, eutrophication, and sediment disturbance. Moreover, they are relatively easy to sample and analyze, making them suitable for long-term environmental monitoring [59].

In the MRB, nematodes are primarily used to assess sediment quality and ecological status, especially in estuarine areas of Vietnam. Standard methods include core sampling (10 cm^2, ≥10 cm depth) in triplicate [60, 61], followed by sieving through a 38 µm mesh and flotation using Ludox TM50 (specific gravity 1.18) to extract both surface and burrowing species [60-62]. This quick method is common among nematologists for extracting specimens in an intact state. Core sampling, sieving, and density gradient extraction yielded a variety of nematode assemblages with epibenthic and endobenthic nematodes.

Most studies are concentrated in Vietnam's Mekong Delta, where nematode diversity and abundance vary with habitat and season. With 244 genera, Nguyen et al. (2012) reported the highest taxonomic richness in the Mekong Delta's estuarine environments [63]. Significant diversity was also reported by other studies; Ngo et al. (2016) identified 230 genera [60], while Nguyen et al. (2020) identified 227 genera in Ben Tre Province, Vietnam [64]. In contrast, Tran et al. (2020) observed lower richness (11-21 genera) in shrimp ponds [65]. There was also a significant variation in nematode density between locations and seasons. Shrimp pond densities varied from 222 to 2,539 individuals/10 cm^2 during the dry season and from 822 to 7,254 individuals/10 cm^2 during the rainy season [61, 62]. While Nguyen et al. (2012) reported a much wider range (64±30 to 3,269±3,928 individuals/10 cm^2) [63], Tran et al. (2020) observed densities ranging from 111±76 to 248±195 individuals /10 cm^2 in estuarine environments, indicating site-specific variability in nematode distribution [65]. Research on nematodes in the Mekong Delta has mostly concentrated on differences in individual density and taxonomic richness among various habitats. These differences underscore the influence of location, season, and anthropogenic impact on nematode distribution.

Nematode community structure has been evaluated using a variety of biological indices. Ngo (2012) found that species diversity was indicated by Shannon-Wiener diversity index (H') values, which varied from 1.30 to 2.70 [66]. Other studies also showed similar trends. For instance, Tran et al. (2020) found similar levels of diversity in the mangrove-shrimp ponds of Ca Mau Province, Vietnam [65], while Nguyen et al. (2020) reported H' values ranging from 1.56 to 2.62 [64]. The differences in community structure across habitats were further supported by the wide range of Margalef's diversity index (D), another indicator of taxa richness, which ranged from 1.10 to 4.70 [64] and 2.20±1.50 to 4.32±0.32 [63]. Pielou's Evenness Index (J'), which measures evenness, displayed comparatively stable values. While Nguyen et al. (2012) discovered a somewhat smaller range of 0.50 to 0.60 [63], Nguyen et al. (2020) reported J' values ranging from 0.62±0.18 to 0.85±0.15 [64]. These results point to a moderate level of species distribution uniformity throughout the regions under study.

Functional and multimetric approaches have further revealed ecological patterns. Nguyen et al. (2012) and Ngo et al. (2016) linked nematode assemblages to environmental conditions in estuarine areas [60, 63]. Tran et al. (2020) examined the role of habitat complexity in structuring biodiversity in mangrove–shrimp pond systems [65]. Nguyen et al. (2020) applied the Trophic Diversity Indicators (TD) in Ben Tre Province ranged from 0.29±0.02 in deeper sediment layers (40-50 cm) to 0.83±0.03 in surface levels (0-10 cm) during the dry season [64]. Tran et al. (2018) used the Abundance–Biomass Comparison (ABC) method to assess ecological quality (EcoQ) in Ca Mau's organic shrimp ponds, reporting stable conditions with improvements during transitional seasons [61, 62].

While primarily applied in estuarine areas, nematodes are reliable indicators of sediment quality and environmental stress. In Vietnam's Mekong Delta, studies have reported high taxonomic richness (up to 244 genera), particularly in estuarine and shrimp pond habitats. Diversity indices (H', D, J') suggest moderate ecological conditions with generally even species distribution. Some sites, such as organic shrimp ponds, showed stable ecological quality across seasons. However, ERHA applications remain concentrated in the lower basin, with limited data from the upper MRB. Nematode density and richness vary by site and season, influenced by habitat and sediment conditions. Lower diversity in intensive farming zones indicates localized stress. Trophic Diversity Index values further suggest organic accumulation in deeper sediments and healthier conditions near the surface.

Zooplankton

As primary consumers, zooplankton link primary producers to higher trophic levels such as fish and serve as effective indicators of ecological stress, nutrient enrichment, and hydrological changes [67]. Their rapid response to environmental variations makes them sensitive bioindicators in the MRB, where flow regimes and sediment loads shape distinct assemblages [32, 68]. Zooplankton sampling commonly employs plankton nets ranging from 20-64 µm mesh, influencing taxonomic capture. The MRC used 20 µm nets [32, 33, 47], while Nguyen et al. (2020) and Le et al. (2019) used 60 µm [69, 70]; Seleznev et al. (2023) applied a 64 µm Juday net [71]. Mesh size influenced species composition, with finer meshes (20 µm) capturing smaller taxa such as Rotifera and Protozoa, while coarser meshes (64 µm) were more effective for larger taxa like Cladocera and Copepoda. For quantitative sampling, water was often collected using buckets and then filtered through plankton nets [32, 33, 47, 72].

