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Technology Readiness Level of biodiversity monitoring with molecular methods - where are we on the road to routine implementation?

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15 Abstract 16

17 Human activities are causing rapid biodiversity loss across ecosystems, affecting human
18 wellbeing and crucial ecosystem services. Traditional biodiversity monitoring tools cannot
19 keep up with the increasing demands of monitoring due to their limited spatial or temporal
20 coverage, high costs, and lack of taxonomic expertise. Thus, implementation of novel
21 molecular monitoring methods (MoMs) such as environmental DNA (eDNA) and DNA
22 metabarcoding, are necessary.
23

24 MoMs offer significant benefits for biodiversity monitoring and environmental assessment:
25 high sensitivity and accuracy, non-invasive sampling, broad taxonomic range and cost and
26 time efficiency. However, the diverse methodological approaches within MoMs lead to poor
27 comparability between studies and surveys, highlighting the need for standardized
28 assessments.
29

30 We used the Technology Readiness Level (TRL) framework to evaluate the maturity of
31 MoMs, providing a structured assessment of their readiness for routine use. In a systematic
32 literature review 420 articles fulfilling the study criteria were assessed and both individual
33 studies and method categories ranked according to the TRL scale. The findings revealed a
34 growing number of studies, particularly in aquatic environments, with most studies validating
35 MoM technologies on a small scale but lacking large-scale system demonstrations. Aquatic
36 eDNA-based methods targeting fish showed overall higher technology readiness compared
37 to other sample types and taxa, and applications of MoMs ranked into the highest TRL were
38 predominantly freshwater studies.
39

40 Key barriers to the broader implementation of MoMs to monitoring include the need for
41 international standards, better quantitative estimates, and comprehensive reference
42 libraries. National and international cooperation is crucial for establishing common
43 standards, ensuring reliable and comparable results, and expediting the routine use of
44 MoMs in biodiversity monitoring. Recent efforts towards international standardization are
45 encouraging, but further coordinated actions are necessary for the global implementation
46 and acceptance of these methods.
47

48 Key words: eDNA, DNA metabarcoding, molecular monitoring methods, standardization,
49 Technology Readiness Level
50
51

52 Introduction

53

54 Major and fast biodiversity losses occur across various ecosystems due to direct and indirect
55 human impacts on nature. Many of these changes have negative and unpredictable
56 reciprocal effects on both human wellbeing and crucial ecosystem services (IPBES 2018)
57 which necessitates the development of methods to accurately measure their extent and
58 provide reliable data about the effectiveness of measures taken in response to detrimental
59 anthropogenic changes. Monitoring biodiversity is essential for the protection, conservation,
60 and restoration of ecosystems, especially given the current challenges such as climate
61 change, habitat loss, and globalization (Baird & Hajibabaei, 2012). The current traditional
62 tools for monitoring ecosystem changes are inappropriate both due to their poor spatial or
63 temporal coverage and the lack of taxonomic expertise and intrinsic time and monetary costs
64 involved. Thus, there is a need for new monitoring methods to fill these gaps and accurately
65 extend our ecosystem level understanding on anthropogenically induced changes.

66

67 The importance of biodiversity monitoring and need for high-quality, accurate, and timely
68 data is becoming increasingly apparent across different sectors in society. A novel
69 development is that in addition to traditional stakeholders, private sector actors from natural
70 resource dependent businesses are also increasingly calling for reliable biodiversity data to
71 assess both their detrimental impacts on nature and to identify emerging positive effects of
72 the use of sustainable practices (Kareiva & Marvier 2012). Novel monitoring methods such
73 as environmental DNA (eDNA) and other molecular monitoring methods (MoMs), sometimes
74 combined with high throughput analysis of in situ samples, have been identified as a very
75 promising future technology (Hering et al. 2018). In addition to vastly improved community
76 data, MoMs can provide additional, previously unavailable data on monitoring genetic
77 diversity and the use and development of indicators on genetic aspects of biodiversity (see
78 Hoban et al. 2020).

