Roadmap for implementing environmental DNA (eDNA) and other molecular monitoring methods in Finland

Vision and action plan for 2022–2025

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Abstract

Roadmap for implementing environmental DNA (eDNA) and other molecular monitoring methods in Finland – Vision and Action Plan for 2022-2025

Technological development in molecular methodology has been extremely fast in the past two decades, and groundbreaking new approaches have been introduced. It is now possible to detect and quantify DNA or RNA of target species or even map the whole species community in environmental samples of water, sediment, soil, air or assemblages of whole organisms. Moreover, the costs of high-throughput sequencing and other advanced molecular methods have decreased and methodological pipelines from sampling to data analysis developed sufficiently to allow large-scale, routine application of the new methods in environmental monitoring. This presents a huge opportunity to improve the coverage, accuracy and cost-efficiency of monitoring, enabling a much more complete picture of biodiversity and the state of the environment and their trends. As the new European Biodiversity Strategy for 2030 and other international policies to halt biodiversity loss and the degradation of habitats are translated into concrete measures, the quality of the monitoring data will play a crucial role in determining their success or failure.

In this roadmap commissioned by the Finnish Ministry of the Environment, we assess the state-of-the-art in molecular monitoring methods in Finland within the international context, identify challenges and development areas that remain to be addressed and propose an action plan for promoting the coordinated implementation of molecular methods in national monitoring programs. Apart from the most recent scientific literature, our analysis is based on survey results, direct enquiries and interviews. Participation of the national community of experts from different sectors was enabled and invited at several stages of the roadmap preparation.

Internationally, molecular monitoring methods are being actively developed and are routinely implemented in monitoring across different taxa and ecosystems. In Finland, molecular monitoring methods have been tested and piloted by all major institutions responsible for environmental monitoring, and the methods are already applied routinely in the monitoring of individual game species such as the wolf and European and Canadian beaver. However, other areas such as the monitoring of biodiversity, threatened species, non-mammalian invasive species or emerging plant or animal pests remain less developed, and national efforts and expertise are scattered across different organizations. Funding and know-how are perceived as the most important factors limiting molecular monitoring method implementation.

We estimate that extensive, routine implementation of a wide range of molecular monitoring methods is conceivable in Finland before 2030. As the primary development areas for reaching this goal, we identify (i) international coordination and standard development, (ii) networking across sectors, (iii) education, (iv) infrastructure, (v) reference sequence libraries and the mapping of whole genomes, and (vi) modelling and analysis tool development. For concrete actions in 2022–2025, we propose (1) a cross-governmental funding instrument, (2) a permanent working group responsible for national and international coordination, (3) a national network and (4) an online platform to enhance interaction and knowledge transfer, as well as (5) a national data management system with collectively agreed data and metadata formats and standards.

Keywords: biodiversity, environmental monitoring, metabarcoding, next-generation sequencing, monitoring strategy

Tiivistelmä

Kansallinen tiekartta ympäristö-DNA:n ja muiden molekyylibiologisten seurantamenetelmien käyttöönotolle – visio ja toimenpidesuunnitelma vuosille 2022-2025

Molekyylibiologisten menetelmien teknologinen kehitys on ollut ennennäkemättömän nopeaa kahden viime vuosikymmenen aikana. Uudet menetelmät mahdollistavat kohdelajien DNA:n tai RNA:n havaitsemisen ja runsausmääritysten tai koko eliöyhteisön kartoittamisen esimerkiksi vesi-, sedimentti-, maaperä- tai ilmanäytteistä tai kokonaisia yksilöitä sisältävistä kokoomanäytteistä. Massiivisen rinnakkaissekvensoinnin ja muiden menetelmien kustannukset ovat merkittävästi laskeneet ja menetelmäketjut näytteenotosta tulosten tulkintaan kehittyneet asteelle, joka mahdollistaa niiden laajamittaisen, rutiininomaisen käytön ympäristön seurannassa. Uusien menetelmien avulla voimme parantaa seurannan kattavuutta, tarkkuutta ja kustannustehokkuutta ja siten täydentää seurannan kautta muodostuvaa kuvaa luonnon monimuotoisuudesta ja sen muutoksista. Tälle tiedolle on suuri tarve – laadukas seuranta on keskeinen edellytys sille, että EU:n uuden biodiversiteettistrategian ja muiden luontokadon ja elinympäristöjen tilan huonontumisen pysäyttämiseen tähtäävien kansainvälisten sitoumusten toimeenpano onnistuu.

Tässä ympäristöministeriön tilaamassa tiekartassa arvioimme molekyylibiologisten seurantamenetelmien nykytilaa Suomessa osana laajempaa kansainvälistä kenttää, tunnistamme huomiota vaativia haasteita ja kehityskohteita ja ehdotamme konkreettisia toimenpiteitä molekyylibiologisten seurantamenetelmien koordinoidun käyttöönoton edistämiseksi lähivuosien aikana. Selvityksemme perustuu uusimman tieteellisen kirjallisuuden lisäksi kyselytutkimukseen sekä suoriin tiedusteluihin ja haastatteluihin. Yhteiskunnan eri sektoreita edustava kansallinen asiantuntijayhteisö osallistui tiekartan valmisteluun työn eri vaiheissa.

Molekyylibiologisia seurantamenetelmiä kehitetään parhaillaan aktiivisesti ympäri maailmaa eri eliöryhmille ja ekosysteemeille, ja yksittäisiä menetelmiä on useissa maissa otettu myös rutiininomaiseen käyttöön. Suomessa menetelmiä on kehitetty ja pilotoitu kaikissa keskeisissä ympäristön seurantaa koordinoivissa laitoksissa, ja yksittäisten riistaeläinten kuten suden ja kanadan- ja euroopanmajavan seurannassa ne ovat jo rutiinikäytössä. Biodiversiteetin, uhanalaisten lajien, vieraslajien (nisäkkäitä lukuun ottamatta) ja muiden haitallisten lajien kansallisessa seurannassa molekyylibiologisten menetelmien käyttö on kuitenkin vielä kokeiluasteella, ja kehittämishankkeiden ja asiantuntijuuden kenttä on hajanainen. Riittämätöntä rahoitusta ja osaamista pidetään alan asiantuntijoiden keskuudessa tärkeimpinä menetelmien käyttöönottoa rajoittavina tekijöinä.

Arviomme mukaan laaja kirjo molekyylibiologisia seurantamenetelmiä olisi mahdollista ottaa laajamittaiseen rutiininomaiseen käyttöön vuoteen 2030 mennessä. Tärkeimmiksi kehityskohteiksi nousivat (i) kansainvälinen koordinaatio ja menetelmien standardointi, (ii) organisaatioiden ja sektoreiden välinen verkostoituminen, (iii) koulutus, (iv) infrastruktuuri, (v) referenssisekvenssikirjastot ja kokonaisten genomien kartoittaminen sekä (vi) malli- ja analyysityökalujen kehittäminen. Konkreettisiksi toimenpiteiksi vuosille 2022-2025 esitämme (1) poikkihallinnollista rahoitusohjelmaa molekyylibiologisten seurantamenetelmien käyttöönottoa edistäville tutkimus- ja kehityshankkeille, (2) pysyvää työryhmää kansallisen ja kansainvälisen koordinaation edistämiseksi, (3) olemassa olevan kansallisen asiantuntijaverkoston laajentamista, (4) internet-pohjaista alustaa vuorovaikutuksen ja tiedonjaon tehostamiseksi sekä (5) kansallista, yhdessä sovittuja data- ja metadatastandardeja noudattavaa molekyylibiologisten seuranta-aineistojen tiedonhallintajärjestelmää.

Asiasanat: biodiversiteetti, ympäristön seuranta, metaviivakoodaus, uuden sukupolven sekvensointi, seurannan strategia

Sammandrag

Färdplan för ibruktagande av miljö-DNA (eDNA) och andra molekylära övervakningsmetoder i Finland – Vision och handlingsplan för 2022-2025

Den teknologiska utvecklingen av molekylära metoder har varit extremt snabb de senaste två årtiondena och nya tillvägagångssätt har introducerats. Det är nu möjligt att detektera och kvantifiera DNA eller RNA från målarter eller till och med från hela artsamhällen i vatten-, jord-, luft- eller organismprover. Kostnaderna för högkapacitetssekvensering och andra avancerade molekylära metoder har sjunkit och de metodologiska rutinerna från provtagning till dataanalyser har utvecklats till ett stadie som möjliggör ett storskaligt och rutinmässigt ibruktagande av dessa nya metoder inom miljöövervakningen. Detta erbjuder en stor möjlighet till förbättring av täckning, precision och kostnadseffektivitet av övervakningen och möjliggör en mer komplett bild av den biologiska mångfalden och miljötillståndet samt trenderna inom dessa. När den nya europeiska strategin för biologisk mångfald 2030 och andra internationella avtal för att stoppa förlusterna av biologisk mångfald och habitatdegradering ska översättas till konkreta handlingar, kommer kvaliteten av övervakningsdatan att ha en betydande roll för att avgöra huruvida man lyckats eller misslyckats med målsättningarna.

I denna färdplan, gjord på uppdrag av miljöministeriet i Finland, bedömer vi utvecklingsnivån av molekylära övervakningsmetoder i Finland i ett internationellt sammanhang, identifierar utmaningar och utvecklingsområden som bör beaktas samt föreslår en handlingsplan för att främja ett koordinerat ibruktagande av molekylära metoder i nationella övervakningsprogram. I tillägg till den senaste vetenskapliga litteraturen baserar sig våra analyser på undersökningsresultat, direkta förfrågningar och intervjuer. Deltagande av nationella experter från olika sektorer möjliggjordes och experterna inbjöds att inverka på förberedelserna av färdplanen under flera skeden.

På internationell nivå utvecklas molekylära metoder aktivt och metoderna används rutinmässigt inom övervakning av arter och ekosystem. I Finland har molekylära metoder testats genom pilotstudier av alla institutioner med ansvar för miljöövervakning och molekylära metoder används redan rutinmässigt i övervakningen av enskilda viltarter så som varg samt europeisk och nordamerikansk bäver. Inom andra områden, så som övervakning av biologisk mångfald, hotade arter, invasiva arter, samt växt- och djurskadegörare är metoderna fortfarande mindre utvecklade och resurserna samt expertisen mer spridda bland olika organisationer. Finansiering och kunnande anses vara de viktigaste faktorerna som begränsar ibruktagandet av molekylära metoder.

Vi uppskattar att ett omfattande, rutinmässigt ibruktagande av ett brett spektrum av molekylära övervakningsmetoder kan uppnås före 2030 i Finland. De primära utvecklingsområdena för att uppnå detta mål anses vara (i) internationell koordinering och standardutveckling, (ii) nätverkande över sektorsgränserna, (iii) utbildning, (iv) infrastruktur, (v) referensbibliotek för sekvenser och kartläggning av hela genom samt (vi) modellerings- och analysverktygsutveckling. Som konkreta handlingar 2022-2025 föreslår vi (1) ett myndighetsövergripande finansieringsinstrument, (2) en permanent arbetsgrupp med ansvar för nationell och internationell koordination, (3) ett nationellt expertnätverk och (4) en online plattform för att befrämja kommunikation och kunskapsöverföring, samt (5) ett nationellt datahanteringssystem med överenskomna data- och metadataformat och -standarder.

Nyckelord: biologisk mångfald, biodiversitet, miljöövervakning, metabarcoding, next-generation sequencing, övervakningsstrategi

Preface

This report is the main output of the eDNA roadmap project *("Kansallinen tiekartta eDNA:n ja muiden molekyylibiologisten seurantamenetelmien tehokkaan, luotettavan ja rutiininomaisen käyttöönoton eri vaiheista"),* proposed by researchers at the Finnish Environment Institute (SYKE) and commissioned by the Finnish Ministry of the Environment (YM) in December 2020. The roadmap project is one milestone but also a convergence point of several lines of development with SYKE involvement, including the international DNAqua-Net (2017-) and SCANDNAnet (2018-) networks, inclusion of DNA metabarcoding in the national monitoring of the environmental impact of agriculture and forestry (MaaMet), the establishment of an informal eDNA network and the launch of the Finnish Ecosystem Observatory (FEO) project (2020). The roadmap work has been conducted in close collaboration with FEO (funded by YM) and the eDNA pilot project ("*A pilot for implementing environmental DNA (eDNA) based methods into environmental and biomonitoring*", funded by SYKE).

The original aims of the eDNA roadmap project were to describe the national state-of-the art in the use of molecular monitoring methods, identify the main possibilities and challenges and development needs, formulate medium- and short-term aims and provide a concrete action plan for the next four years. It quickly became obvious that reaching these goals would be impossible without major input from other organizations, particularly the National Resources Institute Finland (Luke; represented in our team by Eeva Vainio, Terhi Iso-Touru, Taina Pennanen and Sannakajsa Velmala). Utilizing particularly the DNAqua-Net and national eDNA networks, we reached out to the expert community, which contributed essential insight into ongoing research and development projects, the overall state of the field and the possibilities and challenges involved in the implementation of molecular monitoring methods. In addition, the community was invited to comment and discuss the first draft of this report, which resulted in significant improvements in the coverage of different topics and viewpoints. We are deeply indebted to all national and international experts involved in the process – respondents of our Webropol survey, participants of our national workshops on March 25th and November 12th 2021 and all others who gave insightful comments or otherwise contributed to the preparation of this report. We would also like to thank eDNA roadmap project supervisor, Senior specialist Joona Lehtomäki from Finnish Ministry of the Environment for all his valuable guidance during this project.

Contents

Executive summary

The technological leaps achieved in the past 15 years make it possible to detect, identify and quantify DNA or RNA of target species or even map entire species communities from environmental samples. This novel capability extends to water, air, soil, sediment and diverse samples containing a multitude of trapped organisms such as insects. These molecular monitoring methods (MoMM) are revolutionizing the field of biological and environmental monitoring. They offer highly accurate, repeatable and cost-efficient species identification to anyone regardless of operator taxonomic expertise and show great potential for automatization. MoMM have already been implemented in some Finnish national monitoring programs, particularly for individual game species. Further, several pilot projects have been started e.g. in environmental impact monitoring in freshwater systems, marine phytoplankton, fisheries and threatened species monitoring. At the same time, the field is fragmented, with limited links between different organizations and no national coordination. Knowledge and know-how of the new methods is highly scattered in organizations that conduct monitoring and this currently limits the implementation of MoMM more severely than any technical challenges. This roadmap seeks to launch a national discussion on and provide actionable recommendations for the coordinated implementation of MoMM in Finland. Moreover, the roadmap emphasizes that actions should not proceed only on a national level but stresses the need to take an active role also in ongoing international coordination efforts and initiatives.

New biological monitoring tools to track biodiversity trends and ecosystem health

Data from biological monitoring is the primary source of information for detecting and quantifying biodiversity loss, as well as finding and evaluating ways to halt it. Molecular techniques have great potential to benefit, improve and extend current biological monitoring in all types of habitats. They can produce objective, easily comparable and reproducible species identification, and can be applied also to many currently hard-to-detect and poorly known groups of organisms such as microbes and fungi. Present-day methods include species-specific PCR assays based on **DNA barcoding**, **quantitative PCR** methods (qPCR, dPCR, ddPCR) that also determine the abundance of the target species and **DNA metabarcoding** based on the sequencing of massive numbers of individual DNA molecules from a single sample. DNA can be analysed from environmental samples (**eDNA**) or samples containing entire organisms, their parts or feces. **RNA-based methods** provide information on the activity of organisms in the environment.

In many cases, molecular methods can be applied similarly to traditional monitoring methods, with taxonomic identification based on comparison of the DNA sequence to a **reference sequence database** of confirmed specimens. Alternatively, in **taxonomy-free methods**, assemblages of DNA (or RNA) sequences are compared e.g. by machine learning approaches to reveal changes in biodiversity without species identification.

National forerunners in molecular monitoring: game, fisheries, freshwater and more

As a basis for our recommendations, we conducted an analysis of the national state-of-the-art in MoMM implementation within the international context. Our analysis was based on the international **scientific literature** from the past five years, an internationally distributed **Webropol survey**, direct **enquiries and interviews** of persons responsible for national monitoring programs and the results of two **national workshops**.

Internationally, MoMM are being very actively developed across the tree of life and for most ecosystems, from the deep sea to mountains and from tropical forest to polar regions. Generally, eDNA-based methods for biodiversity monitoring are the most actively studied and the readiest for routine implementation in aquatic systems, especially for fish and benthic invertebrates. While most scientific studies report significant advantages offered by MoMM, systematic large-scale demonstrations are still scarce. This result was supported also by the Webropol survey: MoMM were estimated to be in the testing or planning stages, with only some examples of routine implementation. In Finland, examples of implementation in routine monitoring include game species monitoring such as for large carnivores and the European and Canadian Beaver. In addition, advanced pilot projects have included environmental impact monitoring in freshwater systems, fishery assessments and soil quality and microbial biodiversity monitoring. Smaller-scale pilots cover a variety of taxa from liverworts to mussels. Compared to the international level, the national readiness of MoMM is high in managed populations such as game and fisheries, but lags behind in biodiversity, threatened species and invasive species monitoring.

