

## MACROINVERTEBRATES AS BIOINDICATORS OF WATER RESOURCES

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**Abstract:** This research focuses on evaluating the usefulness of aquatic macroinvertebrates as bioindicators for detecting changes in water quality associated with anthropogenic disturbances, as well as understanding the influence of biotic and abiotic factors on their communities. The potential of indices based on the presence or absence of these organisms for low-cost biomonitoring will be highlighted, emphasizing the need to expand their application in regions with limited knowledge such as Venezuela. This work focused on the review of the most recent literature to support the application of macroinvertebrates as bioindicators both nationally and internationally and to point out the lack of information in the area. It will also address the different methodologies applied in the studies, the explanation and usefulness of these biotic indices to reflect tolerance to disturbance, the limitations and challenges that the use of macroinvertebrates as bioindicators addresses.

**Keywords:** macroinvertebrates; biotic indices; bioindicators; water quality

## **1. Introduction**

The bioassessment of water is based on the natural capacity of aquatic biota to respond to the effects of eventual or permanent disturbances (Sawyer *et al.*, 2004). Aquatic macroinvertebrates are a useful tool to evaluate the health of these ecosystems and have a relatively long lifespan (Domínguez and Fernández, 2009), which allows them to be one of the most used biological indicators (Figueroa *et al.*, 2007). This is due to its great diversity within these ecosystems and the ease of sampling the different taxa. Sampling protocols and biotic indices are well standardized, allowing the effects of contamination to be identified over time (Fernández, 2012). Knowing the sensitivity of aquatic biota to different types of disturbances makes it easier to identify pollution problems and take preventive measures (Pino-Selles and Bernal-Vega, 2009).

Among the most used indices in Venezuela are the Family Biotic Index (IBF) and the BMWP' (Biological Monitoring Working Party) method, Shannon-Weaver Equity Index. These indices summarize and classify the arrangement of aquatic insect communities according to a gradient of organic pollution (Segnini, 2003).

In Venezuela, research focused on the use of macroinvertebrates as bioindicators to evaluate the quality of water resources is limited with water bodies that have not covered this aspect (Barrios and Márquez, 2023). The main objective of this study is to learn about aquatic macroinvertebrates as effective bioindicators of water quality. It reviewed recent studies, including national and international research, to address their diversity, adaptations, factors affecting them, their application as bioindicators, sampling methodologies, distribution and abundance factors, use of indices, limitations, and challenges.

## **2. Materials and Methods**

A systematic review of the scientific literature was conducted to collect relevant information regarding macroinvertebrates as bioindicators, applied methodologies, recent advancements, and current challenges in the field.

### *Data Search and Collection*

A comprehensive literature search was performed across several scientific databases, including PubMed, Frontiers, Wiley Online Library, the Royal Society of Chemistry, MDPI, ScienceDirect, SCOPUS, RedALyC, and Google Scholar. The search utilized key terms such as “water quality”,

“macroinvertebrates as bioindicators” “biotic indices”, and “sampling methodologies” to ensure relevant coverage. Information Selection and Refinement: The selected literature spanned the period from 2024 to 2025 to ensure an up-to-date and representative overview of the current state of the field. Reference management was performed using Mendeley (Elsevier, 2021), enabling the organization of sources by relevance and the exclusion of entries that did not meet predefined inclusion criteria.

#### *Organization of Subtopics*

Following the refinement of the collected data, a structured research framework was developed by organizing the content into thematic categories to facilitate a comprehensive analysis of the most relevant aspects of the study.

#### *Data Analysis and Interpretation*

A critical assessment of the selected studies was carried out, identifying emerging trends, knowledge gaps, and potential research opportunities. The insights obtained supported the formulation of evidence-based conclusions.

### **3. Results and discussion**

#### *Bioindicators*

The concept of bioindicator applied to the evaluation of water quality, defines a species (or assembly of species) that has particular requirements in relation to one or a set of physical or chemical variables, indicating that they are within normal values or close to their tolerance limits (Rosenberg and Resh, 1994). Pollution bioindicators measure the quality of the ecosystem through information that is collected in the water, the atmosphere or the soil, and allow the level of environmental deterioration to be identified, within a quality framework (Gamboa *et al.*, 2008). Within this approach, benthic macroinvertebrates are established as efficient bioindicators for assessing water resources. Their wide distribution, functional diversity, and sensitivity to environmental disturbances make them key tools for ecological monitoring (Villela y Delavira 2025). In particular, the aquatic insect fauna stands out for its high biological diversity and abundance, which reinforces its value as an indicator of water quality (Segnini, 2003).

The relevance of these organisms becomes even more evident in the face of the numerous modifications that anthropogenic activities (urban planning and agriculture) have caused in aquatic

ecosystems. These activities are recognized as the main causes of alteration in the structure and functioning of these environments (Lu *et al.*, 2022; Alexandre *et al.*, 2024).

