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REIMAGINING FRESHWATER BIOMONITORING: INTEGRATING FUNCTIONAL, MOLECULAR, AND ECOLOGICAL SIGNALS FROM AQUATIC INSECTS

Ramnikant Kumar[1], Sunil Kumar[2], Santosh Vasantrao Rankhamb[3*], Nitin Devendra Padwal[4], Purushottam Rambhau More[5], Balaji Panchal[6]. Binay Kumar Chakraborty[7], T. S. Pathan[8]

[1]Research scholar MJPRU (Bareilly college) (UP) India
[2]Professor, Department MJPRU (Bareilly College) (UP) India
[3*]Associate Professor, Department of Zoology, Late Ramesh Warpudkar ACS College Sonpeth District Parbhani (Maharashtra) India, Email: santosh.rankhamb@gmail.com
[4]Professor and Head, Department of Zoology, Shankarrao Patil Mahavidyalaya Bhoom Osmanabad, (Maharashtra) India
[5]Assistant Prof. Department of Zoology, Kai. Rasika Mahavidyalaya Deoni Dist. Latur (Maharashtra) India
[6]Biodiesel lab, Mit Satara Parisar, Aurangabad, India
[7]CIRDAP, Visiting Professor, S V. Subharti University, Meerut, UP, India.
[8]Professor, Department of zoology, Kalikadevi Arts, Science and Commerce Collegee, Shirur Kasar Dist. Beed Maharashtra, India
*Corresponding Author: Santosh Vasantrao Rankhamb, santosh.rankhamb@gmail.com

ABSTRACT

Aquatic insects have historically been essential bioindicators in freshwater biomonitoring owing to their ecological sensitivity, specific habitat requirements, and crucial roles in ecosystem functioning. However, conventional taxonomy-based assessment frameworks are often inadequate in identifying cryptic biodiversity loss, functional disruption, and early-stage ecological instability in increasingly disturbed freshwater environments. This review repositions freshwater biomonitoring within a comprehensive framework that integrates functional, molecular, and ecological signals from aquatic insect communities. It emphasis functional trait-based approaches, including life-history, physiological, and trophic characteristics that reveal mechanistic responses to environmental stress, ecosystem resilience, and ecological reorganisation. Concurrently, advancements in molecular ecology, including DNA barcoding, environmental DNA (eDNA), metabarcoding, and emerging multi-omics technologies, are discussed as transformative tools for high-resolution biodiversity assessment and ecological diagnostics. Beyond species-level evaluation, the review synthesises ecological signals arising from trophic interactions, community assembly dynamics, ecological networks, and landscape-scale processes that collectively govern freshwater ecosystem integrity. Additionally, emerging integrative frameworks incorporating artificial intelligence, machine learning, remote sensing, and real-time environmental surveillance are further highlighted as critical components of next-generation biomonitoring. Collectively, these interdisciplinary advances support a transition from reactive biodiversity assessment toward predictive, ecosystem-centred freshwater monitoring capable of strengthening ecological forecasting, conservation prioritisation, and adaptive environmental management.

KEYWORDS: Freshwater biomonitoring, Aquatic insects, Functional traits, Environmental DNA (eDNA), Ecological networks, Freshwater ecology

1. INTRODUCTION

Freshwater ecosystems are among the most ecologically critical and biologically diverse on the planet and serve as a home for vast biodiversity and source for critical ecosystem services like drinking water, nutrient cycling, fisheries, irrigation and climate regulation. Freshwater ecosystems are a global crisis for biodiversity due to the ongoing degradation, which has caused widespread biodiversity loss, ecological process changes and decreases in ecosystem resilience (Albert et al., 2021). Rivers, lakes, streams and wetlands cover only a small fraction of the earth's surface, but account for a disproportionately large amount of species diversity and ecological productivity. These ecosystems are now under growing threat from a variety of anthropogenic pressures such as pollution, fragmentation, alteration of hydrological flow regimes, invasive species, urbanization, agricultural intensification and climate change. Also, a combination of multiple stressors often has synergistic impacts at both spatial and temporal scales that exacerbate ecological instability and make conservation and management in freshwater systems more challenging (Birk et al., 2020).

Today's ecological fragility of freshwater ecosystems has made it more critical than ever to have an effective biomonitoring program to identify ecological degradation and to help guide environmental management based on science. Traditional monitoring of freshwater heavily involved physicochemical assessments based on dissolved oxygen and nutrient concentrations, pH and conductivity. While these methods are still useful, they can only offer a snapshot of environmental conditions and can't reflect cumulative responses of biology to ecological disturbance. Biological evaluation methods therefore became more useful and informative methods to assess freshwater ecosystem health. Aquatic insects are considered important in biomonitoring as they are sensitive to habitat alterations and pollution and at the same time they provide some integration of the environment over time, especially in Macroinvertebrate-based monitoring (Babafemi et al., 2024). Biomonitoring became more important for freshwater conservation in the aftermath of historical water quality legislation and environmental governance developments, particularly after the enactment of several key legislation, such as the Clean Water Act, that paved the way for long-term improvements in aquatic ecosystem assessment and restoration practices (Courtemanch et al., 2023).

Aquatic insects play an important ecological role in freshwater food webs and models, are sensitive to environmental changes and are characteristic of habitats when considering freshwater bioindicators. Some of the better-known groups that exhibit differential tolerances to environmental stress and represent ecological integrity along with disturbance gradients are the Ephemeroptera, Plecoptera and Trichoptera. In addition to their taxonomic importance, aquatic insects play a significant role in the functioning of ecosystems in the processes of organic matter decomposition, nutrient cycling, energy transfer and trophic regulation. They are thus important ecological indicators of ecosystem health and resilience when they respond to environmental change (Arthington et al., 2018). Further, there is growing focus on the role of biological invasions and the loss of functional diversity on the structure and stability of freshwater ecosystems, and a need to integrate more ecological dimensions in biomonitoring (Aleixo, 2025).

Although the traditional biomonitoring methods using taxonomic data have proven to be effective, there are still significant limitations. Conventional approaches have difficulties in capturing cryptic biodiversity loss, functional changes and early signs of ecological changes due to complex environmental stressors. In many freshwater ecosystems, there are several factors that affect the biological community, and an evaluation based on one indicator alone is not enough to interpret the ecology of an ecosystem. Macroinvertebrate community and ecosystem attributes have been shown to be dynamic response to the combination of stressors—such as nutrient enrichment, hydrological modification, and land-use change—and therefore will require more integrative approaches to monitoring (Calabrese et al., 2020). As a result, freshwater biomonitoring is moving towards a more multi-faceted approach to better capture the functional, molecular, and ecological processes at the ecosystem level and to better assess the environment in more precise, predictive, and mechanistic ways.

In this context, the present review aims to investigate how freshwater biomonitoring is evolving by integrating the functional, molecular and ecological signals provided by aquatic insect assemblage. The review discusses new developments in functional trait ecology, environmental DNA technologies, ecological network analysis and predictive ecosystem assessment and emphasizes new interdisciplinary approaches that will enable next generation freshwater ecosystem monitoring and adaptive conservation.