Zooplankton diversity across the MRB varies significantly by region and habitat. The MRC (2008) reported 207 taxa across multiple sites from 2004-2007 [32], whereas other surveys recorded from 137 to 148 species in the Hau River [69] and 138 taxa in the Lower Mekong River [73]. Coastal and estuarine areas showed lower diversity (e.g., only from 30 to 71 species in Tien Giang Province, Vietnam) [72, 74]. Species richness correlated with sampling effort and habitat complexity. Rotifers were dominant in many sites, making up 55.7% of species in U Minh Ha National Park [70] and 50% in Tonle Sap Lake [75]. Copepods dominated in shrimp ponds in Ca Mau Province, Vietnam (45.83%) [76], while Decapods prevailed in Tien Giang's coastal waters [72, 74]. The abundance of zooplankton in the MRB showed spatial and seasonal variability, increasing downstream due to intensified human activities [32]. Densities ranged from 2,158 to 16,592 individuals/m^3 in shrimp ponds [76], 51,447 to 722,553 individuals/m^3 in the Hau River [69, 73], and up to 24,993,634 individuals/m^3 in Tien Giang's coastal waters [74], reflecting strong links to nutrient availability and environmental conditions.

Biodiversity indices varied widely (Table S1). The Shannon-Wiener Index (H') ranged from 0.63-2.91 in the Lower Mekong River to 3.30-4.23 in the Hau River [42, 46, 69]. The highest values suggest a more balanced community structure, while lower values may indicate environmental stress. The Margalef Diversity Index (D) varied from 2.26-5.27 in U Minh Ha National Park [70] to 0.9-4.6 in the Hau River [69]. Evenness indices such as Pielou's (J') ranged from 0.22-0.94, reflecting variations in species distribution. Simpson's Dominance Index (S), which measures dominance by a few species, ranged from 0.15 to 0.80 in Ben Tre Province [74], indicating diverse community structures across sites. Moreover, the ATSPT, an index used to assess human impact, ranges from 39 to 48 and tends to increase in the downstream direction, indicating higher levels of disturbance [32, 33, 46, 47]. Zooplankton-based ABC method was also used. In addition, Tran (2020) likewise employed a zooplankton-based method to assess ecological status in shrimp ponds [75]; and Le et al. (2019) and Nguyen et al. (2020) looked at relationships between zooplankton diversity and physicochemical factors in the Hau River [70, 72].

The results show that zooplankton communities are sensitive indicators of nutrient enrichment, hydrological changes, and ecological stress. While some areas, such as Cambodia, still meet recommended abundance and diversity thresholds, high biodiversity indices (e.g., H' up to 4.23, D up to 5.27) in regions like the Hau River suggest relatively good ecological conditions. However, declining richness and abundance since 2013, particularly in Thailand, point to increasing stress and habitat degradation. Rising ATSPT values and low evenness (J' as low as 0.22) further reflect imbalanced communities, often associated with eutrophication and simplified habitats.

Macroinvertebrates

Macroinvertebrate communities are widely regarded as key indicators for the ERHA due to their sensitivity to water quality, habitat conditions, and flow alterations [25, 32]. Sampling methods varied depending on the habitat and target macroinvertebrate groups, with a focus on littoral and benthic communities. Littoral macroinvertebrates were typically sampled with D-frame nets (30×20 cm, 450-500 µm mesh) using 6-20 sweeps across various microhabitats [32, 33, 47, 77-80]. In shallow ponds, 1 mm mesh hand nets were used [81]. For benthic macroinvertebrates, Petersen grab samplers (0.025 m^2 sampling area) were used, with multiple grabs pooled into composite samples covering 0.1 m^2 [33, 72, 82]. In deeper waters, Ekman grab samplers (0.025 m^2 area, 0.5 mm mesh sieve) were applied [66, 83], while Petit Poinar grab samplers with 200×200 µm mesh sieves were used in the Mekong

Delta [78]. A multi-habitat approach within 500 m reaches ensured comprehensive coverage [79]. These methods enabled the collection of diverse taxa, including Crustacea, Mollusca, Ephemeroptera, Plecoptera, Diptera, and other aquatic insects, allowing for comprehensive assessments of ecosystem health.