Added information, reliability, speed, and cost-effectiveness are perceived as the biggest **possibilities** offered by MoMM. As the most important **challenges** limiting MoMM implementation, we identified the lack of funding, expertise and method standards, incompleteness of reference sequence libraries, limitations in producing abundance information and the complicated interpretation of results.
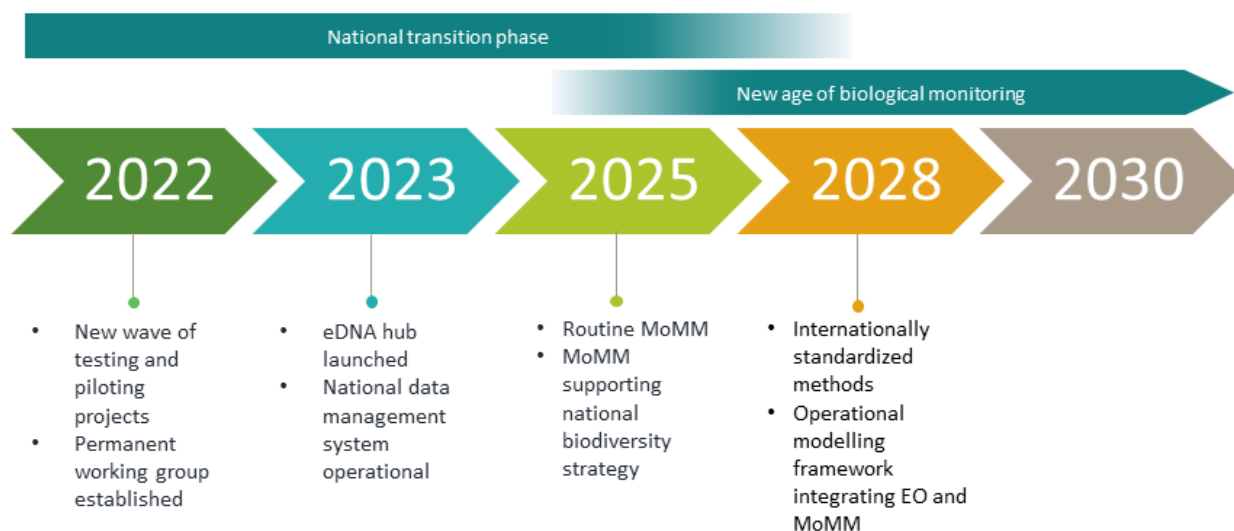

Figure 1. Timeline for the transition into the new age of biological monitoring.

The new age of biological monitoring

Given the speed of the international method development and the highly promising results from the first MoMM programs and pilots, we envision that reliable, internationally standardized molecular methods are routinely used in national biological monitoring before 2030 (Fig. 1). Traditional taxonomic monitoring complements the new methods, ensuring the continuity of valuable long-term data sets and facets of biodiversity not yet captured by molecular methods. As the new methods gain ground, the role of citizen observations in providing additional taxonomic data increases. Where appropriate, citizen science is also routinely applied to sample collection for MoMM. Like traditional monitoring data, MoMM-based data should be Findable, Accessible, Interoperable and Reusable according to the FAIR Guiding Principles for scientific data management and stewardship.

In the new age of biological monitoring, MoMM have developed alongside Earth observation and other remote sensing methodology as well as ecological modelling and machine learning applications. Validated modelling and analysis tools are used to assist the interpretation of DNA-based observations and in sampling design.

Large-scale molecular monitoring requires improvements in standardization, knowledge base and transfer of know-how

Based on our analysis of the state-of-the-art and the chief roadblocks to MoMM implementation, we have identified six development areas that merit particular attention in the coming years.

1) International coordination and standard development are paramount to ensure comparability of data in time and space, to avoid unnecessary duplication of efforts and to minimize time until full implementation on a national, European and international scale.

2) Networking across sectors is essential to increase awareness and knowledge of the new methods, to establish the connections that form the basis of a viable market – and to provide platforms for critical discussion of when, where and how the new methods should be applied.

3) Education plays a key role in solving the lack of up-to-date know-how, one of the most pressing challenges identified in our status assessment. Further, education is strongly related to quality control and overall comparability.

4) Infrastructure to support MoMM implementation largely exists but is somewhat scattered. Existing infrastructure should be used more efficiently and should be developed, updated and extended in a coordinated manner.

5) Reference sequence libraries should be extended not only in designated projects but as a running task in monitoring programs and pilots. Also, efforts towards mapping whole genomes of organisms across different environments should be increased.

6) Modelling and analysis tools should be applied and developed alongside the MoMM methods to fully utilize the wealth of the information they provide.

Concrete actions: transition funding, coordination, networking, data management

We propose five concrete actions for 2022-2025 to promote the implementation of MoMM in Finland (Table 1).

A1: Directed funding for the transition, particularly from the Ministry of the Environment (YM) and the Ministry of Agriculture and Forestry (MMM), is required to enable goal-oriented development and implementation of the methods. We suggest that future ministerial research and infrastructure funding models should use co-funding and entail a cross-governmental and cross-departmental requirement, namely that developments towards MoMM implementation need to include at least two

agencies under the control of different ministries and that developments are tied to the national environmental monitoring strategy framework and implementation program. The need for new biodiversity indicators to monitor and assess the objectives of policies such as the new National Biodiversity Strategy and Action Plan for 2035 provides one concrete goal for research and development efforts (Table 2).

A2: The establishment of a permanent working group within the national environmental monitoring strategy implementation program to increase national MoMM coordination and to strengthen the role of Finland in international MoMM related networks and initiatives.

A3: Expand and promote the national cross-governmental eDNA network established in 2020 to strengthen its role as a national community of practice that produces opportunities for networking, exchange of information and experiences, education and critical discussion on MoMM implementation.

A4: The launch of an open access, subscription based, online platform to enable continuous real-time discussions and co-creative workflows across organizational boundaries and an easy access to MoMM related information, events and commercial services.

A5: Develop a national molecular data management system with data and metadata standards and automated links to international databases.

Table 1. Action plan for promoting MoMM implementation in 2022-2025.

Action	Coordination responsibility	Suggested timing	Cost estimate
A1: Directed R&D funding for transition to MoMM	YM, MMM (VM, TEM, STM)	2022-2025	1 M€ yearly
A2: Establishment of a permanent working group (eDNA embassy)	YM	2022	50 k€ yearly
A3: Expansion and promotion of the eDNA network	SYKE	2022	30 k€
A4: Launch of an online interaction platform (eDNA hub)	SYKE	2022-2023	100 k€
A5: Development of a national data management system	SYKE	2022-2024	500 k€

Table 2. The possibilities and limitations of MoMM in the monitoring and assessment of the objectives of the National Biodiversity Strategy and Action Plan (NBSAP). The primary aim of NBSAP is no net loss of nature in 2020-2035, in other words, a nature positive Finland by 2035.

	CONSERVATION		SUSTAINABLE USE			GENES
	Threat status	**Protection and restoration**	**Species**	**Habitats**	**Ecosystem functioning**	**Genetic diversity**
NBSAP objective by 2035	The level of threat to species and habitats is no longer increasing.	The coverage of protected areas has reached 30%, one third of which is strictly protected. Restoration measures cover 15% of degraded habitats.	Populations of declined indicator species groups of different habitats have increased [20%].	The quantity and quality of habitats under land use pressure have increased [10%].	The capacity of ecosystems to provide central ecosystem services has improved [10%].	Genetic diversity has not decreased (effective population sizes of species have not decreased).
Possibilities offered by MoMM	• Improved detection of threatened species by eDNA-based targeted monitoring (e.g. from water, soil, sediment, air, stomach contents, honey). Sampling feasible also for citizen scientists without taxonomic expertise. • Delimitation of populations of closely related species • Detection of hybridization	• Assessment of the impact of restoration using DNA-based community composition (taxonomy-based/taxonomy-free) or presence of indicator species.	• Monitoring of some poorly detectable groups feasible only with MoMM • eDNA-based targeted monitoring of indicator species. Sampling feasible also for citizen scientists without taxonomic expertise. • Cost-efficient monitoring of whole communities (incl. estimates of relative abundances) based on metabarcoding	• Assessment of habitat quality using DNA-based community composition e.g. in soil, water or air (taxonomy-based/taxonomy-free). • Assessment of habitat quality by DNA-based detection of indicator species and groups (e.g. microbes, fungi). • Assessment of habitat quality based on gene expression (eRNA)	• Inference of functional composition: DNA-based community composition and trait data • Assessment of ecosystem functioning based on gene expression (eRNA)	• Monitoring genetic diversity of target species (key/umbrella species, threatened species) • Estimation of effective vs. census population size • Delimitation of populations within species / assessing population structure
Challenges / limitations of MoMM	• Limited information on species abundance • Indirect observation (DNA transported e.g. by water or air) • Gaps in reference sequence libraries	• Representative sampling (high local variability in soil; DNA transported e.g. by water or air) • Sufficient reference data needed to establish DNA-based habitat quality measures.	• Comparability of MoMM-based and traditional abundance data • Standardized methods crucial for reliable and consistent monitoring	• Representative sampling (high local variability in soil; DNA transported e.g. by water or air) • Sufficient reference data needed to establish MoMM-based habitat quality measures.	• Representative sampling (high local variability in soil; DNA transported e.g. by water or air) • Sufficient reference data needed to establish MoMM-based measures for ecosystem functioning.	• Estimating genetic diversity is by necessity species specific, and therefore significant investments are needed to increase the number of species with sufficient genomic knowledge

1 Introduction

Human activities have completely transformed major parts of natural habitats and affect all life on Earth. The adverse effects of this development for ecosystems and species, including our own, are becoming increasingly evident. National, European and global efforts to conserve biodiversity are fast proceeding (e.g. Global Biodiversity Framework to 2030, EU Biodiversity Strategy for 2030). At the same time, large gaps remain both in the basic mapping and in our ability to monitor changes in biodiversity at relevant scales and resolution. In this chapter, we define the scope of this report and give a general introduction to the aims and challenges of environmental monitoring and the potential that new molecular methods present to the monitoring field.

1.1 Scope and definitions

We define monitoring as systematic, repeated observation particularly aimed at detecting current system status and any temporal changes therein. The scope of this report covers all monitoring of outdoor environments that targets biological organisms either directly (e.g. species monitoring) or indirectly as indicators of the general state of the system (e.g. environmental impact assessment). A particular emphasis is placed on efforts that fit under the umbrella of biodiversity monitoring, such as monitoring community composition or the distribution, population size and population structure of rare and threatened species, EU directive species, invasive alien species or species of economic interest. In addition to natural organisms, we also consider genetically modified organism (GMO) monitoring required by European and national legislation and international agreements. Other types of monitoring considered in this report include water, soil and air quality monitoring.

We use the umbrella term "molecular monitoring methods" (MoMM) to refer to all environmental monitoring methods that base their analysis on DNA or RNA. We focus primarily on methods related to species identification. While MoMM are also central in assessing population genetic metrics (e.g. intraspecific genetic diversity, level of inbreeding, population genetic structure and gene flow), we cover intraspecific genetic diversity only very briefly, recognizing that this topic would merit a separate in-depth assessment. Many MoMM covered here are based on the analysis of environmental DNA (eDNA): DNA found in samples of e.g. water, sediment, soil or air. eDNA includes both DNA released from organisms into their environment and DNA bound to microscopic organisms or their parts (as e.g. in the case of biofilm or plankton samples). However, this report is not restricted to eDNA applications but also considers methods based on different types of samples, such as assemblages of whole organisms (e.g. kick-net samples of stream invertebrates), their parts or fecal samples. Different present-day molecular methods are introduced in section 1.5.

1.2 Policies and legislation

Biodiversity monitoring is embedded in many policies and legislations. The Convention on Biological Diversity (CBD) has influenced opinions on biodiversity conservation and sustainable use at both the EU and the national level. Currently the CBD is negotiating towards a Global Biodiversity Framework for 2030. The EU Biodiversity Strategy for 2030 has already been adopted, and Finland is currently preparing its own national National Biodiversity Strategy and Action Plan for 2035. These international and national action plans will be implemented through upcoming national legislation.

The use of eDNA and other molecular methods is not specifically included in current legislation, but it is a promising methodology to provide the required data that currently is not feasibly attainable

through other means. For instance, eDNA has recently been recommended for use as a standardized biodiversity barometer tool of anthropogenic pressures in coastal ecosystems (DiBattista et al. 2020). Similarly, MoMM can be used for reporting on ecosystem condition and state indicators, requested e.g. by the EU Water Framework Directive (European Union 2000) and the EU Marine Strategy Framework Directive (European Union 2008, 2016, 2017) and can provide accurate data for the EU Habitat and Bird Directive (EU 1992, 2009) as well as for national assessments of threatened species (e.g. Red Lists, see Hyvärinen et al. 2019). Routine MoMM implementation would also benefit the monitoring of threatened species from the field samples and according to international agreements such as Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), monitoring invasive and other harmful species e.g. in adherence to the Ballast Water Management Convention (see Box 1 at the end of this report), and monitoring of GMOs (see Box 2 at the end of this report).

The Finnish environmental protection legislation includes several acts that require knowledge of the state of biodiversity all of which could be supported by eDNA methods (Act on Environmental Impact Assessment Procedure 252/2017, Land Use and Building Act 132/1999 (amendment 222/2003 included)). The current revision of the national nature conservation legislation includes three elements: i) an update of the Nature Conservation Act and the Nature Conservation Decree; ii) the drafting of a new act on compensations for damages caused by protected species; and iii) developing a plan for ecological compensation. The Nature Conservation Act and Nature Conservation Decree cause an increased demand for accurate and near real-time data which could be easily satisfied through the routine implementation of MoMM.

1.3 Biodiversity crisis and the new age of biological monitoring

Biodiversity loss has been recognized among the most serious threats for human well-being (IPBES 2019). It is difficult to conserve, manage and restore biodiversity if we do not know its state and trends. Long-term monitoring is pivotal to understand ecological changes in nature. Traditional biological methods cannot provide all the information that is needed to reliably measure all levels of biological diversity or provide required extensive taxonomic, spatial and temporal resolution. There is a dire need for harmonized monitoring of various dimensions of biodiversity, such as intraspecific genetic diversity, species populations and abundance, species traits, and species communities (Navarro et al. 2017). Novel methods such as remote sensing and eDNA could enable massive improvements with regards to the spatial extent and resolution of monitoring and provide insightful new knowledge on poorly known taxonomic groups and other facets of biodiversity (Bush et al. 2017). However, an abrupt adoption of new methods carries the risk of producing largely incompatible data. Novel method uptake ideally is carefully planned and encompasses method validation processes and transition periods with stepwise implementation. New monitoring technologies need not necessarily replace traditional long-term monitoring but sometimes help to fill existing information gaps. MoMM implementation have clear potential to enhance current biodiversity monitoring, as well as to improve development of biodiversity indicators.

Several international initiatives have recently been launched to promote the transition into the new age of biological monitoring. Internationally, relevant networks supporting MoMM are GEO BON and iBOL. GEO BON is supporting biodiversity monitoring networks at national, regional and global scales and eDNA methods are one important source of harmonized data. Further, iBOL (BIOSCAN) is working to establish an earth observation system that will reveal species, including their dynamics and interactions, based on barcoding. The EuropaBON project (under the umbrella of GEO BON) and the European Biodiversity Partnership (Biodiversa+) are developing harmonized biodiversity monitoring schemes where MoMM will have an important role. In addition to the aforementioned initiatives, the eBioAtlas, a joint cooperation between the IUCN and a UK based company NatureMetrics seek to leverage eDNA assessments of biodiversity through crowd sourced citizen science initiatives. In

Finland, SYKE has launched a new development project <u>Finnish Ecosystem Observatory (FEO)</u> which aims at modernizing legislative biodiversity and ecosystem monitoring with novel methods such as remote sensing, artificial intelligence, and eDNA and other molecular biological methods.

In general, the use of eDNA and other molecular biological methods has been extensively piloted in a range of freshwater, marine and terrestrial ecosystems and is getting sufficiently mature to allow standardization of techniques for certain specific sample types and target groups such as freshwater invertebrates. There is now a strong need for a comprehensive overview of the current situation – how molecular methods are used, their potential and future for biological monitoring. While the focus of this report is on Finland, we discuss also the international status of the field and Finland's role in the European and global context. Moreover, we strongly believe that both national and international coordination are crucial to avoid unnecessary repetition of efforts and extensive intercalibration across a fragmented field in subsequent years. Thus, we direct this national roadmap also towards a wider international audience interested in international cooperation on the implementation of eDNA and other molecular methods in the next generation of biodiversity and environmental monitoring.

1.4 Monitoring development needs

Molecular identification techniques have great potential to benefit, improve and extend current biological monitoring in all types of habitats. Particularly, currently unmonitored changes in patterns of biodiversity in response to global megatrends (e.g. climate change, urbanization, invasive alien species, increasing chemical stress on soils and groundwaters) stand to benefit from the speedy uptake of these methods. Further, their application to more traditional monitoring and assessments would greatly improve the accuracy of monitoring results and ensuing management actions and potentially increase spatial coverage. Molecular methods can produce objective, easily comparable and reproducible species identification, and can be used in large-scale monitoring. They can detect and monitor currently hard-to-detect and poorly known groups of organisms (e.g. aquatic and soil microbes, fungi, certain groups of insects) that are currently excluded from monitoring based on traditional taxonomic identification methods. Furthermore, molecular genetic methods provide the only tools for reliable mapping of intraspecific genetic diversity, the conservation of which is increasingly acknowledged internationally (Convention on Biological Diversity 2020, Hoban et al. 2020). To produce meaningful results, reliable reference databases and species-specific genetic tools for a large range of organisms are needed.

Several methods (e.g. metabarcoding) have evolved from the Technology Readiness Level (TRL; European Association of Research & Technology Organisations 2014) of the prototype stage (TRL 3-4) to the TRL level 6-8 where the technical operation has been demonstrated in relevant settings. Despite their demonstrated success and benefits, applications of molecular identification methods have nationally mainly been limited to proof of concept or validation projects (e.g. WFD related monitoring of the ecological status of water bodies and monitoring of soil fauna biodiversity). Currently their uptake into routine use is restricted by the lack of commonly agreed internationally standardized sampling and analysis protocols, the lack of unified meta- and data standards as well as shortcomings of existing reference libraries. There is an urgent need for national cooperation to implement these methods in the future. At present there is little coordination between different research organizations and other end-users.

The development of national guidelines without concurrent international coordination of efforts entails the risk of creating noncompatible solutions in a quickly evolving field. To counteract this threat Finland has taken an active role in the standardization of these methods for biological monitoring. In the past few years, these efforts have spawned European work to standardize sampling of eDNA from water and progressed to a first draft of a CEN standard. This indicates the level of international interest and commitment of countries to the future routine implementation of these methods. However, further work

is needed to ensure unified application and interpretation of molecular methods in European and national legislative monitoring implementation.