Bioindicators play an important role in the interpretation and management of water resources, as they have certain advantages, among which are their integrative level, low cost, wide distribution, adaptation to different physical-biotic variables, methodological simplicity, speed of results and a retrospective view of pollution events, which makes them an ideal tool for routine monitoring of water quality (Ortiz, 2005). However, these also present certain disadvantages such as not detecting subtle impacts, they do not have a quantitative term (although they are environmentally integrative) compared to physical and chemical analyses; Requires experienced personnel. It is difficult to establish an effect relationship between the presence or absence of a bioindicator and a specific environmental factor. Data analysis requires knowledge in statistics and ecology, so this evaluation has different levels of precision (Roldán-Pérez, 2016).

#### *Diversity of taxonomic groups*

The diversity of aquatic macroinvertebrates is a biological indicator that comprises a large part of biological diversity, often being the main animal component of lotic systems (Esteves, 1988). These organisms play an important role in the food web of freshwater systems, controlling the quantity and distribution of their prey, constituting a food source for terrestrial and aquatic consumers (Wade *et al.*, 1989).

Studies based on their taxonomy and distribution provide important information to understand the ecology and the role they play in the environment (Roldán, 2001). The diversity of aquatic macroinvertebrates is not affected by hydrology, aquatic vegetation or anthropogenic disturbances, among others (Rico-Sánchez *et al.*, 2014). The use of indicator families of specific conditions is efficient, confirming a clear difference between the quality of the surface water and the quality of the substrate, with the surface layer being the one that has the most suitable conditions for various organisms with different levels of tolerance to inhabit it, while the bottom restricts the presence to a few families tolerant to lower conditions (Núñez and Fragoso-Castilla, 2019). Among its most notable groups are:

#### *Phylum Arthropoda: Subphylum Hexapoda*

Insects constitute the most diverse and abundant group of aquatic macroinvertebrates. The Collembola and Insecta classes have adaptations to aquatic and semi-aquatic life, which has

generated an extraordinary morphological and functional variety. Various orders, such as Ephemeroptera (Mayflies), Plecoptera (stoneflies) and Trichoptera (Friganeas), present a high sensitivity to environmental disturbances, making them excellent bioindicators to evaluate the quality of aquatic ecosystems ([Hanson et al., 2010](#)). Chironomids (Chironomidae) can tolerate low oxygen concentrations and high organic load ([Figueroa et al., 2007](#)).

#### Phylum *Annelida*

Within freshwater annelids, the Clitellata class includes oligochaetes and leeches, these are groups of macroinvertebrates of great ecological importance, playing key roles in decomposition processes and forming part of the trophic networks of these ecosystems. Freshwater oligochaetes, generally small and detritivorous, inhabit bottom sediments, while leeches, mostly ectoparasitic or predatory, have a greater diversity of feeding habits ([Martin et al., 2008](#)).

#### Phylum *Mollusca*

Mollusks constitute a diverse group of aquatic macroinvertebrates, notable for their soft body and, in many cases, for the presence of a calcareous shell that provides them with protection. Taxa such as gastropods (snails) and bivalves (clams) are representative examples of this phylum, and play crucial ecological roles in diverse aquatic ecosystems ([Hanson et al., 2010](#)).

#### Phylum *Arthropoda*: Subphylum *Crustacea*

Crustaceans are also used as bioindicator macroinvertebrates; they have a hard exoskeleton and articulated appendages, which are easily recognizable. Decapods are examples of larger macroinvertebrates, while amphipods and isopods are smaller and more abundant forms ([Gonzabay, 2008](#)).

#### Phylum *Platyhelminthes*

Turbellarians belonging to this phylum constitute a diverse group of aquatic macroinvertebrates. Among them, the tricladidae stand out for their ecological importance. As benthic macroinvertebrates, they play a crucial role in aquatic food webs. Their sensitivity to environmental changes makes them valuable bioindicators of water quality. Planarians, a group of larger tricladidae, have been extensively studied due to their relevance in ecological research ([Schockaert et al., 2008](#)).

#### Phylum *Nematoda*

They are macroinvertebrates of great functional diversity, ranging from decomposers of organic matter to predators of other microorganisms such as roundworms, making them key indicators of water quality and the health of ecosystems. Their presence and abundance in a body of water can reveal organic contamination, sediment quality, eutrophication, and the dynamics of biological communities (Martínez-Gallardo *et al.*, 2015).

#### *Adaptations of macroinvertebrates to different aquatic environments*

The relationship between habitats and macroinvertebrates is multifactorial; although environmental characteristics are an important determinant in the distribution and abundance of organisms, they are not the only factor (Townsend and Hildrew, 1994). Dispersal, competitive interactions and mutualistic relationships, among others, also influence the structure and dynamics of ecological communities (Poff and Alan, 1995). Traits of macroinvertebrate communities are related to associated microhabitat conditions (Statzner and Higler, 1986).

Macroinvertebrates have strategies that reflect their adaptations to environmental conditions and their functions can be described by biological traits such as breathing pattern, body shape, mobility, etc. (Domínguez and Fernández, 2009). These attributes are specifically adapted to a segment of an ecosystem (microhabitat); some of these adaptations are directly related to the physical space they occupy, and the environments in which they move or maintain (Torres-Zambrano and Torres-Zambrano, 2016). These adaptations are developed to save energy (behavioral, morphological, etc.), increasing their probability of survival and reproduction (Townsend and Hildrew, 1994).