Macroinvertebrate diversity across the Mekong River and its tributaries varies considerably by region, sampling method, and habitat type. The highest taxa richness was recorded by the MRC (2008), reporting 361 taxa of littoral macroinvertebrates and 177 taxa of benthic macroinvertebrates from 2004-2007 across multiple sites in the Lower Mekong River and its tributaries [32]. Similarly, Sor et al. (2017) identified 299 taxa of benthic macroinvertebrates in the Lower Mekong Basin [82], while Sripanya et al. (2023) recorded 241 taxa of aquatic insects in Laotian streams and rivers [80]. These comparisons suggest that certain local habitats (e.g., biodiverse streams in Laos) can harbor nearly as many taxa as the entire lower basin, though differences in taxonomic resolution (species vs family level) likely contribute. Overall, the Mekong mainstream and major tributaries consistently show high macroinvertebrate diversity, with the MRC (2008) survey serving as a benchmark for maximum taxa richness [32]. Among dominant taxa, Mollusca were prevalent in littoral macroinvertebrate communities, comprising 45% of species in the Mekong River [79] and 44% in benthic communities [82]. In contrast, Diptera dominated in the Nam Gnom Basin, accounting for 37.3% of species [83]. Insect taxa were particularly abundant in certain regions, with Ephemeroptera comprising 19.3% of species in Laotian streams and 18.6% in a subsequent study [84]. In summary, the Mekong's mainstream and delta sites are often mollusk-rich, while tributary streams (e.g., Nam Gnom) show insect (Diptera) dominance, reflecting habitat and flow differences.

The total number of macroinvertebrate individuals varied significantly across locations and sampling years. The highest recorded abundance was 81,186 individuals for littoral macroinvertebrates and 23,470 individuals for benthic macroinvertebrates in the Lower Mekong River from 2004 to 2007 [32]. Similarly, Suriyawong et al. (2018) reported 40,178 individuals of aquatic insects in northern Thailand [85], while Sirisinthuwanich et al. (2017) recorded 35,391 individuals of benthic macroinvertebrates in the Phong River, northeastern Thailand [79]. Density also varied greatly at each sampling site. The macroinvertebrate densities in the Mekong River varied from 1 to 1,251 individuals/m² in the littoral and from 202 to 3,353 individuals/m² in the benthic [78]. Seasonal and environmental f actors affect benthic macroinvertebrate density patterns, with estuarine waters having 30 to 120 individuals/m² [72] and the Lower Mekong River having 4 to 876 individuals/m² [32]. On the estuary delta, macroinvertebrate density is low, but upstream/ downstream freshwater areas of the Mekong have high densities (sometimes thousands per m²). This enormous gradient represents the river's extreme environmental variations from mouth to interior. Environmental variables and seasons affect Mekong macroinvertebrate distribution and density. Season and flow regime are important: monsoon floods and dam discharges scour ecosystems, reducing macroinvertebrate density. Due to habitat damage, Suriyawong et al. (2018) found that insect numbers declined during wet-season high flow, especially above dams. When flows dropped, their study's reference site had more insects [85].

Diversity indices revealed considerable variation in macroinvertebrate community structure across different sites. The Shannon-Wiener Diversity Index (H') ranged from 0.24 to 3.27 for littoral macroinvertebrates and 1.24 to 2.55 for benthic macroinvertebrates in the Lower Mekong River [42]. Comparatively, H' values for benthic macroinvertebrates in the Mekong River ranged from 1.3 to 3.2 [82], while those in mountain streams of northern Thailand varied between 0.31 and 2.69 [85]. The Lower Mekong River and its tributaries generally exhibit higher species diversity in some sections but also show substantial variability, reflecting fluctuating environmental conditions. In contrast, mountain streams in northern Thailand tend to have lower diversity indices, likely due to differences in hydrology, substrate composition, and higher natural disturbances, such as seasonal fluctuations in flow. The Berger-Parker Dominance Index (Do) ranged from 0.05 to 0.88 for littoral macroinvertebrates and 0.37 to 0.86 for benthic macroinvertebrates [42]. The greater variability in D values for littoral macroinvertebrates suggests strong differences in habitat quality and the potential impact of pollution across sites. In contrast, benthic communities generally exhibited higher dominance values, indicating that benthic habitats may be more sensitive to environmental pressures, leading to reduced species evenness. Similarly, Pielou's Evenness Index (J') varied from 0.17 to 0.94 in mountain streams of northern Thailand [85], highlighting regional differences in species distribution and dominance. Furthermore, the ATSPT of littoral macroinvertebrates ranged from 24 to 46, while values for benthic macroinvertebrates varied from 23 to 64, showing an increasing trend downstream that indicates greater anthropogenic pressure [32, 33].