2. Ecological Foundations of Aquatic Insect Biomonitoring

2.1 Diversity and Functional Roles of Aquatic Insects

One of the most diverse and ecologically important groups of insects found in freshwater ecosystems is aquatic insects, which are found in rivers, streams, wetlands, ponds, and lakes under various environmental conditions (Dudgeon, 2024). Major orders of freshwater insects such as Ephemeroptera, Plecoptera, Trichoptera, Diptera, Coleoptera, and Odonata have remarkable adaptations that allow them to survive different hydrological, thermal and chemical conditions. They have a great ecological diversity, enabling them to occupy several trophic levels and habitat niche which makes them a very sensitive indicator of ecosystem condition and biodiversity change in freshwater environments.

In addition to their taxonomic diversity, aquatic insects serve critical trophic and ecological functions which help govern ecosystem productivity and energy transfers. Organic matter decomposition, periphyton regulation, sediment processing and nutrient transformation in freshwater food webs are facilitated by the functional feeding groups like shredders, scrapers, collectors, filter feeders, and predators. They can directly participate in the biogeochemical cycling processes by enhancing the degradation of allochthonous and autochthonous organic matter, thus maintaining the metabolism and ecological resilience of an ecosystem. Their interactions with microbial communities and higher trophic levels further boost ecosystem connectivity and freshwater ecological stability (Enuneku & Isibor, 2021).

2.2 Traditional Biomonitoring Metrics and Indices

Traditional freshwater biomonitoring programs have heavily used macroinvertebrate-based indices that are used to assess ecological quality and environmental disturbance. One of the most common methods is EPT richness, which is the number of species and individuals of Ephemeroptera, Plecoptera, and Trichoptera (EPT) which are sensitive to pollution and habitat degradation. Taxa-specific tolerance values are used to give a standardised assessment of water quality through complementary indices including the Biological Monitoring Working Party (BMWP) score and the Average Score Per Taxon (ASPT). Diversity metrics also help assess the ecological heterogeneity and biological integrity of freshwater habitats (Curd et al., 2024).

Use of these indices has greatly advanced ecological assessment, providing a cost-effective, biologically meaningful approach to monitoring a wide variety of freshwater systems. Biotic integrity indices combine several ecological features, such as species composition, tolerance, and trophic diversity, and provide an ecosystem-level interpretation that is more comprehensive than an interpretation based on purely physicochemical measurements. The use of molecular tools and the development of reference libraries in conjunction with traditional taxonomic indices is becoming increasingly common in contemporary ecological monitoring for greater taxonomic resolution and ecological accuracy. New bioinformatic tools also help to make the assessment of freshwater biodiversity more standardized, increasing the reliability of biomonitoring and freshwater ecological diagnostics.

2.3 Strengths and Limitations of Conventional Approaches

The ecological sensitivity, methodological frameworks and ability to accumulate environmental conditions over time makes conventional aquatic insect biomonitoring approaches very useful. There is often a predictable response of aquatic macroinvertebrates to nutrient enrichment, toxic contamination, habitat fragmentation and hydrological disturbance, and this may be used to reliably interpret ecological condition of freshwater systems. Taxonomic identification also enables long-term ecological comparisons and analyses of temporal trends in freshwater ecosystems. In the field of freshwater ecological research, these approaches are relatively inexpensive, readily available, and well scientifically grounded, and are therefore still used in regulatory assessment programs and for the implementation of environmental policies.

Conventional taxonomy-based biomonitoring, however, has a number of significant methodological and ecological constraints. Species-level identification often demands specific expertise and morphological similarity of cryptic species can limit taxonomy accuracy and ecological interpretation (Deiner et al., 2016). The variation in species composition, environmental conditions, and sampling across regions also adds to the difficulty of making comparisons and standardizing across systems. Community structure may also be affected independently of anthropogenic stress by temporal changes that occur as a result of seasonal emergence patterns and hydrological changes. Molecular approaches to ecology, including the use of environmental DNA, are growing in use and promise to complement traditional monitoring and improve the detection of biodiversity and the spatial ecological resolution.

2.4 Ecological Interpretation of Community Responses

The communities of aquatic invertebrates are highly dynamic when the environment changes and are good indicators of the integrity of the ecosystem and changes of the environment. Often a gradient of pollution, habitat alteration, eutrophication, salinization and hydrological stress can be seen as reflected in changes in community composition. Under poor environmental conditions, sensitive species may decrease in abundance, while the more tolerant species that can survive low oxygen levels or high levels of contaminants may increase in abundance. These ecological changes offer valuable information on the functioning and resilience of freshwater ecosystems and their biological communities, which in turn will help researchers understand the cumulative effects of anthropogenic activities on biological communities and ecological stability.

Pollution tolerance and the restructuring of communities due to disturbances are of particular significance for interpretation of ecosystem health in increasingly stressed freshwater ecosystems. Multiple stressors (chemical, physical, and/or biological) can interact and combine to affect ecological responses by impacting the quality of habitats and trophic organization. Considerable changes in macroinvertebrate assemblage and ecosystem processes may occur as a result of industrial discharge, desalination by-products, changes in salinity regimes or nutrient enrichment, which in turn may impact ecological integrity and biomonitoring success. Therefore, integrated ecological interpretation that is able to capture the complex gradient of disturbance and multidimensional ecosystem response across space and time is increasingly becoming the focus of contemporary freshwater assessment (DE NORMALISATION & NORMUNG, 2024).

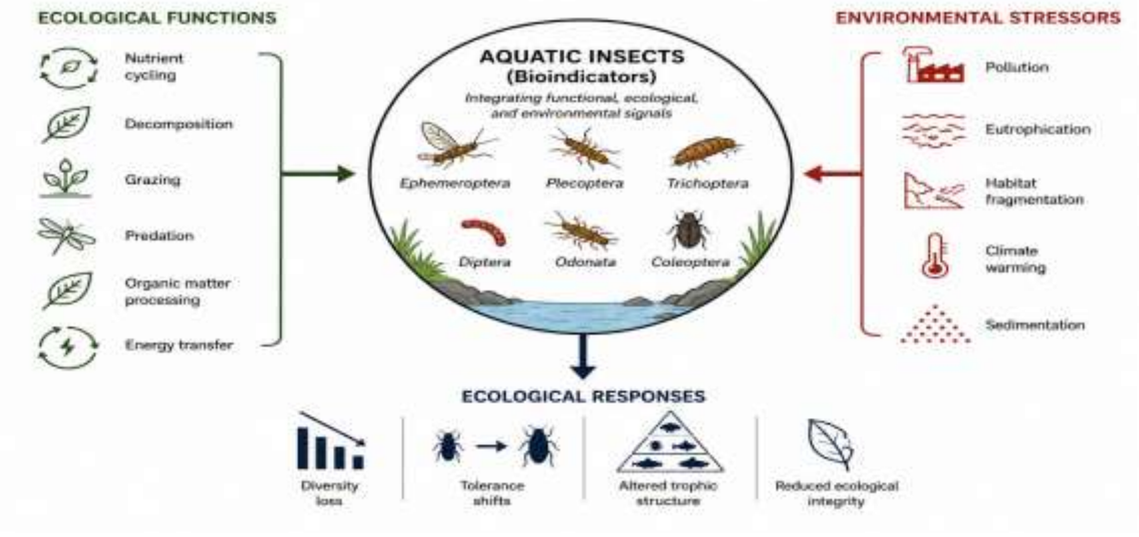

Figure 1. Aquatic Insects as Multi-Dimensional Bioindicators in Freshwater Ecosystems

Figure 1 shows the ecological function, environment stressors and ecosystem responses of aquatic insects providing functional, ecological and environmental signals for comprehensive freshwater biomonitoring and ecosystem assessment.