79

80 The use of MoMs for various environmental and biodiversity monitoring cases has grown
81 exponentially during recent years. Concurrently prices of lab analysis costs have declined
82 from thousands of euros to a few cents per sample (Wetterstrand 2023). A downside of this
83 booming of diverse MoM approaches is that the inherent methodological differences related
84 to the diversity of methods used can result in weak overall comparability between studies,
85 monitoring programs and surveys. Diminished comparability due to methodological
86 pluralism affects all process steps, from field sampling (design and protocols) to lab
87 (pipelines and protocols), bioinformatics (analysis and protocols), data repositories and their
88 use (metadata and FAIR Findable, Accessible, Interoperable, and Reusable standards).

89

90 It is important to understand and assess advantages and disadvantages of different methods
91 chosen for each process step in a systematic way to estimate their maturity (i.e., technology
92 readiness) and to provide recommendations for their applicability to existing traditional
93 monitoring schemes. The Technology Readiness Level (TRL) is a commonly used approach
94 to estimate maturity of any technology for routine use (European Association of Research &
95 Technology Organizations 2014). In our specific context, TRL provides a logical template
96 for an objective assessment of the maturity of various methodological steps for MoMs
97 monitoring schemes and helps to assess the degree of progress made towards
98 implementation of MoMs into routine use for biodiversity monitoring.

99

100 In this review, we aim to provide a comprehensive, literature based situational overview of
101 the use of MoMs in biodiversity monitoring using a systematic approach. Specifically, our

102 objectives are to i) map eDNA and MoM applications in recent biodiversity monitoring, ii)
103 identify forerunners and potential best practices that are ready for transnational uptake, and
104 iii) outline pathways for standardization of mature novel methods, which improve their
105 comparability across various biodiversity monitoring activities.

106
107 We conducted an evaluation of the TRL (European Association of Research & Technology
108 Organizations 2014) through a systematic review of the scientific literature published within
109 the past seven years to i) establish a comprehensive overview on how molecular methods
110 have been recently used and ii) to evaluate their potential for further development and
111 uptake into biological monitoring.

112 **Material and methods**

113 *Systematic literature review*

114
115 We conducted a search for publications published between April 15th, 2017 - November
116 6th, 2023, in the Web of Science database using the search string: “(TS = ((eDNA OR
117 (environmental AND DNA)) AND monitoring AND biodiversity)) AND LANGUAGE:(English)
118 AND DOCUMENT TYPES: (Article)” which resulted in 641 articles.

119
120 To facilitate team review of the research literature corpus we used the systematic review
121 protocol implemented in the CADIMA tool (<https://www.cadima.info>), which addresses the
122 main issues commonly associated with literature reviews (Kohl et al. 2018). Articles were
123 screened against predetermined study selection criteria and cross validation of reviewers
124 was undertaken to harmonize results. Study selection was performed primarily based on
125 the abstract but augmented by referring to the full text where necessary. We set the following
126 inclusion criteria:

- 127
128
129
130 (i) The article is an original research paper.
131 (ii) The study applies molecular methodology.
132 (iii) The molecular methodology is used to assess the presence and / or abundance of one
133 or several target taxa or to assess the status of the environment. By contrast, population
134 genetic studies of individual target species were not included.
135 (iv) The study discusses the topic of applying the adopted methodology in monitoring.
136 (v) At least some of the analyzed samples have been collected from an outdoor
137 environment.

138
139 For each paper meeting the study selection criteria, we extracted a predetermined set of
140 data fields based on the full text that were used to evaluate the TRL that the paper
141 represents. The extracted data included key parameters such as but not limited to i) the
142 methodology used, ii) the taxonomic, iii) spatial and iv) temporal scope of the study, v)
143 whether the molecular methodology was compared to another methodology (e.g.
144 morphology-based identification), vi) whether the molecular methodology was
145 recommended by the authors for routine monitoring and vii) under what conditions (e.g.
146 methodological challenges that still need addressing). An example of the extracted data
147 sheet can be found in Fig 1 of the supplementary information.