On a national scale, an informal network of scientists working on molecular method development and research with the aim of subsequent implementation into legislative environmental monitoring has been formed on a purely voluntary, bottom-up basis (i.e. the so-called eDNA network, coordinated by SYKE). The network has quickly become very popular (currently 60 members) and attracts researchers from many (currently eight) national organizations, as well as occasional international visitors. Thus far it has mainly served as a platform for information exchange but has the potential to become more permanent and goal oriented e.g. if formalized under the umbrella of the national environmental monitoring strategy.

1.5 State-of-the-art and recent development in molecular methodology

Conventional monitoring methods that rely on expert microscopy identification have inherent drawbacks, such as their dependency on personal identification skills, and are prone to human fatigue and error. Moreover, cryptic taxa are impossible to separate into species using expert-based taxa identification methods. Ideally, molecular taxon identification methods allow better harmonization and provide more accurate estimates of biodiversity. The new molecular biology-based tools overcome some limitations of traditional monitoring and allow using non-invasive sampling, achieve broad taxonomic coverage, have high sensitivity, and can be automated. These methods provide cost-effective means to produce reliable information for monitoring biodiversity in terrestrial and aquatic environments.
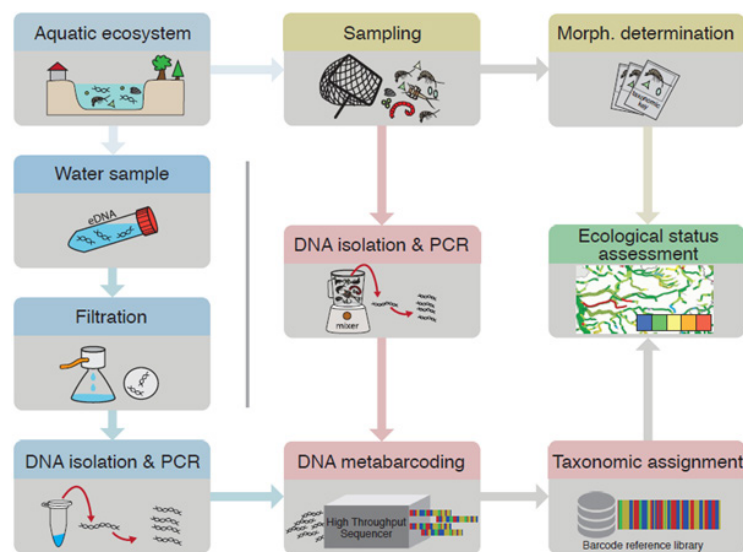
Figure 2. Different approaches for environmental monitoring based on community data: traditional morphological species determination (here from a kick-net sample of invertebrates), bulk DNA metabarcoding from a homogenized sample and eDNA metabarcoding (here from a water sample). Reproduced from Hering et al. (2018).

New molecular techniques in the field and in the laboratory have enabled sampling and identification of much of terrestrial, marine and freshwater biodiversity. These include environmental DNA (eDNA), bulk-sample DNA metabarcoding approaches (Fig. 2) and targeted RNA-based methods (e.g. Valentini et al. 2016; Elbrecht et al. 2017; Mäki & Tiirola 2018) as well as eRNA, useful e.g. for estimating the age of genomic material (Marshall et al. 2021). The eDNA technique uses DNA that is released from

organisms into their environment, from which a signal of organisms' presence in the system can be obtained. For example, in aquatic ecosystems, eDNA is typically extracted from sediment or filtered water samples (e.g. Deiner et al. 2016), and this approach is distinguished from bulk DNA metabarcoding, where organisms are directly identified from e.g. complete biological monitoring samples (e.g. Elbrecht et al. 2017). Despite the demonstrated potential of environmental and bulk-sample DNA metabarcoding approaches in recent years, there are still significant bottlenecks to their routine use that need to be addressed (e.g. Pawlowski et al. 2020).

While the sampling for MoMM is generally straightforward and in many cases comparable to traditional monitoring methods (Fig. 2, Fig. 3), each subsequent analysis step (e.g. in metabarcoding DNA/RNA extraction, PCR amplification, sequencing, bioinformatic analysis) involves numerous technical choices requiring a high level of expertise and usually laborious testing for each new sample type and taxonomic group. In taxonomy-based approaches, DNA sequences from the monitoring sample are identified against a reference library of sequences from confirmed specimens, resulting in a species list and an estimate of species' relative abundances reminiscent of traditional methods. However, MoMM also allow fundamentally different approaches (implementation strategies according to Cordier et al. 2021): taxonomy-free methods based on unidentified groupings of similar sequences (Operational Taxonomic Units or Amplicon Sequence Variants), inferring ecological networks e.g. by identifying DNA in feces or gut contents, and studying the presence and activity of genes linked to specific functions using metagenomics or metatranscriptomics. Furthermore, viruses with RNA genomes (vast proportion of plant, insect and fungal viruses) can be detected using RNA-based methods.

The type of the ecosystem, study questions and the target taxonomic group affects the choice of which MoMM should be used. Different habitats and taxa require different types of samples and different protocols. For example, in aquatic monitoring DNA or RNA can be isolated from water, sediment, biofilm or from bulk sample (e.g. macroinvertebrates) (e.g. Pawlowski et al. 2020). PCR-based single-species detection methods (qPCR, dPCR, ddPCR) are cheaper, faster, and easier compared to high-throughput next-generation sequencing (NGS)-based metabarcoding, which requires more specialized equipment and higher-level expertise in generating and analyzing sequence data with different bioinformatic tools. NGS offers information about multiple species at a time, useful for monitoring biodiversity or the state of the environment based on the whole community, while single-species detection is suited for a few focal organisms (e.g., rare or invasive species, targeted pathogens) with well validated single-species primers (Pawlowski et al. 2020). For a more detailed review of the methods, see e.g. Garlapati et al. (2019) and Ruppert et al. (2019).

Figure 3. Preanalytic conditions are a key point to access high quality data. Here, eDNA samples have been stored on ice immediately after sampling. © Tiina Laamanen, SYKE

2 Status assessment of the implementation of molecular methods – Finland against the backdrop of international development

The recommendations presented in this report are based on a comprehensive analysis of the current state of the implementation of MoMM in Finland within the international context. This chapter presents the data sources, analysis methods and results of this status assessment.

2.1 Data sources

To assess the current status of the implementation of MoMM, we started with an evaluation of the international readiness level of MoMM through a systematic review of the scientific literature published within the past five years. Second, we assessed the present-day international status of MoMM implementation by preparing a Webropol survey to the international community of scientists and officials working in this field. Third, to get a comprehensive overview of the Finnish situation, we made direct enquiries to all relevant institutions and conducted interviews with the key persons responsible for national species monitoring programs. Finally, we organized a national workshop for professionals working in different sectors to interactively identify the possibilities and challenges related to MoMM implementation. Data sources are described in more detail in the following subsections (2.1.1-2.1.4) and results relevant for this report are presented in sections 2.2 and 2.3. The full results of the systematic literature review and the Webropol survey will be published in scientific articles (Laamanen et al., in prep.).

2.1.1 Systematic literature review

A search of the Web of Science database was performed on April 15th 2021 using relevant search strings, resulting in 320 separate articles published no more than five years before the search. Following the systematic review protocol implemented in the CADIMA tool (https://www.cadima.info), the articles were screened against predetermined study selection criteria primarily based on the abstract but referring to the full text where necessary. These criteria included the requirement that the study discusses the topic of applying/implementing the adopted methodology in monitoring, and that at least some of the analyzed samples were collected from an outdoor environment. Note that the literature review did not cover population genetic studies; we recommend that a corresponding assessment of the state-of-the-art in population genetic monitoring be conducted soon. For a more detailed description of the systematic review procedure as well as a list of the reviewed papers, see Appendix 1.

Data was extracted from the selected articles based on the full text. This report uses the information of a sample of 70 original research papers; the full results of the review will be presented in a scientific article (Laamanen et al. in prep.). Based on the extracted data, we assessed the Technology Readiness Level (TRL) of the method presented or applied in each paper from the point of view of its implementation in routine monitoring. In this context, we interpreted the TRLs using the following progressively applied criteria:

- TRL5 (Technology validated in relevant environment): The study selection criteria.
- TRL6 (Technology demonstrated in relevant environment): The molecular method is compared to the traditional method and shown to have advantages.

- TRL7 (System prototype demonstration in operational environment): The molecular method is applied at a medium or large spatial scale (>10 km) and its implementation in monitoring is at least conditionally recommended.
- TRL8 (System complete and qualified): The molecular method is directly compared to the traditional method (i.e. with comparable samples) and its implementation is recommended without major conditions.
- TRL9 (Actual system proven in operational environment): The method is already implemented in an existing monitoring program.

2.1.2 International Webropol survey

The Webropol survey to experts was titled "*Survey on the current state and future needs of the use of molecular* methods *in monitoring*" and openly accessible online in February-April 2021. We advertised the survey actively within our national and international networks, most notably in the DNAQUA International Conference March 9th-11th 2021 organized by the EU COST action DNAqua-Net. This virtual conference attracted over 1,400 participants worldwide. The survey was mentioned in several presentations, including keynotes; moreover, it was included on the agenda of the associated national workshops organized in 17 European countries: Belgium and Luxembourg, Bosnia-Herzegovina, Cyprus and Greece, France, Germany, Hungary, Italy, Netherlands, Norway, Portugal, Slovakia, Sweden, Switzerland, Turkey and Finland. Our international collaborators translated the originally English survey into national languages to widen the audience reached.

The survey consisted of 24 questions divided into two sections: national level questions specifically on the implementation of MoMM and individual level questions on the personal work and involvement of each respondent in this field. All survey questions are listed in Appendix 2. All responses were anonymous.

2.1.3 Enquiries and interviews

Based on the recently updated national Strategy for Environmental Monitoring (Ympäristön seurannan strategia / YSS), we identified the following institutions as the key coordinators of national environmental monitoring programs: Finnish Environment Institute (SYKE), National Resources Institute Finland (Luke), Metsähallitus (MH; Parks & Wildlife Finland), the Finnish Museum of Natural History (LUOMUS), Finnish Institute for Health and Welfare (THL), Finnish Food Authority (RV), National Supervisory Authority for Welfare and Health (Valvira), Finnish Meteorological Institute (FMI) and the Aerobiology Unit at the University of Turku (AU). We contacted all these institutes and enquired about the use of molecular methods in recent, ongoing and soon launching monitoring projects and programs conducted or coordinated by the institute. In addition to actual monitoring, we also enquired about and listed research and development projects conducted by these institutes in which MoMM are piloted. As the focus of this report is on the national-scale implementation and not basic scientific research and the earlier stages of method development, all research projects at Finnish universities applying and developing molecular methods were not systematically mapped. However, we selectively listed university-led research projects that produce extensive data comparable to monitoring or specifically aim at developing methods for routine monitoring.

Interviews of 30 persons responsible for systematic species monitoring at SYKE, Luke, MH and LUOMUS were conducted as part of the status assessment of systematic species monitoring performed within the Finnish Ecosystem Observatory project in 2021. The interviews were based on a predetermined list of topics and questions, centered on the current state and development needs of monitoring in each group of organisms. The interviews included one question specifically on the use and on the future potential of using molecular methods in species monitoring or piloting new monitoring methods.

2.1.4 National workshops on possibilities, challenges and best practices

On March 25th 2021, SYKE organized a half-day national workshop on the current state, possibilities and challenges of MoMM ("Kansallinen työpaja molekyylibiologisten seurantamenetelmien nykytilasta, mahdollisuuksista ja haasteista"), which was part of the series of national workshops associated with the DNAQUA International Conference and funded by DNAqua-Net. The workshop was directed at a wide audience of professionals in different sectors whose work is related to or relevant for environmental monitoring, molecular methods or both. All but three (RV, Valvira, FMI) of the monitoring institutions listed in section 3.1.3 were represented among the >70 participants, SYKE (32%) and Luke (12%) with the highest number of participants. Universities (23%) and private companies (13%) were also well represented.

Following introductory presentations, the participants were requested to identify and discuss different possibilities, challenges and best practices related to the implementation of MoMM. The participants were randomly divided into six groups, and each group collected their ideas on a virtual whiteboard using the Jamboard application. The whiteboards remained accessible and editable to all participants for one week after the workshop. The ideas in the whiteboards were then listed and categorized in order to define and rank general possibilities, challenges and best practices by the number of individual mentions in the whiteboards.

On 12th November 2021, SYKE organized another workshop which provided input for this report. The first part of the workshop was intended for an international audience and gathered over 130 participants. In the second part, ca. 60 national participants discussed the development areas identified in the roadmap work so far. The results of the second workshop are not presented below as figures but the issues raised were incorporated in our recommendations in Chapters 3-5.

2.2 Implementation stage of molecular methodology in monitoring

The internationally published scientific research within the last five years is heavily dominated by aquatic environments (Fig. 4A). Aquatic environments also provided the only examples of the highest Technology Readiness Level (TRL9, already implemented), which included invasive fish species (Carim et al. 2020) and benthic invertebrates (Aylagas et al. 2018). Another famous example of routine eDNA-based monitoring is the great crested newt (*Triturus cristatus*) in Great Britain (Biggs et al. 2015), although this case did not happen to be included in our sample of 70 analyzed papers. Fish and invertebrates are the two most actively studied groups (Fig. 4B). The most common type of monitoring in the reviewed papers was biodiversity monitoring, followed by invasive and threatened species monitoring (Fig. 4C). It should be noted that this distribution also reflects the literature search criteria, as "biodiversity" was one of the search terms.

Among the TRL classes 5-9 included in our review, most of the studies represented the lower end of the spectrum (Fig. 4), indicating that while the methods are broadly validated in small-scale field studies, systematic large-scale demonstrations are still scarce. Among the well-represented taxa, the technology is the most developed in fish (mode TRL7) and other vertebrates (mode TRL6), while taxonomically universal methods remain the least ready (TRL5 only).
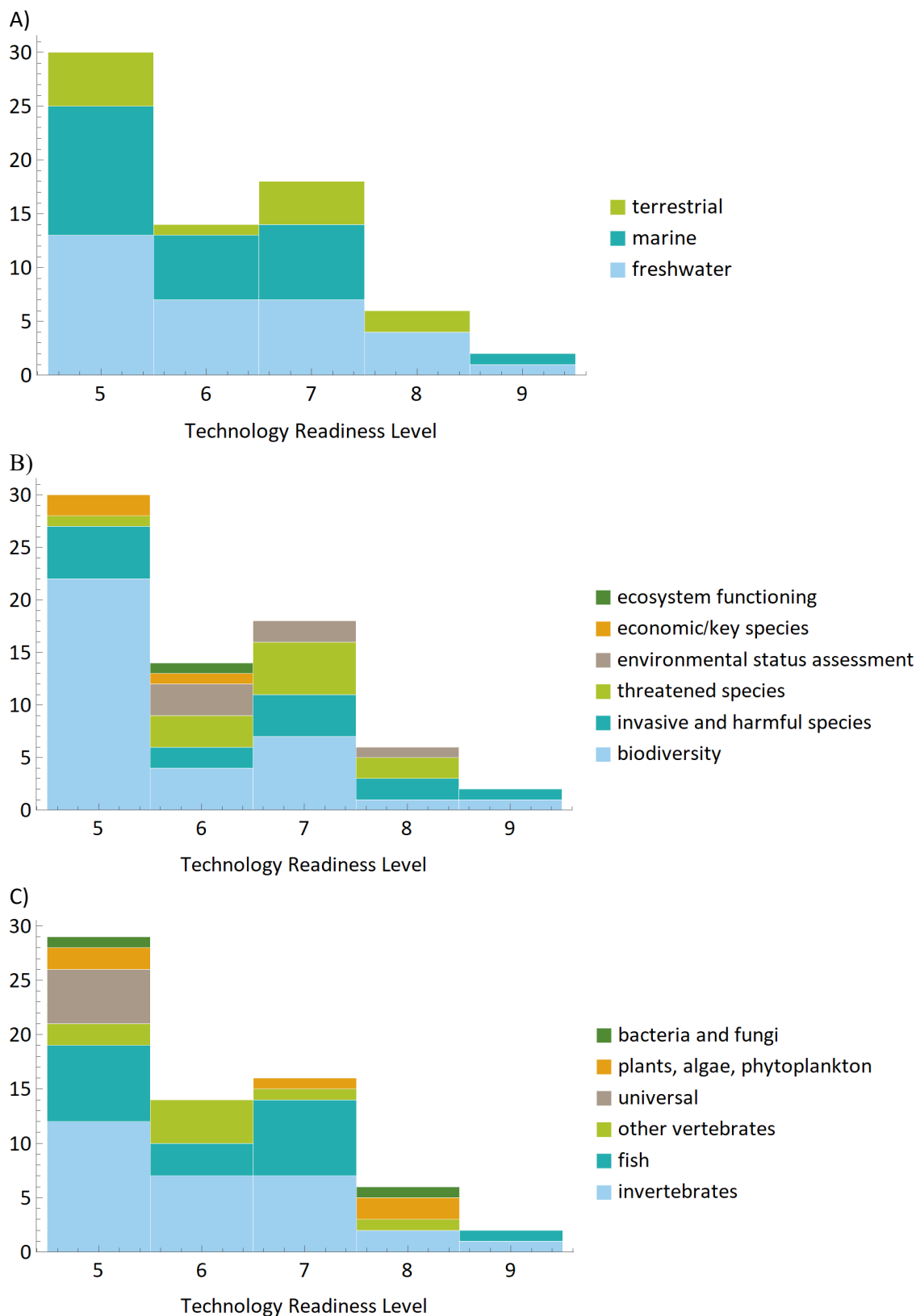
Figure 4. Technology Readiness Level (TRL) of molecular monitoring methods based on a sample of 70 original research papers published in 2016-2021, analyzed in the systematic literature review (section 2.1.1).

