

Benthic Macroinvertebrates as Indicators of Ecological Integrity in Aquatic Ecosystems

Macroinvertebrados Bentônicos como Indicadores da Integridade Ecológica em Ecossistemas Aquáticos

Macroinvertebrados Bentónicos como Indicadores de la Integridad Ecológica en Ecosistemas Acuáticos

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Abstract: This review article aimed to synthesize the scientific knowledge on the use of benthic macroinvertebrates as indicators of ecological integrity in aquatic ecosystems, highlighting their theoretical foundations, assessment methodologies, and responses to anthropogenic environmental pressures. A systematic literature review was conducted using national and international databases, including Web of Science, Scopus, Google Scholar, and SciELO. Studies employing benthic macroinvertebrates to assess the environmental quality of aquatic ecosystems were selected based on their objectives, methodologies, findings, and limitations. The results demonstrate that these organisms are highly effective bioindicators due to their high sensitivity to environmental changes, relatively long life cycles, and limited mobility, allowing them to reflect the cumulative impacts affecting aquatic ecosystems. The most commonly applied methodologies include biotic indices, multimetric indices, and multivariate analyses, which are capable of detecting changes caused by organic pollution, habitat degradation, chemical contamination, and climate change. Advances related to taxonomic sufficiency, the application of molecular techniques, and the adaptation of assessment protocols for Neotropical regions, characterized by high biodiversity and hydrological complexity, were also identified. It is concluded that benthic macroinvertebrates remain essential for biomonitoring programs, supporting conservation initiatives, environmental management, and the development of public policies aimed at protecting and restoring aquatic ecosystems.

Palavras-chave: Benthic Macroinvertebrates; Bioindicators; Biomonitoring; Ecological Integrity; Aquatic Ecosystems.

Resumo: Este artigo de revisão teve como objetivo sintetizar o conhecimento científico sobre a utilização de macroinvertebrados bentônicos como indicadores da integridade ecológica em ecossistemas aquáticos, destacando seus fundamentos teóricos, metodologias de avaliação e respostas às pressões ambientais de origem antrópica. Para isso, foi realizada uma revisão sistemática da literatura em bases de dados nacionais e internacionais, incluindo Web of Science, Scopus, Google Scholar e SciELO. Foram selecionados estudos que empregaram macroinvertebrados bentônicos na avaliação da qualidade ambiental de ambientes aquáticos, considerando objetivos, métodos, resultados e limitações. Os achados demonstram que esses organismos constituem bioindicadores altamente eficientes devido à elevada sensibilidade às alterações ambientais, ao ciclo de vida relativamente longo e à baixa mobilidade, refletindo de forma integrada os impactos acumulados nos ecossistemas. As metodologias mais utilizadas envolvem índices bióticos, índices multimétricos e análises multivariadas, capazes de detectar alterações decorrentes da poluição orgânica, degradação do habitat, contaminação química e mudanças climáticas. Também foram identificados avanços relacionados à suficiência taxonômica, ao uso de técnicas moleculares e à adaptação de protocolos para regiões Neotropicais, caracterizadas por elevada biodiversidade e complexidade hidrológica. Conclui-se que os macroinvertebrados bentônicos

permanecem essenciais para programas de biomonitoramento, subsidiando ações de conservação, manejo ambiental e formulação de políticas públicas voltadas à proteção e restauração dos ecossistemas aquáticos.

Keywords: Macroinvertebrados Bentônicos; Bioindicadores; Biomonitoramento; Integridade Ecológica; Ecossistemas Aquáticos.

Resumen: Este artículo de revisión tuvo como objetivo sintetizar el conocimiento científico sobre el uso de macroinvertebrados bentónicos como indicadores de la integridad ecológica en los ecosistemas acuáticos, destacando sus fundamentos teóricos, metodologías de evaluación y respuestas a las presiones ambientales de origen antropogénico. Para ello, se realizó una revisión sistemática de la literatura en bases de datos nacionales e internacionales, incluyendo Web of Science, Scopus, Google Scholar y SciELO. Se seleccionaron estudios que utilizaron macroinvertebrados bentónicos para evaluar la calidad ambiental de los ecosistemas acuáticos, considerando sus objetivos, metodologías, resultados y limitaciones. Los hallazgos demuestran que estos organismos constituyen bioindicadores altamente eficaces debido a su elevada sensibilidad a las alteraciones ambientales, su ciclo de vida relativamente largo y su limitada movilidad, reflejando de manera integrada los impactos acumulados en los ecosistemas. Las metodologías más utilizadas incluyen índices bióticos, índices multimétricos y análisis multivariados, capaces de detectar cambios derivados de la contaminación orgánica, la degradación del hábitat, la contaminación química y el cambio climático. También se identificaron avances relacionados con la suficiencia taxonómica, el uso de técnicas moleculares y la adaptación de protocolos para las regiones neotropicales, caracterizadas por una elevada biodiversidad y complejidad hidrológica. Se concluye que los macroinvertebrados bentónicos siguen siendo herramientas esenciales para los programas de biomonitoreo, apoyando acciones de conservación, gestión ambiental y la formulación de políticas públicas dirigidas a la protección y restauración de los ecosistemas acuáticos.

Palabras clave: Macroinvertebrados Bentônicos; Bioindicadores; Biomonitoreo; Integridad Ecológica; Ecossistemas Acuáticos.