3. Functional Signals in Freshwater Biomonitoring

3.1 Transition from Taxonomic to Functional Approaches

The practice of freshwater biomonitoring has been steadily moving from a more traditional taxonomy-driven approach to an approach focused on ecosystem processes and organismal responses to environmental change. Taxonomic methods are still useful tools for biodiversity assessment, but they might fail to give a mechanistic understanding of how complex ecosystem functions under particular disturbance regimes (Hamilton et al., 2020). To overcome these shortcomings, functional ecology arose, emphasizing species traits that control ecological interactions, resource use and adaptation to the environment. Disturbance sensitivity and ecological reorganization can thus be assessed more effectively by functional traits in heterogeneous freshwater systems, for which they provide greater predictive power for ecosystem resilience.

As more and more species are known to be essential to ecosystem processes and functional organization, there has been a growing emphasis on functional approaches. Functional traits provide ecological indicators by directly connecting the characteristics of organisms with gradients and ecosystem functioning. Characteristics related to respiration, locomotion, feeding behaviour, thermal tolerance and reproduction give valuable clues as to community adaptation under anthropogenic stress. Thus, functional ecology can improve the ecological interpretation of freshwater biomonitoring to better support freshwater adaptive ecosystem management, conservation prioritisation and restoration planning in highly variable freshwater systems, (Gaiser, 2024).

3.2 Functional Traits of Aquatic Insects

Aquatic insects have a variety of ecological roles and sensitivities, which are reflected in their morphology, physiology, behavior and life history traits. Morphological characteristics (body size, type of gills, composition of exoskeleton, mechanism of attachment) affect the occupation of habitat, mobility and resistance to the hydrological stress. Under varying environmental conditions, physiological traits such as respiration strategies, thermal tolerance and contaminant sensitivity determine survival. Behavioral traits related to drift activity, feeding specialization, and predator avoidance also influence ecological interactions and life-history traits like voltinism, emergence timing, and reproductive strategy influence the resilience of populations and persistence of communities along gradients of disturbance.

Trait-based ecological assessment gives the mechanistic understanding of the response of freshwater communities to the environmental stress and ecosystem transformation. Functional traits are able to incorporate ecological processes at multiple biological scales and thus can be used to assess ecosystems more predictively than taxonomic metrics alone. In the field of freshwater research, there is a growing trend of using molecular ecology and next-generation sequencing techniques to enhance trait characterization and biodiversity resolution in biomonitoring contexts (Esser et al., 2024). These integrative approaches improve the ability to detect ecological change by connecting the dots between genetic diversity, functional adaptation, and ecosystem functioning, and therefore further our robust capacity to conserve freshwater and to forecast ecological responses to growing environmental pressures.

Table 1. Functional Traits of Aquatic Insects in Freshwater Biomonitoring

Trait Category	**Example Traits**	**Ecological Function**	**Biomonitoring Significance**	**Supporting Reference**
Morphological	Body size, gills	Habitat adaptation	Detect flow disturbance	Hamilton et al. (2020)
Physiological	Thermal tolerance	Stress survival	Indicate pollution effects	Escobar Restrepo et al. (2025)
Behavioral	Drift, feeding behavior	Resource utilization	Reflect habitat alteration	Gaiser (2024)
Life-history	Voltinism, emergence timing	Population persistence	Assess ecosystem stability	Esser et al. (2024)
Functional integration	Trait combinations	Ecosystem resilience	Improve predictive monitoring	Guareschi et al. (2021)

The key functional trait categories of aquatic insects are summarized in Table 1, along with their importance to freshwater biomonitoring. Morphological, physiological, behavioural and life-history traits all contribute to habitat adaptation, ecosystem functioning and responses to environmental stress. These traits are useful for ecological integrity assessment, disturbance gradients, assessing ecosystem resilience and predictive freshwater environmental monitoring.

3.3 Functional Feeding Groups and Ecosystem Processes

Since they positively control the energy flow, organic matter decomposition and trophic relations in aquatic food webs, functional feeding groups form a fundamental aspect of freshwater ecosystem organization. Shredders break up large organic matter, like leaf litter, allowing microbes to colonize and nutrients to be released, while scrapers eat algae and biofilms growing on underwater surfaces. For fine particulate organic matter, collectors remove the fine POM in the water column or in the sediments, and filter feeders retain the suspended POM, which is involved in the nutrient cycle. Predatory taxa control prey populations and trophic balance, thus strengthening the ecological stability and ecosystem functions.

The distribution and abundance of functional feeding groups serves as a valuable ecological signal to habitat quality, resource availability and ecosystem disturbance. Changes in hydrology, substrate, riparian plants, and nutrient regime often affect the structure of the trophic organization and the metabolism of freshwater ecosystems. Thus a sensitive indicator of ecological change and environmental degradation is the functional feeding structure. As a result, there is a growing recognition of the need to include trophic and functional evaluations to assess ecosystem recovery and ecological resilience in the development of freshwater ecology restoration programmes, and the importance of maintaining a balanced trophic organisation to sustain freshwater biodiversity and ecosystem services under changing environmental conditions.

3.4 Functional Diversity and Ecosystem Resilience

Functional diversity encompasses the diversity of organismal traits within biological communities, as well as their range and distribution and ecological complementarity, and is a key factor in the resilience of ecosystems. Functional richness is an indicator of how diverse communities are in terms of the variety of ecological strategies they contain; functional redundancy is an indicator of how similar the ecological roles of different species are. Functional evenness is also a measure of the balance of trait distribution among taxa and adds to the stability of the ecosystem under disturbance. Together, these aspects of functional diversity affect the resistance, recovery, and sustainability of freshwater ecosystems. In general, the higher the level of functional diversity, the more resilient the ecosystem, since ecological processes are stabilized and ecosystems are more resilient to environmental fluctuations (Guareschi et al., 2021). But, anthropogenic disturbances often diminish functional heterogeneity and modify ecosystem functioning, through biological invasions, ecosystem modifications, and pollution. The presence of invasive species can affect native diversity, alter food webs and ecological dynamics, and affect functional diversity metrics and biomonitoring interpretation. Therefore, the understanding of the change in functional diversity has been gaining importance to assess ecological integrity, predict ecosystem response to disturbance and inform conservation efforts for sustaining freshwater ecosystem resilience.