Multimetric indices (MMIs) have been widely applied for biomonitoring. Rattanachan et al. (2016) developed a biological multimetric index (BMI) based on reference conditions, incorporating total taxa, Ephemeroptera-Plecoptera-Trichoptera (EPT) taxa, and tolerance metrics [86]. Sripanya et al. (2023) refined this approach by selecting 11 core metrics, including % Diptera, Beck's Biotic Index, and % Burrowers, for biomonitoring programs in Laos [80]. Several studies also explored functional feeding groups (FFGs) to assess ecosystem processes. Sor et al. (2017) found that collector-gatherers and filter-feeders dominated in undammed sites of the Lower Mekong Basin, while predators, shredders, and scrapers were less abundant

[82]. In urban environments, Sirisinthuwanich et al. (2017) observed that collectors (20.52%) and scavengers (15.84%) were the most dominant groups in the Phong River, Thailand [79]. Recent ecological surveys of the Lower Mekong Basin have found worrying trends in littoral and benthic macroinvertebrate communities [16, 87]. Littoral macroinvertebrate richness decreased in the 2017 biomonitoring study. Over 15% of monitoring sites had poor species diversity, and 20% failed MRC ecological standards. The ATSPT exceeded guideline levels at most sites from Laos to Vietnam, consistent with other biological indicators. Benthic macroinvertebrates dropped 25% over the previous monitoring period. Some sites in Thailand and Cambodia did not fulfill the MRC's average abundance and richness requirements; however, only 20% fell below the ATSPT criterion.

An integrated evaluation of diatom, zooplankton, and macroinvertebrate data revealed that 60% of sites were classified as having good ecological health (Class B). Approximately 7% achieved Class A status, indicating excellent conditions, all located in Cambodian tributaries. Conversely, about 5% of sites in Thailand were rated Class D, reflecting poor ecological quality per MRC guidelines. The remaining 28% were categorized as moderate (Class C). Overall, around 15% of all biological indicators fell below their respective ATSPT threshold values, predominantly in Cambodia, suggesting increasing ecological stress in the river system. Particularly, sites such as Tonle Sap Lake, the Mekong River in northern Thailand, and the Mekong Delta in Vietnam have shown rising ATSPT values.

In brief, macroinvertebrate-based ERHA in the MRB reflects moderate to declining ecological health. While species richness remains high in some areas with 361 littoral and 177 benthic taxa reported by the recent MRC survey, there is a noticeable decline in abundance and diversity, especially in downstream and urbanized regions. In 2017, benthic macroinvertebrate numbers dropped by 25%, with over 15% of sites showing poor diversity and 20% failing to meet MRC ecological standards. Elevated ATSPT values from Laos to Vietnam, along with uneven species dominance, indicate increasing environmental stress. Despite the use of effective biomonitoring tools (e.g., EPT taxa, Biotic Indices), continued standardized monitoring is crucial for sustaining and restoring river health.

Fish

Because they are sensitive to changes in the aquatic ecosystem and reflect the cumulative effects of several environmental stresses, fish communities are seen as critical indicators of river health [1]. One of the most biodiverse freshwater systems in the world, the Mekong River supports a great diversity of fish species, many of which are vital for local livelihoods [88]. The rich fish diversity of the Mekong indicates its ecological complexity and general health. Any loss in this variety acts as an early warning of ecological deterioration, indicating problems like habitat loss, pollution, or less river connection [89].

Fish diversity in the MRB is substantial, but species richness varies by study, location, and sampling method. Recent estimates put Lower Mekong Basin fish diversity at 1,148 species [15, 90, 91]. The MRC (2003) found 923 species [92], while Baran et al. (2012) found 781 [93]. Localized studies like Vu et al. (2020) found 176 species in the Hau River [94] and Pool et al. (2019) found 53 species in the Tonle Sap Great Lake [95]. Most differences in species richness come from habitat types, e.g., rivers, floodplains, lakes, sampling methods, and local environmental gradients. Geographical fish order and family dominance show different patterns. Many studies found cypriniformes to be the most numerous group, comprising 41.6% of total species in the Tonle Sap Great Lake [96], 52.6% of total species in the Upper Mekong [97, 98]. Perciformes dominated the Hau River with 28.8% of total species [94], while Cyprinidae dominated the Lower Mekong Basin. Fish abundance varies widely between studies due to habitat, fishing pressure, and seasonal dynamics. Noun et al. (2020) reported over 28 million individuals in the Lower Mekong [99], while Pool et al. (2019) recorded just 1,584 individuals in Tonle Sap [95]. Vu et al. (2020) found low catch rates in the Hau River, with CPUE ranging from 0.53 to 26.3 kg/ha/h [94]. Habitat deterioration, overfishing, and hydrological changes affect fish populations, as seen by these variations. Few studies have employed fish community analysis to assess ecological health and water quality, although many have examined species diversity and commercially important species.

For the fish community, Vu et al. (2020, 2021) found that the Shannon-Wiener Diversity Index (H') values were highest in the top portion (4.33), lowest in the middle (4.08), and slightly higher in the bottom half (4.10), indicating significant species variety across the river [94, 100]. The MRC's Fish Abundance and Diversity Monitoring Report (2018-2022) has begun to fill this gap by assessing fish abundance and diversity across the Lower Mekong Basin. The large floodplains in southern Lao PDR, Cambodia, and Viet Nam have the most species richness [16, 101]. A high Pielou's Evenness Index (J') (0.93) also indicated a reasonably even species distribution [16, 101]. However, 131 native fish species (15% of those assessed) are now listed as threatened, mostly in the middle and lower Mekong [101]. Fish assemblages and environmental gradients have been examined using cluster and ordination techniques. Noun et al. (2020) used SOM and hierarchical clustering to divide fish communities into four categories along an upstream-downstream gradient [99], supporting Chea et al. (2016) [102]. Pool et al. (2019) discovered that canopy coverage, water depth, water transparency, and pH affected Tonle Sap Great Lake fish assemblage composition [94]. However, in the MRB, few conclusions have been drawn regarding ecological classification based on fish assemblages, as most studies have primarily focused on fish diversity and community composition.