1. INTRODUCTION

Aquatic ecosystems are fundamental components of the global environment, providing essential ecosystem services such as water purification, nutrient cycling, and habitat for diverse flora and fauna (Costanza *et al.*, 1997). However, these ecosystems are increasingly threatened by anthropogenic activities, including pollution, habitat destruction, and climate change (Darwall *et al.*, 2008). Therefore, assessing and monitoring the ecological integrity of aquatic ecosystems is essential for supporting effective conservation and environmental management strategies. Among the available biological indicators, benthic macroinvertebrates have emerged as powerful tools for this purpose due to their widespread distribution, diverse responses to environmental stressors, and relatively sedentary nature (Rosenberg; Resh, 1992).

Benthic macroinvertebrates are organisms visible to the naked eye (typically >0.5 mm) that inhabit the bottom substrates of aquatic environments, such as rivers, lakes, and wetlands (Barbour *et al.*, 1996). This taxonomically diverse group includes aquatic insects (e.g., Ephemeroptera, Plecoptera, and Trichoptera), crustaceans, mollusks, and worms, each exhibiting specific tolerances and sensitivities to various environmental conditions (Merritt; Cummins; Berg, 1996). Their life cycles often span several months to years, allowing them to reflect cumulative impacts of pollution and habitat degradation rather than transient environmental events (Hynes, 2001). Consequently, changes in macroinvertebrate community

structure, composition, richness, and abundance provide reliable insights into the long-term ecological condition of aquatic ecosystems (Palmer *et al.*, 2000).

The application of benthic macroinvertebrates as bioindicators dates back to the early twentieth century, with significant advances in methodologies and applications over the past decades (Kolkwitz; Marsson, 1908). Early approaches focused primarily on species presence or absence and diversity indices, whereas more recent developments have incorporated multimetric indices (MMIs) and advanced statistical techniques (Metcalf, 1989; Rosenberg; Resh, 1992). MMIs integrate multiple biological metrics, including taxa richness, community composition, and pollution tolerance, into a single score that reflects the overall ecological condition of a waterbody (Barbour *et al.*, 1996). These indices have demonstrated high effectiveness across different geographic regions, providing comprehensive and scientifically robust assessments of aquatic ecosystem health (Voulvoulis; Arpon; Giakoumis, 2017).

Despite their widespread application, the use of benthic macroinvertebrates as bioindicators still presents important methodological and ecological challenges. Factors such as taxonomic resolution, sampling effort, habitat heterogeneity, and regional biogeographic differences may influence the accuracy, consistency, and comparability of ecological assessments (Chessman, 2003). Furthermore, the increasing complexity of environmental stressors, including emerging contaminants and climate change, requires the continuous refinement of biomonitoring approaches (Durance; Ormerod, 2007). Understanding the ecological responses of different macroinvertebrate taxa to these stressors is fundamental for developing robust and reliable bioassessment programs capable of supporting environmental monitoring and management (Giller; Malmqvist, 1998).

This review aims to synthesize the current knowledge on the use of benthic macroinvertebrates as indicators of ecological integrity in aquatic ecosystems. Specifically, it discusses the theoretical basis supporting their application, reviews the principal methodologies and biological indices employed in biomonitoring, and examines the influence of anthropogenic impacts on macroinvertebrate communities. Particular attention is given to recent developments in bioindication, including taxonomic sufficiency and the challenges and opportunities associated with Neotropical regions. By providing a comprehensive synthesis of the available literature, this review seeks to contribute to the advancement of biomonitoring methodologies and to support conservation policies and environmental management strategies for aquatic ecosystems worldwide.

2. MATERIAL AND METHODS

This review was conducted following a systematic literature review approach to identify, select, and synthesize scientific publications addressing the use of benthic macroinvertebrates as indicators of ecological integrity in aquatic ecosystems. The review aimed to provide a comprehensive overview of the theoretical foundations, methodological approaches, ecological applications, and current challenges associated with the use of these organisms in biomonitoring programs.

2.1. Search Strategy

The literature search was performed using the scientific databases Web of Science, Scopus, Google Scholar, and SciELO. Search terms were selected to encompass the principal concepts related to aquatic biomonitoring and benthic macroinvertebrates. The following keywords, combined using the Boolean operators AND and OR, were employed: “benthic macroinvertebrates,” “bioindicators,” “ecological integrity,” “aquatic ecosystems,” “biomonitoring,” “multimetric indices,” “anthropogenic impacts,” “pollution,” “habitat degradation,” “climate change,” “taxonomic sufficiency,”* and * “Neotropical regions”. The search included classical and contemporary publications relevant to the subject, prioritizing studies that have significantly contributed to the development of aquatic ecological assessment methodologies and biomonitoring practices.

2.2. Inclusion and Exclusion Criteria

Publications were selected according to their relevance to the objectives of this review. The inclusion criteria comprised: (i) original research articles, review articles, books, and book chapters; (ii) studies investigating the application of benthic macroinvertebrates as indicators of ecological integrity or water quality; (iii) publications addressing biomonitoring methodologies, biological indices, ecological responses to environmental disturbances, or anthropogenic impacts on aquatic ecosystems; and (iv) articles published in English or Portuguese.

The exclusion criteria comprised: (i) publications unrelated to the ecological application of benthic macroinvertebrates; (ii) studies focused exclusively on taxonomy, species descriptions, or geographic distribution without ecological assessment; (iii) conference abstracts, dissertations, theses, and technical reports that had not undergone peer review, unless they provided essential information unavailable in the scientific literature; and (iv)

duplicate publications.

2.3. Data Extraction and Synthesis

The selected publications were analyzed in detail, and information regarding study objectives, methodological approaches, biological indices, sampling procedures, principal findings, and identified limitations was extracted. The information was subsequently organized through thematic synthesis, encompassing the theoretical foundations of biomonitoring, methodological developments, anthropogenic impacts on aquatic ecosystems, and applications in different environmental contexts, with particular emphasis on Neotropical regions. The collected evidence was interpreted comparatively to identify common patterns, methodological advances, current challenges, and knowledge gaps, thereby providing an integrated overview of the role of benthic macroinvertebrates as indicators of ecological integrity in aquatic ecosystems (Braga; Braga, 2026).