3.5 Functional Responses to Environmental Stressors

Aquatic insect functional traits are dynamic and sensitive to a variety of environmental stressors such as pollution, eutrophication, hydrological alteration, urbanization and climate warming. Heavy metals, pesticides and industrial contaminants are pollutants that are often responsible for respiratory dysfunction, reproductive issues, feeding efficiency and physiological performance in freshwater invertebrate communities (Escobar Restrepo et al., 2025). Nutrient enrichment and eutrophication also affect oxygen regimes and the trophic structure, with the result that nutrient tolerant species replace ecologically sensitive species. The hydrological changes linked to dams, channelisation and changes in water flow regimes further break the connectivity, sediment transport and community assembly, changing the functioning of freshwater ecosystems and biological integrity.

Climate change and industrial disturbances are becoming more intertwined and add to the ecological stressors in freshwater ecosystems, leading to complex functional responses in the aquatic organisms. Thermal warming affects metabolism, emergence time, dispersal patterns and species distribution, and urbanization adds other chemical and hydrological stresses that disrupt ecological communities. Mining-induced contamination might also result in bioaccumulation effects and microbial adaptation of the freshwater food web, thereby changing the ecological interactions and the function of the ecosystem. These responses to stress in multiple dimensions underscore the need for trait-based ecological assessment to identify incipient ecosystem instability, and to enhance predictive freshwater biomonitoring in the context of rapid environmental change.

3.6 Advantages and Constraints of Functional Biomonitoring

The use of functional biomonitoring has several important benefits over traditional taxonomic approaches, including its focus on ecosystem function, mechanistic responses and ecological processes, instead of just species composition. Functional traits allow for more generalisations over geographic space and have greater predictive power for assessing ecosystem resilience to environmental perturbations. Trait-based approaches also allow for integration across trophic levels and ecological scales and thus better interpretation of stress and recovery processes in ecosystems. Such methods are increasingly being understood as necessary tools in 21st century freshwater monitoring and are effective in providing more holistic and process-based ecological monitoring.

However, functional biomonitoring has methodological and conceptual shortcomings that limit its application. For many freshwater taxa, trait databases may not be complete, and traits may be expressed differently in various environmental contexts and spatial scales. Functional redundancy may also mask ecological responses, where similar ecosystem functions are provided by different species. Also, associating traits directly to stressors can be difficult when multiple stressors are affecting a system at the same time. Limiting the use of functional biomonitoring in freshwater ecological assessment programs universally is still hindered by standardization challenges, data integration problems, and trait assignment uncertainties.

4. Molecular Signals and the Genomic Transformation of Biomonitoring

4.1 Emergence of Molecular Ecology in Freshwater Monitoring

Freshwater biomonitoring has been revolutionised by molecular ecology, which allows the rapid, large-scale and very sensitive assessment of biodiversity, across aquatic ecosystems. Morphological identification techniques are often limited by cryptic taxa, damaged specimens and lack of taxonomic knowledge, especially in the highly diverse freshwater environment. Molecular methods have the ability to circumvent many of these limitations by directly identifying genetic signals of species in the environment. The growing use of genomic tools in ecological assessment is a common theme around the world, with a global effort to enhance the conservation, surveillance and sustainability of freshwater systems that are all the more imperiled (IPBES, 2019).

The advent of molecular monitoring of freshwater ecosystems has been particularly significant in areas where ecological deterioration, a lack of infrastructure and taxonomic expertise prevent traditional biomonitoring. DNA-based technologies enable better evaluation of biodiversity and a higher sensitivity for detecting ecologies under various conditions. Molecular ecology also offers fast and cheap ecological monitoring of less studied and distant freshwater environments, adding to the conservation planning and environmental management. Advancements in sequencing technologies and bioinformatic tools have further boosted the use of molecular biomonitoring in global freshwater ecosystems management and ecological restoration efforts (Izah et al., 2024).

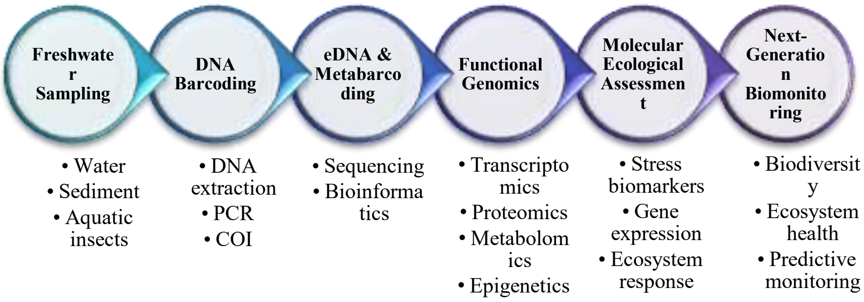

Figure 2. Molecular and Genomic Transformation of Freshwater Biomonitoring

The next generation of freshwater biomonitoring frameworks and predictive ecosystem evaluation starts with freshwater sampling, and then moves on to DNA barcoding, eDNA metabarcoding, functional genomics and molecular ecological assessment (Figure 2).

4.2 DNA Barcoding and Species Identification

As one of the most powerful molecular methods, DNA barcoding has become a major tool in freshwater biomonitoring as it allows the identification of species based on the same standardized genetic markers. The gene coding for the cytochrome c oxidase (COI) has been extensively used as a universal barcode for freshwater taxa, allowing identification of cryptic biodiversity which may not be identified by morphology. DNA barcoding has proved to be a powerful tool for increasing the level of taxonomic resolution in aquatic insect assemblages and has enabled more accurate ecological assessment in all types of freshwater ecosystem (Kajee et al., 2023). The creation of complete reference libraries also improves accuracy and repeatability in the identification of biodiversity in freshwater monitoring program.

DNA barcoding has been a major contributor to the ecological knowledge of freshwater biodiversity patterns, species distributions and conservation priorities. Molecular identification methods help reduce the errors caused by the morphological ambiguity and incomplete preservation of specimens, and help to conduct rapid biodiversity surveys. The correct identification of species is essential to the detection of ecological change, invasive species and declining biodiversity in increasingly disturbed freshwater systems. Spatial biodiversity assessments, also using molecular tools, also improve the protection of ecosystems by delineating high priority conservation areas and freshwater communities ecologically at risk at large spatial scales.

4.3 Environmental DNA (eDNA) Approaches

A major new step in freshwater biomonitoring is the use of environmental DNA (eDNA) approaches, which allow the detection of biodiversity based on DNA excreted, shed as tissue, gametes or during decomposition in aquatic environments. Thus, water samples can contain valuable information about the ecology of the system, and can be used to determine the presence of species without actually capturing organisms. eDNA monitoring is a great alternative to traditional methods of sampling since it decreases the disturbance of the habitat, increases detection sensitivity, and allows for large-scale assessment of biodiversity in a variety of freshwater habitats. These are increasingly used for monitoring aquatic insects, fishes, amphibians and microbial communities in freshwater environments.