148 *The Technology Readiness Level*

149
150
151 Based on the extracted data, we assessed the TRL of the method used in each paper with
152 respect to its implementation in routine monitoring. We used original TRL classes as
153 described in European Association of Research & Technology Organisations (2014) (Fig. 1)

154 as a cornerstone of our classification but adopted more specific criteria for the assignment
 155 into each TRL class to assess the current technology readiness of molecular monitoring
 156 methods as reflected by the scientific literature.
 157

Works in theory & lab	<ul style="list-style-type: none"> • TRL 1 – basic principles observed • TRL 2 – technology concept formulated • TRL 3 – experimental proof of concept • TRL 4 – technology validated in lab 	Not included
Works in the field	<ul style="list-style-type: none"> • TRL 5 – technology validated in relevant environment (industrially relevant environment in the case of key enabling technologies) • TRL 6 – technology demonstrated in relevant environment (industrially relevant environment in the case of key enabling technologies) • TRL 7 – system prototype demonstration in operational environment • TRL 8 – system complete and qualified • TRL 9 – actual system proven in operational environment (competitive manufacturing in the case of key enabling technologies; or in space) 	Included

158
 159 Figure 1. Original TRL classes as described in European Association of Research &
 160 Technology Organisations (2014). TRL class 5 was the cut-off for study selection and only
 161 TRL classes 5-9 were included in our study.

162
 163 TRL class 5 was the cut-off for study selection. For the review, TRLs included were
 164 interpreted using the following progressively applied criteria:

165
 166 **TRL5 (Technology validated in relevant environment).** *Criteria:* This was the minimum
 167 level reached by all studies that met our study selection criteria. *Interpretation:* The methods
 168 used in these studies are relevant for monitoring and technically feasible under relevant
 169 outdoor conditions.

170
 171 **TRL6 (Technology demonstrated in a relevant environment).** *Criteria:* The molecular
 172 monitoring method is compared to another established (“traditional”) method and is
 173 considered to have advantages (e.g., cost-efficiency, improved detection probability of
 174 species or more comprehensive monitoring of the species community). *Interpretation:* The
 175 method has been shown to produce meaningful results in the relevant environment.

176
 177 **TRL7 (System prototype demonstrated in operational environment).** *Criteria:* The
 178 molecular method is applied at a medium or large spatial scale (>10 km maximum distance
 179 between sampling sites), is based on at least 20 samples, and its implementation in
 180 monitoring is at least conditionally recommended. *Interpretation:* The jump in the TRL scale
 181 from a “technology” to a “system” has been interpreted in terms of scale, i.e., a technology
 182 can be demonstrated by sampling at individual locations but to meet the criteria of a system,
 183 the method should be scalable. To qualify for this level requires that the study demonstrates
 184 scalability.

185
 186 **TRL8 (System complete and qualified)** *Criteria:* The molecular method is directly
 187 compared to the prevailing traditional method (i.e., with comparable samples) and its

188 implementation is recommended without major limitation. *Interpretation:* To qualify, the
189 results of the monitoring system should be compared directly to an existing method.
190 Consideration was given that for some taxa readily identified by molecular methods a
191 comparison to traditional methods is not feasible. Thus, a recommendation of
192 implementation without major limitations e.g., on environmental conditions was interpreted
193 to reflect the required technology readiness.

194
195 **TRL9 (Actual system proven in the operational environment).** *Criteria:* The paper states
196 that the molecular method is already implemented in an existing monitoring program.
197 *Interpretation:* The molecular method is used in actual operational monitoring, proving the
198 feasibility of the method at scale, also including solutions for representative sampling design,
199 data recording and organization of sampling.