3. THEORETICAL FOUNDATION

Aquatic ecosystems represent some of the most dynamic and biologically productive environments on Earth, playing a fundamental role in maintaining ecological balance and supporting human well-being. Rivers, streams, lakes, wetlands, and reservoirs provide essential ecosystem services, including water purification, nutrient cycling, flood regulation, carbon sequestration, and habitat for an immense diversity of organisms (Costanza *et al.*, 1997). The ecological integrity of these environments depends on the maintenance of their physical, chemical, and biological characteristics, allowing natural ecological processes to occur while sustaining biodiversity and ecosystem functionality. However, increasing anthropogenic pressures have altered these systems worldwide, emphasizing the need for reliable methods capable of evaluating environmental quality and supporting conservation and restoration initiatives (Allan, 2004; Allan; Castillo, 2007).

Ecological integrity refers to the capacity of an ecosystem to maintain its structure, composition, functions, and resilience under natural environmental conditions while preserving its biological diversity and ecological processes. Unlike traditional water quality assessments based exclusively on physicochemical parameters, ecological integrity incorporates biological communities as direct indicators of ecosystem condition because living organisms integrate environmental disturbances over time (Rosenberg; Resh, 1992). Consequently, biological assessments have become essential components of freshwater

monitoring programs, providing a broader understanding of environmental degradation than isolated chemical measurements.

Among the biological communities used for environmental assessment, benthic macroinvertebrates have gained particular importance because of their ecological characteristics and their close relationship with aquatic habitats. These organisms inhabit the bottom substrates of freshwater ecosystems, where they occupy a wide variety of ecological niches and participate in numerous ecosystem processes (Barbour *et al.*, 1996). Their communities include aquatic insects, crustaceans, mollusks, annelids, and other invertebrates that differ considerably in their ecological requirements and tolerance to environmental disturbances (Merritt; Cummins; Berg, 1996). Such diversity enables researchers to evaluate ecological conditions through changes in species composition, abundance, richness, and functional organization.

The ecological importance of benthic macroinvertebrates extends beyond their role as biological indicators. These organisms actively participate in nutrient recycling, decomposition of organic matter, sediment stabilization, and energy transfer throughout aquatic food webs (Cummins; Klug, 1979; Wallace; Webster, 1996). Functional feeding groups, including shredders, collectors, scrapers, filter feeders, and predators, contribute differently to ecosystem functioning by processing organic materials and regulating biological interactions. Consequently, alterations in macroinvertebrate communities frequently reflect disruptions in ecosystem processes, providing valuable ecological information that cannot be obtained solely through physicochemical analyses.

Another characteristic that makes benthic macroinvertebrates particularly suitable for biomonitoring is their relatively limited mobility. Unlike fish, which may migrate considerable distances, most benthic macroinvertebrates remain associated with specific substrates during most stages of their life cycle (Hynes, 2001). Their life cycles often extend over several months or even years, allowing these organisms to integrate environmental conditions over extended periods. As a result, they reflect cumulative environmental impacts rather than temporary fluctuations caused by short-term disturbances, making them reliable indicators of long-term ecosystem health (Palmer *et al.*, 2000).

The application of benthic macroinvertebrates as indicators of environmental quality has evolved considerably over the last century. The earliest biological assessments were based on the saprobic system proposed by Kolkwitz and Marsson (1908), who demonstrated that aquatic organisms respond predictably to gradients of organic pollution. This pioneering work

established the conceptual foundation for biological water quality assessment by relating species occurrence to environmental conditions. Since then, numerous biological indices have been developed to improve the evaluation of aquatic ecosystems, incorporating different ecological attributes and methodological approaches (Metcalf, 1989).

Among the most widely applied biological assessment tools are biotic indices based on pollution tolerance. The Hilsenhoff Biotic Index (Hilsenhoff, 1987) and the Biotic Index proposed by Lenat (1993) represent important advances in freshwater biomonitoring because they assign tolerance values to different macroinvertebrate taxa according to their sensitivity to organic pollution. These indices allow the calculation of numerical scores that facilitate comparisons among sampling sites and provide objective measures of environmental quality. Although initially developed for specific geographic regions, these approaches have inspired numerous adaptations worldwide.

More recently, multimetric indices (MMIs) have become one of the most comprehensive approaches for evaluating ecological integrity. Instead of relying on a single ecological attribute, MMIs combine several complementary biological metrics, including taxa richness, diversity, abundance, functional composition, and pollution tolerance, into a unified assessment framework (Barbour *et al.*, 1996). This integrated approach increases the sensitivity of ecological assessments by considering multiple dimensions of community organization simultaneously. The application of MMIs has demonstrated high effectiveness across different environmental conditions and geographic regions, contributing significantly to freshwater biomonitoring programs (Voulvoulis; Arpon; Giakoumis, 2017).

Taxonomic resolution also represents an important consideration in biomonitoring studies. Identifying organisms to species level generally provides greater ecological precision; however, this approach demands specialized taxonomic expertise and considerable financial resources. Consequently, several investigations have evaluated whether higher taxonomic levels, such as family or genus, provide sufficient ecological information for environmental assessments. Bowman and Bailey (1997) demonstrated that reduced taxonomic resolution may still preserve meaningful ecological patterns under certain environmental conditions, while Chessman (2003) proposed sensitivity classifications that facilitate biological assessments even when species-level identification is unavailable. These findings support the concept of taxonomic sufficiency, which seeks to balance scientific accuracy with practical feasibility in routine monitoring programs.

