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Review

Advancing River Health Assessments: Integrating Microscopy and Molecular Techniques through Diatom Indices

Ha-Kyung Kim [1,2], In-Hwan Cho [2,3], Eun-A Hwang [2,4], Byung-Hun Han [2] and Baik-Ho Kim [2,5,*]

[1] Water Environmental Engineering Research Division, National Institute of Environmental Research, Inchon 22689, Republic of Korea; hosang9022@naver.com
[2] Department of Environmental Science, Hanyang University, Seoul 04763, Republic of Korea; crice5237@naver.com (I.-H.C.); euna1602@naver.com (E.-A.H.); tttyy3@naver.com (B.-H.H.)
[3] Migang E&C Co., Ltd., Gyeonggi 14057, Republic of Korea
[4] Han River Environment Research Center, National Institute of Environmental Research, Gyeonggi 12585, Republic of Korea
[5] Department of Life Science and Research Institute for Natural Sciences, Hanyang University, Seoul 04763, Republic of Korea
* Correspondence: tigerk@hanyang.ac.kr; Tel.: +82-2-2220-0960

Abstract: This paper reviews the evolution and integration of diatom-based water quality assessments with environmental DNA (eDNA) techniques for advancing river ecosystem health evaluations. Traditional methods, relying on microscopy and diatom indices, have significantly contributed to our understanding of aquatic ecosystems but face challenges such as the need for taxonomic expertise and the labor-intensive nature of sample collection. Recent advancements in molecular biology, particularly eDNA analysis, offer opportunities to overcome these limitations, providing more accurate and comprehensive assessments. This study highlights the benefits of combining traditional microscopy with modern molecular techniques, enhancing the precision and efficiency of water quality evaluations. By addressing the challenges of standardizing methods and improving species identification through comprehensive reference libraries and advanced bioinformatics tools, this integrated approach aims to refine and advance the effectiveness of diatom-based strategies in monitoring and managing river health amidst environmental changes.

Keywords: diatom-based assessment; environmental DNA (eDNA); river ecosystem health; molecular techniques; biodiversity conservation

Citation: Kim, H.-K.; Cho, I.-H.; Hwang, E.-A.; Han, B.-H.; Kim, B.-H. Advancing River Health Assessments: Integrating Microscopy and Molecular Techniques through Diatom Indices. *Water* **2024**, *16*, 853. https://doi.org/10.3390/w16060853

Academic Editors: Marina Marcella Manca, Ryszard Gołdyn and Kun Shan

Received: 27 January 2024
Revised: 21 February 2024
Accepted: 12 March 2024
Published: 15 March 2024

1. Introduction

Recent research has highlighted the severe challenges that rapid urbanization, industrialization, and economic growth pose to biodiversity and ecological balance. Studies have together underscored the grave consequences of these developments, including loss of biodiversity and deterioration of ecosystems [1–7]. This evidence has spurred global efforts to rejuvenate aquatic ecosystems, focusing mainly on addressing eutrophication and restoring biodiversity and ecological equilibrium [8–10].

The escalation of human activities, characterized by higher population densities and industrial activities, has resulted in an increase in pollutants discharged into aquatic environments, thereby endangering the health of river ecosystems. This situation highlights the urgent need for improved detection of hazardous substances and the establishment of stricter chemical pollutant standards [11–15]. Furthermore, the significance of biological indicators, such as fish, aquatic insects, periphyton, and aquatic plants, in the global evaluation of aquatic ecosystems has been recognized, emphasizing the necessity of maintaining ecological integrity in the management of aquatic ecosystems [16–19].

Specifically, diatoms have been identified as crucial indicators for assessing the ecological health of water bodies, providing essential insights into disturbances impacting river ecosystems [20–23]. Despite their importance, traditional methods for benthic diatom-based water quality assessment in river ecosystems are hampered by challenges including subjective interpretations, the need for specialized taxonomic expertise, and difficulties in processing samples. These issues mirror the technical obstacles encountered in environmental DNA (eDNA) analysis, such as contamination and taxonomic inconsistencies [24–27].