200
201 *Data analysis*

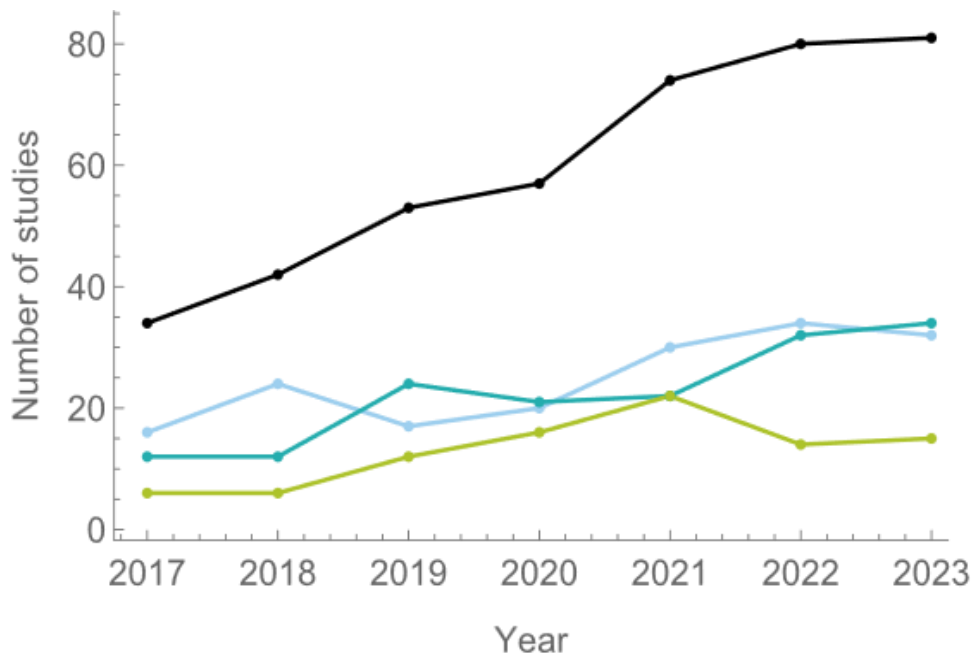
202
203 **Paper-based analysis.** Using the above criteria, each of the reviewed papers was assigned
204 a TRL value. We then visualized the distribution of TRL values in relation to different
205 ecosystems (freshwater, marine and terrestrial), application categories (e.g., biodiversity,
206 threatened species or harmful species monitoring) and organism groups, as well as the
207 change in TRL over time. For illustrative and statistical purposes, we manually classified
208 the specific descriptions of the application and target group recorded for each paper into
209 broader categories for both the “application” and “organisms’ group” categories. s. To
210 assess the statistical significance of the factors explaining paper-specific TRL, we fitted the
211 linear model $TRL \sim publication\ year + ecosystem + application\ category + organism\ group$
212 to the paper-specific data (N=420) assuming normally distributed errors using the
213 LinearModelFit function of the Wolfram Mathematica software (Wolfram Research, Inc.).
214 Publication year was modelled as a continuous variable while ecosystem, application and
215 organism group were categorical variables with 3, 6, and 6 different levels, respectively. In
216 addition, we fitted three alternative models each including an additional interaction term
217 ($year*ecosystem$, $year*application\ category$ or $year*organism\ group$). However, as the
218 interaction term was never significant according to an analysis of variance and the
219 performance of the models with interaction terms was lower than that of the additive model
220 as measured by the AIC and BIC criteria, we report only the results of the additive model.

221
222 **Methodology-based TRL.** In addition to assessing the TRL of each individual paper, we
223 also classified papers representing different methodologies and determined the TRL
224 reached by each methodology as the maximum paper-specific TRL value within the class.
225 Here, a methodology was defined as a unique combination of (i) the broad molecular
226 methodology (DNA metabarcoding or a PCR-based approach such as qPCR or ddPCR), (ii)
227 sample type (e.g., water, soil, sediment) and (iii) organism group. Molecular methods other
228 than DNA metabarcoding and PCR-based methods (e.g., DNA metagenomics or RNA-
229 based methods) were represented only by a small number of papers and were not included
230 in the methodology-based TRL assessment. The methodology-based TRL values were
231 examined visually and not statistically analyzed.