#### *Factors affecting the distribution and abundance of macroinvertebrates*

##### *Abiotic factors*

**Dissolved oxygen:** Dissolved oxygen is a key factor in the distribution of macroinvertebrates. Some groups can survive in low-oxygen environments, while others require oxygen-rich waters. This difference in oxygen tolerances reflects the different life strategies and adaptations of these organisms (Sánchez, 2007).

**Temperature:** Temperature affects the metabolic processes of macroinvertebrates. The increase in temperature reduces the concentration of dissolved oxygen, limits the availability of suitable habitats, altering the structure of macroinvertebrate communities (Mesa, 2010).

pH: Low pH values in water can have negative effects on aquatic life, especially macroinvertebrates. These organisms have difficulties absorbing essential nutrients and are more vulnerable to heavy metal toxicity ([Tripole et al., 2008](#)).

Pollutants: Toxic substances present in water can exert lethal or sublethal effects on macroinvertebrates, resulting in a decrease in species richness in the most contaminated ecosystems. The presence or absence of these species allows the quality of the water to be evaluated and the presence of toxic substances that can negatively affect aquatic ecosystems to be detected ([Merino et al., 2020](#)).

#### Biotic factors

Presence and abundance of predators: The presence of predators significantly influences the composition of macroinvertebrate communities. Altering this factor can generate unexpected changes in the ecosystem, affecting habitat structure and interactions between species. Ecosystem complexity and environmental variability also influence predation intensity ([Rosenfeld, 2000](#)).

Competition: The coexistence of multiple species of macroinvertebrates in the same habitat generates intense competition for resources such as food and shelter. This competition influences the structure of aquatic communities and can lead to the suppression of prey populations ([Lewin et al., 2015](#)). The scarcity of shelter intensifies this competition and alters the movement and distribution patterns of macroinvertebrates. Habitat changes, such as vegetation loss, can exacerbate this situation ([Knowlton, 2004](#)).

Symbiotic relationships: Symbiotic interactions of macroinvertebrates are classified mainly by the effects on the participating organisms, identifying mutualism, commensalism and parasitism as key relationships ([Mackenzie et al., 1998](#)).

Mutualism: Positive interactions between macroinvertebrates and other species are essential for the functioning of ecosystems. For example, some macroinvertebrates use crab burrows as shelter, while crabs benefit from macroinvertebrate activity by improving habitat quality ([Creed et al., 2021](#)).

Commensalism: Some macroinvertebrates use other organisms as a substrate for their development, taking advantage of existing structures as shelters or food sources. This strategy allows them to access specific microhabitats and reduce competition with other species ([Tokeshi, 1993](#)).



Parasitism: Some macroinvertebrates, such as flies from the Nycteribiidae and Streblidae families, are exclusive parasites of bats. These flies have developed special adaptations to live on their hosts, such as modified legs and highly specialized life cycles. This close relationship has given rise to a great diversity of parasitic fly species (Whitaker, 1998).

#### Applications of macroinvertebrates as bioindicators

Macroinvertebrates are widely used as bioindicators due to their high sensitivity to environmental changes. The application of these organisms as sentinels has demonstrated their high sensitivity to climate changes, making them suitable for evaluating the quality of the environment, especially in rapid assessments, where their ease of collection and strong responses to change make them indicated for analysis (Ruaro *et al.*, 2016).

Among the various bioindicators used to evaluate water quality, benthic macroinvertebrates stand out for their representativeness and usefulness. Studies such as that of Girogio *et al.* (2016) have demonstrated a notable sensitivity to pollution, reflected in the significant impact on the composition of the communities of these organisms along a river, reflecting the quality of the water. In the headwaters, where pollution is usually lower, good quality indicator species predominate, while in the more degraded sections, less diverse communities are observed, dominated by species tolerant to adverse conditions. This variation in community composition has been used in numerous studies to develop water quality indices, which allow the ecological status of rivers to be evaluated quickly and efficiently. It is important to highlight that certain species of macroinvertebrates are adapted to living in contaminated environments, as demonstrated by the work of Rizo-Patrón *et al.* (2013). Most macroinvertebrates are sedentary, meaning that they spend most of their lives in one place (De la Lanza-Espino, 2014).

Aquatic macroinvertebrates are valuable bioindicators due to their relative ease of identification and the abundant existing literature (Roldán, 2016). The analysis of its taxonomic composition allows evaluating the ecological state of a body of water (Soria-Reinoso, 2016), although it is crucial to complement this with the evaluation of physical-chemical parameters and factors such as sedimentation (Martínez, 2012). Their ability to reflect changes in water quality makes them effective tools for environmental monitoring, early detection of alterations and sustainable management of ecosystems, impacting both environmental and human health (Damborenea, 2016).