The national state-of-the art summarized in Table 3 is broadly consistent with the international situation based on scientific literature. While there is a relatively high number and diversity of pilot projects, there are relatively few examples of routine implementation. These include the following: 1) The monitoring of large carnivore populations (one of the data sources based on fecal DNA) (Luke). The most recent development of non-invasive DNA-methods used in large carnivore monitoring has been conducted in collaboration with Scandinavian countries. The role of non-invasive DNA-methods has been recognized as increasingly important in monitoring large carnivores as the traditional census methods based on snow tracks are becoming unreliable due to climate change. 2) The monitoring of the distributions of the two beaver species (the domestic European beaver and the invasive Canadian beaver) based on DNA extracted from wood chips collected by citizen scientists (Luke; Iso-Touru et al. 2021). In this case, molecular methods improve markedly the status of monitoring, as for this species pair no other sufficiently reliable non-invasive monitoring method is available. The national monitoring of land use effects on freshwater systems (*Maa- ja metsätalouden kuormituksen ja sen vesistövaikutusten seuranta* (MaaMet)) led by SYKE has also been extended by pilots of DNA-based monitoring of macroinvertebrates and benthic diatoms (e.g. Elbrecht et al. 2017; Meissner et al. 2020; Kahlert et al. 2021; Turunen et al. 2021a) and currently has a DNA metabarcoding sampling method (preservation of benthic invertebrate samples in lakes and rivers) in routine use, operated by consultants.

In contrast to the international TRL assessment, terrestrial systems are comparatively well represented among national monitoring projects and pilots, while examples from the Baltic Sea are few compared to the international status of the marine MoMM field. It is also notable that apart from the Canadian beaver, use of molecular methods in the monitoring of invasive alien species has not been implemented despite the encouraging international examples. However, several method tests and pilots using molecular methods in the monitoring of invasive species have been started, e.g. wild boar, signal crayfish (Mäkinen et al. 2021; Luke, UEF, JYU), wolf-dog hybrids, several fish species (Luke) and mud crab (University of Turku). Bacteria and fungi are better represented in the national pilot projects than in the international scientific literature, suggesting forerunner potential in this field. While almost all national institutes responsible for monitoring have taken some steps towards the implementation of MoMM, Luke stands out in the consistency and scale of the implementation in a variety of monitoring programs.

We received a total of 171 responses to the international Webropol survey, of which 35 % were from Italy and 19 % from Finland, followed by Greece (10%), France (10%), Sweden (5%), the Netherlands (5%), Germany (3%), Norway (2%), Hungary (2%), Estonia (1%) and Cyprus (1%). We also obtained one response each from Poland, Denmark, Canada, Cameroon, Greece and Latvia.

The responses to the individual level questions indicated that most respondents had personal experience in applying molecular methods but were not directly involved in setting up a national strategy for DNA-based monitoring. Both aquatic and terrestrial systems were well represented in the respondents' work. It is also notable that while taxonomy-based methods were the most popular out of the classes defined by Cordier et al. (2020), other strategies (taxonomy-free, structural, functional) were also mentioned by over half of the respondents (53%). This is in clear contrast with the reviewed scientific literature, where only 10% of the papers applied other than taxonomy-based methods. It seems that the interest in alternative implementation strategies is currently fast increasing.

According to the survey results, the overall state of MoMM implementation is roughly similar in Finland compared to other countries (Fig. 5), and consistent with the TRL analysis (Fig. 4) and the summary of national monitoring projects (Table 3).

Table 3. Summary of recent and ongoing Finnish monitoring campaigns and pilots utilizing molecular methods. Research projects have been interpreted as "pilots" if they are comparable to monitoring in methodology and scale. Taxa for which methods are in the earlier development stages are not listed. Invasive alien species (IAS) and nationally red-listed species (Hyvärinen et al. 2019) are indicated.

Species/group	System	Methods	Stage	Conducted by
Viruses	terrestrial, freshwater, marine	eDNA metabarcoding, qPCR, eRNA (water, air, wastewater, ticks, fungi, plants, insects)	Pilot	THL, SYKE, FMI, Luke, universities
Bacteria	terrestrial, freshwater, marine	eDNA metabarcoding, qPCR (soil, water, air, wastewater, ticks)	Pilot	Luke, SYKE, FMI, THL, RV, universities
Endophytic microbes	terrestrial	eDNA metabarcoding of bacteria and fungi within plant tissues	Pilot	Luke
Benthic diatoms	freshwater	eDNA (biofilm) metabarcoding	Pilot	SYKE / Swedish Univ. of Agricultural Sciences
Phytoplankton	freshwater, marine	eDNA metabarcoding	Pilot	SYKE
Liverworts	terrestrial	Bulk DNA metabarcoding	Pilot	Univ. Turku, MH, SYKE
Vascular plants	terrestrial	eDNA metabarcoding/metagenomics (airborne pollen)	Pilot	FMI
Fungi	terrestrial, freshwater	eDNA metabarcoding/metagenomics (soil, water, air)	Pilot	Luke, SYKE, FMI, RV, universities
Freshwater pearl mussel (EN)	freshwater	eDNA + specific PCR	Pilot	Univ. Jyväskylä, MH
Benthic macroinvertebrates	freshwater, marine	Bulk DNA metabarcoding	Pilot	SYKE
Soil invertebrates	terrestrial	eDNA metabarcoding	Pilot	Luke
Arthropods	terrestrial	Bulk DNA metabarcoding	Pilot	Universities
Glanville fritillary butterfly (EN)	terrestrial	240 SNP panel, whole genome re-sequencing	Pilot (long-term research)	Univ. Helsinki
Noble crayfish (EN), signal crayfish (IAS)	freshwater	eDNA + dPCR	Pilot	Luke, Univ. Eastern Finland
Atlantic salmon (VU), Baltic salmon (VU)	freshwater, marine	An array of 220k SNPs	Routine	Univ. Helsinki, Luke
Fish	freshwater, marine (coastal)	eDNA + qPCR, eDNA metabarcoding	Pilot	Luke, MMM
Common frog, moor frog	freshwater	eDNA + qPCR	Pilot	Luke, Luomus, MMM
Lesser white fronted goose (CR)	freshwater	eDNA + Sanger sequencing	Pilot	Kiljuhanhi LIFE, MH, Univ. Oulu
Bats	terrestrial	DNA from feces + metabarcoding	Pilot	Luomus
Brown bear (NT)	terrestrial	DNA from feces + 96 Single Nucleotide Polymorphism (SNP) panel	Forthcoming pilot (2022)	Luke
European beaver (NT), Canadian beaver (IAS)	terrestrial	eDNA (wood chips) + PCR assays	Routine	Luke
European lynx	terrestrial	DNA from feces + 96 SNP panel	Forthcoming pilot	Luke
White-tailed deer (IAS)	terrestrial	DNA from feces + microsatelli-tes	Pilot	Luke
Wolf (EN) and wolf-dog hybrids	terrestrial	DNA from feces/urine + 96 SNP panel	Routine 2022-	Luke
Wolverine (EN)	terrestrial	14 microsatellites and mtDNA control region (579 bp)	Pilot	Univ. Oulu, Luke

The rate of routine implementation is estimated to be generally low and the highest for health-related microbial monitoring (sewage, drinking and swimming waters). A clearly higher proportion of respondents stated that the implementation of MoMM is currently in the testing and planning stages, while only a few respondents stated that implementation is not yet planned. Considering their involvement in the field, surprisingly many respondents were unaware of the state of the implementation in their country, demonstrating the need for active networking and knowledge transfer.

According to the responses, the implementation of MoMM in managed populations such as game and fisheries appears more advanced in Finland than in other countries. Indeed, several monitoring examples listed in Table 3 involve managed populations, while this type of monitoring forms only a small minority in the reviewed international scientific literature (Fig. 4C; note that this is also affected by the search criteria). By contrast, the national implementation of molecular methods lags somewhat behind the international level in biodiversity, threatened species and invasive species monitoring, which is in agreement with results in Fig. 4 vs. Table 3. For a more thorough discussion on the monitoring of invasive species, pests and pathogens with eDNA/eRNA, see Box 1 at the end of this report.

Both nationally and internationally, implementation of MoMM in the monitoring of genetically modified organisms (GMOs) is the least well known. We treat this topic in more detail in Box 2 at the end of this report.

A) International status of implementation (excluding Finland)

	Routine implementation	Testing phase	Planned	Not planned	I do not know
GMO	14%	9%	5%	9%	63%
Managed populations (game, fisheries)	7%	16%	8%	8%	61%
Invasive species	7%	34%	19%	7%	34%
Threatened species	9%	30%	15%	8%	38%
Microbial monitoring (sewage, drinking,…)	25%	16%	8%	4%	46%
Environmental status	6%	32%	24%	9%	30%
Biodiversity	11%	40%	19%	6%	24%

B) National status of implementation

	Routine implementation	Testing phase	Planned	Not planned	I do not know
GMO	4%	4%	7%	4%	81%
Managed populations (game, fisheries)	22%	15%	7%		59%
Invasive species		30%	33%		48%
Threatened species	4%	33%	15%		59%
Microbial monitoring (sewage, drinking, swimming water)	26%	11%	4%	4%	56%
Environmental status	11%	15%	41%		33%
Biodiversity	15%	26%	33%		33%

Figure 5. The status of the implementation of molecular methods in different fields of monitoring, internationally (A; excluding Finland) and nationally (B), as assessed by the respondents of an international Webropol survey (section 2.1.2).

2.3 Possibilities and challenges for implementation

Although different studies have addressed the benefit of implementing MoMM into national monitoring programs compared to current traditional monitoring methods, there are still challenges to the effective use of MoMM at a national level. When the experts participating in our national workshop were asked to estimate the time frame within which molecular methods can be implemented in routine monitoring on a scale of 0 to 10 years, 57 workshop attendees gave the average estimate of 2.8 years, with

individual estimates spanning across the whole range. Clearly, there is high degree of optimism and enthusiasm about the possibilities presented by the new methods, but also important reservations that should be carefully considered. In this section, we discuss the concrete possibilities and challenges of MoMM based on the data sources described in section 2.1.

Possibilities

Based on the output of the national workshop, by far the greatest possibility offered by MoMM is new information – e.g. on rare and evasive taxa, structural and functional aspects of natural communities and the genetic structure of populations (Fig. 6A). Other commonly mentioned possibilities included more reliable, faster and cost-effective monitoring. Using molecular methods does not require taxonomic expertise and identification is more transparent, repeatable and comparable between different environments and countries. Therefore, molecular methods can be seen as more reliable than traditional monitoring methods.

A) Possibilities
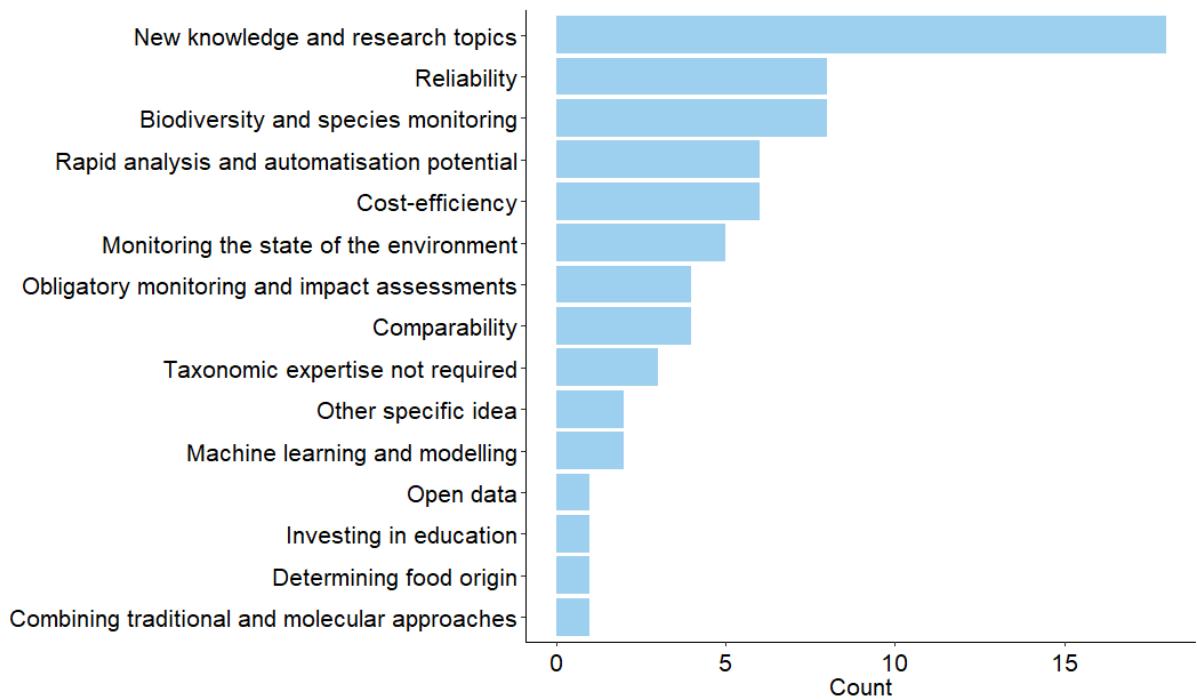
B) Challenges
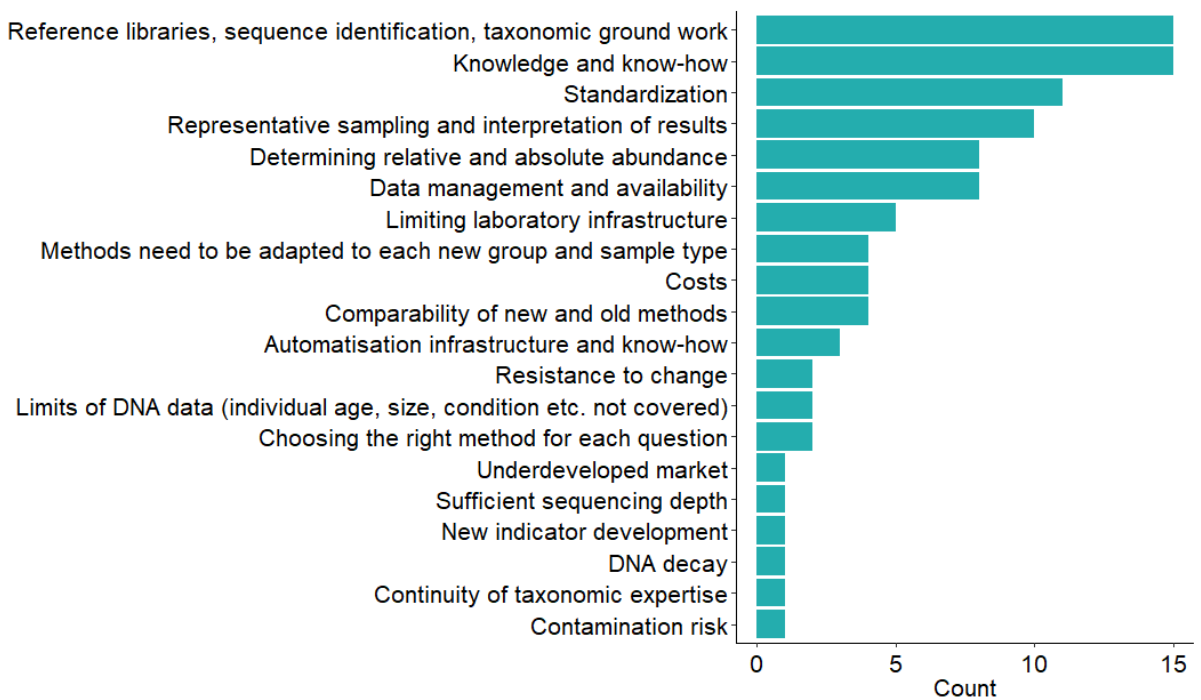
C) Best practices
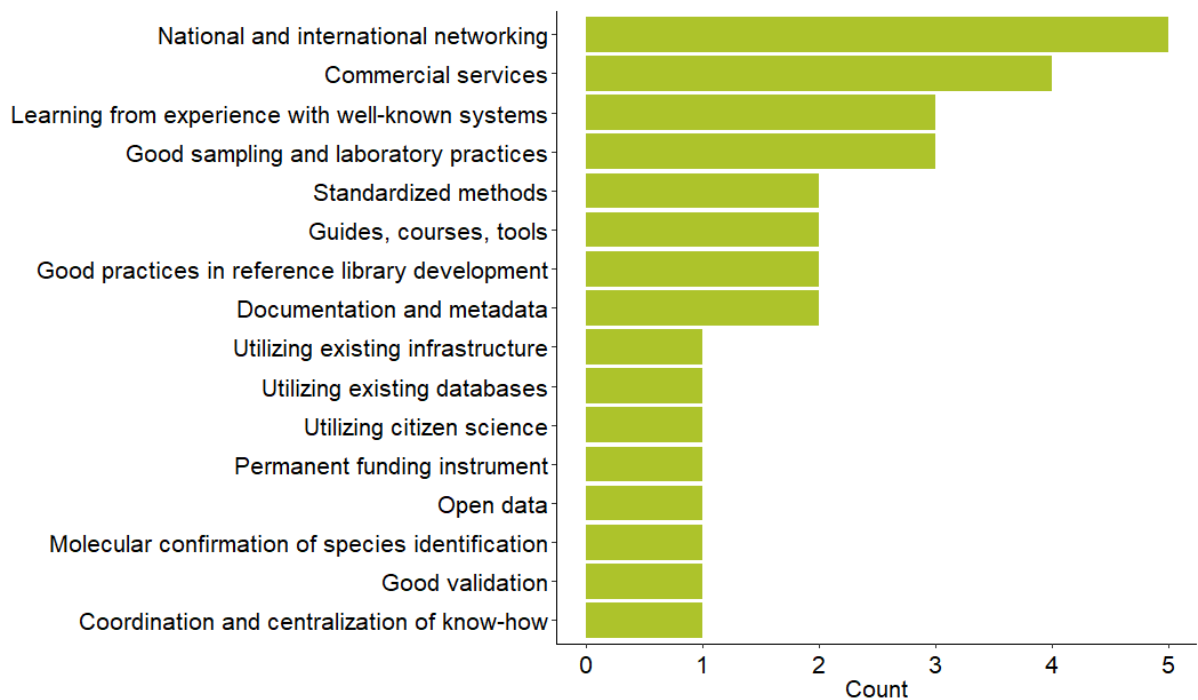
Figure 6. Possibilities (A), challenges (B) and best practices (C) related to molecular monitoring, as identified by ca. 60 experts participating in a national workshop in March 2021 (section 2.1.4).