This study aims to improve the assessment of river ecosystem health by developing and applying rapid and accurate species-specific genetic methods and data collection protocols for diatoms. By comparing traditional diatom analysis techniques with those based on eDNA, this research intends to identify more effective strategies for monitoring and conserving aquatic environments.

2. The Evolution of Microscopy-Enabled Diatom-Based Water Quality Assessment

Since the late 20th century, the development of diatom-based assessments has played a pivotal role in evaluating the health of aquatic ecosystems and the overall quality of water worldwide. Diatom indices, as highlighted by researchers such as Kim et al. [28] and Masouras et al. [29], have significantly improved our capability to analyze diatom cluster data for assessing pollution tolerance, nutrient levels, trophic states, and the general ecological health of water bodies. These methods have been continually refined to enhance the health of aquatic ecosystems across the globe (Supplementary Materials S1).

A major breakthrough came in 1979 with the introduction of Descy's Index, which initiated the quantitative assessment of water quality using diatom data. This breakthrough was followed by the establishment of several key indices, such as Bourelly's Generic Diatom Index (1981), Foerster's German Diatom Index (1982), the Diatom Assemblage Index (DAI) in 1986, and the Trophic Diatom Index (TDI) in 1995. These indices, taking into account ecological preferences and pollution tolerance, are utilized to assess the health of rivers and streams. In 2004, the European Committee for Standardization (CEN), along with updates to the Biological Diatom Index (BDI) in 1996 and 2009, standardized ecological assessment methods using diatom indicators. Moreover, the launch of the Index of Biotic Integrity (IBI) in 2005 and the Acid Mine Drainage-Diatom Index (AMD-DIBI) in 2010 further enhanced assessment capabilities by incorporating multiple indicators and accounting for specific environmental conditions.

From 1979 to 2019, the creation of numerous diatom indices has been critical for evaluating and monitoring aquatic ecosystems globally. Nevertheless, the application of diatom-based assessments encounters challenges such as regional variations in diatom species composition and sensitivity, necessitating the development of region-specific indices. Accurate species identification demands taxonomic expertise and specialized personnel, which can be time-consuming. Additionally, collecting and processing diatom samples is labor-intensive, requiring specialized equipment and preservation methods. The Diatom Index's focus on specific stressors may miss other ecological aspects, and potential delays in diatom response could hinder the detection of recent pollution events.

As illustrated in Table 1, despite these challenges, the Diatom Index continues to be an essential tool for assessing the health of river ecosystems. Its advantages include diatoms' high sensitivity to changes in water quality, making them reliable early indicators of shifts in ecosystem health. Diatom records also offer insights into historical water quality trends, illustrating the effects of human activity and environmental changes over time, as demonstrated in numerous studies [29–31]. However, challenges such as the necessity for region-specific indices due to regional variation and the requirement for taxonomic expertise to avoid misidentification and inaccurate assessments persist. The laborious process of collecting and analyzing diatom samples raises concerns about the practicality of the index and the potential for delays in diatom response, thus affecting the timeliness of ecosystem health assessments [32–34].

Table 1. Simplified advantages and challenges of using the diatom index for river health assessment.

Diatom Index		Description
Advantages	Sensitivity	Diatoms quickly indicate changes in water quality, such as pollution and nutrient levels, signaling ecosystem health shifts.
	Integrative indicator	They reflect cumulative environmental stressors, offering a comprehensive view of river health and long-term trends.
	Historical insights	Diatoms help track historical water quality changes, showing the effects of human and environmental shifts.
	Non-destructive sampling	They are collected harmlessly, preserving river ecosystems while assessing health.
	Early warning	Diatoms alert to water quality issues early, preventing severe problems.
Challenges	Regional variation	Diatom sensitivity varies regionally, necessitating tailored indices for accurate health assessments.
	Taxonomic expertise	Identifying diatoms accurately demands specialized knowledge, posing challenges for assessment accuracy.
	Sample handling	Collecting and processing diatom samples requires effort and specific equipment, with careful handling needed for sample integrity.
	Limited scope	The diatom index mainly detects changes related to diatoms, possibly missing broader ecosystem health indicators.
	Lag time	Environmental changes may not immediately reflect in diatom communities, potentially delaying pollution detection.