232
233 **Results**

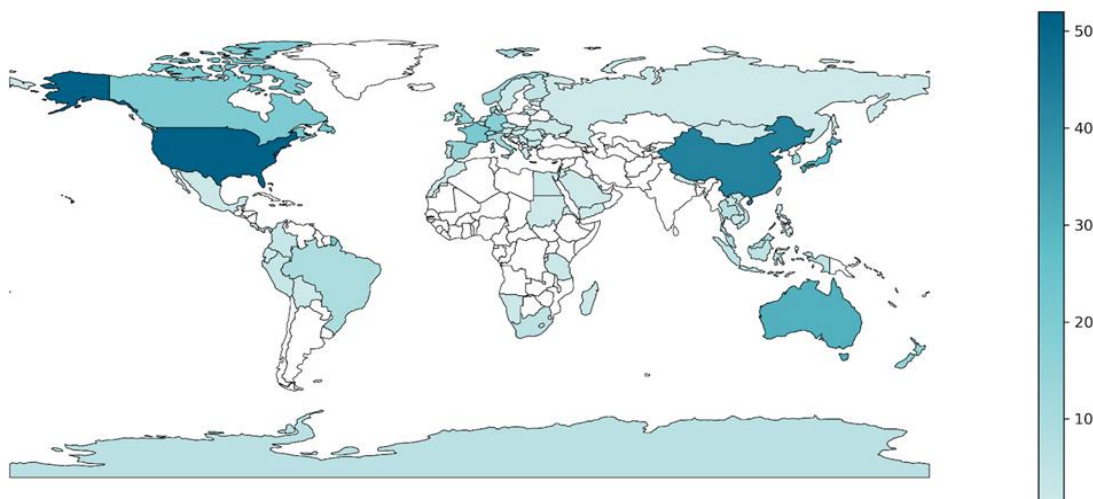
234
235 641 research papers published between 15.4.2017 and 6.11.2023 were screened against
236 predetermined study selection criteria in the CADIMA tool, resulting in 420 research papers
237 fulfilling the criteria. The number of papers published increased annually during the study
238 period. In 2018, the first full year of our search period, 42 papers which met our search
239 criteria, were published. The number of published papers per year has almost doubled within

240 our search window with 80 published papers fulfilling the criteria in 2022, the last full year of
 241 our search period (Fig. 2). The number of studies performed in the freshwater environment
 242 varied from 17 in 2019 to 34 per year in 2022. In marine environments, the minimum number
 243 of papers was 12 all of which were published in 2018 whereas the maximum, i.e. 34 papers
 244 were published in 2023. Comparably, in terrestrial environments only 6 papers were
 245 published in 2018 and the maximum number of published papers for terrestrial environments
 246 was 18 in 2021. Note that the amount of published terrestrial papers per year decreased to
 247 14 and 15 published in 2022 and 2023, respectively.
 248
 249



250
 251 Figure 2. Number of original research papers published per year during the search period
 252 15.4.2017–6.11.2023 in different study environments (black = all, light blue = freshwater,
 253 turquoise = marine, green = terrestrial)
 254

255 Most of the study sites were located in the United States (54 studies), followed by China
 256 (43), Australia (31) Japan (30), France (21), Canada (20), Germany (17), Denmark (17),
 257 New Zealand (15), and The United Kingdom (14) (Fig. 3). Only 30 studies spanned sites
 258 over multiple countries whereas the vast majority of studies were conducted only in one
 259 country.
 260

