PNIE PROCEEDINGS OF THE NATIONAL INSTITUTE OF ECOLOGY OF THE REPUBLIC OF KOREA

Review Article

PNIE 2025;6(1):1-14
https://doi.org/10.22920/PNIE.2025.6.1.1
pISSN 2765-2203, eISSN 2765-2211

International Trends in the Application of Environmental DNA Methods to National Aquatic Ecosystem Monitoring Programs and Challenges for Domestic Implementation

Yerim Choi[1], Hye-Ji Oh[1,2], Ju-Duk Yoon[3*], Kwang-Hyeon Chang[1*]

[1]Department of Environmental Science and Engineering, Kyung Hee University, Yongin, Korea
[2]University-Industry Cooperation Foundation, Kyung Hee University, Yongin, Korea
[3]Research Center for Endangered Species, National Institute of Ecology, Yeongyang, Korea

ABSTRACT

To identify the spatiotemporal distribution of organisms by detecting DNA fragments released into the environment, environmental DNA (eDNA) is readily employed. This technique is receiving significant attention in ecological research, particularly in aquatic ecosystems, as an effective alternative method for national monitoring systems that need to simultaneously investigate diverse biological communities at multiple sites, at low costs and technical discrepancies. However, challenges remain, such as improving poor reliability of genetic information databases, standardizing application of methods, and interpretating of acquired genetic information, including issues of false positives and false negatives. This study explores international cases in which eDNA methods have been actively developed and applied, and summarizes the current state of research in South Korea and the various issues identified in these studies to guide individuals and domestic institutions regarding the application of eDNA technology as a targeted investigation or supplementary tool for future biological surveys.

Keywords: Biological monitoring, Ecosystem health assessment, Fish environmental DNA, Metabarcoding, National policy

Introduction

Because biodiversity loss has emerged as a serious global environmental issue, there is an increasing demand for more extensive data on biodiversity. Traditional survey methods that rely on the direct “identification” or “collection” of organisms are limited by cost, time, and effort. Additionally, these methods face challenges in detecting species with very low population densities, such as endangered species, which are central to the decline in biodiversity (Ruppert *et al.*, 2019). Environmental DNA (eDNA) analysis, which amplifies the DNA of organisms present in

Received October 6, 2024; **Revised** December 10, 2024; **Accepted** December 11, 2024

***Corresponding author:** Ju-Duk Yoon
e-mail grandblue@nie.re.kr
https://orcid.org/0000-0003-1667-327X
Kwang-Hyeon Chang
e-mail chang38@khu.ac.kr
https://orcid.org/0000-0002-7952-4047

the environment to identify biological communities, is a method that complements the limitations of traditional surveys. Recently, remarkable advancements have been made in this field (Ruppert *et al.*, 2019). Accordingly, countries such as the United States with the Clean Water Act, the European Union (EU) with the EU Water Framework Directive (WFD), and environmental authorities in Australia and New Zealand have standardized the application of eDNA methods for monitoring biological resources and biodiversity, while also conducting continuous quality control of these methods (De Brauwer *et al.*, 2023; Kelly *et al.*, 2023; Schenekar, 2023).

Advantages of Environmental DNA in Biological Monitoring

Unlike traditional biological monitoring, the emerging concept of "Monitoring 2.0" or "Next Generation Biomonitoring" represents a novel system that integrates biological monitoring methods with data utilization. This approach incorporates an extensive process that includes the collection of large-scale spatiotemporal data through advanced methods and technologies, the development of quantitative indices (e.g., quantification, scoring, and ranking of ecosystem structure and function), the creation of predictive models using artificial intelligence (AI) and machine learning, and naturally encouraging public participation and community collaboration through citizen science. The results can be utilized in policy decision-making (Derocles *et al.*, 2018; Makiola *et al.*, 2020), which makes it particularly effective for various environmental policymaking processes, offering substantial benefits for government-led environmental monitoring projects and utilizing their results. Within this system, biological surveys employing eDNA techniques have emerged as pivotal for collecting comprehensive data and facilitating citizen science, thereby supporting data collection and policy decisions (Derocles *et al.*, 2018; Larson *et al.*, 2020).

The reasons for the preference of eDNA techniques as a national-level biomonitoring method beyond its use in research, include its economic advantages (such as reduced costs and effort compared to traditional surveys) and its technical advantage of being less dependent on the expertise level of the surveyor (De Brauwer *et al.*, 2023; Kelly *et al.*, 2023). For example, when conducting biological surveys on fish communities in a lake, the eDNA technique is more cost effective than traditional survey methods because it significantly reduces the effort and time required for species identification in the field and laboratory (Table 1). Fish surveys can only be conducted at 2-3 locations per day when using stationary nets, which require transportation and setup time. In contrast, eDNA surveys, which require the collection of just 1-2 liters of water, allow for sampling at significantly more locations, resulting in a substantial cost advantage over stationary nets because of its greater efficiency.

According to studies comparing the total process costs of eDNA and traditional survey methods, Morris *et al.* (2024) found that, for a single replicate survey, the total cost of conventional fish sampling with a fyke net was

Table 1. Comparison between classical fish survey methods and eDNA methods

Parameters	Classical method	eDNA method
Survey method	Fixed Net, Cast Net, Stake Net	Water sampling
Effort required for survey	3 persons×2 days 1 hour for Cast Net, Stake Net Installation and Retrieval of Fixed Net: 24 hours	1 person×10-20 minutes (per site per occasion)
Observer bias	Large	Small
Impact on organisms and habitat during survey	Damage to individuals and habitat disturbance	Minimal
Analytical work	Identification, counting, measurement of weight and length, etc.	MiFish method: metabarcoding analysis etc. Species-specific detection: real-time PCR analysis, etc.
Information on surveyed individuals	Individual number per CPUE[†] Length and weight Presence of malformations and other morphological abnormalities	None
Cost*	300,000 Korean won	600,000 Korean won

eDNA, environmental DNA; PCR, polymerase chain reaction. *Cost is estimated based on the typical expenses incurred when outsourcing to Korean providers. [†]CPUE: catch per unit effort, indirect measure of abundance of target organisms, usually used in fisheries and conservation biology.

$60, while that for eDNA sampling was $193, which was over three times higher. However, in delivering over 95% detection of invasive species, the cost was $1,020 for conventional fish sampling and $965 for eDNA, indicating that eDNA is relatively cost-effective for monitoring invasive species. Additionally, eDNA showed more than double the detection probability of fyke nets, emphasizing its efficiency in monitoring invasive species. Bálint *et al.* (2018) compared the cost-effectiveness of eDNA with traditional methods (visual and audio encounter surveys) for frogs. Their study found that eDNA was more cost-effective in species-rich areas, whereas traditional methods more so in regions with lower species richness per site.

As genetic analysis costs continue to decrease annually and labor-intensive method costs are likely to increase with inflation-adjusted wages, the cost efficiency of eDNA methods is expected to improve further. Moreover, while traditional methods allow taxonomic experts to identify many species immediately, securing sufficient field researchers with specialized taxonomic knowledge can be challenging for broad-scale national or global biodiversity surveys. Therefore, eDNA analysis enables more consistent data collection at a lower cost, resulting in large-scale simultaneous or short time intervals biological surveys becoming more feasible (Agersnap *et al.*, 2022; Berger *et al.*, 2020; Zhang *et al.*, 2020). Its key advantage is the ability to significantly expand or finely adjust the spatial and temporal scope of a survey (Kelly *et al.*, 2023).

Ultimately, large amounts of biological information can be collected in the form of big data to ensure its utility and applicability of the data (Navarro *et al.*, 2017). Furthermore, by establishing and promoting standardized methods for water sampling and filtration in eDNA analysis for surveys of target species, it is possible to expand the scope of surveys and participants in the form of citizen science. This process facilitates natural community building as well as objective information sharing with stakeholders or citizen groups from diverse perspectives, allowing more flexible progress in environmental policy- and decision-making processes.