#### *Sampling methodology for aquatic macroinvertebrates*



The choice of method depends on various factors such as the type of habitat, depth, current speed and the objectives of the study. For its collection, various methods are used such as the Surber net, dredges and hand collectors, the choice of which depends on the type of habitat and the objectives of the study. The collected macroinvertebrates are identified taxonomically and parameters such as specific richness, abundance and biotic indices are analyzed to evaluate water quality and detect alterations in the ecosystem (Sermeño *et al.*, 2010).

**Surber Networks:** Used in multi-habitat sampling, using this network with a mesh aperture of 250 µm to sample three types of substrates: stone, leaf litter and roots. At each sampling site, at least three replicates are collected. The collected biological material is preserved in 96% alcohol and stored in labeled bags for subsequent identification and analysis in the laboratory (Sermeño *et al.*, 2010).

**Artificial substrate:** Artificial substrates are valuable tools in the study of aquatic macroinvertebrates, they provide heterogeneity of microhabitats with substrates such as wood bricks and clay. These substrates create ecological niches that allow the coexistence of various species with specific requirements. Harvesting is done by hand weekly and stored in glass containers with 70% alcohol (López *et al.*, 2023).

**Kick sampling:** it is a simple but effective sampling method to evaluate the biological quality of water bodies. It consists of gently removing the substrate with your feet or a brush, concentrating the organisms in a hand net. This technique, widely recognized in the scientific community, allows obtaining a representative sample of the benthic macroinvertebrate community (Czerniawska-Kusza, 2005).

**D-shaped or rectangular hand net sampling:** Hand net sampling is a technique commonly used in aquatic ecology to collect benthic macroinvertebrates, especially in those bodies of water where access is limited or depth is low. This type of sampling technique allows semiquantitative and quantitative surveys to be carried out in a particular habitat or multihabitats. One of the advantages of using the D or rectangular network is its easy handling in any type of habitat (Baque, 2021).

**Use of the dredge:** The Ekman dredge consists of two steel buckets that close against each other using a mechanism of levers and hinges. It is especially useful for collecting benthic macroinvertebrates that live in fine sediments such as gravel, sand or silt, and that are found at depths greater than 2 meters, mainly in lentic water bodies (Chacón-Vélez, 2017).

Direct collection: It is a complementary method based on the collection of samples of accumulated leaf litter with branches, lifting stones of different sizes, trunks or hollow canes. All the collected material is placed in plastic or metal trays, for manual collection with the use of entomological tweezers of all the invertebrates found in this material or those that differ from those found with the other techniques. It is a purely qualitative method (Nugra *et al.*, 2016).

#### *Use of macroinvertebrate indices for water quality sampling*

Initially, biotic indices were developed in which a taxonomic identification of macroinvertebrates was necessary down to the genus or species level, but it has been proven that the most practical indices (due to their ease of obtaining) are those in which only qualitative data (presence or absence) and taxonomic identification down to the family level are necessary (Fierro, 2012). Like, for example:

#### *EPT (Ephemeroptera, Plecoptera and Trichoptera)*

The EPT index (Ephemeroptera, Plecoptera and Trichoptera) is used to demonstrate good water quality (Álvarez, 2007). To calculate the EPT Index, you begin by strategically selecting various points in the body of water, ensuring that they represent different habitat conditions. At these points, sampling nets are used to collect macroinvertebrates from different microhabitats. Subsequently, the collected organisms are identified to the order level, with special attention to the orders Ephemeroptera, Plecoptera and Trichoptera. Una vez contados los individuos de estos órdenes, se calcula el índice dividiendo el número total de individuos de EPT entre el número total de individuos de todas las especies y multiplicando el resultado por 100. Este porcentaje obtenido refleja la abundancia relativa de estos órdenes y sirve como indicador de la calidad del agua. Un porcentaje de EPT superior a 50%, indica una buena calidad del agua (López *et al.*, 2019).

#### *BMWP Index*

The BMWP Index (Biological Monitoring Working Party) is a tool widely used in aquatic ecology to evaluate water quality based on the presence or absence of different families of macroinvertebrates (Gutiérrez-Fonseca and Ramírez, 2016). The BMWP index is calculated through a series of steps. First, samples of macroinvertebrates are collected in the body of water to be evaluated. These organisms are then identified to the family level and assigned a specific score based on their tolerance to organic contamination. The most sensitive families receive higher scores as shown in Table 1, these scores are assigned by the researcher's discretion. Subsequently, the

scores of all the families identified in the sample are added, thus obtaining the total value of the BMWP index. Finally, this value is interpreted: the higher the score, the better the water quality, since it indicates the presence of organisms more sensitive to pollution, which suggests a less altered environment (Chuqui Lema and Manzaba-Jiménez, 2021).