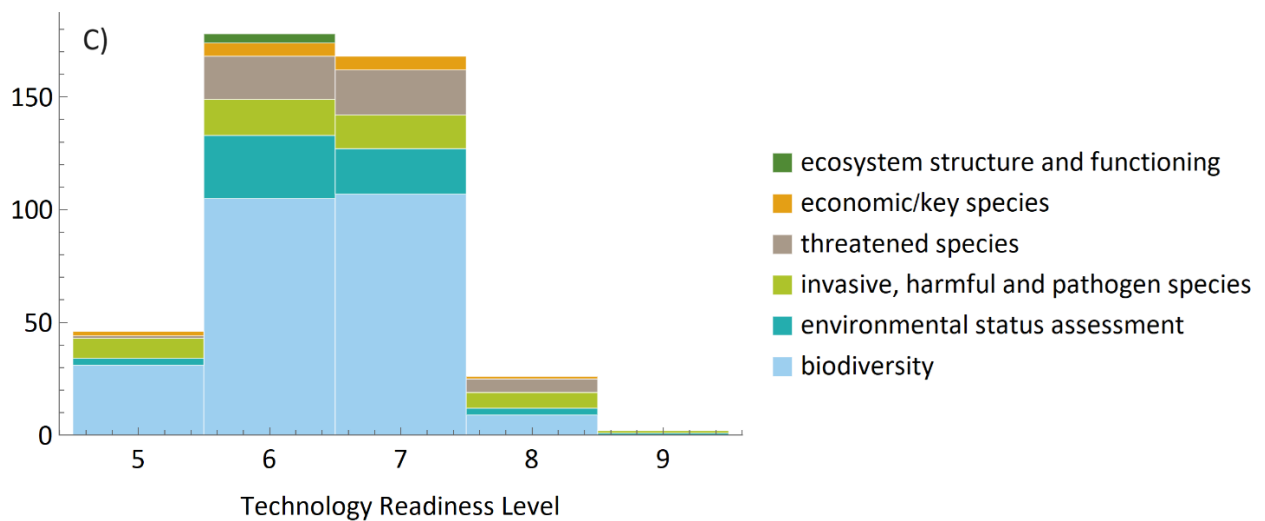
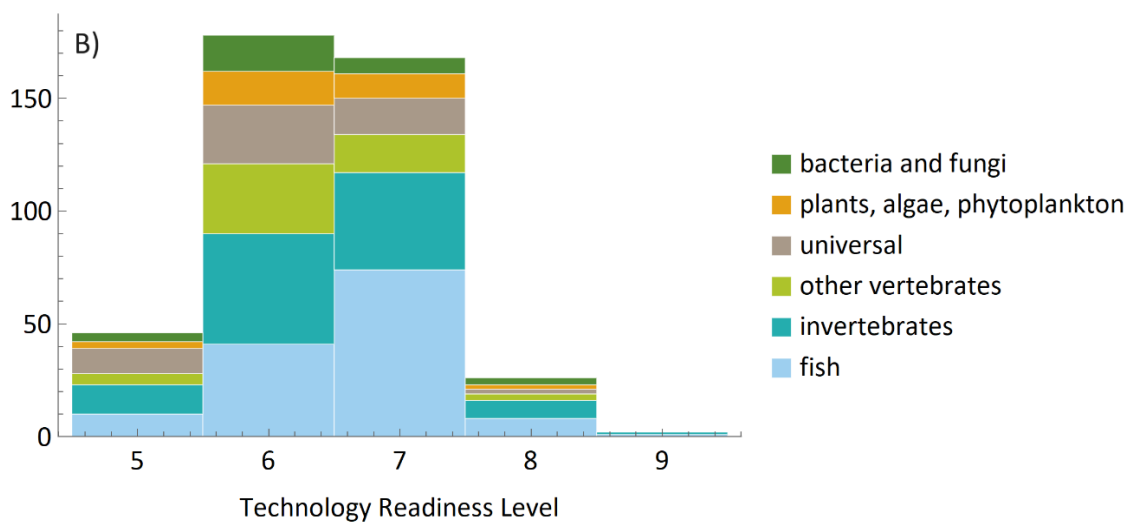
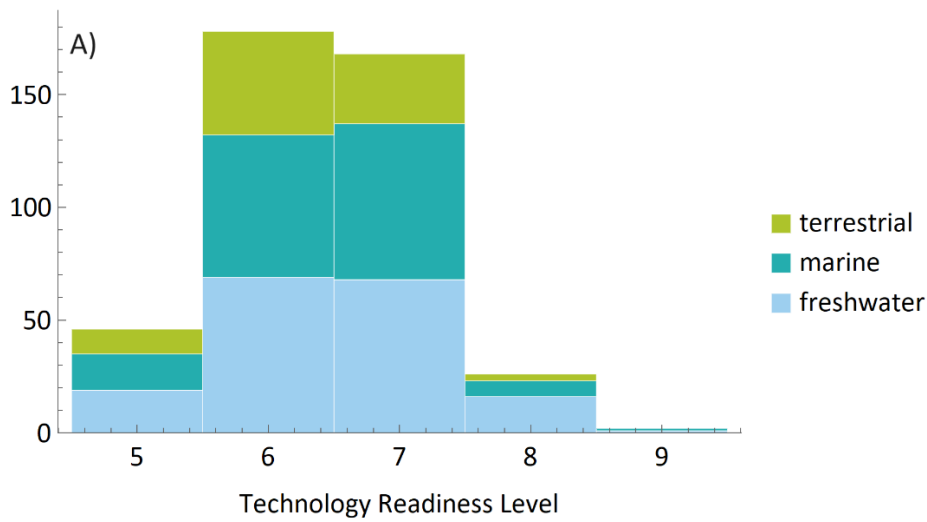


261
 262 Figure 3. Geographical distribution and incidents of the research papers.