Challenges

Based on the survey, lack of funding, expertise and method standards were recognized as the most important limiting factors of the implementation of MoMM both in Finland and internationally (Fig. 7). Lack of expertise was stressed particularly at the national level, possibly partly due to the more diverse backgrounds represented among the Finnish respondents. Indeed, although taxonomic identification expertise is less crucial when using molecular methods, knowledge and skills related to the new methods will be in great demand. It is important to note that this is the case even when the DNA analytics are purchased from external service providers. In the national workshop as well as other networking events, many professionals in the field of biological monitoring have expressed the difficulty in using, selecting or even finding such services due to the lack of the necessary background knowledge. Another challenge emphasized particularly at the national level is the incompleteness of sequence libraries used as reference databases in the identification of DNA sequences detected in samples (Fig. 6B, Fig. 7). Lack of laboratory infrastructure was acknowledged as a limiting factor more often in the international than a national level. IT infrastructures were not seen as the primary factor limiting the implementation.

Figure 7. Factors currently limiting the implementation of molecular methods in monitoring, as perceived by the respondents of an international Webropol survey (section 2.1.2).

One limiting factor of the implementation of MoMM is that many monitoring programs need to produce quantitative abundances, biomass data or occurrence size estimates. Quantitative data is needed f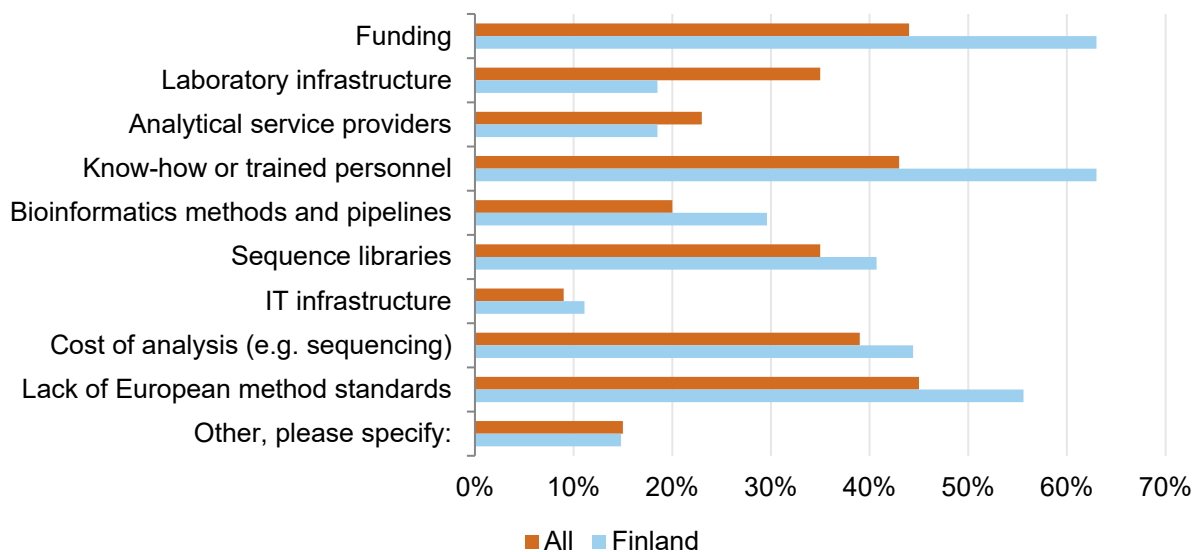or the environmental status indicators, requested by e.g. the EU Water Framework Directive (European Union 2000) and Marine Strategy Framework Directive (European Union 2008, 2010, 2016, 2017). Similarly, abundances and population sizes are needed for many biodiversity monitoring purposes (e.g. EU Habitat and Bird Directives (EU 1992, 2009), national evaluations of red listed species (Hyvärinen et al. 2019), and national state indicators of biodiversity (e.g. Luonnontila.fi)). Quantitative monitoring data is also the foundation for sustainable management of game and fish populations. Many current eDNA methods do not yet produce reliable quantitative data directly comparable to traditional data, and for this reason they cannot be adopted as an exclusive alternative for the existing, standardized monitoring methods in many ongoing monitoring programs. Enhancing the reliability of quantitative MoMM is under very active international development, and e.g. qPCR-based approaches and high-throughput sequencing without PCR amplification show great promise. As a national example, there is an ongoing pilot project on quantitative estimation based on eDNA of fish species in Luke. Relative sequence abundances have also been successfully used in biodiversity surveys of bulk stream macroinvertebrate samples using DNA metabarcoding (Turunen et al. 2021b).

Another example of quantitative MoMM, methods enabling detailed estimation of the number of individuals and territories are those that reveal the identity of individuals or family groups (e.g. from fecal or hair samples), for example the number of wolf packs routinely estimated by Luke. The downside of such methods is that they require either custom-developed genetic markers that vary at the individual or family group level (SNPs or microsatellite markers) or whole genome re-sequencing. Such analyses are therefore laborious and necessarily incur relatively high expenses, making such detailed routine analyses possible only for a small number of species. However, such approaches may become more readily available across species and systems if costs of sequencing continue to lower and genomic resources for non-model organisms continue increasing.

Compensating for the remaining lack of quantitative methods, eDNA methods can already offer additional qualitative information on biodiversity. For example, in cases where a target species is spatially or temporally demanding to observe with traditional monitoring methods, presence observation based on eDNA samples may provide great help in targeting the traditional mapping or monitoring methods in the most potential locations. For example, eDNA methods have the potential to increase

information on biodiversity of single-celled pelagic primary producers of marine and freshwater

methods will likely give a better assessment of the total phytoplankton diversity. In the future, when genetic methods are developed further, they may also provide quantitative data e.g. for the purposes of environmental status indicator analyses and biodiversity monitoring.

In addition to limitations related to quantitative data, implementation of MoMMs in biodiversity monitoring is limited by the lack of information on the life-history phase of the organism, for example, whether it was reproducing or not at the sampling moment. This limitation concerns especially species that migrate, are nomadic or disperse long distances and can be observed in eDNA samples far away from the locations where they reproduce. For example, reporting for the EU Bird Directive and the EU Habitat Directive requires estimates of breeding bird population sizes and locations of breeding and resting places of species of interest, respectively.

While MoMMs would in theory suit species mapping surveys and monitoring well, there are currently practical limitations such as too high expenses of analyzing all samples. In addition, while molecular methods may provide great help in identifying individuals that were not identified with traditional methods in the field, it may also be that already the sampling decisions in the field require special taxonomic expertise. An important category of challenges identified in our national workshop is related to the representativeness of DNA-based sampling and the interpretation of the new types of information (Fig. 6B). Many current monitoring programs focus on locally observed biodiversity. By contrast, an eDNA sample composes the aggregation of locally shed DNA traces and DNA transported from remote sources, and the organisms themselves are never directly observed. Although extracellular DNA typically decays in a matter of days, this time frame allows transport across large distances particularly in the air and in many aquatic systems (e.g. Deiner & Altermatt 2014, Goldberg et al. 2015). Specifically, in the river systems eDNA samples also comprise a number of dendric upstream sources. In such cases, utilizing eDNA methods for local anthropogenic impact assessment of biodiversity comparable to traditional methods is challenging. Moreover, much of the eDNA found in water samples is still bound to cells, which further increases its lifespan and thus the potential transport distance. This shortcoming may apply for many assessments of anthropogenic impact in river systems (e.g. those for WFD) and may support the use of bulk DNA metabarcoding over eDNA. Another possible avenue is complementing eDNA with eRNA analysis, allowing estimation of eDNA age and thus improving the reliability of local eDNA detection (Marshall et al. 2021).

Best practices

Based on the results of the national workshop, collaboration and networking with other experts both nationally and internationally were identified to be important when implementing MoMM (Fig. 6C). The use of commercial services in the analysis of DNA samples was also supported as a good and helpful practice. Standards and practical field/laboratory guides were also mentioned as important practices. In some, particularly microbial systems, next generation molecular methods are already well established in research, thus experience gained in such systems should be utilized more broadly. Moreover, existing infrastructures and databases should be utilized effectively.

3 Vision

In Chapters 1-2, we have set the stage and presented our analysis of the state-of-the-art in the implementation of MoMM. The remaining Chapters 3-6 are future-oriented. Here, we start by outlining our longer-term vision for the role of molecular methods in environmental monitoring. We stress that while the new methods will eventually reduce the costs of monitoring, the primary aim of their implementation should be driven by the need for improved knowledge on biodiversity and the state of the environment and their ability to accurately and quickly detect environmental change facilitating timely application of required management and mitigation measures. Sufficient investment in the transition phase is crucial to ensure the continuity of high-quality data series. As the new methods gain ground, the role of citizen observations in providing additional taxonomic data will increase. At the same time, a minimum set of publicly maintained traditional monitoring efforts performed by professionals should be identified to guarantee the coverage of essential features not captured by the new methods. The commitment of monitoring organizations to follow the FAIR data management principles should cover also MoMM-based data.

Based on our analysis of the state of the field, we envision that by 2030, reliable, internationally standardized molecular methods are routinely used in national biological monitoring (Fig. 1). Traditional taxonomic monitoring may complement the new methods, ensuring the continuity of valuable long-term data sets and facets of biodiversity not captured by molecular methods. At the same time, traditional citizen observations are increasing in number, quality and taxonomic coverage, and significant investments in taxonomic and science education and applications assisting data recording are made in recognition of their value. Where appropriate, citizen science is also routinely applied to sample collection for MoMM. Apart from species occurrence and abundance information, MoMM can provide information also on facets of biodiversity that have previously been underrepresented in monitoring schemes. These include the large-scale mapping of intraspecific genetic diversity across multiple species as well as the functions and biochemical processes active within the environment, as can be observed from the presence and activity of groups of functional genes.

Sequence libraries have reached a sufficiently high coverage for the reliable application of taxonomy-based methods for most taxonomic groups. Sufficient taxonomic expertise is directed to support continuing efforts of mapping unknown biodiversity and developing sequence libraries. Alongside taxonomy-based methods, taxonomy-free methods are routinely applied to monitor environmental status (e.g. water, sediment, air and soil quality) and environmental impacts of human activities. Additionally, whole genomes of species characteristic of different habitat types are made available at an increasing rate, building capacity towards routine implementation of monitoring of intraspecific genetic diversity and spatial genetic structure of natural populations (Formenti et al. 2022, Lewin et al. 2018). The genomes and DNA-based monitoring data is stored, handled and made available following a national data management plan and using internationally agreed upon metadata standards, following the FAIR (Findable, Accessible, Interoperable, Reusable) Guiding Principles for scientific data management and stewardship (Wilkinson et al. 2016). Most of the data (except for strictly protected species) are georeferenced, openly available and made available through Application Programming Interfaces (APIs) or automatically fed into global databases.

Education programs in biological and environmental science and laboratory analytics have been updated to respond to the growing need for expertise in molecular monitoring methods. A significant part of laboratory and bioinformatic analyses for national biological monitoring are carried out by the

private sector and there is a well-developed national as well as international market for state-of-the-art molecular analytics and products. Finland is an international forerunner in providing analytical, bioinformatics and consulting services as well as accredited laboratory services through its accredited reference laboratory framework following internationally agreed standards.

MoMM are developed alongside Earth observation and other remote sensing methodology as well as ecological modelling and machine learning applications. Validated modelling and analysis tools are used to assist in the interpretation of DNA-based observations and in sampling design. Spatiotemporal approaches enable automatically updated distribution maps based on ecological model simulations, as well as the creation of user-defined future scenarios illustrating e.g. the effect of land use activities and climate change on national biodiversity or the spread and impact of invasive species. Uncertainty is quantified and highlighted in all model outputs.

4 General development plan

Based on our analysis of the state-of-the-art and the chief obstacles standing in the way of the envisioned MoMM implementation, we have identified six development areas that merit particular attention in the coming years:

1) International coordination is paramount to ensure comparability of data in time and space and to avoid unnecessary duplication of efforts and resulting delays in overall method uptake.

2) Networking across sectors is essential to increase awareness and knowledge of the new methods, to establish the connections that form the basis of a viable market for services and products – and to provide platforms for critical discussion on when, where and how the new methods should be applied.

3) Education plays a key role in solving the lack of up-to-date know-how, one of the most pressing challenges identified in our status assessment.

4) Infrastructure exists but should be used more efficiently and codeveloped, updated and extended in a co-creative and coordinated manner between relevant government agencies, universities and other organizations.

5) The coverage of sequence libraries should be continuously improved not only in designated projects but as a running task in monitoring programs and pilots. Additionally, in order to allow for comprehensive mapping of intraspecific genetic diversity, the number genomes published for different organisms would need to be increased.

6) Modelling and analysis tools should be applied and developed alongside MoMM and other new monitoring methodologies to fully utilize their information.

4.1 International coordination and standard development

Until recently, there has been little concerted international coordination with respect to implementation of molecular methods into environmental monitoring. While several organizations (e.g. Biodiversity Information Standards, originally Taxonomic Databases Working Group (TDWG)) have long developed guidance for data management, there is little official agreement in the form of international standards. This gap has been realized in Europe and is explicitly addressed for molecular methods used to assess water quality. To fill this gap, in 2018 a working group under the European Committee for Standardization (CEN) was established in the technical committee TC 230 which oversees standards pertaining to water quality. This working group, "WG28 eDNA and DNA methods" is chaired by SYKE as the national representative of the Finnish national standardizing body SFS. WG28 is processing its first work item "Water sampling for capture of macrobial environmental DNA in aquatic environments", which will be finalized in early 2023. Other standards, concerning the analysis of eDNA from other matrices as well as minimum requirements for data and metadata, are planned for the coming years.

Other standards have been developed under the International Organization for Standardization (ISO), such as the standard: "Soil quality — Direct extraction of soil DNA", ISO 11063:2020. A newly formed working group under ISO TC 331 "Biodiversity" is likely to suggest several general standards for the use of molecular methods in biodiversity assessments. SYKE will attempt to set up a liaison between ISO TC 331 and CEN TC 230 / WG28 in the spirit of the Vienna Agreement (1998), the ISO Council resolution 35/2001, and the CEN Administrative Board resolution 2/2001 to avoid duplication of effort.

SYKE has recently established links to the eBioAtlas initiative which originated in the UK through an interaction between the company NatureMetrics and the IUCN. It is in the national interest to both participate and widen the scope of international participation including possibly GeoBON in the eBioAtlas and jointly strive towards the establishment of internationally accepted guidance on the minimal requirements for molecular method and related data use. Similarly, exchanges and more engaged and goal-oriented cooperation between Nordic administrations progressing the implementation as well as with other European forerunners developing molecular methodology are needed to assure a timely uptake of the methods. In Finland this will require formalized fora for information exchange which should be established under the umbrella of the implementation program of the national environmental monitoring strategy.

4.2 Networking and promoting market development

As is characteristic for a new and actively growing area, the Finnish field of molecular monitoring is highly scattered, with several individual actors and groups discovering and applying the new methods independently of each other, while the general knowledge of the methods is relatively scarce even among professionals in biological monitoring. Moreover, many researchers and officials in the monitoring field are unaware of molecular biological services that would be available or lack the background knowledge to make use of them. Lack of knowledge and know-how was the second most often mentioned challenge in the implementation of MoMM both in the national workshop (Fig. 6B) and in the international Webropol survey (Fig. 7), and national and international networking was the most often mentioned best practice in the national workshop (Fig. 6C). Promoting information exchange and encounters between service providers and biological monitoring professionals would be a cost-efficient way to speed up the uptake of molecular methodology in monitoring. One example of such information exchange between biological monitoring professionals is the virtual networking initiated by SYKE in 2021 for phytoplankton experts in the HELCOM area and the Nordic countries. This networking is connected to SYKE's ongoing project "*Implementing genetic methods in phytoplankton monitoring* (GeMeKa)", and there have already been three network meetings in 2021.

In 2020, SYKE established an informal "eDNA network" of scientists working on molecular method development and research with the aim of subsequent implementation of the methods in monitoring. The network now has 60 members representing 8 national organizations and will be further opened and promoted to reach all major national institutions conducting biological monitoring. Apart from the informal virtual meetings that form the backbone of interaction, this network forms an excellent basis also for more directed and goal-oriented activities, such as courses and workshops. We also recommend that a permanent working group promoting, coordinating and monitoring the implementation of MoMM should be established under the umbrella of the national environmental monitoring strategy. This group should be closely linked to the wider, "bottom-up" eDNA network to make sure that the national uptake of molecular methods proceeds in a transparent manner and that key points from critical discussions reach relevant levels in the administration.

Apart from general networking, additional efforts should be spent to increase interactions between sectors, particularly between private companies and other service providers and their potential clientele, such as scientists and monitoring professionals in universities, governmental research institutions and

regional environmental administrations to create an interactive community of practice. According to the international Webropol survey (section 2.1.2), sequencing is the most used commercial service (38% of respondents), but also DNA/RNA extraction (16%), bioinformatics (16%) and sampling (7%) are mentioned. Marketing events such as trade fairs and online resources providing an overview of available services and their providers would help to remove such information blocks between demand and supply. For example, in our workshops, many researchers were surprised to hear that some companies now also offer "full service packages", including consulting at all stages of the work from sampling design to the interpretation of results – even the writing of funding proposals. It should also be noted that international and national standardization of MoMM (section 5.1) as well as the development of accreditation of MoMM services will be essential in mainstreaming the new methods and expanding their market. Apart from the technical solutions, standards would also be needed for common issues in data analysis and interpretation, such as the conclusions made based on semiquantitative metabarcoding data.