The ecological significance of eDNA methods is that they can provide signals of overall spatial biodiversity and allow detection of rare, elusive or low abundance species that cannot be detected using traditional surveys. Upstream water-column DNA transport further allows for the evaluation of upstream ecological conditions and catchment level patterns of biodiversity. eDNA methods thus contribute to more robust and thorough surveillance and ecological forecasting in the face of accelerating environmental change. Their use as part of monitoring in freshwater systems has greatly enhanced the ability to assess ecological integrity, conservation effectiveness, and response of freshwater biodiversity to anthropogenic stressors across freshwater landscapes.

4.4 Metabarcoding and High-Throughput Sequencing

Freshwater biodiversity assessment has been dramatically transformed by metabarcoding and high throughput DNA sequencing which allow simultaneous and rapid analysis of complex communities of organisms from bulk environmental samples. These are based on universal genetic markers and next generation sequencing platforms for the identification of multiple taxa in an efficient and fast manner in aquatic systems. Metabarcoding offers greater taxonomic resolution and ecological coverage than traditional surveys based on morphology. These technologies have gained in significance in the assessment of the ecological integrity, ecological disturbance and biodiversity dynamics in freshwater ecosystems under conditions of several anthropogenic pressures.

High throughput sequencing also helps the design of powerful bioinformatic pipelines for the analysis of big ecological datasets and the identification of fine changes at the community level (Juvigny-Khenafou et al., 2021). Molecular community analyses help to understand species interactions, trophic organization and ecological resilience in freshwater environments. Research on multiple anthropogenic stressors has shown that the composition and functional diversity of the freshwater macroinvertebrate community is dynamically influenced by interacting environmental perturbations and that integrative molecular approaches are important for ecological interpretation and ecosystem management in the face of ever increasing freshwater stressors.

4.5 Beyond Taxonomy: Functional Genomics and Multi-Omics

Biomonitoring in freshwater has gone beyond the usual species identification to functional genomics and multi-omics approaches that can help to understand processes in the ecosystem at the molecular and cellular level. Transcriptomics focuses on gene expression changes in response to the environment and proteomics on physiological adaptation of aquatic organisms in terms of protein synthesis. Biochemical pathways related to stress responses, nutrient dynamics and ecological interactions are also studied through metabolomics. Epigenetic studies also investigate environmentally triggered changes in the regulation of genes, which gives more insights to adaptive mechanisms in freshwater ecosystems under environmental disturbance.

Combining multi-omics technologies with biomonitoring will provide enhanced ecological interpretation, linking genetic processes to organismal function and ecosystem dynamics. The use of functional genomics allows the early detection of molecular responses to environmental stress, even prior to ecological degradation, which can enhance the ability to predict ecosystem impacts of environmental stressors. They are especially useful for the assessment of the ecological adaptation in the face of very fast environmental changes related to pollution, thermal stress, habitat changes, and nutrient changes. Molecular ecosystem diagnostics are now increasingly supporting more accurate freshwater management approaches that can deliver biodiversity and long-term ecosystem sustainability.

4.6 Molecular Biomarkers of Environmental Stress

Molecular biomarkers are highly sensitive indicators of environmental stress in freshwater organisms, and are able to identify physiological and cellular responses of an organism to environmental disturbance. Biomarkers like heat shock proteins, oxidative stress enzymes, and/or changes in gene expression patterns show sublethal effects of pollutants, thermal fluctuations, and chemical exposure prior to the appearance of community-level effects. These molecular responses are also early-warning signs of ecosystem instability and environmental degradation. Using biomarker-based methods to complement freshwater biomonitoring allows more sensitive detection of ecological change and facilitates the understanding of the underlying mechanisms of organismal responses to a changing environment.

Beyond pollution detection, the ecological relevance of molecular biomarkers is also linked to physiological responses, which are often related to more global changes in the ecosystem, such as urbanization, eutrophication and climate change. Biomarker analysis also helps to define ecological thresholds and ecological limits of resilience in freshwater ecosystems exposed to chronic environmental stress. Combining molecular biomarkers with ecological and functional assessment frameworks thus enhances the ability to predict ecosystem trajectories and assess the effectiveness of conservation efforts. These methods are now known to be important tools for future "next-generation" freshwater monitoring and adaptive environmental management in ecologically sensitive freshwater systems.

4.7 Challenges and Limitations of Molecular Biomonitoring

Although these molecular biomonitoring methods have great promise for transformation they also have a number of methodological and technical challenges that limit their more widespread use. The accuracy of detecting biodiversity in an ecosystem and of its representativeness might be decreased by primer bias in the DNA amplification process, which has the potential to selectively amplify certain groups of taxa. Molecular detection reliability is further affected by degradation of DNA from environmental factors like temperature, UV radiation and microbial activity. The databases for species identification are also incomplete, which also hinders the precision of species identification, especially for less explored freshwater species and isolated ecosystems. Cross-study comparisons and ecological interpretation are also confounded by standardization issues with sampling protocols, sequencing methods and bioinformatic analyses.

The accessibility and scalability of molecular biomonitoring programs are still constrained by economic and technological factors, especially in developing regions where there is a shortage of research infrastructure (Kumar et al., 2024). The cost, computing power and knowledge of specialized analysis involved with advanced genomic technologies can limit their uptake. In addition, in highly dynamic freshwater environments, methodologically interpreting the molecular ecological signal is difficult since ecological responses can be mediated by interacting environmental variables. However, with the recent development of new sequencing techniques, bioinformatics tools and sustainable river management strategies, the feasibility and ecological benefit of molecular freshwater biomonitoring systems are constantly moving forward.

5. Ecological Signals and Ecosystem-Level Interpretation

5.1 Ecological Integrity and Ecosystem Health

Ecological integrity is an indicator of the ability of freshwater ecosystems to sustain biological organization, ecosystem function and resilience in the face of natural and anthropogenic stressors. Biodiversity–function relationships are increasingly being used to assess ecosystem stability and sustainability and are being taken into account in ecological condition assessment in addition to species richness. Ecological integrity is a multidimensional concept that is central to freshwater conservation and adaptive environmental management in an increasingly disturbed aquatic landscape where functional connections between aquatic organisms drive the nutrient cycle, productivity and trophic balance (Martini et al., 2021).

5.2 Community Assembly and Environmental Filtering

The niche-based processes, dispersal dynamics, and environmental filtering that control community assembly in freshwater ecosystems act together to structure the composition and ecological interactions of species. Pollution, habitat modification or hydrological alteration can cause disturbance-driven community change where there is an increase in tolerant taxa and a decline in sensitive organisms. This ecological filtering affects functional diversity and ecosystem processes and consequently the resilience of freshwater systems and ecological stability. A knowledge of these assembly mechanisms is thus crucial in interpreting biomonitoring results and modelling ecosystem response to environmental stress.

5.3 Food-Web Dynamics and Trophic Interactions

Complex, predator–prey and trophic interactions influence the flow of energy and the functioning of freshwater ecosystems. Aquatic insects are at a number of trophic levels and play a large role in decomposition, nutrient transformation, and ecosystem metabolism. Changes in the trophic organization, the microbial activity, and ecological efficiency of the aquatic food webs could be affected due to disturbances such as contamination with wastewater or chemical pollution. Thus, trophic interactions are valuable sensitive responses to ecological imbalance and ecosystem condition in freshwater stressed ecosystems (Makut et al., 2022).