Issues of Application Technologies for Community-Level Surveys in Aquatic Ecosystems

Currently, eDNA techniques applied to aquatic ecosystem biological surveys can be categorized into species-specific eDNA detection methods, which target particular species, and metabarcoding methods, which comprehensively analyze all species present at the community level. To distinguish species at the community level, DNA barcoding for species identification relies entirely on existing sequence libraries for specific regions of interest within the genetic fragments of each species (e.g., National Center for Biotechnology Information [NCBI]). Consequently, species identification is entirely dependent on the sequence information currently registered. Therefore, the absence of genetic information for the target taxa is the most fundamental barrier to conducting biological surveys using eDNA. Many countries conduct national and international barcoding projects to enhance the resolution of species identification. However, as of 2018, for many taxa other than fish, particularly annelids, crustaceans, and molluscs, the proportion of species with reference barcodes did not exceed 50% of the actual species (Leese *et al.*, 2018). Japan's Ministry of the Environment has advised caution regarding the presence of genetic information when using eDNA for fish surveys (Biodiversity Center of Japan, 2020). In South Korea, the proportion of genera with genetic information registered in the NCBI remains low for most taxa, excluding fish, regardless of the genetic region. Among the benthic macroinvertebrates, the registration rate averaged 60% for macroinvertebrates and 30% for diatoms (Fig. 1; Kwak *et al.*, 2022).

eDNA analysis identifies species based on sequence differences within relatively short DNA fragments amplified using universal primers, making it difficult to distinguish closely related species. Additionally, most currently registered species-specific genetic information is based on DNA extracted from a small number of individuals collected from specific regions, which can fail to reflect genetic variation across different regions. Errors exist in genetic database systems, such as the Barcode of Life Data System, including species name misidentification, genetic data contamination, and cases in which multiple barcode entries correspond to the same species or single barcode entry points for various species (Bergsten *et al.*, 2012; Leese *et al.*, 2018; Oliveira *et al.*, 2016). Therefore, with the current state of eDNA technology, careful attention is needed when using metabarcoding techniques to confirm species lists at the community level, and significant differences often occur when compared to species compositions identified using traditional survey methods.

Because of these issues, most countries, including the U.S., EU, and Japan, are continuously working to gather genetic information for species that inhabit their regions so that eDNA techniques can be applied in biological surveys. For surveys requiring high accuracy, the use of eDNA is typically limited to specific purposes such as monitoring endangered or invasive species rather being available for than analyzing community composition, but various national network platforms have already been used to implement strategies to overcome these challenges.

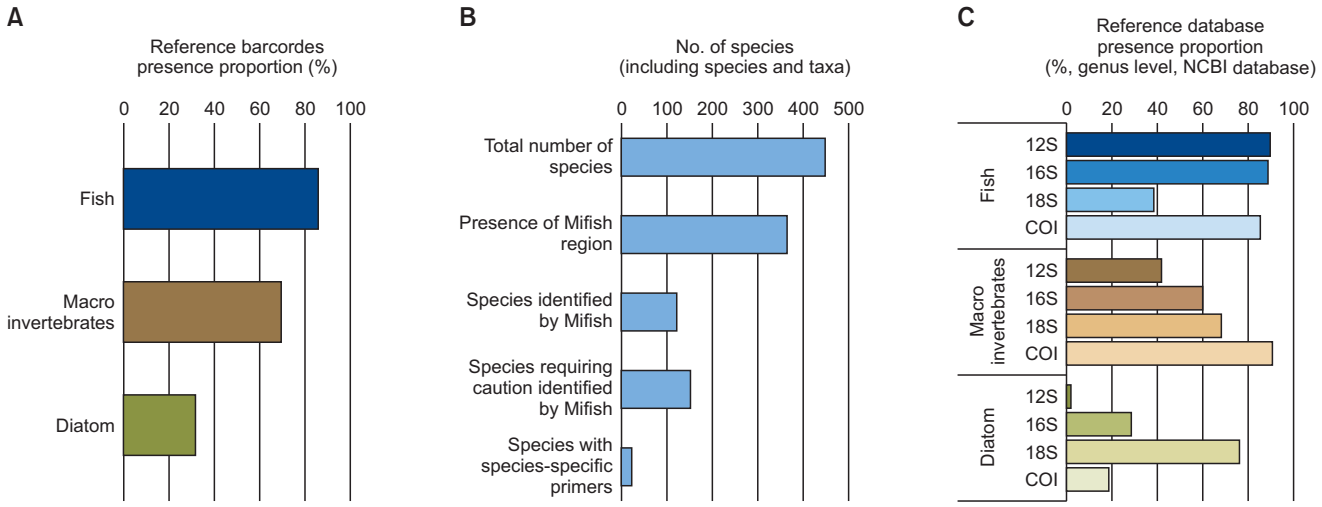

Fig. 1. Current status of genetic information databases used for eDNA analysis. (A) Proportion of species with genetic information (reference barcodes) compared to the total number of resident species in Europe by taxonomic group (Source: Leese *et al*., 2018). (B) Information on species identifiable by eDNA methods and species requiring caution among the total species (including taxa; Source: ME, 2020). (C) Reference database presence proportion for fish, benthic macroinvertebrates, and diatoms (Source: Kwak *et al*., 2022). eDNA, environmental DNA; NCBI, National Center for Biotechnology Information; COI, cytochrome c oxidase subunit I.

International Trends in the Application of Environmental DNA for National Biological Monitoring

The U.S., EU, and Japan are prominent examples of countries strategically implementing eDNA techniques in national biomonitoring. These countries aim to reduce monitoring costs while accumulating and utilizing national-level biological data by applying standardized methods and leveraging years of accumulated genetic research on species inhabiting their regions. Additionally, they are establishing platforms that organically connect biomonitoring, creating genetic libraries and data utilization, and presenting strategies to overcome current technical limitations. In particular, New Zealand introduced standardized eDNA survey methods to restore river continuity. It actively utilized these methods along with citizen science to accumulate data for ecosystem health assessments and to promote public awareness.

United States: development and utilization of integrated biological information from rivers to oceans

The U.S. eDNA initiative is based on 15 years of eDNA research and development. It aims to continuously improve the accuracy, consistency, and sensitivity of eDNA techniques through collaboration across the scientific, technological, and industrial sectors. A standardized methodology was uniformly applied across all government departments to ensure consistent biomonitoring (Kelly *et al*., 2023). This approach is particularly valuable for marine species surveys crucial for fishery management. Field survey methods, such as status assessments and habitat studies, are standardized and commonly used by related departments, and biological and eDNA survey data collected from various investigations are compiled into databases for sharing, verification, and utilization. To support this, standardized eDNA survey methods have been formulated in official guidelines (Laramie *et al*., 2015).

In the U.S., a significant budget is allocated annually to assess biological resources, biodiversity, and aquatic ecosystems because of the need for personnel and equipment (Kelly *et al*., 2023). This cost advantage allows eDNA survey methods to enable large-scale investigations across time and space, with the added benefit of accumulating consistent, high-quality data through standardized methods, making it a crucial technology in national biomonitoring programs (Stepien *et al*., 2023). Currently, the U.S. is designing a comprehensive biological and environmental monitoring system, spanning freshwater and marine ecosystems, by establishing a cross-departmental monitoring framework. To achieve strategic objectives, the application of conceptual models and the development of eDNA-related technologies are emphasized (Fig. 2). The eDNA method has been highlighted as a monitoring tool capable of addressing biological and environmental issues across connected habitats, from lakes to oceans, allowing for a comprehensive assessment of species distribution and movement within these ecosystems.