In sum, while microscopy-enabled diatom-based assessments have significantly advanced our understanding and management of aquatic ecosystem health, they are not without limitations. There is a growing need for complementary molecular biological research, such as eDNA analysis, to overcome these limitations and provide a more comprehensive evaluation of water quality and ecosystem health.

3. Advancements in Diatom Research through Molecular Techniques

Molecular techniques have greatly advanced diatom research, revolutionizing the application of established indices and aligning with environmental standards to enhance the evaluation of aquatic ecosystem health. The development of region-specific, multimetric indices has improved the precision and reliability of data by accounting for regional environmental factors that affect diatom communities (Table 2).

Table 2. Simplified advancing methods and tools for diatom research.

Techniques	Methods (Tools)
Microscopy	Simplified to essential imaging techniques and software for diatom study, focusing on confocal and electron microscopy for morphology analysis (confocal microscopy, electron microscopy, basic image analysis).
Molecular Methods	Streamlined genomic analysis utilizing Next-Generation Sequencing and CRISPR-Cas9 for targeted gene editing, with a focus on essential DNA and RNA analysis (NGS, CRISPR-Cas9, DNA/RNA analysis).
Culture and Automation	Reduced to core automated culture and sample processing techniques for efficient diatom research (automated diatom culture, sample processing, DNA extraction, PCR).
Collaborative Research	Focused on key interdisciplinary collaborations using computational models and data sharing for diatom biology and ecology (interdisciplinary projects, data sharing).
Bioinformatics and Data Analysis	Condensed to fundamental bioinformatics and data analysis techniques, with an emphasis on sequence analysis and taxonomic categorization using machine learning (bioinformatics and machine learning for data analysis).

Despite the ongoing reliance on fossil diatoms due to challenges in DNA extraction, microscopic analysis remains the primary method. The difficulty of culturing diatom species, especially in areas where they are scarce, highlights the necessity for accurate morphological species identification to prevent inaccuracies in DNA sequence databases like GenBank.

Molecular classification techniques, developed to complement morphological identification, address the scarcity of expert taxonomists. However, the vital role of taxonomists with morphological expertise in supporting effective molecular classification underscores the importance of both methods. The adoption of environmental DNA (eDNA) analysis for water quality assessment marks a significant advancement, offering a more accurate and comprehensive alternative to traditional visual identification techniques [32,35–37].

This method overcomes challenges related to direct counting and subjective interpretation, with advancements in eDNA analysis methods for diatoms emphasizing the need for streamlined field surveys and vigilance against contamination risks during DNA extraction. Establishing robust, standardized protocols is crucial to minimizing these risks [38–40].

Recent progress in eDNA analysis has significantly enhanced water quality assessment capabilities in diatom research, highlighting the crucial role of molecular technologies in revealing the ecological importance of diatoms. DNA barcode analysis has become a powerful tool for species identification, overcoming the limitations of morphology-based classifications. Molecular technologies have broadened our understanding of diatom diversity, ecosystem health, and pollution impacts [41–44], providing deeper insights into diatoms' roles in nutrient cycling and aquatic nutrient dynamics [45–49]. Furthermore, eDNA sequencing has offered valuable data on diatoms' historical distribution in coastal regions, providing perspectives on ecosystem changes [50–52].

These technological innovations have not only improved water quality assessments but also deepened our understanding of diatom diversity and ecological dynamics. The focus on molecular analysis, particularly eDNA analysis, in diatom research highlights the accuracy and comprehensiveness of modern methods over traditional identification practices. Moreover, it underscores the ongoing challenges and the critical need for precise species identification, the adoption of DNA barcoding, and the extensive application of molecular technologies in elucidating the ecological significance of diatoms. The limitations of molecular techniques underscore the necessity for morphological studies like microscopy analysis to complement and enhance our understanding of diatom research.