263 The internationally published scientific research on MoMs within the last seven years is
264 heavily dominated by application cases to aquatic environments (Fig. 4). There is an equal
265 proportion of freshwater to marine studies reporting MoMs application that rank at TRL 6
266 and 7. Fewer studies scored higher than TRL 7 and those that did were strongly dominated
267 by freshwater studies. Aquatic environments also provided the only two examples of the
268 highest Technology Readiness Level (TRL 9, already implemented), which included
269 invasive fish species (Carim et al. 2020) and benthic invertebrates (Aylagas et al. 2018).
270 Another example of a high TRL is the routine eDNA-based monitoring of the great crested
271 newt (*Triturus cristatus*) in Great Britain (Biggs et al. 2015).

272
273 Overall, fish and invertebrates were the two most actively studied groups. The dominant
274 pattern in the data is that TRL classes 5-9 seem to roughly follow a normal distribution, with
275 most of the studies falling into categories 6 and 7, indicating that while the methods are now
276 broadly validated in small-scale field studies, systematic large-scale demonstrations are still
277 scarce. This pattern was very robust across different ecosystems, application categories
278 and groups of organisms, and interestingly also did not markedly evolve over the seven
279 years included in our study (Fig. 6). The results of the statistical analysis confirm that year,
280 ecosystem and application category did not significantly explain variation in TRL (Table 1)
281 and that overall, the explanatory power of the statistical model was very low ($R^2 = 0.06$).
282 According to the analysis of variance, the organism group was the only significant factor,
283 and the estimated model parameters show that this effect can be mostly attributed to the
284 higher TRL in studies targeting fish (Table 1, Fig. 4).

285



286

287 Figure 4. Distribution of the 420 original research papers published between 2017-2023
 288 according to Technology Readiness Levels (TRL) of molecular monitoring methods
 289 classified by a) ecosystem, b) organism group c) primary monitoring application represented
 290 by the study.

291 Table 1. Results of the statistical analysis examining the role of different factors in explaining
 292 the Technology Readiness Level represented by individual studies (N=420). Note that the
 293 levels of the categorical variables (ecosystem, application category and organism group)
 294 are the same as those illustrated in Fig. 4.

Model TRL ~ year (continuous) + system (categorical; 3 levels) + application (categorical; 6 levels) + group (categorical; 6 levels)