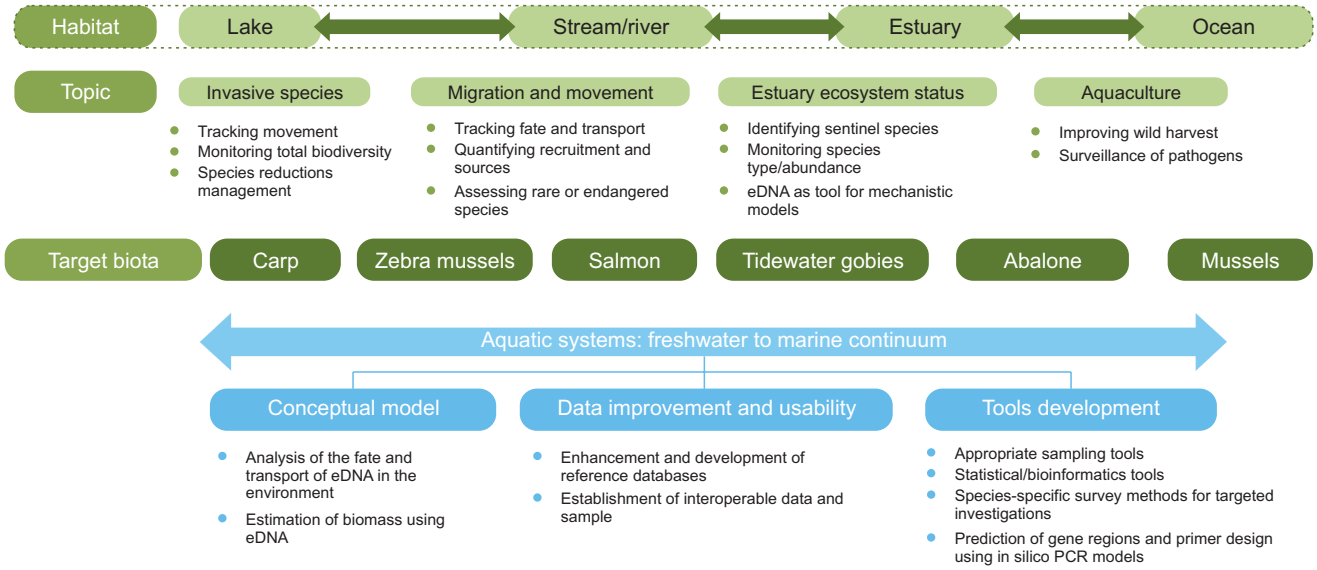

Fig. 2. Illustration of monitoring methods across habitats from lakes to marine environments. eDNA, environmental DNA; PCR, polymerase chain reaction. Source: Kelly *et al*., 2023.

European Union: development of indices for assessing ecosystem integrity

The EU biomonitoring system, established in 2000 and led by the EU WFD to protect and manage water resources within the EU (Kallis & Butler, 2001), focuses on addressing biodiversity loss and ecosystem assessment due to climate change and other human-induced environmental changes, with particular emphasis on establishing evaluation systems related to ecosystem function and health (Leese *et al*., 2018; Stubbington *et al*., 2018). While maintaining traditional biological survey methods, the EU is actively adopting the concept of next-generation biomonitoring and promoting the development of aquatic ecosystem assessment indices using eDNA techniques, particularly through metabarcoding and next-generation sequencing (Derocles *et al*., 2018; Hering *et al*., 2018; Leese *et al*., 2016; 2018).

The aquatic ecosystem assessment system of the EU WFD was designed to identify discrepancies between the expected and observed conditions of water bodies, set reference conditions based on the characteristics of each ecosystem, and compare them to the surveyed sites. Biological Quality Elements (BQEs) are bioindicators for assessing ecological status. In lake ecosystems, BQEs include phytoplankton, aquatic plants, and benthic macroinvertebrate communities, which are composed of individual taxa based on their sensitivity to environmental factors. Currently, the EU is complementing traditional BQEs evaluation methods with metabarcoding and -genomics as supplementary approaches while developing a 'taxonomy-free' index based solely on genetic analysis, without being constrained by matching the results to taxa identified through traditional methods or existing species data (Fig. 3; Leese *et al*., 2018).

The EU's DNAqua-Net (www.DNAqua.net) aims to promote the application of DNA-based tools and develop a roadmap to incorporate these tools into standardized ecological assessments of aquatic ecosystems across Europe and beyond to optimize environmental management and promote nature conservation. Although the technological advancement of genomic tools showing rapid progress in Europe, significant challenges remain for the standardized application of eDNA techniques in environmental monitoring, according to European legislation. The most critical challenges include: 1) method standardization; 2) selective application of DNA barcode references and biomarkers; 3) sequencing platforms; 4) data analysis, storage, and sharing; 5) development of new gene-based indices; and 6) integration of metabarcoding and eDNA methods into biological monitoring protocols and ultimately into current legislation (Leese *et al*., 2016). DNAqua-Net provides a platform for academic, technical, and social collaboration to address these challenges, forming working groups for each task and fostering interactions both within and outside the groups to systematically develop eDNA-based biomonitoring techniques, as well as sharing technology and offering education.

Japan: development of fish environmental DNA survey methods in collaboration with academic societies and consortia

In Japan, the Ministry of Land, Infrastructure, Transport and Tourism conducts biological surveys on river and dam management using the National Census on River Environ-

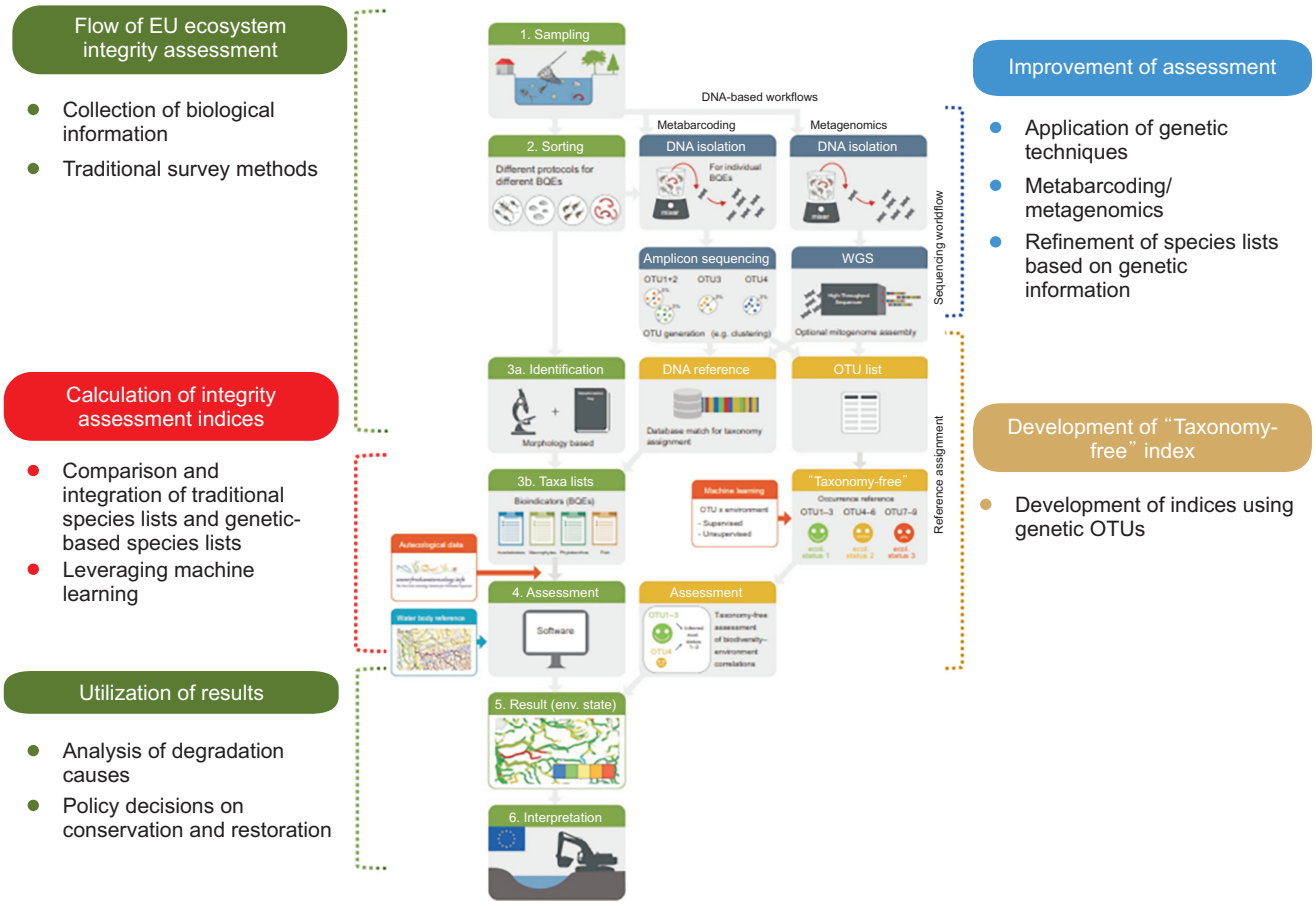

Fig. 3. Flowchart of biological surveys and aquatic ecosystem assessments using eDNA techniques of the EU. EU, European Union; BQE, Biological Quality Elements; WGS, whole genome sequencing; OTU, operational taxanomic unit.