**Table 1.** BMWP table focused on the fauna of Colombia in the rainy season (Nuñez and Fragoso-Castilla, 2019)

| Macroinvertebrates associated with macrophytes |                 |                 | Rainy Period |      |      |      |      |
|--|-----------------|-----------------|--------------|------|------|------|------|
| Class  | Order           | Family          | E1           | E2   | E3   | E4   | E5   |
| Insecta  | Hemiptera       | Gerridae        | 11           | 27   | 10   | 35   | 8    |
|  |                 | Veliidae        | 8            | 21   | 9    | 19   | 11   |
|  |                 | Naucoridae      |              | 7    |      | 5    | 4    |
|  |                 | Notonectidae    | 15           | 23   | 9    | 17   | 8    |
|  | Odonata         | Libellulidae    | 5            | 5    | 4    | 3    |      |
|  |                 | Gomphidae       |              | 4    | 2    | 3    |      |
|  | Coleoptera      | Elmidae         |              |      | 4    | 6    |      |
|  |                 | Hydrophiilidae  | 5            |      |      | 3    | 3    |
|  |                 | Dytiscidae      | 6            | 11   |      | 2    | 6    |
|  |                 | Chironomidae    | 12           | 3    |      |      | 2    |
|  | Diptera         | Culicidae       | 6            |      |      |      |      |
|  |                 | Ceratopogonidae | 4            |      |      |      |      |
|  | Ephemeroptera   | Baetidae        |              | 5    | 7    | 5    | 3    |
| Malacostraca                                   | Decapoda        | Palaemonidae    | 6            |      |      | 4    | 8    |
| Branchiopoda                                   | Diplostraca     | Limnaeidae      |              |      |      |      | 3    |
| Arachnida                                      | Trombidiformes  | Hydrashnidae    |              | 3    | 3    | 2    |      |
| Gastropoda                                     | Basomatophora   | Planorbidae     | 4            | 2    |      | 3    | 5    |
|  |                 | Physidae        |              |      | 2    |      | 3    |
|  | Mesogastropoda  | Hydrobiidae     | 4            |      | 3    | 2    | 2    |
|  | Stylommatophora | Bulimulidae     | 8            | 2    | 2    |      | 2    |
| Total  |                 |                 | 94           | 113  | 55   | 109  | 68   |
| %Abundance                                     |                 |                 | 13           | 15.7 | 7.62 | 15.1 | 9.42 |

E1 (Station 1): Arroyo Paraluz Influence; E2 (Station 2): Northeast Sector; E3 (Station 3): Central Sector; E4 (Station 4): South Sector; E5 (Station 5): Arroyo Garrapata Influence.

### *Family Biotic Index (IBF)*

The Family Biotic Index (IBF) assigns tolerance values to each taxonomic family of macroinvertebrates, weighting their relative abundance. The evaluation of the Family Biotic Index (FBI) involves collecting macroinvertebrates at various points and seasons. These organisms are identified down to the family level and are assigned a pollution tolerance score, based on the criteria of each researcher, governed by international regulations such as those of the IUCN (International Union for Conservation of Nature). The IBF is calculated by adding the scores and dividing by the total number of families. The result obtained is classified into water quality categories and correlated with other physicochemical parameters such as temperature and dissolved oxygen, for a comprehensive evaluation (López *et al.*, 2019).

### *Diversity indices*

Diversity indices are a measure used to evaluate the variety of species and their distribution in a given ecosystem. There are different types of diversity indices, each with its own advantages and disadvantages (Del Carmen Zúñiga and Cardona, 2009). Some of the most used are:

**Shannon-Wiener Index:** Measures both the wealth and equity of a community. The proportions of each taxon in the total sample are calculated and the formula  $H' = - \sum p_i * \ln(p_i)$  is applied to obtain the diversity index, where  $p_i$  is the proportion of individuals of the species in the sample and  $\ln$  is the natural logarithm. High values indicate high species diversity and a relatively equal distribution of individuals among them, and low values suggest low species diversity and dominance of a few species (Huaman, 2019).

**Simpson Index:** The Simpson Index is a measure of diversity that evaluates the probability that two individuals selected at random from a sample belong to the same species. It is calculated with the equation  $D = \frac{1}{\sum (n/N)^2}$ , with  $n$  being the number of organisms that belong to the species and  $N$  being the total number of organisms. This index is particularly useful for identifying dominant species in a community (Pérez-Postigo *et al.*, 2021).

### *Average Score Per Taxon (ASPT)*

It is a biological index that summarizes the sensitivity of the aquatic macroinvertebrate community to organic pollution. Each family collected in the sample receives a score based on its tolerance to environmental stress (for example, from 1 for the most tolerant to 10 for the most sensitive). The ASPT is defined as the arithmetic mean of these taxonomic scores, such that high values indicate

communities dominated by sensitive organisms and, therefore, good water quality, while low values reveal severe environmental impacts (Armitage, 1983). It is identified at the family level and the corresponding sensitivity score is assigned according to published keys. All the scores of the families present are added and then that total is divided by the number of families sampled. This test is simple, reproducible and allows comparisons between sites and time periods (Hilsenhoff, 1988).

#### Hilsenhoff Biotic Index (HBI)

This is a quantitative method designed to assess the abundance of arthropods in streams as an estimator of water quality, based on predetermined tolerances for each taxon to organic pollution. The methodology consists of collecting a sample of at least 100 arthropods and assigning each species or genus a tolerance value ranging from 0 (very sensitive) to 10 (very tolerant). The number of individuals of each taxon ( $n$ ) is multiplied by its tolerance value ( $a$ ), these products are added, and the total is divided by the overall number of specimens ( $N$ ), thus obtaining the HBI value. To reduce bias, confounding variables such as overabundance of dominant species, seasonal stress, and flow are controlled for, and the maximum catch per taxon is limited to 10 (Hilsenhoff, 1977).