Natural and human activities both affect the distribution and quantity of fish species in the MRB. Many studies draw attention to how fish populations are affected by habitat alteration, climate change, and fishing pressure. Using species distribution models (SDMs), Noun et al. (2024) evaluated the possible consequences of population density and climate change on fish species distribution, hence highlighting notable changes in habitat suitability [103]. Pool et al. (2019) underlined, therefore, the importance of conservation initiatives to reduce mesohabitat-scale land alteration for timber cutting and agriculture since these activities endanger flood-pulse fish populations [95]. Changes in fish assemblages have also been connected to hydrological changes, including dam building. While Cyprinidae stayed dominant in the Mekong River, making up 50.8% of species, Phomikong et al. (2014) reported changes in population dynamics in places impacted by hydropower construction [104].

The results indicate a mixed ecological status, with high biodiversity alongside increasing environmental stress, especially in the middle and lower reaches. With approximately 1,148 recorded species, the Mekong River supports one of the world's most diverse freshwater fish communities. High diversity indices (H' up to 4.33, J' = 0.93) and species richness in floodplains of southern Lao PDR, Cambodia, and Vietnam reflect healthy fish assemblages in some areas. However, 15% of native species are now threatened, and declining catch rates in areas like the Hau River signal reduced productivity. Habitat degradation, overexploitation, and hydropower development have disrupted community structure and diminished habitat quality. Although fish are valuable indicators, the limited application of standardized fish-based indices has constrained their role in ERHA. Expanding monitoring programs and adopting unified assessment frameworks are crucial for maintaining and restoring the Mekong River's ecological health.

Comparison of Different Groups Used for Ecological River Health Assessment in the Mekong River Basin

The ERHA program in the MRB often uses benthic diatoms, zooplankton, littoral macroinvertebrates, and benthic macroinvertebrates to assess the aquatic ecosystem [16, 33, 47, 101]. The process of ERHA using different aquatic biological groups is summarized in Fig. 2. The steps include planning the field trip and collecting samples of the designated aquatic organism groups, incorporating both quantitative and qualitative specimens. In the laboratory, the samples were identified to determine species composition and density. These data were subsequently used for data analysis and the calculation of biological indices.

These biological categories have advantages and drawbacks in MRB river health assessments. Microalgae (e.g., benthic diatoms) are sensitive to water quality fluctuations and respond quickly to disturbances, but taxonomic challenges limit their application [29, 31, 32]. Areas generally lack the ability to identify algal taxa. Zooplankton, which indicate trophic status, are easily collected and distributed throughout the river, but their abundance varies with seasonal flow, making assessments difficult [67]. Littoral and Benthic macroinvertebrates are widely used bioindicators in streams due to their sensitivity to pollution and well-established multimetric indices, but inconsistent sampling methodologies, taxonomic resolution issues, and data gaps between studies hinder