Finally, the most concrete way of cross-sectoral networking is collaboration. Both individual research and pilot projects and the whole field would greatly benefit from the inclusion of cross-organizational and cross-sectoral activities. This can be achieved under the umbrella of the national environmental monitoring strategy implementation program. The implementation program will work in a co-creative and open manner in targeted working groups which will genuinely facilitate overall interaction and integration of public sector stakeholders.

An obvious immediate benefit from collaboration is the possibility for organizations with small sample numbers to fill up batches of sequencing runs together, thus obtaining a better price and/or faster throughput. As another concrete example, environmental impact assessments of large facilities or land use activities provide good opportunities for comparing the ability of molecular methods vs. traditional methods in detecting environmental change. Such projects could be realized as collaboration between the consulting company conducting the assessment using traditional methodology and a university or governmental research institute organizing the DNA-based assessment and comparing the results. A third idea rising from our workshops and other cross-sectoral discussions concerns a key concern for many new or potential MoMM users: how quantitative is the data obtained from metabarcoding? Methodological research and development around this question could be another area where collaboration between environmental consulting companies and researchers or practitioners would be of significant mutual benefit.

4.3 Education

The eDNA approaches are based on novel biotechnologies that undergo rapid modifications and improvements. It is very important to develop the best practices by sharing the newest information and knowledge. In the survey and national workshop the lack of know-how was identified as one of the most important factors which is currently limiting implementation of molecular based methods.

In 2021-2022, SYKE's project "*A pilot for implementing environmental DNA (eDNA) based methods into environmental and biomonitoring (eDNA pilot)*" promoted information exchange on molecular methods both within SYKE and with national and international partners. This project provided workshop training on methods by international top-level experts to national experts related to the use of MoMM. The eDNA roadmap, eDNA pilot and the Finnish Ecosystem Observatory projects arranged two online workshops in 2021. SYKE will continue to organize targeted expert training and knowledge sharing events in the future.

A good channel for introducing MoMM to students and future users is the Biodiversity education network of Finnish universities (https://www.biodiversityeducation.fi/), a joint project of the Universities of Eastern Finland, Helsinki, Oulu, Turku and Jyväskylä. The Biodiversity education network develops biodiversity education nationwide by bringing together study content and an

operation model to meet the learning needs in biodiversity issues now and in the future. Co-operating universities bring expertise from their areas of strengths and strategic objectives for the use of the entire network. The network is funded by the Ministry of Education and Culture.

In the future, organizing various training events for research institutes, companies and citizens will be very important. In the medium and long run, the establishment of dedicated curricula in universities, universities of applied sciences or vocational schools may be necessary. This will require cross-sectoral cooperation, long-term planning and funding. For the short term, our workshops identified a particular need for training in the following fields: 1) how to use and choose bioinformatics pipelines, 2) how mature the different methods are for different environments/taxonomic groups/questions), 3) sampling protocols and quality controls, 4) conserving samples and 5) sampling design. These training events could be arranged with international collaboration. Separate events for different taxa and sample types are needed.

4.4 Infrastructure, computing resources and databases

Laboratory infrastructure was mentioned as a factor limiting the implementation of MoMM by only 13% of the Finnish respondents in the Webropol survey. Thus, laboratory infrastructure is not perceived as a primary limiting factor. Indeed, most of the monitoring institutions have a good capacity for basic molecular work, such as DNA extraction and PCR amplification (normal and qPCR). Instruments for more automated analyses (e.g. DNA extraction robots) and some next generation approaches (e.g. dPCR machines and Oxford Nanopore sequencers) are also becoming more affordable. The most expensive equipment such as Illumina high-throughput sequencing platforms have been acquired by some national institutes, including the Institute of Biotechnology, Institute for Molecular Medicine Finland (FIMM), Luke and the Universities of Oulu and Jyväskylä, most of which also offer their services to external customers. Considering also private service providers, high-throughput sequencing as well as other state-of-the-art analysis services can be purchased from a number of sources in Finland and abroad, and the costs are steadily decreasing. The costs of MoMM analyses per sample are naturally highly case specific, depending especially on the analysis platform, specifications such as sequencing depth and the total number of analyzed samples. However, as a rough estimate, the cost of metabarcoding in the international market can be expected to decrease to 20 € per sample within the next five years, including all laboratory steps (prof. Florian Leese 2021, personal communication).

Given the rapid development of the molecular analysis technology, the foreseeable growth of the Finnish and international service market and the high sample numbers typically required for reaching the break-even point, developing a full state-of-the-art molecular laboratory will remain beneficial only for a few national organizations. We recommend active collaboration and communication, particularly between governmental organizations, to fully utilize existing infrastructure and plan new investments in a coordinated manner. This was also the take home message from the second national workshop: coordinated use of existing resources and following commonly (preferably internationally) agreed methodological standards was perceived as more crucial than developing centralized laboratory infrastructure. By contrast, the centralization of data management and computing services received support from the workshop participants.

The handling of massive DNA sequence data is highly CPU-intensive. Nevertheless, IT infrastructure was seen as a factor limiting the implementation by only 9% of the Finnish respondents in the survey. Free computing resources of CSC – IT Center for Science are now available for academic and governmental research institutes for non-profit purposes, and the services can also be purchased for commercial purposes. In addition, several institutes such as universities and the FMI have their own high-performance computing resources, and the performance of ordinary personal computers is also increasing and sufficient for more limited tasks. The know-how of bioinformatics methods and pipelines are seen as a clearly more limiting factor (30% of respondents) than restriction through hardware.

Another area with clear development needs is data management. As yet, data management practices are very heterogeneous, with no national database for DNA-based species and community data, nor commonly agreed standards for data formats or metadata. This is a major practical problem that significantly increases the workload and limits reliability when combining and comparing different data sets. We note that while the development of national databases and data standards is urgently needed, it should be done in close contact with existing national and international initiatives, aiming at smooth interfaces between national and international databases. There is a widely shared agreement that also DNA-based environmental data should be made openly available, and all major monitoring institutions in Finland are committed to the principles of open data and open science. The well-established Finnish Biodiversity Information Facility (FinBIF) and the more recently launched Finnish Ecosystem Observatory (FEO) are internationally recognized forerunners (Schulman et al. 2021, Pennisi 2021) in the open distribution of biological and environmental monitoring data and associated services and provide an excellent basis for developing the national distribution of MoMM-based data for researchers, officials, companies and the general public.

4.5 Sequence library development

When analyzing genetic data, sequences of the studied community are compared with the global sequence library databases in order to conclude which taxa are present in the studied environment (Schallenberg et al. 2020). One of the most commonly used global databases is GenBank, which comprises publicly available nucleotide sequences obtained through submissions from e.g., individual laboratories and from large-scale sequencing projects, including whole-genome shotgun (WGS) and environmental sampling projects. GenBank is part of the International Nucleotide Sequence Database Collaboration, which contains the DNA DataBank of Japan (DDBJ), the European Nucleotide Archive (ENA), and GenBank at NCBI. (Benson et al., 2013). While data are rapidly accumulating in such global resources, reference libraries for many groups of organisms are still incomplete. The scarcity of existing DNA barcodes in global sequence library databases restricts the informative potential of genetic methods and was identified in our surveys as one of the major limiting factors for MoMM implementation (Fig. 6B, Fig. 7). Moreover, the reliability of databases can be unsatisfactory due to mislabeled specimen and other errors that persist due to lack of curation.

Completing the databases with barcodes of all species is laborious and time-consuming. However, the completion of the databases is essential for monitoring species, their distributions and their environmental associations. At the EU level, the aim is to publish DNA sequences of 50% of the organisms in European ecosystems by the year 2030 (Lamy et al. 2020). In Finland, the Finnish Barcode of Life (FinBOL) project has since 2010 made a huge effort towards this aim and is now approaching it in the most species rich group, arthropods (43% of known species barcoded; Roslin et al. 2021). Moreover, FinBOL has set as the national, more ambitious target to complete the reference library of DNA barcodes for all (described) Finnish species by 2026. However, at present many gaps remain, particularly in groups where even the traditional taxonomic knowledge is still very incomplete and a large fraction of the true species diversity remains undescribed. It is also worth noting that in some cases, high intraspecific genetic diversity can challenge the development of reliable barcodes and in such cases species should ideally be represented by individuals from different populations to increase the quality of the species references. On the other hand, estimating intraspecific genetic diversity can also be among the goals of monitoring efforts (see below) and it could be desirable to develop methods for combining species level genetic monitoring with that of intraspecific genetic monitoring across species. Combining a set of markers that not only differ between species but also differ in their levels of intraspecific variability could allow combining both goals at least to some extent. For example, the most comprehensive, well curated databases for phytoplankton DNA barcodes are currently the SILVA high quality ribosomal RNA database and the Protist Ribosomal Reference Database (PR[2]), which combines

data from other important DNA databases, such as from the European Nucleotide Archive (ENA) and The Barcode of Life Data System (BOLD). However, the PR^2 database has severe deficiencies for Finnish phytoplankton taxa; only ca. 35% of our marine phytoplankton taxa were present in the databases. Thus, SYKE has collaborated with FinBOL since autumn 2020 to sequence the DNA barcodes of phytoplankton taxa in the FINMARI Culture Collection of SYKE.

Sequence library development should be included in the projects developing and implementing genetic methods in monitoring programs, whenever possible. This requires additional resources in collecting samples of various organisms, species identification, sequencing, and sharing the information with open global sequence library databases (including standardized metadata). Currently, many international working groups are already collaborating in creating reference databases. For example, the Scientific Committee for Ocean Research (SCOR) Working Group WG157: MetaZooGene is developing The MetaZooGene Barcode Atlas and Database (MZGdb), which includes already >150,000 mitochondrial cytochrome oxidase I (COI) sequences for ~5,600 described species of marine zooplankton. Collaboration with the already existing international or global working groups and internationally curated databases is recommendable whenever possible.

In addition to ensuring continued development and maintenance of high-quality barcode libraries, it will be important to also build capacity for comprehensive mapping of intraspecific genetic diversity. Intraspecific genetic diversity and genetic population structure have been routinely estimated in various research projects and using various methods (e.g. restriction enzymes, microsatellites or SNPs) for some decades. However, the recent fast adoption of high-throughput sequencing methods and bioinformatics pipelines developed for non-model organisms encourages towards building entire reference genomes instead of trying to only develop limited sets of species-specific marker genes. The 2018 initiated Earth Biogenome Project (EBP; Lewin et al. 2018; www.earthbiogenome.org) is an ambitious attempt to map the genomes of all eukaryotic species in only ten years' time which highlights that this is also the trend internationally.

4.6 Modelling and analysis tool development

The two main purposes of applying modelling approaches to biological observations are to help interpret the biological processes behind the observations and to predict new observations. In the case of biological monitoring, the processes of interest typically include general biodiversity patterns (e.g. Antao et al. 2020), dynamic processes such as population growth and decline, colonization-extinction dynamics and adaptation (e.g. Hällfors et al. 2021, Moor et al. 2021) and the responses of species and communities to environmental factors such as habitat features and human-induced pressures (e.g. Häkkilä et al. 2018; PUROHELMI project). Model predictions can be used to characterize the current state of the system, e.g. to estimate species' distributions or national abundances based on available habitat data (e.g. Henckel et al. 2020), or to predict the development of the system under different future scenarios (e.g. Löbel et al. 2021, Mair et al. 2018).

By the sheer volume of information they enable, MoMM offer a vast opportunity to achieve a better understanding of global biodiversity, its patterns and underlying principles – particularly when combined with high-resolution spatial data from remote sensing and multivariate modelling approaches (Bush et al. 2017, Compson et al. 2020, Abrego et al. 2021). At the same time, MoMM produce new kinds of data that must be interpreted carefully (Popa et al. 2020). Indeed, interpretation and designing representative sampling were identified as one of the main challenges in the national workshop (Fig. 6B). A promising approach is to combine molecular data with a probabilistic model that incorporates also other sources of information, such as mechanistic descriptions of eDNA transport and decay (Lugg et al. 2018, Matter et al. 2018, Carraro et al. 2020). Finally, machine learning methods have great potential in the analysis of massive sequence data, particularly in non-taxonomic approaches in which

OTU- or ASV-based data are mined for patterns that demonstrate e.g. responses to environmental degradation (Cordier et al. 2017, 2018).

In acknowledgement of the technical and conceptual challenges involved in the analysis of molecular data, as well as the high rewards of fully utilizing its information, we recommend that some of the resources allocated for the transition to MoMM be invested in the further development of suitable modelling and analysis tools.

5 Concrete action plan 2022-2025

In Chapters 3 and 4, we envisioned the future role of MoMM and described the general development areas that are key to the transition towards this future. Here, we narrow our focus to the next four years and list the concrete actions that are needed to launch the coordinated implementation of MoMM in Finland. Directed funding for the transition, particularly from the Ministry of the Environment and the Ministry of Agriculture and Forestry, is central and necessary to achieve the plotted progress. The conditions of directed funding should be carefully adjusted to instill both cross-governmental cooperation and to ensure necessary progress in the Technology Readiness Level towards large-scale application and to avoid further fragmentation of the field. Other recommended actions include strengthening of national coordination and Finland's role in international networks and initiatives by establishing a permanent working group, building an online interaction platform, and by developing a national data management system with automated links to international databases. Specific suggestions for improving the monitoring of invasive, pathogenic and pest species and GMOs are given in Boxes 1-2 below.

5.1 Funding instruments for applied research and development projects and monitoring pilots

Large-scale development of MoMM will require investments into research and development particularly during the transition phase (Fig. 1). Directed funding would be crucial for building molecular-based monitoring in a goal-oriented manner based on the fast-developing science. Concerning monitoring pilots and implementation in monitoring, funding is currently available mainly from national ministries, such as the Ministry of Environment (YM) and the Ministry of Agriculture and Forestry (MMM). Other potential funding sources include, for example, the EU, the Nordic Council of Ministries, the Academy of Finland, and private foundations. In addition, e.g. HELCOM and ICES (concerning marine habitats) are interested in collecting and utilizing monitoring data, but they do not have their own funding potential.

We propose that YM and MMM jointly coordinate yearly research and development funding calls in 2022-2025 to promote the implementation of MoMM (**A1**; Table 1). We suggest that future ministerial R&I funding models should use co-funding and entail a cross-governmental and cross-departmental requirement, namely that developments towards MoMM implementation need to include at least two agencies under the control of different ministries and that developments are tied to the national environmental monitoring strategy framework and implementation program. Besides YM and MMM, updating environmental monitoring is also a relevant issue for the Ministry of Finance (VM), the Ministry of Economic Affairs and Employment (TEM) and the Ministry of Social Affairs and Health (STM).

While the scope of the funding instrument should span across all taxa and environments and the whole value chain (or circle) from sampling design to DNA analyses, bioinformatics, data analysis and modelling, the focus should be applied. In particular, successful proposals should convincingly demonstrate the potential for a significant advance in Technology Readiness Level (TRL; section 2.1.1, Fig. 4) towards routine implementation. To promote national and international coordination of efforts, collaboration between organizations in Finland and abroad should be highly encouraged by the

evaluation criteria. Contribution to sequence library development should be strongly recommended particularly for projects developing and applying taxonomy-based methods.

One perspective that can be adopted to guide research and development efforts is the establishment of MoMM-based indicators for the monitoring and assessment of the objectives of specific policies. As a topical example, Table 2 presents the possibilities and challenges of MoMM following the proposed indicator categories of the National Biodiversity Strategy and Action Plan for 2035 which is currently under preparation.

5.2 Permanent working group to coordinate national efforts and involvement in international networks

Finland is already participating in several important international activities that promote the uptake of molecular methods. However, the links between the activities of individual national actors have been relatively weak, although the situation is improving. To be effective, Finnish actors need to jointly formulate detailed national objectives and act concertedly through their individual networks. Thus, the formation of a formal working group is required (**A2**; Table 1). Finnish actors should together promote more unified guidance on the use of molecular methods and data based on nationally agreed goals. It cannot be stressed enough that the planned actions of individual national actors need to be openly communicated in the group of actors before they are taken, to avoid both duplication of effort and incompatible or conflicting outcomes of individual actions. This will require open communication and concerted planning. As concrete examples of first steps needed in international coordination, we propose that Finland should actively pursue the idea of establishing an international working group. This group could address common issues and knowledge gaps, e.g. map the international need for quality control of MoMM (e.g. proficiency tests).

5.3 Expanding and promoting the eDNA network

The national eDNA network initiated by SYKE has been described in sections 1.4 and 4.2. We propose that this existing network be expanded and promoted to strengthen its role as a national community of practice that produces opportunities for networking, exchange of information and experiences, education and critical discussion on MoMM implementation (**A3**; Table 1). Active promotion to other organizations, including private companies, more regular meetings (4-6 /year), yearly workshops, courses and bottom-up links to the permanent working group are the crucial next steps.

5.4 Online interaction platform (eDNA hub)

To complement the meeting-based interaction in the eDNA network, we propose the establishment of a national online platform ("eDNA hub"; **A4** in Table 1), a community of practice website enabling more continuous interaction. This website would function as a gateway to curated, up-to-date information on MoMM, a noticeboard of upcoming events and a forum for real-time discussions and co-creative workflows across organizational boundaries. Importantly, the hub could also serve as a bridge between sectors, e.g. maintaining a catalogue of MoMM-related services (including available infrastructure) and service providers and enabling the sharing of experiences and reviews of specific services. The platform could also be used for educational purposes, e.g. for sharing teaching materials.

5.5 National data management system

MoMM are evolving fast and the need to compile sequence-based environmental monitoring data in a way that is as easily available as possible to all users will increase significantly in the coming years. Currently, the storage of sequence data is scattered across different servers (e.g., CSC) and individual researchers' computers. Using the data, especially in retrospect, is currently incredibly challenging. With raw sequence data, information is often not available on how and by which bioinformatics tools the data have been processed, which quality criteria have been used, which sequencing method has been chosen, whether the data is normalized etc. In addition, it would be important that other biological and environmental data collected simultaneously with molecular biological sampling could easily be combined with molecular biological data.