#### *Methodological limitations and challenges of studying macroinvertebrates as bioindicators*

The choice of methodology and experimental design in studies with aquatic macroinvertebrates is crucial and is closely linked to the specific objectives of the research. A fundamental first step is to clearly define what the study seeks to answer. This definition will guide the selection of qualitative or quantitative methods. While qualitative studies are useful to characterize the biodiversity of a site, quantitative studies are necessary to detect changes and make comparisons between different locations or moments in time. However, the implementation of quantitative methodologies requires a rigorous sampling design and considerable sampling effort, which may limit its applicability in certain contexts (Ramírez and Gutiérrez-Fonseca, 2014). The lack of scientific rigor in these studies not only affects the scientific community, but also has direct implications for the management and conservation of our aquatic ecosystems (Roldán, 2016).

Knowledge about the taxonomy of aquatic macroinvertebrates in Latin America still presents significant gaps. Although groups such as Ephemeroptera, Trichoptera, Plecoptera and Coleoptera are the most used in environmental assessments, the information available on other taxa is scarce (Alonso *et al.*, 2014). Despite the great potential of the Latin American region for research on

aquatic macroinvertebrates, these organisms remain poorly studied due to various limitations. However, efforts have been made to improve this, as is the case of the different conferences that have been held on freshwater macroinvertebrates in Latin America, seeking to share knowledge and promote more exhaustive studies on these important indicators of the health of aquatic ecosystems ([Ramírez and Gutiérrez-Fonseca, 2014](#)). Below are two tables with studies based on Macroinvertebrates as bioindicators. Table 2 discusses research carried out with macroinvertebrates as bioindicators in Venezuela using biological indices and Table 3 indicates recent international research on macroinvertebrates as bioindicators using biological indices.

**Table 2.** Research carried out with macroinvertebrates as bioindicators in Venezuela using biological indices

| Most detected families                     | Body of water and location                | Sampling type                                   | Water quality                         | Indices or analysis used  | Reference                              |
|--|---|---|---------------------------------------|---|--|
| Caenogastropoda                            | Lower Las Marias River Basin (Portuguesa) | Manual collection                               | Regular                               | Margalef Diversity Indices, Simpson Dominance Index, Shannon-Wiener Index, IBF Index, BMWP Index, EPT Index | <a href="#">Domerçant et al., 2016</a> |
| Hydraenidae<br>Dytiscidae<br>Hydrobiosidae | River meachiche (Falcón)                  | Grid of Surber 30 x 60 cm                       | Good (E1)<br>Regular (E2)<br>Bad (E3) | Shannon-Wiener Index (H')<br>Biotic family index (IBF) BMWP Index   | <a href="#">Cedeño y Rincón, 2019</a>  |
| Balanidae<br>Tanaididae<br>Neritidae       | Chama river Basin (Mérida)                | Manual collection and bibliographic compilation | Regular                               | BMWP Index  | <a href="#">Segnini et al. 2021</a>    |

*Continuation...* **Table 2.** Research carried out with macroinvertebrates as bioindicators in Venezuela using biological indices

| Most detected families | Body of water and location      | Sampling type                                | Water quality | Indices or analysis used  | Reference                          |
|------------------------|---------------------------------|--|---------------|---------------------------|------------------------------------|
| Balanidae              | Eastern coast of Lake Maracaibo | Manual collection with the help of a spatula | Bad           | Shannon-Wiener Index (H') | <a href="#">Lárez et al., 2021</a> |

|  |  |  |  |            |                                 |
|--|--|--|--|------------|---------------------------------|
| Leptoceridae,<br>Hydropsychidae,<br>Odontoceridae<br>Culicidae | Rivers Aisme<br>Tigre, Chive,<br>Caris, La Peña,<br>San Antonio,<br>Hamaca, Pao,<br>Agua Clara,<br>Carapa, Areo<br>and Urupia<br>(Anzoátegui and<br>Monagas) | D type grid<br>(250,00 µm<br>Mesh opening) | Bad (San<br>Antonio)<br>Regular<br>(Chive,<br>Caris, the<br>peña,<br>Hamaca,<br>Pao, clear<br>water<br>and Urupia)<br>Good (Tigre,<br>Pao, Carapa<br>and Areo) | BMWP index | Barrios and<br>Márquez,<br>2023 |
|--|--|--|--|------------|---------------------------------|