The effectiveness of benthic macroinvertebrates as bioindicators is strongly associated

with their differential responses to anthropogenic disturbances. Organic pollution, excessive nutrient enrichment, habitat degradation, sedimentation, chemical contamination, hydrological alteration, and climate change all produce measurable changes in community composition (Smith; Tilman; Nekola, 1999; Wood; Armitage, 1997). Sensitive taxa, particularly representatives of the Ephemeroptera, Plecoptera, and Trichoptera (EPT) orders, typically decline under degraded environmental conditions, whereas pollution-tolerant organisms often become dominant. Such predictable ecological responses enable researchers to diagnose environmental degradation while identifying the principal stressors affecting aquatic ecosystems.

Human activities influence freshwater ecosystems through multiple interacting stressors rather than isolated disturbances. Land-use changes, urbanization, agricultural expansion, industrial development, and river regulation modify hydrological processes, habitat complexity, and water quality simultaneously (Allan, 2004; Allan; Castillo, 2007). Crain, Kroeker and Halpern (2008) emphasized that cumulative interactions among stressors frequently produce ecological responses that are more complex than the effects of individual disturbances alone. Therefore, comprehensive biomonitoring programs should consider multiple environmental variables to accurately evaluate ecological integrity and support effective environmental management.

4. RESULTS

The effectiveness of benthic macroinvertebrates as bioindicators stems from several key ecological principles. Firstly, their position in the food web, often as primary consumers or detritivores, makes them sensitive to changes in lower trophic levels and nutrient availability (Wallace; Webster, 1996). Secondly, their relatively long life cycles (from months to several years) mean that their community structure reflects integrated environmental conditions over time, rather than transient fluctuations (Allan; Castillo, 2007).

Thirdly, their limited mobility, especially for many larval forms, ensures that they are exposed to local environmental conditions, making them excellent indicators of site-specific impacts (Giller; Malmqvist, 1998). Finally, the high diversity of macroinvertebrate taxa, each with specific physiological tolerances to physical and chemical stressors, allows for a nuanced assessment of water quality and habitat integrity (Rosenberg; Resh, 1992). For instance, taxa like Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies), collectively known as EPT, are generally sensitive to pollution and are often used as indicators

of good water quality, while oligochaetes and chironomids are more tolerant and tend to dominate in degraded environments (Lenat, 1993).

Over the decades, various methodological approaches and indices have been developed to utilize benthic macroinvertebrates for biomonitoring. These range from simple biotic indices to complex multimetric indices and multivariate statistical analyses. Early methods, such as the Saprobien System, focused on the presence or absence of indicator species to classify water quality (Kolkwitz; Marsson, 1908). While foundational, these systems often lacked the resolution to detect subtle changes or differentiate between multiple stressors.

Biotic indices assign tolerance scores to different macroinvertebrate taxa based on their sensitivity to organic pollution. Examples include the Trent Biotic Index (TBI) and the Biological Monitoring Working Party (BMWP) score system, widely used in Europe (Metcalf, 1989). In North America, the Hilsenhoff Biotic Index (HBI) is commonly employed, assigning values from 0 (very intolerant) to 10 (very tolerant) to individual taxa (Hilsenhoff, 1987). These indices provide a single numerical score that reflects the overall organic pollution level of a waterbody. While effective for detecting organic enrichment, biotic indices may not fully capture other forms of degradation, such as habitat alteration or chemical contamination.

Diversity indices, such as the Shannon-Wiener index and Simpson's index, quantify the richness and evenness of macroinvertebrate communities. High diversity is generally associated with healthy ecosystems, while reduced diversity can indicate environmental stress. However, diversity indices alone can be misleading, as some forms of pollution might increase the abundance of a few tolerant species, leading to seemingly high diversity despite ecological degradation. Therefore, they are often used in conjunction with other metrics.

Multimetric indices represent a significant advancement in biomonitoring, integrating multiple biological attributes into a single, comprehensive score. MMIs typically combine metrics related to: (i) Taxa Richness (e.g., total number of taxa, EPT richness); (ii) Community Composition (e.g., percentage of EPT individuals, percentage of tolerant individuals); (iii) Tolerance/Intolerance (e.g., HBI, average tolerance value); (iv) Trophic Structure (e.g., percentage of shredders, scrapers); and (v) Functional Feeding Groups (FFGs) (Barbour *et al.*, 1996; Palmer *et al.*, 2000). The development of an MMI involves selecting a suite of metrics that respond predictably to environmental degradation, standardizing them, and combining them into a final index score. The Index of Biotic Integrity (IBI), originally developed for fish communities, has been adapted for macroinvertebrates (B-IBI) and is widely used globally (Palmer *et al.*, 2000; Voulvoulis; Arpon; Giakoumis, 2017). MMIs are particularly robust

because they capture different facets of ecological health and are less susceptible to the limitations of individual metrics.

Advanced statistical techniques, such as Principal Component Analysis (PCA), Correspondence Analysis (CA), and Non-metric Multidimensional Scaling (NMDS), are increasingly used to analyze complex macroinvertebrate data sets. These methods help in identifying patterns in community structure, correlating them with environmental variables, and distinguishing between natural variability and anthropogenic impacts. They are particularly useful for exploring relationships between multiple stressors and biological responses, providing a more nuanced understanding of ecosystem dynamics.