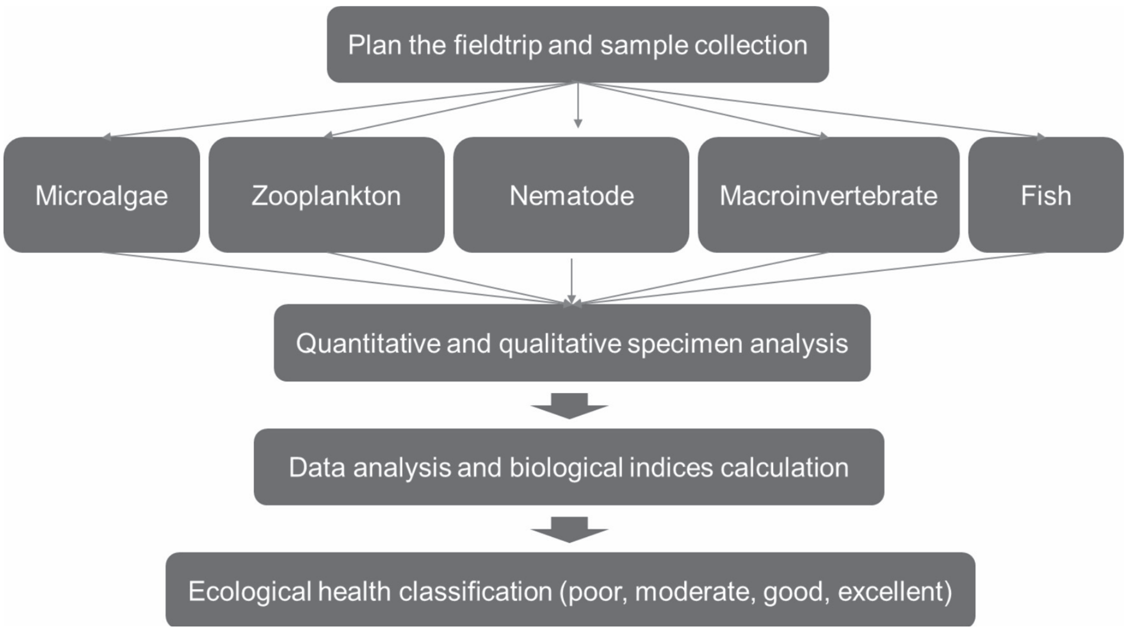

Fig. 2. The process of EHM using different aquatic biological groups in MRB.

comparability across sites and countries in the MRB [1]. Due to logistical and resource restrictions, fish are not routinely used in biomonitoring despite their ecological and socioeconomic relevance. Regular assessments are complicated by their migratory nature and representative sampling effort. Adding fish by electrofishing or eDNA would improve ERHA by collecting ecosystem changes. All of these features should be tested and evaluated throughout time to enhance, develop, and add to them. By integrating these bioindicator groups, a comprehensive assessment of river health in the MRB can be achieved. This approach is crucial for effective management and conservation strategies, mitigating environmental threats, and ensuring the long-term sustainability of the aquatic ecosystems of the MRB.

The MRB can be divided into three main sections: the upper, middle, and lower sections, each characterized by distinct geographical, ecological, and hydrological features. The upper section originates in the Tibetan Plateau, where the river flows through steep mountainous terrain in China, known as the Lancang River [15, 105, 106]. This part of the basin is dominated by high altitudes, rapid flow, and deep valleys, contributing significantly to the river's overall discharge. The middle section covers parts of northern Thailand, Laos, and Myanmar, where the river slows and meanders through a more complex landscape of hills and floodplains [20]. This section is crucial for sediment transport and plays a vital role in supporting biodiversity and agriculture through seasonal flood cycles. Finally, the lower section spans across Cambodia and Vietnam, including the famous Mekong Delta. Our results suggest that each biological group offers distinct advantages in ERHA within the MRB. Microalgae and zooplankton, being primarily planktonic organisms, rely on the water column for their life cycles, making them effective indicators of short-term water quality changes. In contrast, nematodes inhabit sediments and serve as reliable indicators of sediment conditions. On the other hand, macroinvertebrates, with their longer life cycles and bottom-dwelling nature, serve as reliable long-term indicators of habitat integrity, while fish reflect broader ecosystem health.

The ERHA of the MRB using different biological groups is summarized in Table 1 and Fig. 3. Ecosystem health classification data are concentrated in the middle and lower MRB due to stronger institutional cooperation, better accessibility, and centralized ecological monitoring. In contrast, the upper basin has limited assessment data, despite numerous studies and widespread local-language publications. Additionally, ecological health assessments based on nematode communities are available exclusively for the lower section, with no corresponding data for the middle or upper sections (Table S1). Furthermore, there are no ecological health classifications available based on fish communities across any section of the MRB. This highlights significant data gaps, particularly

Table 1. Comparative summary of bioindicator groups and biomonitoring methods in the Mekong River Basin.

Bioindicator groups	Key roles in the ecosystem	Sensitivity to environmental changes	Common biomonitoring methods	Ecological health classification	Key references
Microalgae (Phytoplankton, Periphyton, Diatoms)	Primary producers; form the base of the aquatic food web	Sensitive to nutrient levels, pollution, and hydrological changes	Taxonomic diversity, community composition, multivariate analyses; Saprobic Index, IBI	Laos: Moderate to Good; Thailand, Cambodia, and Vietnam: Moderate to Good	[32, 33, 35, 36, 39, 40, 47]
Nematodes	Sediment-dwelling organisms involved in nutrient cycling	Respond rapidly to pollution, sediment quality, and organic enrichment	Diversity indices (H', J', D); FFG; ABC	Vietnam: Poor to Good	[60, 62, 63, 64, 65, 66]
Zooplankton	Link between primary producers (algae) and higher trophic levels (fish)	Sensitive to nutrient levels, water quality changes, and flow regime alterations	Biotic indices (IBI, ATSPT); diversity indices (D, H')	Laos: Moderate to Good; Thailand, Cambodia, and Vietnam: Moderate to Good	[32, 33, 47, 67, 69, 70, 72, 73, 74]
Macroinvertebrates	Key indicators of water quality; important in detrital processing and food webs	Highly sensitive to pollution, habitat degradation, and hydrological changes	Biotic indices (BMWP, EPT Index, ASPT); FFG; MMIs	Laos, Thailand, Cambodia: Moderate to Good; Vietnam: Poor to Good	[32, 33, 47, 72, 77, 79, 80, 82, 85, 86]
Fish	Indicators of habitat quality, ecosystem connectivity, and long-term environmental changes	Affected by habitat fragmentation, pollution, climate change, and overfishing	CPUE; cluster analysis; species distribution models (SDMs)		[94, 96, 98, 99]