5.4 Ecological Networks and System Resilience

Ecological networks are complex webs of interdependent biological interactions, which govern ecosystem resilience, stability and functional connectivity in freshwater systems. Predator/prey, decomposer and microbial interactions are important in determining the resistance of ecosystems to environmental perturbations and ecological persistence. For a stable network, there should be ecological balance and functional redundancy between trophic levels. An ecological network analysis of a biomonitoring system has become increasingly common to understand the dynamics of ecosystems and freshwater ecological reorganization in anthropogenically disturbed environments.

5.5 Landscape and Catchment-Level Influences

Watershed land use, riparian vegetation structure, and hydrological connectivity are key landscape-scale processes that have a strong influence on freshwater ecological condition. Freshwater catchments are often subject to changes in sediment transfers, nutrient inputs, and habitat complexity in the context of land use change, particularly agricultural expansion, urbanization, and deforestation (Masese et al., 2025). Riparian vegetation also helps to control temperature, organic matter input and bank stability, which helps to support aquatic biodiversity and ecosystem functions. Catchment-scale ecological management is therefore needed to address the complex landscape drivers to ecosystem degradation and biodiversity loss and is essential for effective freshwater conservation.

Table 2. Landscape and Catchment-Level Drivers Affecting Freshwater Ecosystem Integrity

Landscape Factor	Ecological Impact	Effect on Aquatic Insects	Ecosystem Consequence	Biomonitoring Significance	Supporting Reference
Agricultural land use	Nutrient enrichment	Decline of sensitive taxa	Eutrophication	Indicates water quality degradation	Masese et al. (2025)
Urbanization	Habitat alteration	Reduced biodiversity	Ecological instability	Reflects anthropogenic stress	Martini et al. (2021)
Riparian vegetation loss	Increased temperature	Altered feeding structure	Reduced ecosystem resilience	Assesses habitat integrity	Lamy et al. (2020)
Hydrological fragmentation	Disrupted connectivity	Impaired dispersal	Community restructuring	Detects flow disturbance	Machuca-Sepúlveda et al. (2023)
Deforestation	Increased sedimentation	Habitat smothering	Reduced ecological function	Evaluates catchment disturbance	Makut et al. (2022)

Table 2 provides an overview of the main landscape and catchment drivers affecting the integrity of freshwater ecosystems and aquatic insect assemblage. Various factors, including agricultural land use, urbanization, loss of riparian vegetation, fragmentation of hydrology, and deforestation have a profound impact on habitat, biodiversity, and ecosystems. All are environmental factors that are important ecological indicators for freshwater biomonitoring and ecosystem assessment.

5.6 Climate Change and Ecological Reorganization

Extensive ecological reorganization of freshwater system is being caused by the thermal stress, hydrological change, phenological change, and distribution range change due to climate change. Aquatic organisms' metabolic performance, reproductive timing, and dispersal patterns are affected by rising temperatures, often with negative effects on biodiversity and community homogenization. International freshwater sustainability frameworks are currently increasingly focusing on integrated governance of inland water ecosystems, and adaptive ecological monitoring to enhance resilience in inland waters facing increased climatic pressures (Lamy et al., 2020).

Demonstrating the change of ecosystems in reaction to climate change is being increasingly achieved using new molecular and omics-based ecological assessment tools. A transcriptomic, metabolomic and genomic approach can identify physiological stress responses and biodiversity changes at the community level, before ecological degradation can be seen (Machuca-Sepúlveda et al., 2023). A system like this integrative monitoring will enhance the ability to make predictions on how freshwaters are managed and conserved by connecting molecular responses with the ecological dynamics of the ecosystem. As such, omics technologies are increasingly gaining in significance for the understanding of ecological reorganization and ecological resilience in the face of a continuous change in the global environment.

6. Integrating Functional, Molecular, and Ecological Signals

6.1 Rationale for Multi-Signal Biomonitoring

Traditional one-metric biomonitoring systems are often inadequate to adequately reflect the ecological complexity of freshwater systems subjected to multiple stressors that interact. Taxonomic indices do not necessarily capture functional disruption, molecular adaptation and ecosystem level instability that can precede a detectable drop in biodiversity (Menzie et al., 2024). As a result, multi-signal biomonitoring systems to assess ecosystems using functional, molecular and ecological indicators are more complete. Combining several ecological signals helps increase the accuracy in diagnostics, ecological forecasts and environmental risk assessments in a more dynamic freshwater environment.

6.2 Linking Traits, Genes, and Ecological Processes

Combining functional traits with genomic and ecological data allows interpretation of the dynamics of freshwater systems and organism responses to environmental disturbance across scales. Traits from metabolism, dispersal, reproduction and trophic interactions can be related to gene expression patterns and ecological processes to find mechanistic pathways leading to stress in an ecosystem. This integration enhances the knowledge of adaptive capacity, resilience and ecological reorganization of aquatic communities. Thus, molecular and genomic technologies enhance predictive ecosystem assessment and improve biodiversity conservation strategies amidst the fast-changing environment (Ramzan et al., 2025).

6.3 Integrated Biomonitoring Frameworks

Integrated biomonitoring frameworks integrate ecological, functional and molecular indicators in multidimensional systems that can provide insights into ecosystem complexity over ecological and temporal scales. These frameworks support ecosystem intelligence approaches which combine patterns of biodiversity, ecological interactions and environmental stress responses into synergic monitoring strategies (Michez et al., 2025). Effective implementation further depends on the standardisation of data management systems that are capable of guaranteeing interoperability, transparency and long-term ecological accessibility of data. Today, there is a growing need for integrated ecological databases and coordinated monitoring infrastructures to be a critical part of next-generation freshwater biomonitoring programs.

6.4 Artificial Intelligence and Predictive Biomonitoring

AI and machine learning are revolutionizing freshwater biomonitoring, enhancing automated taxa identification, ecological forecasting, and the interpretation of environmental data. Ecological data, generated in vast quantities, can be analysed using machine learning algorithms to find patterns in biodiversity, to predict how ecosystems will react and to detect subtle changes in an ecosystem that could be linked to environmental disturbance. Automated image recognition systems also enable fast identification of aquatic taxa and minimize taxonomy uncertainty. These predictive ecological modeling methods enhance real-time ecological assessment and adaptive management in dynamic freshwater ecosystems.

6.5 Remote Sensing and Digital Freshwater Monitoring

The use of remote sensing technologies and digital monitoring systems is becoming more common in freshwater ecological assessment, providing greater spatial extent and near real-time monitoring of ecosystems. Geographic Information Systems (GIS), environmental sensors and satellite-based observations provide the capability to monitor and track hydrology, land use, temperature, vegetation and water quality on a continuous basis at the freshwater catchment scale. These technologies can assist in the detection of ecosystem degradation and habitat alteration on a large scale. This means that digital monitoring platforms enhance the freshwater conservation planning process and enable more dynamic freshwater ecosystem management under growing anthropogenic pressures.