Benthic macroinvertebrate communities are highly susceptible to a wide range of anthropogenic disturbances, which can alter their structure, composition, and functional roles within aquatic ecosystems. Understanding these impacts is crucial for effective environmental management and restoration efforts. Organic pollution, often originating from untreated sewage, agricultural runoff, and industrial discharges, leads to increased nutrient loads (eutrophication) and reduced dissolved oxygen levels in aquatic environments (Smith; Tilman; Nekola, 1999). Macroinvertebrate communities respond predictably to these changes. In severely polluted conditions, sensitive taxa (e.g., EPT) are replaced by tolerant species, such as chironomids and oligochaetes, which can thrive in low-oxygen environments (Lenat, 1993). This shift results in a decrease in species richness and diversity, and an alteration in functional feeding groups, with a dominance of collectors and filter-feeders that can utilize abundant organic matter (Cummins; Klug, 1979).

Physical alterations to aquatic habitats, such as channelization, dam construction, deforestation of riparian zones, and sediment deposition, significantly impact macroinvertebrate communities. Channelization reduces habitat complexity, leading to a loss of diverse microhabitats (e.g., riffles, pools, snags) that support a variety of macroinvertebrate taxa. Dams alter flow regimes, temperature, and sediment transport, creating conditions unfavorable for many lotic species (Poff *et al.*, 2007). Deforestation increases light penetration and water temperature, and enhances sediment and nutrient runoff, negatively affecting sensitive taxa that require stable, shaded, and clean substrates (Naiman; Décamps; McClain, 2006). Increased fine sediment deposition can smother benthic habitats, reducing interstitial spaces and food resources, thereby impacting species that rely on these spaces for shelter and feeding (Wood; Armitage, 1997).

Aquatic ecosystems are exposed to a myriad of chemical contaminants, including heavy

metals, pesticides, pharmaceuticals, and industrial chemicals. These substances can have direct toxic effects on macroinvertebrates, leading to mortality, reduced growth, impaired reproduction, and behavioral changes (Malaj *et al.*, 2014). Different taxa exhibit varying sensitivities to specific chemicals, making macroinvertebrates valuable indicators of chemical pollution. For example, some mayfly species are highly sensitive to heavy metals, while others may tolerate certain levels. The cumulative and synergistic effects of multiple contaminants, often present in complex mixtures, pose a significant challenge for biomonitoring and risk assessment (Crain *et al.*, 2008).

Climate change is emerging as a major driver of ecological change in aquatic ecosystems, with significant implications for benthic macroinvertebrate communities (Durance; Ormerod, 2007). Rising water temperatures can directly affect metabolic rates, growth, and reproduction of ectothermic macroinvertebrates, potentially leading to shifts in species distribution and community composition (Durance; Ormerod, 2007). Altered precipitation patterns can lead to more frequent and intense floods or droughts, which can scour habitats, reduce water availability, and increase pollutant concentrations, respectively (Poff *et al.*, 2007). Changes in phenology, such as earlier emergence of aquatic insects, can also disrupt trophic interactions and ecosystem processes (Parmesan, 2006). The interaction between climate change and other anthropogenic stressors can exacerbate negative impacts on macroinvertebrate communities, making them even more vulnerable.

Recent advancements in biomonitoring have focused on refining existing methods and exploring new approaches to enhance the accuracy and efficiency of ecological assessments. Two notable trends include the continuous development of multimetric indices and the debate surrounding taxonomic sufficiency. The development of MMIs continues to evolve, with efforts to create region-specific indices that better reflect local ecological conditions and stressors (Voulvoulis; Arpon; Giakoumis, 2017). Researchers are increasingly incorporating a wider range of metrics, including those related to functional feeding groups, life history traits, and genetic diversity, to provide a more holistic assessment of ecosystem health (Palmer *et al.*, 2000). The use of machine learning algorithms and artificial intelligence is also gaining traction in MMI development, allowing for the identification of optimal metric combinations and the prediction of ecological status with greater accuracy. Furthermore, there is a growing emphasis on developing MMIs that are sensitive to specific types of stressors, such as acidification or salinization, to provide targeted management recommendations.

Taxonomic sufficiency refers to the level of taxonomic resolution required for effective

biomonitoring. Traditionally, identification to the species level has been considered the gold standard, providing the most detailed ecological information. However, species-level identification can be time-consuming, expensive, and requires specialized taxonomic expertise, which may not always be available. The concept of taxonomic sufficiency proposes that identification to higher taxonomic levels (e.g., family, order) may be sufficient for detecting significant environmental impacts, particularly in routine monitoring programs (Rosenberg; Resh, 1992).

Studies have shown that for many biomonitoring objectives, family-level identification can provide comparable results to species-level identification, especially when assessing broad-scale impacts like organic pollution (Bowman; Bailey, 1997). This approach can significantly reduce processing time and costs, making biomonitoring more feasible for resource-limited regions. However, for detecting subtle impacts, assessing specific stressors, or understanding fine-scale ecological processes, species-level identification often remains crucial. The optimal level of taxonomic resolution depends on the specific research question, the type of stressor, and the ecological characteristics of the study area. A balanced approach, combining different levels of taxonomic resolution as appropriate, is often recommended.

The application of benthic macroinvertebrates as bioindicators in Neotropical regions presents unique challenges and opportunities. These regions are characterized by exceptionally high biodiversity, complex hydrological regimes, and a wide array of anthropogenic pressures, often coupled with limited resources for environmental monitoring.

Neotropical aquatic ecosystems harbor an immense diversity of macroinvertebrates, many of which are still undescribed or poorly known taxonomically (Domínguez; Fernández, 2009). This high biodiversity poses a significant challenge for species-level identification, as it requires extensive taxonomic expertise and comprehensive reference collections, which are often lacking. The development of regional taxonomic keys and the training of local taxonomists are crucial for overcoming this hurdle. Furthermore, the high species turnover across different basins and ecoregions means that indices developed in one area may not be directly applicable to another, necessitating the development of region-specific assessment tools.