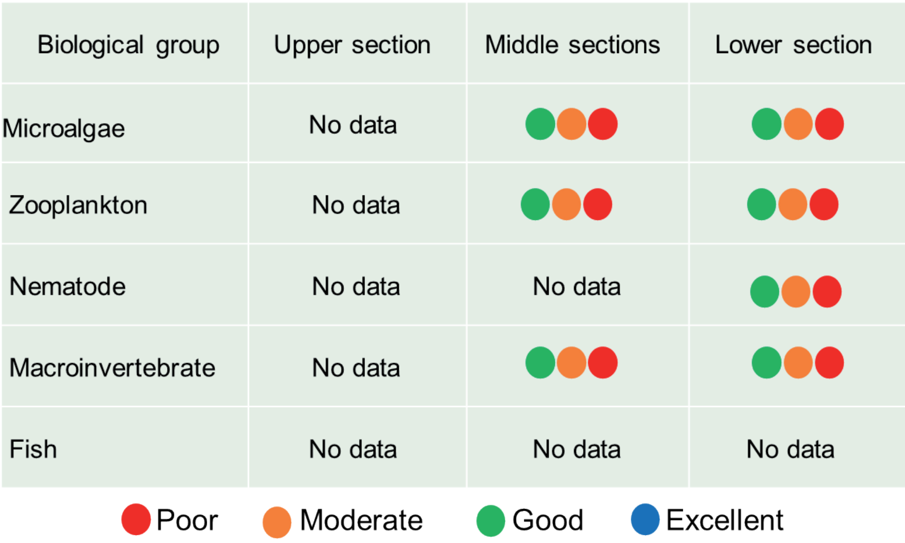

Fig. 3. The ecological health assessment using different biological groups for the MRB. The blue circle indicates excellent, the green circle indicates good or high, the orange circle indicates moderate or medium, and the red circle indicates poor levels.

in the upper reaches of the basin and for certain biological indicators, underscoring the need for more comprehensive and regionally inclusive monitoring efforts.

Challenges and Perspectives

Challenges

There are still several obstacles to overcome to ensure the accuracy, consistency, and applicability of these methodologies, despite the tremendous progress made in utilizing bioindicators to evaluate ecological health in the MRB. Variability in sampling methodologies, such as changes in plankton net mesh sizes or sediment coring depths for nematodes, may have an impact on estimates of species richness and may make it more difficult to compare data [32, 33, 36]. The ability to identify long-term ecological patterns and environmental stressors is further hindered by inconsistencies in sample frequency and spatial coverage, as stated by Parmar et al. (2016) [26]. Because of the extraordinary variety of the MRB, taxonomic identification is made even more difficult. This is especially true for microorganisms such as diatoms and nematodes, which require specialized knowledge [34]. In spite of the fact that functional techniques offer useful ecological insights, standardized frameworks are required in order to guarantee uniformity in the evaluation of ecosystem processes [66, 79].

In addition, considerable problems are posed by anthropogenic activities and emissions, which include hydrological changes and pollution. The construction of dams causes disruptions in the communities of fish and zooplankton, which in turn leads to changes in the species composition and a reduction in the connectivity between habitats [99, 104]. Pollution, which includes nutrient enrichment and the discharge of industrial waste, can have a variety of impacts on macroinvertebrates and algae, which makes it more difficult to understand the results of biomonitoring [44, 76]. Moreover, established biomonitoring indicators have deficiencies in their capabilities. Although traditional biotic indices provide valuable insights, they may not fully capture ecosystem changes or serve as early warning indicators in the Mekong River and its tributaries [42, 46], as noted by Vu et al. (2020) [98]. Similarly, indices such as the ATSPT, BMWP, and Saprobic Index require refinement to better reflect the ecological conditions of the MRB. This includes accounting for regional taxa adaptations, the basin's diverse hydrology, and the impacts of human activities. Adapting these indices to local ecological contexts will improve their reliability and relevance for river health assessment in the MRB [27].

Future Perspectives

A comparative summary of the challenges and potential future solutions for ERHA in the MRB is presented in Table 2. The integration of modern approaches, the expansion of long-term monitoring activities, and the reinforcement of conservation policies should be the primary focuses of future research in order to improve ERHA in the MRB. It is possible to increase the comparability of different research by developing a uniform framework for sampling designs, taxonomic resolution, and data reporting [33, 47]. In addition, the establishment of a consolidated database for aquatic biodiversity in the MRB, which will include both historical and contemporary records, would make it easier to conduct ecological assessments on a wide scale. The utilization of molecular methods, such as DNA metabarcoding and environmental DNA (eDNA), has the potential to improve the identification

Table 2. Comparative summary of challenges and future solutions.