ment (NCRE). This survey covered 109 river systems managed by the national government and 93 dam lakes managed by either the government or the Water Resources Development Public Corporation, targeting fish, benthic organisms, phytoplankton, zooplankton, plants, birds, amphibians, reptiles, mammals, and terrestrial insects (Kobayashi *et al.*, 2013; Osugi *et al.*, 2019). In 2006, which can be considered the early stages of eDNA research, the Ministry of Agriculture, Forestry, and Fisheries launched the eDNA Project to analyze soil biota using eDNA extracted from soil (Fujii *et al.*, 2009). Since then, eDNA research on aquatic ecosystems, mainly fish, has been actively conducted (Takahara *et al.*, 2013). The extensive biological data accumulated through the NCRE have been used as an excellent reference for the development of eDNA research methods (Jo *et al.*, 2021). The eDNA Society was established in 2018, and the first field survey guidelines for eDNA were published in 2019. These guidelines have since been revised, with the updated edition released (Minamoto *et al.*, 2021). In 2020, based on this work, the Biodiversity Center of Japan published a guide on freshwater fish survey methods in secondary natural environments (Biodiversity Center of Japan, 2020). Since 2019, thematic surveys on the potential of eDNA for identifying river biota have been conducted, and the local government departments responsible for surveys have actively explored eDNA methods, particularly for fish studies. These active research efforts on eDNA applications enhance NCRE and explore its use in citizen science (Doi & Nakamura, 2023).

Additionally, All Nippon eDNA Monitoring Network (ANEMONE; db.anemone.bio), a collaboration between universities, companies, public institutions, and private organizations, operates as a platform to promote nature-positive initiatives, advance and disseminate eDNA technology, and strengthen and stabilize eDNA observation networks within Japan. ANEMONE also provides a platform for consortiums in the industrial and economic sectors related to the potential value of biodiversity. The eDNA observation network produced fish-related eDNA data (site information, primer information, detected species composition, etc.) from 77 regular observation sites,

including 55 coastal, 18 rivers, and four lake locations. In addition to the data provided by professional researchers, the network collects and shares various citizen science data and contributions from the public (Kondoh *et al.*, 2024).

New Zealand: environmental DNA application to assessment of habitat connectivity and citizen science through development of a Taxon-Independent Index

eDNA can effectively assess the spatial distribution of migratory fish in rivers, enabling the evaluation of river habitat continuity (Yamanaka & Minamoto, 2016). In New Zealand, continuous research is being conducted on the application of eDNA methods for river health assessments (Suren *et al.*, 2024), mainly to evaluate the restoration of river continuity through fish movement and to assess the effectiveness of fish passages installed on longitudinal structures. Detailed survey methods for evaluating fish passage efficiency are presented in the Ministry of Environment guidelines. These guidelines suggest the use of Before-After-Control-Impact surveys to assess the impact of longitudinal structures before and after improvements by examining fish species diversity and the presence or absence of specific species upstream and downstream of the structures to evaluate the effectiveness of reducing fish migration barriers (NIWA, 2018). Recently, New Zealand's Ministry for the Environment standardized eDNA survey methods for fish and benthic macroinvertebrates, including fish eDNA survey methods, in their guidelines for evaluating the improvement of longitudinal structures and fish passages (NIWA, 2023).

Although eDNA survey methods are able to analyze fish communities' species-composition through techniques such as metabarcoding, their application is limited because they cannot determine the abundance of detected species or assess the size and life stage of individuals. eDNA surveys using species-specific primers for indicator species are recommended for evaluating fish passage through longitudinal structures. Indicator species are typically weak-swimming migratory species; if these species are detected upstream after passing through the structure, it is assumed that other fish species can also migrate through the fish passage.

A representative indicator species used was Inanga (Whitebait, *Galaxias maculatus*), which has weak swimming abilities, migrates to estuaries for spawning in au-

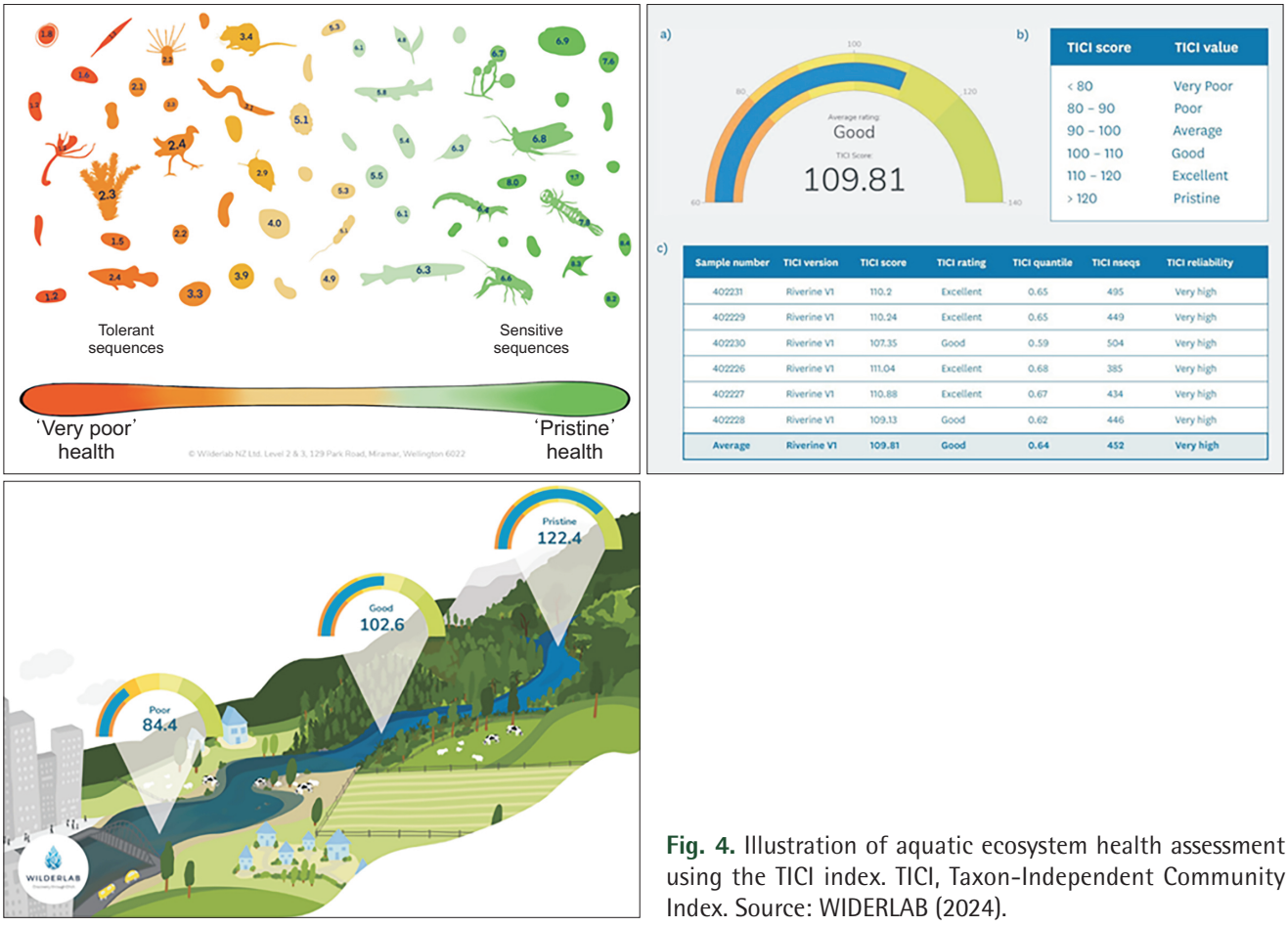

Fig. 4. Illustration of aquatic ecosystem health assessment using the TICI index. TICI, Taxon-Independent Community Index. Source: WIDERLAB (2024).