Neotropical rivers and streams often exhibit high hydrological variability, with pronounced wet and dry seasons, which can significantly influence macroinvertebrate communities (Poff *et al.*, 2007). This natural variability can mask or confound the effects of anthropogenic impacts, making it challenging to distinguish between natural fluctuations and human-induced degradation. The high habitat heterogeneity, including diverse substrate types,

riparian vegetation, and flow conditions, further complicates sampling and data interpretation. Standardized sampling protocols adapted to the specific conditions of Neotropical ecosystems are essential for reliable biomonitoring.

Aquatic ecosystems in Neotropical regions are frequently exposed to multiple anthropogenic stressors acting synergistically, including deforestation, agricultural runoff, mining activities, urbanization, and climate change (Darwall *et al.*, 2008). Disentangling the individual and combined effects of these stressors on macroinvertebrate communities is a complex task. Moreover, there is a significant lack of long-term ecological data and baseline studies in many Neotropical areas, which hinders the establishment of reference conditions and the assessment of temporal trends in ecological integrity (Callisto *et al.*, 2002).

Despite these challenges, the Neotropical region offers significant opportunities for advancing biomonitoring using benthic macroinvertebrates. The high biodiversity itself provides a rich source of potential bioindicators, with many species exhibiting narrow ecological tolerances that could be highly informative (Orozco-González; Ocasio-Torres, 2023). The development of molecular tools, such as DNA barcoding and metabarcoding, offers promising avenues for rapid and cost-effective identification of macroinvertebrates, even in species-rich and taxonomically challenging groups (Baird; Hajibabaei, 2012). Furthermore, community-based monitoring programs, involving local stakeholders and citizen scientists, can help to overcome resource limitations and generate valuable data for ecological assessment and conservation. Collaborative efforts among researchers, governmental agencies, and local communities are vital for developing robust and sustainable biomonitoring programs tailored to the unique characteristics of Neotropical aquatic ecosystems.

The concept of Functional Feeding Groups (FFGs) categorizes macroinvertebrates based on their primary mode of food acquisition, such as shredders (consume coarse particulate organic matter), collectors (gather fine particulate organic matter), scrapers (graze on periphyton), and predators (consume other invertebrates) (Cummins; Klug, 1979). Changes in the relative abundance of these FFGs can provide valuable insights into the functional integrity of aquatic ecosystems and the availability of different food resources. For instance, a shift from a balanced FFG distribution to a dominance of collectors often indicates increased fine sediment deposition or organic enrichment, as these groups thrive on abundant particulate organic matter (Wallace; Webster, 1996). Conversely, a reduction in shredders can signal a decrease in allochthonous organic matter input, often due to riparian deforestation. The analysis of FFGs complements traditional taxonomic approaches by providing a functional perspective on

ecosystem health, highlighting how disturbances alter energy flow and nutrient cycling within the aquatic food web.

While often associated with rivers and streams, benthic macroinvertebrates are also crucial bioindicators in other aquatic environments, including lakes, wetlands, and estuaries. Each ecosystem type presents unique challenges and opportunities for biomonitoring. In lentic systems like lakes and reservoirs, macroinvertebrates respond to factors such as oxygen depletion in the hypolimnion, sediment quality, and trophic status. Profundal macroinvertebrates, particularly chironomids and oligochaetes, are commonly used to assess the trophic state of lakes, with specific species indicating oligotrophic, mesotrophic, or eutrophic conditions (Wiederholm, 1980). The profundal zone, being less affected by short-term disturbances, reflects long-term changes in water quality. Littoral macroinvertebrates, on the other hand, are influenced by substrate type, macrophyte abundance, and shoreline development, providing insights into habitat degradation in shallower areas. Multimetric indices adapted for lakes often incorporate metrics related to species richness, diversity, and the presence of pollution-tolerant taxa.

Wetlands, including marshes, swamps, and bogs, are highly diverse and productive ecosystems that provide critical ecological services. Macroinvertebrates in wetlands are influenced by hydroperiod, vegetation structure, water chemistry, and connectivity to other aquatic systems (Batzler; Wissinger, 1996). They play vital roles in decomposition, nutrient cycling, and as a food source for higher trophic levels. Biomonitoring in wetlands often focuses on assessing changes in community composition in response to hydrological alterations, pollution, and habitat loss. The development of wetland-specific bioassessment tools is ongoing, given the unique characteristics and high variability of these ecosystems (Daniel; Rader, 2005).

Estuaries, where freshwater meets saltwater, are dynamic environments characterized by fluctuating salinity, tides, and sediment regimes. Benthic macroinvertebrates in these systems are adapted to these variable conditions and are sensitive to changes in salinity, dissolved oxygen, and sediment contamination. They are widely used to assess the impacts of urbanization, industrial discharges, and agricultural runoff on estuarine health. Indices such as the Benthic Index of Biotic Integrity (B-IBI) have been adapted for estuarine environments, incorporating metrics that reflect species richness, diversity, and the abundance of pollution-tolerant and sensitive taxa (Engle; Summers, 1999). The challenges in estuarine biomonitoring include high natural variability and the complex interplay of multiple stressors.