6.6 Conceptual Framework for Next-Generation Biomonitoring

Upcoming freshwater biomonitoring systems focus on the inclusion of ecosystem level interpretation, molecular diagnostics and ecological function within a single predictive system. All four functional diversity metrics, genomic analyses, ecological networks, and digital surveillance give multidimensional information on ecosystem resilience and environmental stress responses. The effectiveness of these arrangements hinges on the ability to accurately describe the diversity of traits and interactions among ecological communities. Studies on the correlation of traits and functional diversity metrics are thus a valuable addition to ecological interpretation and robustifying integrative freshwater biomonitoring approaches (Ohlert et al., 2024).

Advanced integrative biomonitoring also provides ecosystem restoration and environmental remediation through the use of ecosystem diagnostics and adaptive intervention strategies. Microbial systems have become more and more important for environmental management by developing microbial systems that could support pollutant degradation and support the ecosystem's recovery (Nikel & de Lorenzo, 2021). These multidisciplinary strategies lead to increased ecological resilience and better freshwater sustainability in the context of ever-increasing anthropogenic disturbances. Thus, future biomonitoring programs are likely to increasingly be predictive, data-based and ecosystem-oriented in the context of global conservation of freshwater ecosystems.

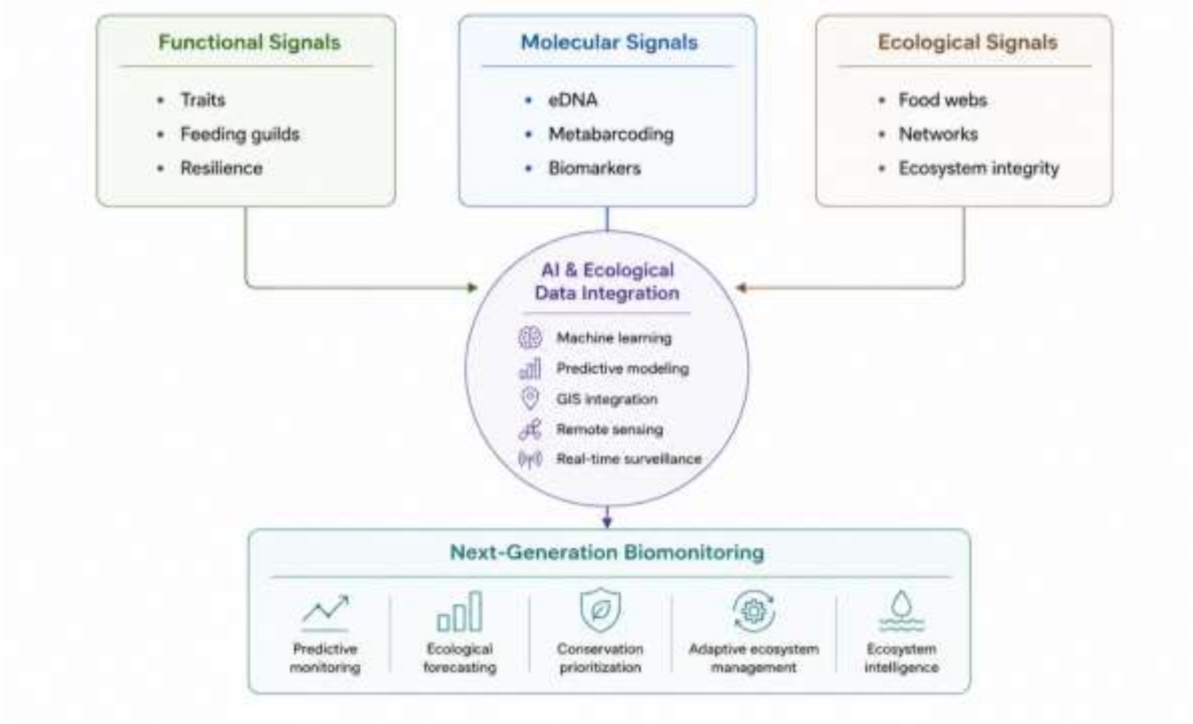

Figure 3. Integrated Framework for Predictive Freshwater Biomonitoring

A conceptual framework of how functional, molecular and ecological signals can be combined using artificial intelligence, predictive modelling, GIS integration and remote sensing to enable next generation freshwater biomonitoring, ecological forecasting, conservation prioritisation and adaptive ecosystem management is presented in Figure 3.

7. Challenges, Knowledge Gaps, and Methodological Constraints

7.1 Taxonomic and Geographic Biases

There are still many taxonomic and geographic limitations in freshwater biomonitoring that hamper interpretation and comparability of the results on an international scale. A lot of freshwater habitats are poorly studied, especially in tropical and developing regions and many aquatic groups are not fully described and have no reference database. Monitoring schemes tend to focus in the more developed parts of the world, leading to a bias in biodiversity representation and a poor understanding of global patterns of freshwater ecology. These differences limit the ability to create a common biomonitoring approach and conservation plan for ecologically sensitive freshwater systems (Tickner et al., 2020).

7.2 Standardization and Reproducibility Issues

One of the difficulties in freshwater biomonitoring is the lack of standardization across studies in sampling design, laboratory practices, taxonomy, and analytical methods, which can limit the ability to be reproducible. There are heterogeneities in ecological indicators and environmental assessment protocols, which make it difficult to carry out cross-system comparisons and long-term environmental ecological interpretation (Su et al., 2021). Comparisons of methods for assessing river health using biological data and indicator-based data have revealed significant method differences that can impact ecological findings and decision making. To improve freshwater ecological reliability and biomonitoring consistency, harmonized monitoring frameworks are needed, as are standardized analytical protocols.

7.3 Data Integration and Computational Challenges

Multidimensional biomonitoring data are becoming very complex and are a significant computational and analytical challenge for freshwater ecological research. The integration of this taxonomic, molecular, functional, spatial and environmental information demands cutting-edge computer systems as well as complex bioinformatic analysis methods that can process large ecological datasets. Data differences in their formats, scales and metadata standards also exacerbate the challenges of interoperability and synthesis for ecology. Thus, the ability to effectively integrate requires the development of strong digital platforms and analytical tools that enable ecological interpretation and predictive environmental assessment at the ecosystem scale.

7.4 Economic and Technological Barriers

Advanced freshwater biomonitoring methods are still limited by economic and technological factors for implementation, especially in resource poor areas. The technologies used for molecular sequencing, environmental sensors, remote sensing systems and the computational infrastructure typically demand significant investment and technical expertise. Emerging technologies to address environmental issues like bioengineering algae and nanoscale systems to monitor the environment show significant ecological promise, but are expensive and technically challenging for widespread adoption. These barriers will impact equal access to innovative biomonitoring systems and may reduce global freshwater conservation effectiveness (Selvaratnam et al., 2025).

7.5 Policy and Regulatory Constraints

The effective implementation of integrated frameworks for freshwater biomonitoring at the local, national and international levels is often constrained by policy and regulatory barriers. Environmental governance systems might fail to be coordinated, to commit to long-term monitoring or to apply an ecosystem-based approach to tackle multifaceted freshwater issues (Stocco et al., 2020). In many places, the approach to regulatory assessment continues to be focussed on physicochemical rather than multidimensional ecological measurements. This has led to the growing appreciation of the importance of enhanced policy integration, interdisciplinary governance, and environmental planning based on ecosystem services for sustainable freshwater management and ecological restoration.