We propose the development of a national molecular data management system (**A5**), with commonly agreed data and metadata standards and automated links to international databases. For example, the Global Biodiversity Information Facility (GBIF) now includes also DNA-based biodiversity data following explicit guidelines (Andersson et al. 2020), and several countries are taking steps in this direction e.g. by implementing the Living Atlases following the example of the Atlas of Living Australia. The national data standards should be carefully considered for international compatibility, and broadly discussed with the most relevant user groups, once these are identified, aiming at a consensus on minimum requirements that are acceptable to all while still allowing some flexibility in recognition of different data types and needs by different actors. In addition, keen attention should be paid to the data quality assignment and the classification for smooth utilization by different user groups – e.g. researchers, public authorities, private sector, education. An important step in the co-creation of the data management system is a thorough scrutiny of the relevant functioning Finnish RIs, e.g. the Finnish Biodiversity Information Facility (FinBIF), to guarantee the best practice of the national development and implementation. For assuring the FAIR compatibility, the data management system should be designed to demonstrate the critical functions needed. As all national monitoring data, molecular data should be open-access by default, with exceptions for special circumstances such as strictly protected species. The data management system should also aim at recognized certification to increase the trustworthiness and credibility in the eyes of international collaborators and e.g. funders to further collaborative sustainable financing. Data management expertise will be secured by investigating the need of Data Steward training or recruitment.

6 Monitoring the progress of the implementation

In Chapters 4-5, we presented general development areas and concrete actions to promote the implementation of MoMM. Finally, the realization of this roadmap and the effectiveness of the measures we suggest should be monitored based on predetermined criteria. Here, we propose a set of criteria corresponding to the actions listed in Chapter 5.

To monitor the level of funding (A1, 5.1), we encourage that organizations aspiring to utilize MoMM would in subsequent years follow the amount of funding available for MoMM research and development against the 2021 level. Similarly, the number of projects an organization participates in that use or develop MoMM should be monitored. Finally, the number of staff that is involved with the promotion or use of molecular methods should be followed. As a measure of effectiveness, the progress of molecular method readiness for environmental monitoring should be evaluated in the degree change in Technology Readiness Level observed for methods for different taxa.

For the permanent working group (A2, 5.2), the establishment of the group is an evaluation criterion. To monitor the international activities, the number of initiated molecular method standards for environmental monitoring that Finnish actors are involved in should be followed.

For the eDNA network development, (A3, 5.3), the number of events (workshops, conferences, summer schools etc.) that include more than two Finnish organizations should be monitored.

For the online platform (A4, 5.4), the launch of the website is the primary monitoring criterion. In addition, the number of visitors to the site should be monitored.

For the data management target (A5, 5.5) the evaluation should be twofold: a) narrative evaluation of the role of Finnish actors and b) the state of development of a national federated resource that has active links to international data infrastructure.

Box 1. Monitoring invasive species, pests and pathogens with eDNA/eRNA

Early detection is crucial

An **alien species** is defined as any live organism introduced outside its natural range by human activity, whether accidentally or deliberately. However, not all alien species are invasive. An **invasive alien species** (IAS) is a species which has been found to threaten the biodiversity at a given site. Once IAS have established, their eradication is often nearly impossible. The risk of emerging IAS in Finland is predicted to increase due to climate change and constantly expanding global trade of goods and products.

Quarantine pests and **protected zone quarantine pests** are significant pests throughout the territory of the European Union or in certain restricted areas. **Priority pests** refer to the most harmful pests that are not known to occur in the territory of the European Union or that have very minor, irregular occurrences in some parts. A recent study (Allen et al. 2021) concluded that eDNA is likely to become an increasingly useful and powerful addition to the toolbox of pest detection.

Besides invasive alien species and quarantine pests, there is a need to monitor the occurrence of **indigenous pests and pathogens** as their distribution areas and disease severity is expected to change due to climate change. For example, forest pathogens are already monitored during the National Forest Inventory (VMI), but the monitoring does not yet include MoMM. The risks of occurrence of invasive forest pathogens have increased exponentially in Europe (Santini et al. 2013).

Overall, invasive species are one of the main global threats to biodiversity and ecosystem stability in natural environments, productivity of crop species, and human and animal health (Larson et al. 2020, Farrell et al. 2021). Early detection of invading organisms is crucial in terms of effectiveness and cost-efficiency of the actions. Thereby new technological tools such as MoMM, remote sensing and smartphone and internet applications-enabled citizen science are proposed to strengthen the early detection and surveillance of environments.

Expert organizations in Finland

Various ministries, institutes, organizations and associations (Table Box2.1) provide statutory and expert services in Finland. They are responsible for collecting observations and reports and taking elimination measures.

Table Box2.1 Expert bodies responsible for monitoring various treats for biodiversity and health.

Topic	Expert body	In colloboration with
Invasive alien species: The Finnish Advisory Board for Invasive Alien Species	Ministry of Agriculture and Forestry	Ministry of Transport and Communications, Ministry of the Environment, The Finnish Food Authority, The Finnish Transport Infrastructure Agency, The Finnish Transport and Communications Agency , The advisory board of pet Animal welfare, Natural Resources Institute Finland, Finnish Wildlife Agency, Finnish Environment Institute?, Finnish Forest and Park Service, Customs, Regional Centres for Business, Transport and the Environment, Cities of Helsinki and Lahti, Universities of Helsinki and Eastern Finland, Central Museum of Natural History, Association of Finnish Municipalities, The Central Union of Agricultural Producers and Forest Owners, Finnish Forest Industries, Central Organization for Finnish Horticulture, The Finnish Association for Nature Conservation, BirdLife, WWF, Rural Women's Advisory Organisation
Animal and human health, Risk assesment and pandemics: Zoonotic diseases, food safety antimicrobial resistance, Cross-border health threats, and international treaties	The Ministry of Social Affairs and Health	Ministry of Agriculture and Forestry, Finnish Food Authority, Finnish Institute for Health and Welfare, The Ministry of the Interior, The Ministry of the Environment
Plant and forest health	Ministry of Agriculture and Forestry	Finnish Food Authority, Natural Resources Institute Finland

Pilot use of MoMM, examples and potential pitfalls

MoMM (see Table 3) are effective for detecting fungal, bacterial, invertebrate/arthropod and viral pathogens from terrestrial and aquatic habitats, as well as microbes residing inside tissues. MoMM are especially suitable for the detection of so-called **cryptic species** that cannot be identified based on morphological characters, for example many fungal and bacterial pathogens (van der Wal et al. 2017), as well as monitoring the situation of emerging pathogens and **uncultivable organisms** such as obligate biotrophic pathogens. In most cases, MoMM used for pathogen detection are still in the pilot phase. However, in **aquatic environments,** MoMM are used widely (Deiner et al. 2017; Cristescu & Hebert 2018), for both targeted search of unrecorded or newly emerging populations (e.g. IAS) (Chucholl et al. 2021), and also to monitor the presence-absence of aquatic animal diseases such as crayfish plague (Johnsen et al. 2020).

    Airborne eDNA can offer a new tool to monitor the terrestrial vertebrate community (Lynggaard et al. 2021) but the usefulness of airborne eDNA for invasive species detection has not yet been demonstrated in natural environments. However, spatial and temporal variation in airborne microbial communities was recently reported from eDNA retrieved from archived air filters initially collected and deposited to monitor radioactive fallout in Sweden (Karlsson et al. 2020), and potential of the archived filters to provide insights into pathogens and invasive species was suggested.

    Any MoMM is ultimately dependent on the representativeness of the **sampling scheme**. Therefore, species absence in a laboratory analysed sample should not be taken as direct evidence that the organism of interest is absent from the sampled environment. On the other hand, analysis **artefacts** and **contaminations** may bring risks for false identifications. This is particularly important to take into account when a MoMM is developed as a validated method for pest and pathogen detection. Moreover, the estimation of abundance of organisms by eDNA is typically challenging, but errors and uncertainties associated with eDNA metabarcoding studies can be mitigated by thoughtful study design, sampling, replication and appropriate primer choice (Deiner et al. 2017).

Future work and funding

Taken together, MoMM can be successfully and effectively used to detect organisms and populations from numerous substrates. As metagenomics-based MoMM are capable of detecting a wide range of organisms, they can be utilized for the investigation of biodiversity and pathogen presence simultaneously. Molecular laboratory infrastructures and facilities are prerequisites for methodological development needed in applying analyses into practice and finally into routine use. Adapting and applying methods to local conditions, species-specific detections or screening of cryptic species demands primary research and adequate human resources before cost-efficient high flowthrough of samples can be established. Strong national and international collaboration in improving reference databases, bioinformatic workspaces and analysis packages is also needed. To enable the future monitoring of emerging threats, stable and sufficient resources and funding need to be secured.

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Box 2. Monitoring genetically modified organisms

Genetically modified organisms

A genetically modified organism (GMO) is any organism whose genetic material has been altered using techniques in a way that do not occur naturally by mating or by natural recombination. The most common aim of the modification is to give a cultivated plant a new trait, such as disease resistance or tolerance to an herbicide, and thus to get more profitable cultivars. The so-called first-generation GMOs contain new genetic material, often deriving from different organisms (recombinant-DNA). Genetic modification can also be achieved by using the so-called new genomic techniques where in some cases no new DNA is inserted into the genome of the organism. Gene editing is a group of technologies that enable scientists to change an organism's DNA, i.e. material to be added, removed, or altered at particular locations in the genome. A recent application is known as CRISPR-Cas9, which is short for clustered regularly interspaced short palindromic repeats and CRISPR-associated protein 9.

Synthetic biology refers to the application of science, technology and engineering to facilitate and accelerate the design, manufacture and/or modification of genetic materials in living organisms to alter living or non-living materials (European Commission 2014). This includes the use of so-called synthetic gene drives. A synthetic gene drive is a technology of genetic engineering that propagates a particular suite of genes throughout a population by altering the probability that a specific allele will be transmitted to offspring (instead of the Mendelian 50% probability).

So far, the only GM crop accepted for cultivation in the EU is the insect resistant GM maize MON810, also known as YieldGard maize. However, numerous food and feed products consisting of genetically modified cotton, maize, soybean of oilseed rape are registered for the import in the EU. There are no GM-animals on the market in the EU at the moment, and only one aquaculture species, salmon, has been genetically modified for production in the US (Waltz 2017). However, the current situation might change in the future due to new genomic modification techniques and the changing climate.

At this point, no synthetic biology applications have been introduced outside laboratories. Experimental laboratory work has been carried out to use gene drives in order to suppress populations of malarial mosquitoes.

GMO monitoring

According to the EU-legislation, commercial cultivation or food/feed import and use of genetically modified organisms have to be monitored. The aim is to identify potential adverse effects of the genetically modified organisms and their use on human health and the environment. Therefore, applications for GMOs and GM food/feed products must contain a monitoring plan which forms part of the authorization decision. There are different kinds of monitoring procedures depending on the scope of the application (see EFSA 2020).

- For the applications for GM-plant cultivation, post-market environmental monitoring plan (PMEM) must fulfil the requirements of Annex VII of the Directive 2001/18/EC. Case-specific monitoring needs to be done to detect direct and indirect effects, which have been identified in the environmental risk assessment. General surveillance is for detecting unanticipated adverse effects.
- Post-market monitoring plan (PMM) is requested in cases where it is appropriate to verify that the conditions of GM food/feed use are properly applied.

The consent holder of the approved GMO must implement the monitoring plan and report on it regularly. Monitoring methods must be suitable to detect direct and indirect, immediate and long-term effects, as well as unforeseen effects.

Currently there is no legislation on monitoring synthetic biology applications if they fall outside the GMO-legislation. There are still major gaps in knowledge to be filled for performing a reliable risk assessment on synthetic biology applications. Therefore, no release to the environment can be expected in the near future. Many ideas on how to apply synthetic biology are still at a theoretical level and the few applications are still in a very preliminary phase.

Current situation

For general surveillance the Directive 2001/18/EC recommends to make use of existing agricultural and environmental monitoring programmes. For this purpose, a survey was done in 2008 by the European Commission and also Finland participated in the survey. A comprehensive survey was carried out by the Finnish Environment Institute. Several monitoring programmes on e.g. birds, butterflies and threatened plant species were identified as possible candidates to be used also in GM-plant cultivation monitoring. However, it was clear that none of these programmes would be directly suitable, but some could be developed for that purpose by choosing carefully the relevant monitoring areas and species and sometimes by also increasing the monitoring frequency, which should in our opinion have minimum 1-3 year-intervals and extend to at least 2-3 generations in general (see ENV_08_13_General Surveillance and ENV_08_13_01_Appdx 1_General Surveillance). Further work and updating information would be needed to improve the preparedness for environmental GMO-monitoring in Finland.

The control of genetically modified food is part of the regular food control that is based on the operator's in-house control. The practical side of food control is carried out by municipal food control authorities and Finnish Food Authority's inspection veterinarians. Genetically modified food is also controlled by the Finnish Customs. Both Finnish Food Authority and the Finnish Customs have technical capacity to detect GM-material by molecular methods.

Future work

GM-monitoring with eDNA methods is still to be developed. However, the use of eDNA in different environmental monitoring programmes might prove to be useful for GMO-surveillance, especially detecting changes in the environment (e.g. changes in soil microbial diversity or genetic diversity) for general surveillance and post-market monitoring of GM-organisms. eDNA methods can prove to be applicable for monitoring synthetic biology applications, including gene drive organisms, in the future.

eDNA can be used for identifying GM-material but also for detecting potential changes in the environment caused by GMOs. For example, Hecht et al. (2014) found feral GM plants along railway lines and in port areas in Switzerland using real-time PCR. Nakajima et al. (2020) conducted a roadside survey of GM oilseed rape in Japan and Kim et al. (2020) monitored GM oilseed rape using an efficient multiplex PCR assay in natural environments in South Korea. Similar studies on detecting GM-oilseed rape have been planned in Finland by a task force under the Board of Gene Technology, but no funding has been applied yet. The aim of the study is to find out, whether GM oilseed rape plants have already been established in the environment, e.g. roadsides. The result would provide a baseline for future monitoring. Monitoring would be compulsory in the case GM-oilseed rape would be accepted for cultivation in the EU.

Furthermore, GM pollen monitoring by bioaerosol sampling and PCR screening might be useful surveillance methods (Folloni et al. 2012). Organisms whose CRISPR-Cas (see above) altered DNA has no externally visible phenotypic expression would also require genetic monitoring, either of trapped organisms or of eDNA (Power 2021). It might also be informative to obtain baseline eDNA data before any GM- (and synthetic biology; see EFSA 2021 and Dolezel et al. 2020) applications have been introduced into our environment.

A recent study has used eDNA methods in detecting transgenes of GM animals from terrestrial and aquatic sources in invertebrate and vertebrate systems. The authors extracted eDNA from food media of GM fruit flies (*Drosophila melanogaster*), feces, urine, and saliva of GM laboratory mice (*Mus musculus*), and aquarium water of GM tetra fish (*Gymnocorymbus ternetzi*) (Xu et al. 2021).

All these studies show that eDNA has great potential to improve the GMO-monitoring in the future.

Funding needs

It is clear that Finland should increase its preparedness to carry out GMO-monitoring. At present, no GM-plants are cultivated in Finland. However, this may change rapidly, especially when the new genomic techniques are further developed and used. It would be important to start the oilseed rape pilot study mentioned above. Further, a desk-study should be carried out to update the information on the usefulness of

biodiversity monitoring programmes for GMO-monitoring. Both studies would benefit from eDNA techniques.

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Appendices

Appendix 1. Literature search, study selection and data extraction procedure and the resulting data set of the systematic literature review (section 2.1.1).

This appendix describes the procedure adopted for the systematic literature review and presents the data subset that was used in this report (a sample of 70 papers; Table A1). Full results of the review are to be published as a scientific article (Laamanen et al. in prep).

Database search:
A search of the Web of Science database was performed on April 15th 2021 using the search string:
 "(TS = ((eDNA OR (environmental AND DNA)) AND monitoring AND biodiversity)) AND LANGUAGE: (English) AND DOCUMENT TYPES: (Article)" This resulted in 320 separate articles published no more than five years before the search.

Study selection:
Following the systematic review protocol implemented in the CADIMA tool (https://www.cadima.info), the articles were screened against predetermined study selection criteria. Study selection was performed primarily based on the abstract but referring to the full text where necessary. We included the following criteria:
(i) The article is an original research paper.
(ii) The study applies molecular methodology.
(iii) The molecular methodology is used to assess the presence and/or abundance of one or several target taxa or to assess the status of the environment. (Note: population genetic studies not included.)
(iv) The study discusses the topic of applying/implementing the adopted methodology in monitoring.
(v) At least some of the analyzed samples have been collected from an outdoor environment.

Data extraction and TRL assessment:
Data was extracted from the selected articles based on the full text. Based on the extracted data, we assessed the Technology Readiness Level (TRL) of the method presented or applied in each paper. We interpreted the TRLs using the following progressively applied criteria:

- TRL5 (Technology validated in relevant environment): The study selection criteria.
- TRL6 (Technology demonstrated in relevant environment): The molecular method is compared to the traditional method and shown to have advantages.
- TRL7 (System prototype demonstration in operational environment): The molecular method is applied at a medium or large spatial scale (>10 km) and its implementation in monitoring is at least conditionally recommended.
- TRL8 (System complete and qualified): The molecular method is directly compared to the traditional method (i.e. with comparable samples) and its implementation is recommended without major conditions.
- TRL9 (Actual system proven in operational environment): The method is already implemented in an existing monitoring program.

Table A1. The data set of the literature review.