The field of macroinvertebrate biomonitoring is continuously evolving with the integration of new technologies and analytical approaches, enhancing the efficiency and accuracy of ecological assessments. Traditional morphological identification of macroinvertebrates is labor-intensive, requires specialized taxonomic expertise, and can be challenging for cryptic species or early life stages. Molecular techniques, particularly DNA barcoding and metabarcoding, offer promising alternatives (Baird; Hajibabaei, 2012). DNA barcoding involves sequencing a standardized gene region (e.g., cytochrome c oxidase subunit I - COI) to identify individual specimens to species level. Metabarcoding, on the other hand, involves sequencing DNA from bulk environmental samples (e.g., water, sediment, or bulk invertebrate samples) to identify multiple taxa simultaneously. These techniques can significantly accelerate the identification process, reduce taxonomic uncertainty, and provide a more comprehensive picture of biodiversity, especially in species-rich regions like the Neotropics (Taberlet *et al.*, 2012). While still facing challenges related to reference libraries, primer bias, and quantitative accuracy, molecular methods are rapidly becoming indispensable tools in modern biomonitoring.

Automated image analysis systems, combined with machine learning algorithms, are being developed to streamline the processing of macroinvertebrate samples. These systems can automatically count, measure, and even identify macroinvertebrates from digital images, reducing the need for manual sorting and identification. This technology has the potential to significantly increase the throughput of samples, making large-scale and long-term monitoring programs more feasible. Machine learning can also be applied to analyze complex ecological datasets, identify patterns, predict ecological status, and develop more robust multimetric indices.

Remote sensing technologies (e.g., satellite imagery, aerial photography) and Geographic Information Systems (GIS) are increasingly used to characterize aquatic habitats and identify potential stressors at broader spatial scales. These tools can provide information on land use, riparian vegetation, water quality parameters (e.g., chlorophyll-a, suspended solids), and hydrological patterns, which can then be correlated with macroinvertebrate community data (Allan, 2004). Integrating remote sensing and GIS with in-situ biological monitoring allows for a more comprehensive assessment of ecological integrity, enabling the identification of critical areas for conservation and restoration.

The field of benthic macroinvertebrate biomonitoring is dynamic, with several areas requiring further research and development to enhance its effectiveness and applicability. Most

aquatic ecosystems are affected by multiple interacting stressors, yet many bioassessment approaches still focus on single stressors. Future research needs to develop methodologies that can effectively disentangle and quantify the individual and cumulative impacts of multiple stressors (e.g., pollution, habitat alteration, climate change) on macroinvertebrate communities (Crain *et al.*, 2008). This requires a better understanding of synergistic and antagonistic effects and the development of more sophisticated ecological models.

While many biotic indices and MMIs have been developed, there is a continuous need to refine and validate them for specific biogeographic regions and ecosystem types, particularly in highly diverse and understudied areas like the Neotropics. This involves developing region-specific tolerance values, identifying appropriate reference conditions, and ensuring that indices are sensitive to local stressors. The development of flexible frameworks that can be adapted to different contexts will be crucial.

Despite advancements in molecular techniques, the fundamental importance of taxonomic expertise remains. There is a need to bridge the gap between traditional taxonomy and modern ecological applications, fostering collaboration between taxonomists, ecologists, and molecular biologists. Investment in taxonomic training and the development of comprehensive, publicly accessible reference libraries (both morphological and genetic) are essential for advancing biomonitoring efforts globally.

Moving beyond taxonomic composition, future biomonitoring efforts should increasingly incorporate functional traits of macroinvertebrates (e.g., body size, reproductive strategies, dispersal ability) to better understand how communities respond to environmental change and how these changes impact ecosystem functions and services (Poff *et al.*, 2007). Linking macroinvertebrate community changes to alterations in ecosystem services (e.g., water purification, decomposition) will provide a more compelling argument for conservation and management actions.

Engaging local communities and citizen scientists in biomonitoring programs offers a cost-effective way to collect large-scale data and raise environmental awareness. Future research should focus on developing robust protocols and training programs for citizen science initiatives, ensuring data quality and integrating citizen-generated data into broader ecological assessments. This approach can foster a sense of ownership and promote sustainable management practices at the local level.

5. DISCUSSION

The comprehensive review of benthic macroinvertebrates as indicators of ecological integrity in aquatic ecosystems underscores their indispensable role in environmental assessment and management. Their widespread distribution, diverse responses to stressors, and relatively sedentary nature make them ideal biological sentinels, reflecting the cumulative impacts of environmental degradation over time. The evolution of biomonitoring methodologies, from simple biotic indices to sophisticated multimetric approaches and molecular techniques, demonstrates a continuous effort to enhance the accuracy, efficiency, and ecological relevance of these assessments.

The theoretical underpinnings of macroinvertebrate bioindication are robust, rooted in fundamental ecological principles such as trophic interactions, life history strategies, and species-specific tolerances. These principles allow for a predictable response of macroinvertebrate communities to various anthropogenic impacts, including organic pollution, habitat degradation, chemical contamination, and climate change. The shift in community composition from sensitive to tolerant taxa, alterations in functional feeding groups, and changes in diversity metrics consistently signal environmental stress, providing clear indicators of ecological impairment.

Multimetric indices (MMIs) represent a significant advancement, offering a holistic assessment of ecosystem health by integrating multiple biological attributes. Their adaptability, as evidenced by region-specific developments across North America, Europe, Australia, and emerging efforts in Neotropical regions, highlights their utility in diverse ecological contexts. However, the effectiveness of MMIs is contingent upon careful selection of metrics, calibration against regional reference conditions, and continuous validation. The debate surrounding taxonomic sufficiency also points to a pragmatic need to balance scientific rigor with practical feasibility, particularly in resource-limited or highly biodiverse regions where species-level identification can be prohibitive.

The integration of habitat assessment with macroinvertebrate data is crucial for a comprehensive understanding of ecological integrity. Physical habitat quality profoundly influences macroinvertebrate communities, and a combined approach allows for a more nuanced diagnosis of impairment, distinguishing between water quality issues and physical habitat limitations. This integrated perspective is vital for designing effective restoration and management strategies that address the root causes of degradation.