4. Advancing River Water Quality Assessment through Integrated Microscopy and Molecular Techniques via Diatom Indices

The shift toward integrating diatom-based indices with environmental DNA (eDNA) analysis marks a pivotal change in the methods used for assessing river ecosystem health. This synthesis explores a holistic approach that merges traditional diatom microscopy with the latest molecular biology advancements. Moving from reliance on diatom indices to the inclusion of molecular techniques has significantly enhanced our understanding and assessment capabilities of river water quality.

Table 3 outlines the strengths and challenges of both traditional diatom analysis and eDNA analysis methods. Our research aims to refine our understanding of these methods' unique attributes and how they complement one another for a more precise ecosystem evaluation.

Table 3. Simplified strengths and challenges of diatom and eDNA analyses.

Aspect	Diatom Analysis	eDNA Analysis
Strengths	Provides comprehensive insights from specific to broad ecosystem views with enhanced sensitivity Offers efficient, precise evaluation by combining both methods	Potential for diverse species detection using DNA markers Can detect a wider range of organisms beyond diatoms Non-invasive, reducing ecological disruption Suitable for long-term monitoring Provides a more comprehensive view of ecosystem health Utilizes DNA sequencing with the potential for automated identification Offers potential for more standardized protocols
Challenges	Dependent on taxonomic skills Requires investment in microscopes and personnel Demands taxonomic expertise\ Labor-intensive with potential identification errors Involves physical sample collection with potential ecological disruption Requires consistent access to skilled personnel Shows variable sample representativeness Subject to variability and taxonomic revisions	Requires lab setup and molecular biology equipment costs Requires bioinformatics skills for DNA sequence analysis Faces technical challenges in DNA analysis, an evolving field with sequencing challenges Detects species presence, potentially with less contextual information

Historically, diatom indices like Descy's Index and the Biological Diatom Index (BDI) have been foundational in quantitative assessments of water quality. These indices utilize the ecological preferences and pollution tolerance of diatoms for nuanced evaluations of nutrient levels, trophic states, and overall ecological health, thus improving the monitoring of aquatic ecosystems. Nonetheless, traditional diatom-based assessments face challenges such as the need for extensive taxonomic expertise and the labor-intensive nature of sample collection and processing. Additionally, the variability in diatom species composition across regions necessitates the development of region-specific indices, highlighting the limitations of traditional methods in capturing the full spectrum of diatom diversity and its implications for water quality.

The adoption of eDNA for water quality assessment represents a major leap in diatom research, courtesy of molecular techniques. Molecular classification and DNA barcode analysis now facilitate species identification, addressing the limitations of morphological classification and the shortage of taxonomic experts [53,54]. These molecular methods enable a more detailed and accurate analysis of diatom communities, shedding light on their ecological roles, diversity, and pollution impacts. The field has been revolutionized by recent advances in eDNA analysis and Next-Generation Sequencing (NGS) technologies, allowing for the precise detection and characterization of diatom communities [55–57].

Combining these advancements with traditional microscopy offers a comprehensive view of aquatic ecosystem health, enabling highly accurate and efficient water quality assessments. This integrated approach not only utilizes the strengths of both traditional and modern methodologies but also highlights the importance of advanced bioinformatics tools, standardized quality control protocols, and collaborative research. The development of reference databases and the application of metabarcoding techniques are crucial for ensuring the reliability and comprehensiveness of diatom-based assessments [58–62]. Moreover, cross-validating eDNA results with traditional methods emphasizes the value of a multifaceted approach to river water quality evaluation [41,63,64].

Therefore, the integration of microscopic examination with molecular analysis in diatom research signifies a major advancement in our ability to assess and monitor river ecosystem health. By leveraging the benefits of both traditional and modern methods, researchers and environmental managers can achieve a more nuanced and effective water quality assessment methods [25,62,65]. This integrated approach underscores the ongoing need for advanced bioinformatics expertise, collaborative research, and continuous innovation in the pursuit of sustainable water management and conservation strategies.