Reference	Type of monitoring	System	Taxonomic group	Methodology	Sample type	Geographic scope (km)	Temporal scope	Method comparison	Advantages	Implemented	Implementation recommended	TRL
Abrams et al. 2019	biodiversity	terrestrial	invertebrates	leech-iDNA metabarcoding	leech iDNA	100 - 1000	1-12 months	direct	yes	no	yes, condtionally	7
Alexander et al. 2020	biodiversity	marine	invertebrates	Metabarcoding	water	1-10	<1	indirect	yes	no	yes	6
Andujar et al. 2018	environmental status assessment	freshwater	invertebrates	Metabarcoding	water	<1	1-12 months	Direct	yes	no	yes	6
Apotheloz-Perret-Gentil et al. 2017	environmental status assessment	freshwater	plankton and algae	PCR, qPCR	biofilm of submerged stones	100-1000	1-2 years	direct	yes	no	yes	8
Archana & Baker 2020	ecosystem functioning	marine	fish	eDNA metabarcoding	water	Not specified	1 month	Indirect	yes	no	not mentioned	6
Aylagas et al. 2018	biodiversity	marine	invertebrates	Metabarcoding	sediment, bulk	10-100	1 month	direct	yes	yes	yes	9
Bagley et al. 2019	biodiversity	freshwater	fish	eDNA metabarcoding	water, sediment	1-10	1-12 months	indirect	yes	no	yes, condtionally	6
Bakker et al. 2017	biodiversity	marine	fish	eDNA metabarcoding	water	>1000	1-12mo	indirect	yes	no	indirectly recommend	7
Barsoum et al. 2019	biodiversity	terrestrial	invertebrates	metabarcoding	bulk, malaise traps	10-100	1-12	none	yes	no	yes	5
Bernardino et al. 2019	environmental status assessment	marine	invertebrates	Metabarcoding	sediment	<1	< 1 month	none	yes	no	yes	6
Blackman et al. 2020	invasive and harmful species	freshwater	molluscs	standard PCR	water	100-1000	1-12	direct	yes	no	yes, indirectly	8
Bombin et al. 2021	biodiversity	marine	plankton and algae	Metabarcoding	water and benthic samples	100-1000	?	no	yes	no	yes, conditional	5
Borrell et al. 2017	invasive and harmful species	marine	invertebrates	Metabarcoding	water	<1	Single sampling occasion	direct	yes	no	yes	6
Boussarie et al. 2018	threatened species	marine	fish	eDNA metabarcoding	water	100-1000	1-12 months	indirect	yes	no	yes	7
Boyd et al. 2020	threatened species	freshwater	crustaceans	qPCR	water / groundwater	100-1000	1-12mo	direct	yes	no	yes	8
Bracken et al. 2019	threatened species	freshwater	fish	qPCR	water	100-1000	<2 years	indirect	yes	no	yes	7

Reference	Type of monitoring	System	Taxonomic group	Methodology	Sample type	Geographic scope (km)	Temporal scope	Method comparison	Advantages	Implemented	Implementation recommended	TRL
Broman et al. 2021	biodiversity	marine	invertebrates	eRNA shotgun sequencing	sediment	1-10	<1 month	none	no	no	yes	5
Brunbjerg et al. 2018	environmental status assessment	terrestrial		Metabarcoding	soil	100-1000	1-2 years (?)	indirect	yes	no	yes, condtionally	7
Brys et al. 2021	biodiversity	freshwater	fish	PCR, qPCR and sequencing	water	<1	< 1 month	none	no	no	yes	5
Brys et al. 2021	threatened species	freshwater	fish	ddPCR	water	100-1000	< 1 month	none	no	no	yes	5
Bucklin et al. 2019	biodiversity	marine	plankton and algae	Metabarcoding	water	> 1000	> 2 years	None	no	no	yes	5
Bylemans et al. 2018	biodiversity	freshwater	fish	Metabarcoding primer design	water	10-100	1-12 months	None	no	yes	yes	5
Carew et al. 2018	biodiversity	freshwater	invertebrates	Metabarcoding	Invertebrate samples	10-100	> 2 years	None	no	no	yes	5
Carim et al. 2020	invasive and harmful species	freshwater	fish	PCR	water	100-1000	> 2 years	Direct	yes	yes	yes	9
Clark et al. 2020	environmental status assessment	marine		eDNA metabarcoding	sediment	10-100	< 1 month	indirect	yes	no	yes, condtionally	7
Closek et al. 2019	biodiversity	marine	fish	Metabarcoding	water	100-1000	1-2 years	indirect	yes	no	yes	7
Clusa et al. 2017	invasive and harmful species	freshwater	invertebrates	PCR	water	100-1000	1-12 months	none	yes	no	yes	5
Cornman et al. 2021	biodiversity	freshwater	fish	eDNA metabarcoding	water	10-100	1-12 months	indirect	yes	no	yes, condtionally	7
Cowart et al. 2020	biodiversity	marine	invertebrates	eDNA metabarcoding	sediment	< 1	1-2 years	direct	yes	no	yes. condtionally	6
Cowart et al. 2018	biodiversity	marine	invertebrates	metagenomic shotgun sequencing	water	100-1000	1-12 months	none	no	no	yes	5
Crookes et al. 2020	invasive and harmful species	freshwater	fish	qPCR	water	100-1000	<1 month	none	yes	no	yes, condtionally	5
Curd et al. 2019	biodiversity	marine	universal	Metabarcoding	water	10-100	none	none	no	yes	yes	5
Currier et al. 2018	threatened species	freshwater	molluscs	qPCR	water	100-1000	1-12 months	direct	yes	no	yes, condtionally	7

Reference	Type of monitoring	System	Taxonomic group	Methodology	Sample type	Geographic scope (km)	Temporal scope	Method comparison	Advantages	Implemented	Implementation recommended	TRL
Day et al. 2019	threatened species	terrestrial	birds	qPCR	water	?	<1 month	indirect	yes	no	yes	6
De Ventura et al. 2017	invasive and harmful species	freshwater	molluscs	qPCR/PCR	water	100-1000	1-12 months	indirect	yes	no	yes	7
Deagle et al. 2018	biodiversity	marine	plankton and algae	metabarcoding	water	>1000	1 month	direct	yes	yes	Yes,condtionally	7
Deutschmann et al. 2019	economic/key species	freshwater	fish	qPCR	water	1-10	<1 month	indirect	yes	no	yes, condtionally	6
DiBattista et al. 2019	biodiversity	marine	universal	Metabarcoding	sediment	100-1000	1-2 years	none	yes	no	no	5
Drinkwater et al. 2021	biodiversity	terrestrial	mammals	Metabarcoding	leech iDNA	10-100	1-12 months	no	yes	no	yes, condtionally	5
Drinkwater et al. 2019	biodiversity	terrestrial	mammals	metabarcoding	leech iDNA	10-100	1-12 months	none	yes	no	no	5
Dysthe et al. 2018	threatened species	freshwater	molluscs	qPCR	water and tissue	10-100	1-2 years	indirect	yes	no	yes, conditionally	7
Dzhembekova et al. 2017	invasive and harmful species	marine	plankton and algae	metabarcoding	water	< 1	1-2 years	none	yes	no	no	5
Eiler et al. 2018	threatened species	freshwater	amphibians	qPCR	water	1-10	1-12 months	indirect	yes	no	yes conditionally	6
Elberri et al. 2020	economic/key species	freshwater	fish	qPCR	water	10-100	unclear (1-12 months?)	none	yes	no	yes, condtionally	5
Fais et al. 2020	biodiversity	marine	invertebrates	metabarcoding	sediment	10-100	< 1 month	none	yes	no	no	5
Froslev et al. 2017	biodiversity	terrestrial	plants	metabarcoding	soil	100-1000	unclear (1-12 months?)	direct	yes	no	yes	8
Gerhard & Gunsch 2019	invasive and harmful species	marine	bacteria and fungi	high throughput sequencing	water	>1000	>2 years	none	no	no	no	5
Guerrieri et al. 2021	biodiversity	terrestrial	universal	Metabarcoding	sediment	10-100	<1 month	none	no	no	no	5
Hajibabaei et al. 2019	biodiversity	freshwater	invertebrates	Metabarcoding	water and bulk	10-100	<1 month	none	not discus-sed	no	no	5
Hardulak et al. 2020	invasive and harmful species	terrestrial	invertebrates	bulk DNA metabarcoding	bulk	10-100	> 2 years	indirect	yes	no	yes, condtionally	7

Reference	Type of monitoring	System	Taxonomic group	Methodology	Sample type	Geographic scope (km)	Temporal scope	Method comparison	Advantages	Implemented	Implementation recommended	TRL
Harper et al. 2018	invasive and harmful species	freshwater	crustaceans	qPCR	water	10-100	>2 years	indirect	yes	no	yes conditionally	7
Hayami et al. 2020	biodiversity	freshwater	fish	PCR	water	100-1000	>2 years	indirect	no	no	yes conditionally	5
Jo et al. 2020	threatened species	freshwater	amphibians	eDNA + real-time PCR	water	< 1	1-12 months	direct	yes	no	yes, condtionally	6
Klymus et al. 2017	invasive and harmful species	freshwater	molluscs	eDNA metabarcoding	water	unclear	unclear; up to 12 months	direct	yes	no	yes	6
Leasi et al. 2018	biodiversity	marine	invertebrates	Metabarcoding	sediment	100-1000	between February 22 and March 9, 2016	direct	inconclusive			5
Loit et al. 2019	invasive and harmful species	terrestrial	bacteria and fungi	3rd generation sequencing / metabarcoding	plant material	100-1000	> 2 years	direct	yes	no	yes	8
Lopes et al. 2021	threatened species	terrestrial	amphibians	eDNA metabarcoding	water	100-1000	1-12 months	indirect	yes	no	yes	7
Lopes et al. 2017	biodiversity	freshwater	amphibians	metabarcoding	water	1.loka	< 1 month	direct	yes	no	yes	6
Lugg et al. 2018	threatened species	freshwater	mammals	qPCR	water	100-1000	1-12 months	direct and indirect	yes	no	yes	8
Macher et al. 2018	biodiversity	freshwater	invertebrates	eDNA / bulk DNA metabarcoding	water / bulk (invertebrates)	100-1000	< 1 month	none	yes	no	yes	5
Machler et al. 2021	biodiversity	freshwater	universal	Metabarcoding	water	10-100	< 1 month	none	no	no	yes, condtionally	5
Mariani et al. 2021	biodiversity	marine	fish	eDNA metabarcoding	water	10-100	1-12 months	indirect	yes	no	yes	7
Matter et al. 2018	economic/key species	freshwater	fish	qPCR	water	100-1000	1-2 years	none	yes	no	yes, condtionally	5
Mauffrey et al. 2021	environmental status assessment	marine	invertebrates	Metabarcoding	sediment	1-10	< 1 month	direct	yes	no	yes	6
Mauvisseau et al. 2018	invasive and harmful species	freshwater	crustaceans	qPCR	water	10-100	1-2 years	indirect	yes	no	yes	7
McClenaghan et al. 2020	biodiversity	marine	fish	eDNA metabarcoding	water	100-1000	1-2 years	direct	yes	no	yes, condtionally	7

Reference	Type of monitoring	System	Taxonomic group	Methodology	Sample type	Geographic scope (km)	Temporal scope	Method comparison	Advantages	Implemented	Implementation recommended	TRL
McGee et al. 2020	biodiversity	terrestrial	invertebrates	metabarcoding	soil	1.loka	< 1 month	none	yes	no	yes	5
Pansu et al. 2021	biodiversity	freshwater	universal	Metabarcoding	sediment	10-100	1-12 months	none	no	no	yes	5
Stat et al. 2019	biodiversity	marine		eDNA metabarcoding	water	10						5
van den Heu-vel-Greve et al. 2021	invasive and harmful species	marine	invertebrates	Metabarcoding	sediment	10-100	< 1 month	none	no	no	yes	5

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Appendix 2. International survey on the current state and future needs of the use of molecular methods in monitoring (section 2.1.2).

Survey on the current state and future needs of the use of molecular methods in monitoring

We warmly welcome you to participate in our survey on the current state and future needs regarding the use of molecular methods in routine monitoring.

The survey contains two parts: national level questions (specifically on the implementation in routine monitoring) and individual level questions (including research; and the level of your involvement).

All the answers of the survey will be used anonymously to outline the current state and future needs of the molecular methods in monitoring at both national and international levels. It takes approximately 15-20 minutes to complete the survey. We like to kindly thank you in advance for your time and your valuable answers.

If you have already filled in this survey in the spring 2021, please do not respond again.

The first part contains the national level questions.

1. Which country do you represent?

_____

2. What kind of organization do you work for? You can choose multiple options.

☐ Academic

☐ Goverment

☐ Private

☐ Other, please specify: _____

3. Are you directly involved in setting up a national strategy for the implementation of DNA methods in monitoring?

◯ Yes, please specify your role: _____

◯ No

4. Are there national guidelines for the use of molecular methods in monitoring? You can choose multiple answers.

☐ Sampling

☐ Laboratory procedures

☐ Bioinformatics

☐ In preparation

☐ I do not know

☐ No

☐ Please provide a link to the guidelines mentioned above, if possible: _____

5. Is there separate funding available specifically for the implementation of molecular methods?

◯ Yes, please specify: _____

◯ No

◯ I do not know

6. What is the status of the molecular method implementation in these different fields in your country?

	Routine implementation	Testing phase	Planned	Not planned	I do not know

	Routine implementation	Testing phase	Planned	Not planned	I do not know
Biodiversity	☐	☐	☐	☐	☐
Environmental status	☐	☐	☐	☐	☐
Microbial monitoring (sewage, drinking, swimming water)	☐	☐	☐	☐	☐
Threatened species	☐	☐	☐	☐	☐
Invasive species	☐	☐	☐	☐	☐
Managed populations (game, fisheries)	☐	☐	☐	☐	☐
GMO	☐	☐	☐	☐	☐

7. What is currently limiting the implementation? You can choose multiple answers.

☐ Funding

☐ Laboratory infrastructure

☐ Analytical service providers

☐ Know-how or trained personnel

☐ Bioinformatics methods and pipelines

☐ Sequence libraries

☐ IT infrastructure

☐ Cost of analysis (e.g. sequencing)

☐ Lack of European method standards

☐ Other, please specify: _____

8. Are there national metadata standards for the management of sequence data in use?

◯ Yes, please specify: _____

◯ No

◯ I do not know

9. Are there computing resources available for handling the sequence data?

☐ Yes, please specify: _____

☐ No

☐ Planned, please specify: _____

☐ I do not know

10. If molecular methods are already applied in monitoring or if they will be implemented in the future, services will be provided by: (You can choose multiple answers.)

	Governmental operator	Commercial operator	University	Foreign operator	I do not know
Sampling is / will be provided by	☐	☐	☐	☐	☐
Analytical service is / will be provided by	☐	☐	☐	☐	☐
Bioinformatics is / will be provided by	☐	☐	☐	☐	☐

11. If molecular methods are already applied or if they will be implemented in the future, will data storage be: (You can choose multiple answers.)

☐ Internal to your organization

☐ On a dedicated national platform, please provide name or link: _____

☐ On an international remote storage site, please provide name or link: _____

☐ I do not know

12. If molecular methods are already applied or if they will be implemented in the future, will data be: (You can choose multiple answers.)

☐ Open access

☐ Partially open

☐ Restricted access

☐ I do not know

☐ You can specify your answer: _____

13. To your knowledge are the authorities in your country cooperating and coordinating the uptake of molecular methods?

☐ Yes, nationally

☐ Yes, internationally

☐ No

☐ I do not know

☐ Planned nationally

☐ Planned internationally

☐ You can specify your answer: _____

14. Have there been national citizen science sampling campaigns for molecular analysis?

☐ Yes, please specify and provide links: _____

☐ No

☐ I do not know

The second part explores the "Individual level", i.e. how do you apply molecular methods in biodiversity/environmental monitoring/research?

15. What has been your role in the use of molecular methods? You can choose multiple options.

☐ Planning

☐ Sampling

☐ Laboratory work

☐ Sequencing

☐ Bioinformatics

☐ Analysis of results

☐ End user of results

☐ Other, specify _____

16. What kind of monitoring/research do you work with or is your work applicable to? You can choose multiple options.

☐ Biodiversity

☐ Environmental status

☐ Microbial monitoring (sewage, drinking, swimming water)

☐ Threatened species

☐ Invasive species

☐ Managed populations (game, fisheries)

☐ GMO

☐ Other, please specify _____

17. What kind of environment is your work related to? You can choose multiple options.

☐ Marine

☐ Freshwater

☐ Terrestrial

☐ Other, please specify: _____

18. Which taxa are you targeting? You can choose multiple options.

☐ Individual species, please specify taxa: _____

☐ Whole community, please specify: _____

19. What is studied? You can choose multiple options.

- ☐ Occurrence
- ☐ Abundance
- ☐ Population structure
- ☐ Community structure
- ☐ Other, please specify _____

20. Which implementation strategy is your work related to? You can choose multiple options.

- ☐ Taxonomy-based (Morphologically identified species lists are substituted by molecular methods)
- ☐ De novo (developing new monitoring indices for molecular community data, including also unidentified OTUs/ASVs)
- ☐ Structural (developing monitoring indices based on structural properties such as alpha diversity or association network features)
- ☐ Functional (developing monitoring indices based on transcriptome data, directly addressing ecosystem functioning)
- ☐ Open space for specifying your answer: _____

21. What kind of samples do you use? You can choose multiple options.

- ☐ Water
- ☐ Sediment
- ☐ Soil
- ☐ Air
- ☐ Bulk samples of whole organisms
- ☐ Part of organisms
- ☐ Other, please specify: _____

22. Who is primarily responsible for the following steps? You can choose multiple options.

	I do it myself	Our research group	Collaboration	Purchased service

	I do it myself	Our research group	Collaboration	Purchased service
Collecting the samples	☐	☐	☐	☐
DNA/RNA extraction	☐	☐	☐	☐
Sequencing	☐	☐	☐	☐
Bioinformatics	☐	☐	☐	☐
Other (e.g. statistical) analysis of the data	☐	☐	☐	☐

23. Is there anything that was not covered by this survey that you would like to share or if you want to provide feedback on this survey please use the space below.

_____
_____
_____
_____
_____

You have now answered all the questions and we would like to warmly thank you for your time.

Are you interested in replying to a detailed query on laboratory, sequencing etc. procedures, to be used for the development of methodological standards? If yes, please send email: tiina.laamanen@syke.fi and we will contact you later.

Roadmap for implementing environmental DNA (eDNA)
and other molecular monitoring methods in Finland – Vision and Action Plan for 2022-2025

Finnish Environment Institute

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