tumn and dies after spawning. As a result, eDNA was not detected upstream of river structures above the spawning grounds during winter. However, if eDNA was detected upstream during the juvenile migration season, it can be concluded that the Inanga juveniles successfully passed through the structure. In addition to monitoring the movement of native species, such as Inanga, the spread of non-native species has also been investigated to detect, at an early stage, species that could have undesirable effects on the country's native ecosystems. The application of eDNA survey methods for such evaluations should be carried out through comparative analyses with reference rivers that have fish community characteristics and habitat environments similar to those of the surveyed site to ensure a more accurate assessment.

In New Zealand, the Taxon-Independent Community Index (TICI) has been developed to utilize eDNA metabarcoding techniques more widely for biodiversity surveys and ecosystem health assessments in key aquatic ecosystems, integrating these surveys into citizen science initiatives (Bird *et al.*, 2024). Through the 'Open Waters Aotearoa' program, the New Zealand Environmental Protection Authority selected citizen groups or organizations interested in aquatic ecosystem biodiversity surveys and provided them with eDNA survey tools, with the results compiled into a database. This program provides information on eDNA and aquatic ecosystems through educational materials available on the Environmental Protection Authority website and regular webinars. For biodiversity surveys of aquatic ecosystems, project proposals submitted by applicant groups outlining survey objectives and plans were evaluated and selected to enable data collection. The collected survey results were mapped using ArcGIS through 'Open Waters Aotearoa,' and detailed results from each project are available. The results included species detected by metabarcoding and ecosystem health assessments based on TICI derived from genetic sequences and community composition (Fig. 4).

Table 2. Domestic journals' review papers on eDNA technology and methods for freshwater ecosystem surveys and research

Study	Topic	Main content
Kim *et al.*, 2021b	Comparison of efficiency between methods for eDNA metabarcoding of freshwater fish	• Comparison of detection results according to filter, extraction kit, and primer combinations • Comparison of read number and OTU according to primer • 12S rDNA (MiFish 4 primer combinations) • 16S rDNA (AquaAmp, Fish META Universal PCR Kit)
Oh *et al.*, 2021	Application of eDNA methods for zooplankton community analysis	• Sample collection, preservation, and DNA extraction methods for individual barcoding • Primer and PCR methods • Metabarcoding methods for community and summary of primers by taxon • Analysis method for ietary sources through rotifers gut content DNA analysis
Kwak *et al.*, 2021	Water sampling filtration system for eDNA collection	• Design and fabrication of water sampling filtration system for on-site raw water collection • Proposal of a collection method without damaging filters (voltage and pressure control system) • Review of the efficiency of eDNA monitoring methods from intake sources in collection reservoirs
Kim *et al.*, 2021a	eDNA collection methods	• Overall process of eDNA collection • Water sampling method: sampling tools operable from the water's edge • Filtration: selection of filter paper according to target organisms, filtration methods, and comparison of filtration volume • Comparison of various preservation methods • Comparison of DNA extraction methods
Yoo *et al.*, 2023	Analysis of the potential for harmful algal blooms using sediment eDNA	• Introduction to sediment pretreatment and eDNA extraction methods • Physical disruption separation method (silica bead beating method) • Density gradient centrifugation method

eDNA, environmental DNA; OTU, operational taxanomic unit; PCR, polymerase chain reaction.

The TICI aims to resolve the discrepancies between the presence of indicator species identified through eDNA metabarcoding and their actual occurrence confirmed by traditional surveys. To achieve this, the TICI calculates the community index using specific sequences of indicator species detected in the metabarcoding process, classifying them as sensitive or tolerant, regardless of whether they match a particular species. This approach equates nucleotide sequences with species occurrence to calculate the index. eDNA surveys are repeatedly being conducted at well-documented sites, and the results are quantified into an index using modeling and machine learning methods (Wilkinson *et al.*, 2024). Scores are assigned to organisms that represent healthy aquatic ecosystems owing to their environmental sensitivity and species that inhabit polluted environments due to their tolerance. The evaluation was performed based on the distribution of the overall scores, using a method that assigns scores to genetic sequences verified to correspond with biological indices to calculate and assess the index (Chessman, 2003).

Environmental DNA Research in Korea and Further Application for National Monitoring Program

In Korea, various studies on aquatic ecosystems using eDNA methods have been conducted, and the results from metabarcoding analyses of freshwater fish communities in rivers have been published in international journals (Alam *et al.*, 2020; An *et al.*, 2023; Kang *et al.*, 2024). However, compared with fish in freshwater ecosystems, the number of species registered in databases for benthic macroinvertebrates and other plankton communities is significantly lower, which limits community analysis that uses metabarcoding techniques (Kwak *et al.*, 2022). Various research findings on the potential application of eDNA technology in surveys of aquatic ecosystem biota in Korea have been introduced in domestic journals (Kim *et al*., 2021a; 2021b; Kwak *et al*., 2021; Oh *et al*., 2021). Table 2 summarizes papers on technical approaches related to eDNA and review articles on the methods of application that have been published in domestic journals.

Recently, studies that simultaneously applied traditional methods and eDNA technology to freshwater ecosystems and analyzed their differences have been actively reported in domestic journals (Kang *et al.*, 2023). Notably, most of these studies focused on fish community surveys, which have the most complete databases, and the results provide important insights into areas that require improvement for the successful application of metabarcoding to native species in Korea. Repeated reports of false negatives may point to issues related to the misapplication of methods based on habitat characteristics (Burian *et al.*, 2021) while also highlighting the need to supplement genetic information for certain species and improve existing databases (Schenekar *et al.*, 2020). Studies that conduct traditional and eDNA surveys simultaneously in various environments commonly show a tendency for eDNA methods to detect more species. However, false negatives, especially those involving native and similar species, indicate that further research is necessary for a more accurate application of metabarcoding in community surveys (Table 3) (Eum *et al*., 2023; Kim & Song, 2021; Kim *et al*., 2020; Mun *et al*., 2024; Yoo *et al*., 2023).

Species-specific primers have been developed for endangered species such as *Liobagrus obesus* (Yun *et al.*,

Table 3. False-negative freshwater fish species reported in papers that applied and compared eDNA methods and traditional sampling methods simultaneously (using MiFish-U-F and MiFish-U-R primers)

Study	Study site	False negative species	Potentially misidentified species
Kim & Song, 2021	Aquarium (Gyeonggi freshwater fish ecology learning center)	*Erythroculter erythropterus* *Hemiculter eigenmanni* *Sarcocheilichthys wakiyae* *Rhodeus notatus* *Gnathopogon strigatus*	-
Kim *et al*., 2020	River (Geum River, Hwangji River, Seomjin River)	*Koreocobitis naktongensis* (Hwangji River) *Acheilognathus majusculus* (Seomjin River)	*Koreocobitis rotundicaudata*
Mun *et al*., 2024	River (Han River)	*Hyporhamphus sajori* *Sarcocheilichthys nigripinnis*	-
Eum *et al*., 2023	CCZ	*Rhynchocypris oxycephalus* *Orthrias nudus*	*Rhynchocypris lagowskii* *Orthrias toni*

eDNA, environmental DNA; -, not available; CCZ, civilian control zone.