5. Advancing River Health Assessments: The Future of Diatom-Based Strategies

This comprehensive review sheds light on the development and underlying principles of diatom-based water quality assessments, identifying the lack of universal standards as a critical challenge. This issue stems from difficulties in standardizing methods and the limited taxonomic resolution in environmental DNA (eDNA) analyses. It underscores the urgent need for comprehensive reference libraries that reflect the diversity and ecological intricacies of diatom species across various regions [66,67]. It also emphasizes the importance of enhancing DNA barcoding and metabarcoding techniques to identify diatom-specific unique barcode regions, thereby improving species identification precision and ecological assessments [68].

This review highlights the transformative potential of Next-Generation Sequencing (NGS) technologies, including metagenomics and metatranscriptomics, in advancing diatom-based evaluations. Automating the sampling and analysis process is crucial for reducing subjective biases and streamlining research. The necessity for interdisciplinary collaboration is accentuated, combining expertise from ecology, molecular biology, bioinformatics, and environmental science to address the complex challenges of aquatic ecosystem assessment [69,70]. The significance of long-term studies in capturing the spatial and temporal dynamics of ecosystems and their role in documenting the effects of seasonal changes, climate variations, and human activities on water bodies is also highlighted.

Despite molecular advancements, the importance of traditional morphological taxonomy expertise is acknowledged, with a call for focused training and educational efforts to maintain diatom identification accuracy. The reliance on conventional methods and the subjective nature of microscopic analysis highlight the need for more objective and reproducible assessment tools. Furthermore, there is an advocacy for developing automated imaging and analysis systems to minimize human error [71–73].

This paper acknowledges the challenges related to contamination and the complexity of processing environmental samples in eDNA studies. It calls for research into more effective sample processing techniques and contamination control strategies. Moreover, the high costs and time requirements of comprehensive diatom and eDNA analyses are recognized as barriers, necessitating the development of more cost-effective and efficient methods and technologies [62,74,75].

While recognizing advances in understanding diatom biology and ecology, this article points out remaining gaps in knowledge about diatom responses to environmental stressors and their roles in nutrient cycling. It advocates for expanded research efforts to address these gaps, aiming to enhance our understanding of aquatic ecosystems and refine assessment methodologies [1,76,77].

Collectively, the significant progress made in assessing river ecosystem health and emphasizes the ongoing need for research. By addressing the identified challenges and employing innovative strategies, collaborative efforts, and cutting-edge technologies, the precision, accuracy, and relevance of diatom-based water quality assessments can be greatly enhanced, ensuring their effectiveness amidst environmental changes.

6. Conclusions

This review underscores the significant advancements in river ecosystem health assessment achieved through the integration of traditional diatom-based methodologies and modern molecular techniques, particularly environmental DNA (eDNA) analysis. By leveraging the strengths of both approaches, this integrated strategy enhances the precision, accuracy, and comprehensiveness of aquatic ecosystem evaluations. This study emphasizes the importance of developing comprehensive reference libraries, advanced bioinformatics tools, and standardized protocols to address current challenges in species identification and assessment standardization. Furthermore, it calls for continued innovation, interdisciplinary collaboration, and research to refine and enhance diatom-based water quality assessments. This approach not only improves our understanding of aquatic ecosystems but also ensures the effectiveness of monitoring and management strategies in response to ongoing environmental transformations, highlighting the critical role of diatoms as indicators of ecosystem health.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/w16060853/s1, Supplement S1: Advancements in diatom indices for evaluating aquatic ecological health and water quality. The table references data from studies conducted by Kim et al. [28] and Masouras et al. [29] for all mentioned indices.

Author Contributions: H.-K.K., I.-H.C., E.-A.H., B.-H.H. and B.-H.K. conceptualized the research; H.-K.K., I.-H.C., E.-A.H., B.-H.H. and B.-H.K. conducted the fieldwork; H.-K.K., I.-H.C., E.-A.H., B.-H.H. and B.-H.K. analyzed the data; H.-K.K. and B.-H.K. wrote and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This study did not receive external funding.

Acknowledgments: We extend our gratitude to the anonymous reviewers for their valuable and constructive comments.

Conflicts of Interest: Author In-Hwan Cho was employed by the company Migang E&C Co. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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