**Table 3.** Recent international investigations of macroinvertebrates as bioindicators using biological indices

| Most detected families                         | Body of water and location                                    | Sampling type   | Water quality   | Indices or analysis used                       | Reference  |
|--|---|---|---|--|--|
| Chironomidae<br>Hydropsychidae<br>Heptagenidae | Ganges river<br>(Uttarakhand,<br>India)                       | Manual<br>collection  | Good<br>(Haridwar)<br>Regular<br>(Jagjeetpur<br>upstream) Bad<br>(Jagjeetpur<br>downstream) | BMW index                                      | Agrawal <i>et al.</i> , 2019                     |
| Chironomidae<br>Ephemeroptera<br>Trichoptera   | Raba river,<br>Dunajec river<br>and Vístula<br>river (Poland) | Surber grid of<br>40 cm × 40<br>cm.                             | Bad (Vístula<br>river)<br>Regular<br>(Dunajec river)<br>Good (Raba<br>river)                | Shannon-Wiener<br>Index (H')                   | Kownacki<br>and Szarek-<br>Gwiazda,<br>2022      |
| Leptophlebiidae<br>Elmidae                     | Lapa river<br>(Cayey and<br>Salinas, Puerto<br>Rico)          | Manual<br>collection and<br>D type» Grid<br>(mesh of 250<br>µm) | Bad (place C y<br>D) Regular (B<br>place)<br>Good (A place)                                 | Biotic family index<br>(IBF) and BMWP<br>index | Orozco-<br>González and<br>Casio-Torres,<br>2023 |

*Continuation...* **Table 3.** Recent international investigations of macroinvertebrates as bioindicators using biological indices

| Most detected families  | Body of water and location                              | Sampling type  | Water quality             | Indices or analysis used  | Reference                    |
|---|---|--|---------------------------|---|------------------------------|
| Nemouridae<br>Rhyacophilidae                                      | Ibar river<br>(Kosovo)                                  | Manual<br>collection<br>hand grid                                      | Regular in<br>most places | BMWP index,<br>ASPT, EPT  | Buçinca <i>et al.</i> , 2024 |
| Baetidae,<br>Lepidostomatidae,<br>Hydropsychidae<br>Chilrinominae | Brantasen<br>Malang river<br>basin<br>(Java, Indonesia) | Surber grid<br>with a mesh<br>size of 0.5 mm,<br>of 30 cm by 30<br>cm. | Bad in most<br>seasons    | Uniformity<br>indices, Shannon-<br>Wiener Index<br>(H'), Dominance<br>(C), BMWP and | Hertika <i>et al.</i> , 2024 |



|                                       |                                      |  |   | ASPT (Average Score per Taxon)   |
|---------------------------------------|--------------------------------------|--|---|--|
| Palaemonidae<br>Dytiscidae            | Missolé stream<br>(Gabón, África)    | Dit net of 30 cm (malla de 400 µm) conical grid to 50 cm of depth.   | Very good   | Biotic index of Hilsenhoff (HBI), EPT and Shannon-Wiener (H') index<br><a href="#">Nyamsi et al., 2024</a>   |
| Cerithiidae<br>Veneridae<br>Muricidae | The Jambeli Archipelago<br>(Ecuador) | Manual collection with cylindrical container (25 cm x 25 cm x 30 cm) | Good  | Dominance index (Y), Shannon-Wiener diversity index (H), Pielou's evenness index (J), trophic index of functional feeding groups, Marine Biotic Index (AMBI)<br><a href="#">Vidal et al., 2025</a> |
| Chironomidae<br>Thrycorithidae        | Amojú River<br>(Peru)                | 30 cm x 30 cm wooden Surber net                                      | Good (Place 8, Place 7)<br>Regular (Place 6, Place 5, Place 4, Place 3)<br>Bad (Place 2, Place 1) | Shannon-Wiener H' index, Simpson index, BMWP index, Bray-Curtis index<br><a href="#">Tapia and García 2025</a>   |

### *Research on macroinvertebrates as bioindicators of water resources carried out in Venezuela*

Table 2 presents the results of various studies carried out in different bodies of water in different regions of the country. In each study, the most abundant family of macroinvertebrates, the type of sampling used, the body of water analyzed, the quality of the water and the indices or statistical analyzes used to evaluate the quality of the aquatic ecosystem were identified. This set of studies offers a valuable perspective on the application of this methodology in the national context, allowing the identification of trends, patterns and knowledge gaps, showing a compilation of some works presented from 2001 to 2022, which highlights the notable diversity in terms of the ecosystems studied, the sampling methods and the taxonomic groups analyzed. This heterogeneity reflects the complexity of Venezuelan aquatic ecosystems and the versatility of macroinvertebrates as bioindicators.

To evaluate the ecological quality of different bodies of water, researchers have used a variety of biological indices: Margalef, Shannon–Weaver, and Simpson's diversity was applied to evaluate

community richness and equity. The BMWP, EPT and IBF indices allowed classifying water quality according to taxonomic sensitivity. Among the most used are the Shannon-Weaver index, which measures species diversity, and the BMWP (Biological Monitoring Working Party) index, which assigns values to families of macroinvertebrates based on their tolerance to pollution. Therefore, the table 1 shows that the quality of the water varies from good in some sections of the Macubache River and certain sub-basins of the Apure, to average or poor in sectors of the Chama basin and Lake Maracaibo. These differences reflect both local anthropogenic pressure and watershed management in each region. In each study, the most abundant family of macroinvertebrates was identified. In the Las Marías basin, Caenogastropoda dominated, a frequent indicator of less disturbed ecosystems. In the Macubache River and other Andean rivers, Hydracarina, Odonatidae and Hydroscoidea stand out, groups with a certain tolerance to quality variations. The Ephemeroptera (Baetidae) appear constantly, reflecting an intermediate degree of conservation, while Leptoceridae and Hydropsychidae are detected in plain rivers, linked to more stable substrates and better oxygenation.