Emerging contaminants (ECs) pose a new frontier in macroinvertebrate biomonitoring.

While traditional approaches have focused on well-known pollutants, the subtle and chronic effects of PPCPs, pesticides, and microplastics necessitate the development of novel biomarkers and ecotoxicological studies. The challenge lies in detecting these low-concentration, often synergistic impacts, and integrating them into existing biomonitoring frameworks. Molecular tools and advanced analytical techniques will be instrumental in addressing this complex issue.

Long-term monitoring programs are the backbone of effective environmental management. They provide the necessary temporal context to differentiate natural variability from anthropogenic impacts, establish ecological baselines, and track the success of restoration efforts. Without sustained monitoring, the subtle, gradual changes indicative of chronic stress or the slow recovery trajectories post-restoration can be easily overlooked. The data generated from these programs are invaluable for informing policy, supporting adaptive management, and fostering a deeper understanding of aquatic ecosystem dynamics.

Neotropical regions, with their unparalleled biodiversity and unique environmental challenges, represent both a critical area for biomonitoring and a significant opportunity for scientific advancement. The taxonomic hurdles and hydrological variability demand innovative approaches, including the development of region-specific indices, the application of molecular tools, and the engagement of local communities. Overcoming these challenges will not only enhance our understanding of tropical aquatic ecosystems but also contribute to global efforts in biodiversity conservation and sustainable resource management.

In conclusion, benthic macroinvertebrates remain a cornerstone of aquatic ecological assessment. Their continued utility is ensured by ongoing advancements in methodology, the integration of new technologies, and a growing understanding of their responses to a complex array of environmental stressors. Future research must focus on refining multi-stressor assessment, tailoring indices to specific regional contexts, bridging taxonomic and ecological expertise, and leveraging citizen science to build robust and globally comparable biomonitoring programs. The ultimate goal is to translate scientific understanding into effective conservation actions that safeguard the ecological integrity of our precious aquatic resources.

Benthic macroinvertebrates serve as invaluable bioindicators for assessing the ecological integrity of aquatic ecosystems worldwide. Their diverse responses to environmental stressors, relatively sedentary nature, and integration of cumulative impacts make them powerful tools for biomonitoring. This review has highlighted the theoretical foundations, methodological advancements, and the critical role of macroinvertebrates in detecting and evaluating the effects of various anthropogenic impacts, including organic pollution, habitat

degradation, chemical contamination, and climate change.

The evolution of biomonitoring techniques, from simple biotic indices to sophisticated multimetric approaches and the integration of molecular tools, underscores the continuous effort to enhance the precision and applicability of these assessments. While challenges persist, particularly in highly biodiverse and resource-limited regions like the Neotropics, ongoing research and technological innovations are paving the way for more comprehensive and efficient monitoring programs. The emphasis on long-term monitoring, integrated habitat assessment, and the consideration of emerging contaminants are crucial for developing robust strategies for aquatic ecosystem protection and restoration.

Ultimately, the continued development and application of macroinvertebrate-based biomonitoring are essential for informing evidence-based conservation policies, guiding adaptive management practices, and ensuring the sustainable health of our global aquatic resources. By understanding and leveraging the ecological insights provided by these small but significant organisms, we can better safeguard the vital services that aquatic ecosystems provide to both nature and humanity.

6. CONCLUSION

Benthic macroinvertebrates remain among the most reliable biological indicators for assessing the ecological integrity of aquatic ecosystems due to their ecological diversity, relatively limited mobility, and predictable responses to environmental disturbances. As demonstrated throughout this review, these organisms provide valuable information on the cumulative effects of anthropogenic pressures, including organic pollution, habitat degradation, chemical contamination, hydrological alterations, and climate change. Their ability to integrate environmental conditions over extended periods makes them indispensable tools for evaluating ecosystem health and supporting evidence-based environmental management.

The evolution of biomonitoring methodologies has significantly improved the capacity to detect ecological impairment. The development of biotic indices, multimetric indices, functional approaches, and advanced statistical analyses has expanded the accuracy and applicability of macroinvertebrate-based assessments across different aquatic environments. Furthermore, emerging technologies such as DNA barcoding, DNA metabarcoding, machine learning, and geographic information systems have created new opportunities to enhance taxonomic resolution, increase analytical efficiency, and improve large-scale monitoring programs.

Despite these advances, several challenges remain, particularly in highly biodiverse regions such as the Neotropics. Taxonomic limitations, natural hydrological variability, habitat heterogeneity, and the interaction of multiple environmental stressors continue to complicate ecological assessments. These limitations reinforce the need for standardized sampling protocols, region-specific assessment indices, long-term monitoring programs, and greater investment in taxonomic research and scientific capacity building. Integrating biological indicators with habitat assessment and physicochemical analyses also remains essential for obtaining comprehensive evaluations of ecological integrity.

Future research should prioritize the refinement of biomonitoring methodologies capable of addressing multiple environmental stressors simultaneously while incorporating functional and molecular approaches into routine ecological assessments. The expansion of regional reference databases, the development of adaptive multimetric indices, and the promotion of collaborative monitoring initiatives involving researchers, environmental agencies, and local communities will further strengthen conservation efforts. In this context, benthic macroinvertebrates will continue to play a central role in freshwater ecological assessment, contributing to biodiversity conservation, sustainable water resource management, and the formulation of public policies aimed at protecting and restoring aquatic ecosystems.

7. CONFLICT OF INTEREST

The author declares that she has no personal, financial, academic, professional, or institutional conflicts of interest related to the conduct of this research, the analysis of the data, the interpretation of the results, or the preparation of this manuscript.

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