2022) and *Gobiobotia nakdongensis* (ME, 2020; 2021; 2022). These primers were used to determine the presence of these species while combining environmental data and statistical analysis techniques to study their habitat requirements.

In South Korea, national biological monitoring projects are being conducted, such as assessments of river, estuary, and lake aquatic ecosystem health and river continuity.

Table 4. Summary of eDNA survey guidelines of major countries for the national biological survey

Study	Target	Collection method	Filtering method	Filter volume	Filter membrane	DNA extraction and primer
Laramie *et al.*, 2015	Aquatic ecosystems - Fish - Amphibians - Others	- Collect water directly using filtering device - Collect water using bottle and pour to filtering device	- Hand-driven vacuum pump - Rechargeable cordless driver with peristaltic pump head - 120-V AC motor with peristaltic pump head	- 250-2,000 mL - 1 L in general	- Cellulose nitrate filter membrane - 0.45 μm	- Not suggested
Carim *et al.*, 2016	Streams - Fish	- Collect water directly from stream using filter equipped hose	- The eDNA Sampling Kit includes designated pump - Filter 5 L (8-9 minutes)	- 5 L - Minimum 2-3 L (when the filter is clogged)	- Glass microfiber binder-free filter - 1.5 μm	- Not suggested
Biodiversity Center of Japan, 2020	Secondary natural waters - Fish	- Collect water directly using bottle - Long-distance water collection using a rope-attached bucket - Water collection from bridge using a rope-connected collector	- Transport to laboratory and filter using a pump	- 1,000 mL	- Glass-fiber membrane - 0.7 μm	- Use of cartridge filter - DNeasy Blood and Tissue kit - MiFish-L-F - MiFish-L-R
NIWA, 2023	Streams - Fish - Benthic macroinvertebrates	- Riverbank of the stream (minimize contamination) - Collect upstream raw water while standing downstream - Use of device capable of long-distance water collection	- Use of syringes or hand pump on site - Active filtering with applied pressure - Passive filtering without artificial pressure	- 0.5-5 L	- Cellulose acetate syringe filter - 0.2-0.6 μm - 0.45-1.2 μm - 5 μm for turbid water	- Not suggested
Pawlowski *et al.*, 2020	Aquatic ecosystems - Fish - Amphibians - Others	- Water, bulk sample of sediment, biofilm, benthic macroinvertebrates - Filtration and sedimentation methods for water collection - Collect water using a bottle considering the water flow	- Syringe - Peristaltic pump - Manual vacuum pump	- 50-100 mL - Several litters using peristaltic pump	- Encapsulated filter (Sterivex) - 0.22-0.7 μm	- Qiagen, Macherey-Nagel - Provide basic guidelines to separately use according to purpose (protein-coding genes vs. ribosomal genes), but specific primer not suggested

eDNA, environmental DNA; AC, alternating current.

Biological data obtained through these efforts are being used to calculate biological indices applied to health or continuity assessments. As previously mentioned, eDNA analysis is increasingly being employed for large-scale biodiversity surveys (species presence, abundance, and community composition) at the national level (Biodiversity center of Japan, 2020; Carim *et al*., 2016; Laramie *et al*., 2015; NIWA, 2023; Pawlowski *et al*., 2020). Accordingly, there is a need to establish protocols suited to Korea's specific conditions to enable the complementary use of eDNA analysis alongside currently implemented biomonitoring methods (Table 4).

Initially, it is essential to determine the specific evaluation areas for the application of eDNA analysis (e.g., habitat assessments for rare or invasive species and aquatic ecosystem health evaluations) and to prioritize taxonomic groups that are relatively easy to sample, thereby ensuring a minimum level of eDNA information for reliable assessment. Simultaneously, robust, reproducible, and detailed protocols for sampling and analysis must be established to avoid variability among researchers (Dickie *et al*., 2018). Furthermore, a sampling protocol that is simple, cost-effective, long-lived, adaptable to new methods and personnel changes is needed. Therefore, we should promote collaboration with private researchers and pursue government-led projects to enable the effective use of eDNA methods from methodological, cost, and spatiotemporal perspectives.

Conclusion

eDNA analysis techniques enable the consistent collection of data in a cost-efficient way compared to traditional survey methods. This makes them particularly effective for national biological monitoring programs, which require continuous collection of biological information across extensive regions. At the current level of eDNA technology, however, there are areas that require improvement, such as insufficient genetic information, species misidentification, and contamination of genetic data when using metabarcoding techniques to identify species lists at the community level. However, when the purpose and target of detection are clearly defined, such as detecting invasive or endangered species, eDNA analysis offers a significant advantage as a non-destructive and cost-effective alternative to traditional survey methods. To maximize the efficiency of eDNA analysis, it is essential to develop monitoring strategies and standardized methodologies tailored to the specific objectives of eDNA applications in each country. This review comprehensively summarizes various national cases where eDNA has been applied to biological monitoring programs. Insights into strategies aimed at overcoming current technical limitations—such as securing genetic libraries, establishing data utilization platforms, and developing monitoring frameworks—will serve as valuable resources for effectively planning national-scale eDNA-based biological monitoring initiatives in the future.

Author Contributions

Concept: K. H. Chang; Acquisition of data: Y. Choi; Drafting of the manuscript: K. H. Chang, Y. Choi, and H. J. Oh; Critical revision: H. J. Oh, and J. D. Yoon.

Conflict of Interest

The authors declare that they have no competing interests.

References

Agersnap, S., Sigsgaard, E.E., Jensen, M.R., Avila, M.D.P., Carl, H., Møller, P.R., *et al.* (2022). A national scale "BioBlitz" using citizen science and eDNA metabarcoding for monitoring coastal marine fish. *Frontiers in Marine Science*, 9, 824100. https://doi.org/10.3389/fmars.2022.824100

Alam, M.J., Kim, N.K., Andriyono, S., Choi, H.K., Lee, J.H., and Kim, H.W. (2020). Assessment of fish biodiversity in four Korean rivers using environmental DNA metabarcoding. *PeerJ*, 8, e9508. https://doi.org/10.7717/peerj.9508

An, H.E., Mun, M.H., and Kim, C.B. (2023). Metabarcoding by combining environmental DNA with environmental RNA to monitor fish species in the Han River, Korea. *Fishes*, 8, 550. https://doi.org/10.3390/fishes8110550

Bálint, M., Nowak, C., Márton, O., Pauls, S.U., Wittwer, C., Aramayo, J.L., *et al*. (2018). Accuracy, limitations and cost efficiency of eDNA-based community survey in tropical frogs. *Molecular Ecology Resources*, 18, 1415-1426. https://doi.org/10.1111/1755-0998.12934

Berger, C.S., Hernandez, C., Laporte, M., Côté, G., Paradis, Y., Kameni, D.W., *et al*. (2020). Fine-scale environmental heterogeneity shapes fluvial fish communities as revealed by eDNA metabarcoding. *Environmental DNA*, 2, 647-666. https://doi.org/10.1002/edn3.129

Bergsten, J., Bilton, D.T., Fujisawa, T., Elliott, M., Monaghan, M.T., Balke, M., *et al*. (2012). The effect of geographical scale of sampling on DNA barcoding. *Systematic Biology*, 61, 851-869. https://doi.org/10.1093/sysbio/sys037

Biodiversity Center of Japan. (2020). [*A Guide to Freshwater Fish Survey Methods Using Environmental DNA Analysis Technology: For Using Environmental DNA Analysis for the First Time in Secondary Natural Environments*]. Biodiversity Center of Japan, Nature Conservation Bureau, Japanese Ministry of the Environment. *Japanese*.