In the type of sampling, manual collection predominates, complemented in some cases with Surber nets (30×30 cm) or type D (25×25 cm) and A (10×10 cm) nets. This methodological diversity expands the capture of taxa of different sizes and habitats, but imposes comparability biases if common criteria are not established for sampling effort, sample size, size of the water body analyzed, and the statistical indices or analyzes used to evaluate the quality of the aquatic ecosystem.

The results obtained in the studies compiled show a clear relationship between the composition and structure of macroinvertebrate communities and water quality. The presence of species sensitive to pollution, such as those belonging to the orders Ephemeroptera, Plecoptera and Trichoptera, indicates favorable environmental conditions. On the contrary, the dominance of pollution-tolerant taxa such as the Diptera taxa suggests a deterioration in water quality. Despite the progress made, there are still important limitations in the application of bioindication with macroinvertebrates in Venezuela. Among them, the lack of standardization in sampling and analysis protocols, the scarcity of information for some regions of the country and the need to develop specific biological indices for national ecosystems stand out.

*Research on macroinvertebrates as bioindicators of water resources carried out internationally*

Table 3 provides a synthesis of recent research (as of 2019) that has used aquatic macroinvertebrates as bioindicators in various ecosystems worldwide. The geographical diversity of the samples makes it possible to identify global and local patterns in the structure of aquatic communities, reflecting natural variables that modulate the health of river ecosystems. These studies, ranging from small streams to large rivers, have used a variety of biological indices. However, the BMWP Biological Index stands out as the most used, thanks to its simplicity and low cost. A wide range of families of aquatic insects used as bioindicators are observed, among which are: Chironomidae, Hydropsychidae, Heptagenidae, Leptophlebiidae, Elmidae, Nemouridae, Rhyacophilidae, Baetidae, Lepidostomatidae, Hydropsychidae, Chironominae, Palaemonidae and Dytiscidae.

This diversity reflects the adaptation of different groups of insects to different environmental conditions and their sensitivity to different types of pollutants. A clear example is the Baetidae and Chironomidae families, which appear recurrently in studies. In the case of the Hydropsychidae family, it indicates good physical-chemical quality, warning about mild or moderate disturbances. On the other hand, Chironomidae, by resisting more degraded conditions, marks stages of organic contamination or eutrophication. Combining macroinvertebrates with biotic indices allows us to draw a tolerance gradient that enriches the ecological diagnosis and improves the precision of environmental evaluations, even allowing us to see the variability in different microhabitats of the same body of water. International studies show the use of Surber and type D nets to manual collections with 0.25 mm meshes in the methodology, significantly influencing the data. Fine mesh devices and manual collection allow smaller organisms to be captured and provide greater resolution in diversity metrics, however, their application requires more field time. Therefore, it is crucial to standardize procedures or at least document the sampling effort in detail to ensure comparison between studies and regions.

The studies were carried out on various continents and geographic regions, which demonstrates the universal applicability of sampling with macroinvertebrates. The differences in water quality, ranging from optimal in Gabon, Ecuador and Perú to very degraded levels in India and Indonesia, show the need to adapt monitoring programs to each region. It is important to standardize sampling methods and adjust biological indices to local conditions, taking into account local taxonomic richness, consistent sampling protocols and statistical approaches. All of this will allow the results

to be more reliable, facilitating their comparison between different regions on an international scale. This approach also contributes to the strengthening of more complete global databases, essential to sustain comparative studies and guide decision-making in environmental management.

#### **4. Conclusions**

Despite certain limitations, the findings suggest that these organisms have great potential as bioindicators, thanks to their taxonomic diversity. Further research is needed on the relationship between macroinvertebrate communities and the environmental factors that affect their distribution and abundance. A review of national and international studies revealed advances in the application of the BMWP, EPT, and IBF biotic indices, as well as in the variety of sampling methods. However, there is still a lack of information in poorly studied regions due to the absence of uniform protocols and the scarcity of tools calibrated for different local contexts.

The methodology chosen is crucial for obtaining reliable data, and combining qualitative and quantitative approaches with advanced statistical tools will enable the creation of more robust predictive models. Although progress has been made, challenges remain in standardizing protocols, taxonomic identification, and available resources. Fostering collaborative networks is essential to improve research in the region and enrich knowledge about macroinvertebrate biodiversity in Latin America. It is recommended to establish long-term monitoring programs and implement assessment tools that facilitate the sustainable management of aquatic ecosystems. The recognition of macroinvertebrates as key indicators in aquatic ecosystems lays the foundation for future research and conservation strategies in Venezuela and around the world.

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