7.6 Ethical and Conservation Considerations

Biomonitoring in freshwater systems is becoming more and more a concern in terms of the protection of biodiversity, the disturbance of ecosystems and the sustainable management of ecosystems. Intensive sampling, habitat modification and technological intervention could have unintended impacts on sensitive freshwater organisms and ecological interactions. Furthermore, freshwater ecosystems are ecologically complex because of their strong connections to the adjacent terrestrial ecosystems via trophic exchanges and ecological connectivity. Knowledge of such cross-ecosystem interactions is thus essential for ethically sound biomonitoring and sustainable conservation of biodiversity in freshwater ecosystems that are increasingly shaped by humans (Rideout et al., 2024).

Table 3. Major Challenges and Methodological Constraints in Freshwater Biomonitoring

Challenge Category	**Key Issue**	**Ecological/Methodological Impact**	**Biomonitoring Consequence**	**Supporting Reference**
Taxonomic and geographic biases	Incomplete biodiversity records	Uneven ecological representation	Limited global comparability	Tickner et al. (2020)
Standardization issues	Variable sampling protocols	Reduced reproducibility	Inconsistent ecological assessment	Su et al. (2021)
Data integration challenges	Complex multidimensional datasets	Computational difficulties	Limited ecosystem-scale interpretation	Rideout et al. (2024)
Economic and technological barriers	High infrastructure costs	Restricted technology access	Reduced monitoring efficiency	Selvaratnam et al. (2025)
Policy and regulatory constraints	Weak interdisciplinary governance	Fragmented management strategies	Limited conservation effectiveness	Stocco et al. (2020)

8. Future Perspectives in Freshwater Biomonitoring

8.1 Predictive and Real-Time Ecological Assessment

There is a growing trend towards freshwater biomonitoring based on predictive and real-time ecological assessment methods that identify ecosystem instability before destructive changes become irreversible. The development of environmental sensors, automated ecological monitoring systems, and AI (Artificial Intelligence) technologies is facilitating ongoing monitoring of ecosystems on both spatial and temporal scales. Real-time ecological diagnostics enhance adaptive management by helping to respond quickly to environmental conditions and anticipate conservation needs. These strategies should enhance the resilience and sustainability of ecosystems in increasingly vulnerable freshwater systems that are subject to increasing anthropogenic pressures.

8.2 Precision Freshwater Ecology

Precision freshwater ecology involves a highly targeted assessment of the ecology of the ecosystem employing advanced molecular, spatial and computational tools that are able to detect finer scale variation in ecology. The tools of DNA barcoding, genomic surveillance and high-resolution ecological modelling are enhancing the detection and ecological interpretation of biodiversity in complex aquatic systems (Torres et al., 2024). Yet, there are major gaps in the global molecular reference collection that reduce the level of biodiversity resolution in many freshwater areas. It will therefore be crucial to increase the number of molecules and ecological databases available to improve precision-based freshwater monitoring and conservation.

8.3 Citizen Science and Participatory Monitoring

Citizen science and participatory monitoring are now more recognized as a key component of freshwater ecological monitoring and information, and in the empowerment and involvement of the public in conservation of biodiversity. Community-based monitoring programs augment the data collection capacity over wide geographic areas and increase awareness and stewardship of the environment. Decentralized environmental reporting and ecological observation is further facilitated by digital platforms, mobile applications and collaborative ecological networks. The public is increasingly enlisted in participatory science projects, contributing to the enhancement of biodiversity monitoring and ecosystem resilience and showing the importance of linking public engagement to scientific research (Walker et al., 2025).

8.4 Biomonitoring in Conservation and Restoration Ecology

By supporting ecosystem recovery, biodiversity protection, and sustainable environmental management, freshwater biomonitoring is likely to be increasingly at the heart of conservation and restoration ecology. Ecological indicators based on functional, molecular and ecosystem-level assessment can help to enhance the assessment of restoration success and long-term ecosystem resilience (Tomas et al., 2019). The focus of conservation strategies is now increasingly on interfaces between science, policy and local decision-making processes that are grounded in collaboration. Incorporating these integrated approaches is especially relevant in ecologically sensitive wetlands and freshwater landscapes in the midst of rapid environmental change.

8.5 Toward Ecosystem Intelligence Networks

The future of freshwater biomonitoring is heading towards ecosystem intelligence networks, where all ecological data streams, molecular diagnostics, environmental sensors, and predictive analytics become interconnected monitoring systems. These interdisciplinary frameworks seek to offer ongoing ecological interpretation, and in turn to support adaptive environmental governance and ecosystem forecasting. With the development of new challenges like microplastic contamination in the environment, there is a need for integrated monitoring strategies that can connect ecological risk assessment, policy intervention, and mitigation planning for aquatic systems (Vaseashta et al., 2025).

Advanced ecosystem intelligence networks are also likely to include interdisciplinary innovation from biotechnology, environmental humanities and integrated laboratory sciences to enhance ecological problem solving and sustainability planning (Willet, 2021). Scientific experimentation, ecological design and technologies can be integrated in collaborative approaches to help design more inclusive and adaptive freshwater conservation systems. These future-oriented biomonitoring frameworks go beyond simply detecting biodiversity, to also involve society in ecological stewardship and sustainable governance of ecosystems in a fast-changing environment.

9. CONCLUSION

The ecological importance, sensitivity, habitat specificity and role in trophic organisation and ecosystem functioning make aquatic insects still important bioindicators for measuring the integrity of freshwater ecosystems. While applications of conventional taxonomy-based biomonitoring methods have proved to be useful for freshwater assessment and environmental management, the ecological complexity and the increased anthropogenic pressures have shown some shortcomings of single-metric biomonitoring. Today, freshwater systems are impacted by several stressors and factors, such as pollution, climate change, habitat loss, biological invasive species, hydrological changes, and others, which require more holistic and mechanism-driven ecological assessments. Functional, molecular and ecological signals are a huge conceptual and methodological leap for biomonitoring in freshwater environments. Functional trait analysis enhances the understanding of ecosystem processes and resilience, while high resolution taxonomic analysis and early detection through molecular tools (DNA barcoding, eDNA, metabarcoding, and multi-omics technologies) are unprecedented. Ecological interpretation at the ecosystem level, with consideration of trophic interactions, ecological networks and processes at the landscape scale, also contributes to understanding ecosystem dynamics and changes in the environment. All these complementary strategies enhance ecological forecasting, biodiversity protection, and adaptive ecosystem management. The future of freshwater biomonitoring is to increasingly be data-driven, predictive and ecosystem-based, involving artificial intelligence, remote sensing, real-time environmental sensing and integrated ecological intelligence systems. The development of interdisciplinary cooperation, accessibility, and a harmonized monitoring system will have a key role to play in enhancing global freshwater conservation and in the long-term sustainability and resilience of freshwater systems in the face of environmental change.

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