Bioindicator group	Key challenges	Future solutions	Key references
Algae	- High variability in sampling techniques (e.g., mesh size, collection methods) affecting species richness estimates. - Difficulties in taxonomic identification, particularly for diatoms. - Sensitivity to pollution complicates the interpretation of bioassessment results.	- Standardized sampling protocols for consistency in data collection. - Integration of DNA metabarcoding and eDNA for improved species detection. - Functional gene analysis to assess ecological processes beyond traditional diversity indices.	[32, 33, 35, 47]
Nematodes	- Inconsistent sampling depths affecting species diversity estimates. - Taxonomic challenges due to the high diversity of nematode genera. - Limited use of functional approaches in bioassessments.	- Adoption of trait-based approaches and trophic diversity indices for functional assessments. - Expansion of molecular identification techniques to improve taxonomic resolution. - Long-term monitoring to analyze spatial and seasonal patterns.	[56, 60, 63, 64, 65]
Zooplankton	- Impact of hydrological alterations (dams, water regulation) on species composition. - Seasonal variability affecting data consistency. - Difficulty in integrating zooplankton-based indices into ERHA.	- MMIs incorporating zooplankton functional diversity. - Long-term monitoring programs to track climate and hydrological impacts. - SDMs to predict changes in zooplankton communities.	[32, 33, 47, 70]
Macroinvertebrates	- Pollution and habitat degradation reducing biodiversity and altering community structure. - BMWP and EPT may not fully capture functional changes. - Limited data on FFGs in the Mekong region.	- Refinement of biotic indices to account for regional species adaptations. - FFGs assessments for improved ecological evaluations. - Participatory biomonitoring involving local communities.	[32, 33, 80, 82, 86]
Fish	- Declining fish populations due to overfishing, pollution, and dam construction. - Lack of basin-wide conservation strategies for transboundary fish migration. - CPUE may not fully reflect ecosystem changes.	- SDMs to predict shifts in fish populations under climate change. - Transboundary conservation initiatives to mitigate hydropower impacts. - Use of SOM and cluster analysis for fish community assessments.	[16, 94, 98, 103, 104]

of species, particularly for microorganisms and rare species, hence enhancing the accuracy of taxonomic classifications in biomonitoring [107]. According to Losi et al. 2021, functional gene analysis, when combined with community structure assessments, has the potential to offer more profound insights into the processes that occur within ecosystems, particularly with regard to microbial bioindicators such as diatoms and nematodes [60, 80]. Increasing the use of trait-based and functional diversity measurements would further increase assessments of ecosystem resilience and responses to environmental stresses. This will be accomplished by expanding the use of these metrics.

The improvement of ecological evaluations can be achieved by the refinement of multimetric indices (MMIs) through the incorporation of additional ecological indicators. These indicators include trophic diversity indices for nematodes [66] and functional feeding group assessments for macroinvertebrates [82]. According to Noun et al. (2024), SDMs should be deployed in order to forecast changes in fish and macroinvertebrate communities under a variety of climatic and land-use scenarios [103]. This would allow for proactive conservation strategies to be effectively implemented. For the purpose of determining the cumulative effects of hydrological changes, pollution, and habitat fragmentation, long-term ecological monitoring programs should be implemented, particularly in susceptible locations such as the Tonle Sap Great Lake and the Mekong Delta [78, 95, 108]. Participatory biomonitoring, in which local communities are involved, improves data collection, raises awareness about the need for conservation, and incorporates traditional ecological knowledge into scientific evaluations [24]. Finally, it is essential to increase transboundary collaboration among the six nations that share the Mekong River Basin: China, Myanmar, Laos, Thailand, Cambodia, and Vietnam. This is necessary to implement basin-wide conservation plans and reduce the ecological impacts of large-scale development projects [109].

Conclusions

The use of bioindicators is essential for effective freshwater ecosystem management. In the MRB, the interaction between biodiversity, local economies, and infrastructure development creates a complex management landscape. This paper reviews key bioindicator groups used in ERHA, highlighting their advantages and limitations. Microalgae are highly sensitive to water quality changes but require specialized taxonomic expertise. Nematodes reflect sediment conditions but are often overlooked due to limited data and identification challenges. Zooplankton are useful for assessing trophic status but show strong seasonal variability, complicating interpretation. Macroinvertebrates are widely used and sensitive to pollution, though inconsistent sampling hinders cross-study comparisons. Fish provide valuable long-term insights into ecosystem health, but are influenced by migration and fishing pressure.

Inadequate cross-border cooperation and ongoing methodological differences are impeding current efforts to monitor the situation. The most efficient way to achieve ERHA in the MRB is to use a multimetric approach, a consistent protocol, functional metrics that combine techniques, and a combination of multiple indicator groups. It will be helpful to expand genetic reference databases and functional trait analyses to support more thorough assessments. Coordination of basin-wide monitoring is essential given the socioeconomic importance of the Mekong and its abundant biodiversity. Investing in taxonomic capacity, data harmonization, and community-based monitoring will ensure sustainable river management and strengthen regional conservation strategies throughout the MRB.

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Conflict of Interest

The authors declare no conflict of interest.

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Supplementary Material

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