Bird, S., Wilkinson, S., and McGaughran, A. (2024). Wetland biodiversity in Aotearoa New Zealand: an eDNA perspective on exotic and non-exotic species. *New Zealand Journal of Zoology*, 1-18. https://doi.org/10.1080/03014223.2024.2359685

Burian, A., Mauvisseau, Q., Bulling, M., Domisch, S., Qian, S., and Sweet, M. (2021). Improving the reliability of eDNA data

interpretation. *Molecular Ecology Resources*, 21, 1422-1433. https://doi.org/10.1111/1755-0998.13367

Carim, K.J., McKelvey, K.S., Young, M.K., Wilcox, T.M., and Schwartz, M.K. (2016). *A Protocol for Collecting Environmental DNA Samples from Streams*. United States Department of Agriculture.

Chessman, B.C. (2003). New sensitivity grades for Australian river macroinvertebrates. *Marine and Freshwater Research*, 54, 95-103. https://doi.org/10.1071/MF02114

De Brauwer, M., Clarke, L.J., Chariton, A., Cooper, M.K., De Bruyn, M., Furlan, E., *et al*. (2023). Best practice guidelines for environmental DNA biomonitoring in Australia and New Zealand. *Environmental DNA*, 5, 417-423. https://doi.org/10.1002/edn3.395

Derocles, S.A., Bohan, D.A., Dumbrell, A.J., Kitson, J.J., Massol, F., Pauvert, C., *et al*. (2018). Biomonitoring for the 21st century: integrating next-generation sequencing into ecological network analysis. *Advances in Ecological Research*, 58, 1-62. https://doi.org/10.1016/bs.aecr.2017.12.001

Dickie, I.A., Boyer, S., Buckley, H.L., Duncan, R.P., Gardner, P.P., Hogg, I.D., *et al*. (2018). Towards robust and repeatable sampling methods in eDNA-based studies. *Molecular Ecology Resources*, 18, 940-952. https://doi.org/10.1111/1755-0998.12907

Doi, H., and Nakamura, K. (2023). Dominant barriers and the solutions to the social application of environmental DNA. *Landscape and Ecological Engineering*, 19, 305-312. https://doi.org/10.1007/s11355-023-00549-7

Eum, S.J., Kim, N., Seol, M.A., and Kim, J.Y. (2023). Comparative analysis of freshwater fish species in civilian control zone in South Korea: a comparison between direct survey results and indirect assessment via eDNA. *Korean Journal of Ichthyology*, 35, 224-235. https://doi.org/10.35399/isk.35.4.2

Fujii, T., Morimoto, S., Hoshino, Y.T., Okada, H., Wang, Y., Chu, H., *et al*. (2009). *Studies of diversity and functions of soil microbes and nematodes in NIAES using nucleic acids extracted from soil.* Paper presented at Marco Sumposium 2009, Tsukuba, Japan.

Hering, D., Borja, A., Jones, J.I., Pont, D., Boets, P., Bouchez, A., *et al*. (2018). Implementation options for DNA-based identification into ecological status assessment under the European Water Framework Directive. *Water Research*, 138, 192-205. https://doi.org/10.1016/j.watres.2018.03.003

Jo, T., Ikeda, S., Fukuoka, A., Inagawa, T., Okitsu, J., Katano, I., *et al*. (2021). Utility of environmental DNA analysis for effective monitoring of invasive fish species in reservoirs. *Ecosphere*, 12, e03643. https://doi.org/10.1002/ecs2.3643

Kallis, G., and Butler, D. (2001). The EU water framework directive: measures and implications. *Water Policy*, 3, 125-142. https://doi.org/10.1016/S1366-7017(01)00007-1

Kang, Y., Jeon, J., Han, S., Won, S., and Song, Y. (2023). Feasibility of environmental DNA metabarcoding for invasive species detection according to taxa. *Journal of Environmental Impact Assessment*, 32, 94-111. https://doi.org/10.14249/eia.2023.32.2.94

Kang, Y., Shin, W., Kim, Y., and Song, Y. (2024). Land use characteristics affect the sub-basinal scale urban fish community identified by environmental DNA metabarcoding. *Landscape and Ecological Engineering*, 20, 163-185. https://doi.org/10.1007/s11355-023-00587-1

Kelly, R.P., Lodge, D.M., Lee, K.N., Theroux, S., Sepulveda, A.J., Scholin, C.A., *et al*. (2023). Toward a national eDNA strategy for the United States. *Environmental DNA*, 6, e432. https://doi.org/10.1002/edn3.432

Kim, G., and Song, Y. (2021). Identification of freshwater fish species in Korea using environmental DNA technique: from the experiment at the Freshwater Fish Ecological Learning Center in Yangpyeong, Gyeonggi Do. *Journal of Environmental Impact Assessment*, 30, 1-12. https://doi.org/10.14249/EIA.2021.30.1.1

Kim, J.H., Jo, H., Chang, M.H., Woo, S.H., Cho, Y., and Yoon, J.D. (2020). Application of environmental DNA for monitoring of freshwater fish in Korea. *Korean Journal of Ecology and Environment*, 53, 63-72. https://doi.org/10.11614/ksl.2020.53.1.063

Kim, K., Ryu, J., and Hwang, S.J. (2021a). Sampling and extraction method for environmental DNA (eDNA) in freshwater ecosystems. *Korean Journal of Ecology and Environment*, 54, 170-189. https://doi.org/10.11614/KSL.2021.54.3.170

Kim, K.S., Kim, K.Y., and Yoon, J.D. (2021b). Efficiency comparison of environmental DNA metabarcoding of freshwater fishes according to filters, extraction kits, primer sets and PCR methods. *Korean Journal of Ecology and Environment*, 54, 199-208. https://doi.org/10.11614/ksl.2021.54.3.199

Kobayashi, S., Akamatsu, F., Nakanishi, S., Yajima, Y., Miwa, J., and Amano, K. (2013). [Describing benthic invertebrate communities in Japanese rivers using national environmental census data: spatial distributions of total biomass and major taxa]. *Japanese Journal of Limnology*, 74, 129-152. *Japanese*. https://doi.org/10.3739/rikusui.74.129

Kondoh, M., Kasada, M., Abe, T., Kasai, A., Dazai, A., Masuda, R., *et al*. (2024). Community science initiatives utilizing environmental DNA. In Y. Suzuki-Ohno (Ed.), *Community Science in Ecology: Case Studies of Public Participation in Ecological Research in Japan* (pp. 83-99). Springer Singapore.

Kwak, I.S., Ji, C.W., Kim, W.S., and Kong, D. (2022). The list of Korean organisms registered in the NCBI nucleotide database for environmental DNA research. *Korean Journal of Ecology and Environment*, 55, 352-359. https://doi.org/10.11614/KSL.2022.55.4.352

Kwak, T.S., Kim, W.S., Lee, S.H., and Kwak, I.S. (2021). Development of the filterable water sampler system for eDNA filtering and performance evaluation of the system through eDNA monitoring at catchment conduit intake-reservoir. *Korean Journal of Ecology and Environment*, 54, 272-279. https://doi.org/10.11614/ksl.2021.54.4.272

Laramie, M.B., Pilliod, D.S., Goldberg, C.S., and Strickler, K.M. (2015). *Environmental DNA Sampling Protocol: Filtering Water to Capture DNA from Aquatic Organisms*. U.S. Department of the Interior, U.S. Geological Survey.

Larson, E.R., Graham, B.M., Achury, R., Coon, J.J., Daniels, M.K., Gambrell, D.K., *et al*. (2020). From eDNA to citizen science: emerging tools for the early detection of invasive species. *Frontiers in Ecology and the Environment*, 18, 194-202. https://doi.org/10.1002/fee.2162

Leese, F., Altermatt, F., Bouchez, A., Ekrem, T., Hering, D., Meissner, K., *et al*. (2016). DNAqua-Net: developing new genetic tools for bioassessment and monitoring of aquatic eco-

systems in Europe. *Research Ideas and Outcomes*, 2, e11321. https://doi.org/10.3897/rio.2.e11321

Leese, F., Bouchez, A., Abarenkov, K., Altermatt, F., Borja, Á., Bruce, K., *et al*. (2018). Why we need sustainable networks bridging countries, disciplines, cultures and generations for aquatic biomonitoring 2.0: a perspective derived from the *DNAqua-Net* COST action. *Advances in Ecological Research*, 58, 63-99. https://doi.org/10.1016/bs.aecr.2018.01.001

Makiola, A., Compson, Z.G., Baird, D.J., Barnes, M.A., Boerlijst, S.P., Bouchez, A., *et al*. (2020). Key questions for next-generation biomonitoring. *Frontiers in Environmental Science*, 7, 197. https://doi.org/10.3389/fenvs.2019.00197

Minamoto, T., Miya, M., Sado, T., Seino, S., Doi, H., Kondoh, M., *et al.* (2021). An illustrated manual for environmental DNA research: water sampling guidelines and experimental protocols. *Environmental DNA*, 3, 8-13. https://doi.org/10.1002/edn3.121

Ministry of Environment (ME). (2020). *Monitoring of the Natural Ecosystem in the Naeseong Stream Watershed*. ME.

Ministry of Environment (ME). (2021). *Monitoring of the Natural Ecosystem in the Naeseong Stream Watershed*. ME.

Ministry of Environment (ME). (2022). *Monitoring of the Natural Ecosystem in the Naeseong Stream Watershed*. ME.

Morris, L., Beesley, L.S., Stevens, E.R., Gwinn, D.C., Hyde, J., Thompson, S., *et al*. (2024). Active eDNA is more cost-effective than fyke nets or passive eDNA collection when monitoring the invasion of an alien freshwater fish. *Environmental DNA*, 6, e70010. https://doi.org/10.1002/edn3.70010

Mun, M.H., An, H.E., and Kim, C.B. (2024). Examination of the utility of environmental DNA metabarcoding for monitoring fish species in Han Rivr, Korea. *Journal of Environmental Analysis, Health and Toxicology*, 27, 39-48. https://doi.org/10.36278/jeaht.27.1.39

National Institute of Water and Atmospheric Research (NIWA). (2018). *New Zealand: fish passage guidelines: for structures up to 4 metres*. Retrieved October 3, 2024 from https://environment.govt.nz/assets/publications/Files/New-Zealand-fish-passage-guidelines-for-structures-up-to-four-metres.pdf.

National Institute of Water and Atmospheric Research (NIWA). (2023). *eDNA guidelines and field protocols for lotic systems*. Retrieved October 3, 2024 from https://niwa.co.nz/sites/default/files/2024-05/eDNa%20guidelines%20report_12122023%20FINAL%20%283%29.pdf.

Navarro, L.M., Fernández, N., Guerra, C., Guralnick, R., Kissling, W.D., Londoño, M.C., *et al*. (2017). Monitoring biodiversity change through effective global coordination. *Current Opinion in Environmental Sustainability*, 29, 158-169. https://doi.org/10.1016/j.cosust.2018.02.005

Oh, H.J., Chae, Y.J., Choi, Y., Ku, D., Heo, Y.J., Kwak, I.S., *et al*. (2021). Review and suggestions for applying DNA sequencing to zooplankton researches: from taxonomic approaches to biological interaction analysis. *Korean Journal of Ecology and Environment*, 54, 156-169. https://doi.org/10.11614/KSL.2021.54.3.156

Oliveira, L.M., Knebelsberger, T., Landi, M., Soares, P., Raupach, M.J., and Costa, F.O. (2016). Assembling and auditing a comprehensive DNA barcode reference library for European marine fishes. *Journal of Fish Biology*, 89, 2741-2754. https://doi.org/10.1111/jfb.13169

Osugi, T., Akashi, E., Yamaguchi, K., Kanazawa, H., and Nishikawa, M. (2019). National census on river and dam environments in Japan and utilization for appropriate dam management using the results. In J.P. Tournier, T. Bennett, and J. Bibeau (Eds.), *Sustainable and Safe Dams Around the World* (pp. 1590-1602). CRC Press.

Pawlowski, J., Apothéloz-Perret-Gentil, L., Mächler, E., and Altermatt, F. (2020). *Environmental DNA Applications in Biomonitoring and Bio-assessment of Aquatic Ecosystems: Guidelines*. Federal Office for the Environment.

Ruppert, K.M., Kline, R.J., and Rahman, M.S. (2019). Past, present, and future perspectives of environmental DNA (eDNA) metabarcoding: a systematic review in methods, monitoring, and applications of global eDNA. *Global Ecology and Conservation*, 17, e00547. https://doi.org/10.1016/j.gecco.2019.e00547

Schenekar, T. (2023). The current state of eDNA research in freshwater ecosystems: are we shifting from the developmental phase to standard application in biomonitoring? *Hydrobiologia*, 850, 1263-1282. https://doi.org/10.1007/s10750-022-04891-z

Schenekar, T., Schletterer, M., Lecaudey, L.A., and Weiss, S.J. (2020). Reference databases, primer choice, and assay sensitivity for environmental metabarcoding: lessons learnt from a re-evaluation of an eDNA fish assessment in the Volga headwaters. *River Research and Applications*, 36, 1004-1013. https://doi.org/10.1002/rra.3610

Stepien, C.A., Lance, R.F., Klymus, K.E., and Hunter, M.E. (2023). The government eDNA working group 6th Annual eDNA Technical Exchange Workshop. *Environmental DNA*, 5, 1196-1201. https://doi.org/10.1002/edn3.466

Stubbington, R., Chadd, R., Cid, N., Csabai, Z., Miliša, M., Morais, M., *et al*. (2018). Biomonitoring of intermittent rivers and ephemeral streams in Europe: current practice and priorities to enhance ecological status assessments. *The Science of the Total Environment*, 618, 1096-1113. https://doi.org/10.1016/j.scitotenv.2017.09.137

Suren, A.M., Burdon, F.J., and Wilkinson, S.P. (2024). eDNA is a useful environmental monitoring tool for assessing stream ecological health. *Environmental DNA*, 6, e596. https://doi.org/10.1002/edn3.596

Takahara, T., Minamoto, T., and Doi, H. (2013). Using environmental DNA to estimate the distribution of an invasive fish species in ponds. *PloS One*, 8, e56584. https://doi.org/10.1371/journal.pone.005658454

WIDERLAB. (2024). *The taxon-independent community index*. Retrieved January 13, 2025 from https://www.wilderlab.co.nz/tici.

Wilkinson, S.P., Gault, A.A., Welsh, S.A., Smith, J.P., David, B.O., Hicks, A.S., *et al*. (2024). TICI: a taxon-independent community index for eDNA-based ecological health assessment. *PeerJ*, 12, e16963. https://doi.org/10.7717/peerj.16963

Yamanaka, H., and Minamoto, T. (2016). The use of environmental DNA of fishes as an efficient method of determining habitat connectivity. *Ecological Indicators*, 62, 147-153. https://doi.org/10.1016/j.ecolind.2015.11.022

Yoo, K.E., Ho, H.I., Kim, H., Kim, K., and Hwang, S.J. (2023). Efficiency of density gradient centrifugation method (Ludox method) based on eDNA for the analysis of Harm-

ful algal bloom potential. *Korean Journal of Ecology and Environment*, 56, 36-44. https://doi.org/10.11614/KSL.2023.56.1.036

Yun, B.H., Kim, Y.H., Sung, M.S., Han, H., Han, J., and Bang, I.C. (2022). Species-specific marker development for environmental DNA assay of endangered bull-head torrent catfish, Liobagrus obesus. *Korean Journal of Ichthyology*, 34, 208-217. https://doi.org/10.35399/ISK.34.3.7

Zhang, S., Lu, Q., Wang, Y., Wang, X., Zhao, J., and Yao, M. (2020). Assessment of fish communities using environmental DNA: effect of spatial sampling design in lentic systems of different sizes. *Molecular Ecology Resources*, 20, 242-255. https://doi.org/10.1111/1755-0998.13105