R² 0.0594688

295

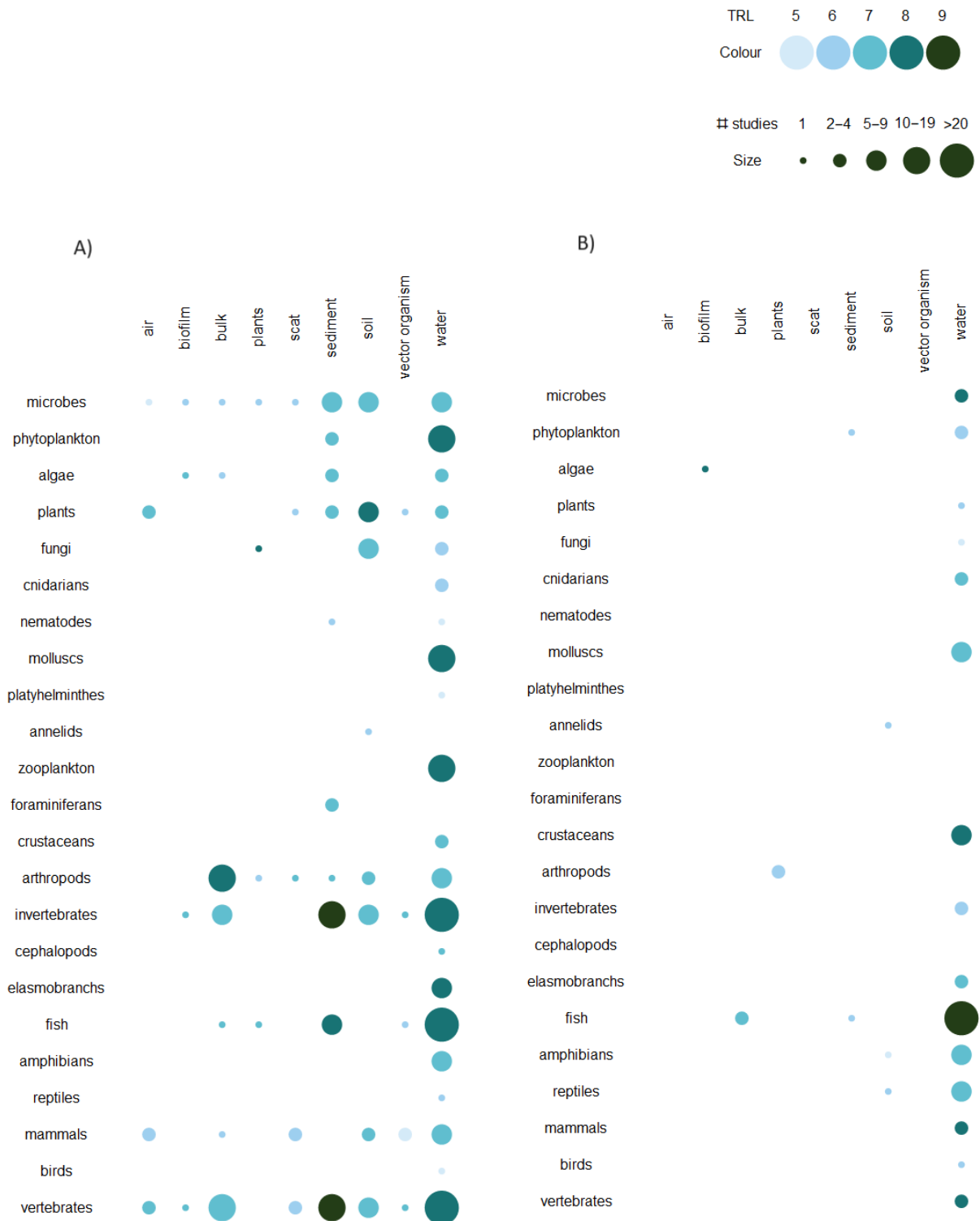
ANOVA table

	DF	SS	MS	F-Statistic	P-Value
year	1	0.555066661	0.555066661	0.925630888	0.336573463
system	2	2.516815336	1.258407668	2.098524536	0.123965509
application	5	4.557215006	0.911443001	1.51992518	0.182392616
group	5	7.764839045	1.552967809	2.589733943	0.025393985
Error	406	243.4632068	0.599663071		
Total	419	258.8571429			

296

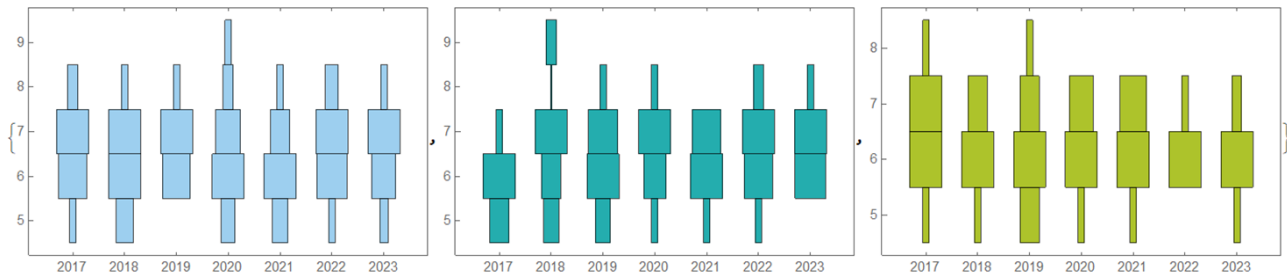
Parameter estimates

	Estimate	Standard Error	t-Statistic	P-Value
1	-27.6412908	42.54276765	-0.649729492	0.516234397
year	0.016819437	0.021062255	0.798558225	0.425013488
system["freshwater"]	0.04468746	0.111175385	0.401954622	0.687928936
system["marine"]	0.069866293	0.111865334	0.624557143	0.532612566
application["biodiversity"]	-0.274156713	0.131149343	-2.090416216	0.037201876
application["economic/key species"]	-0.374838605	0.233073503	-1.608242036	0.108559491
application["ecosystem structure and functioning"]	-0.65834646	0.408514569	-1.611561767	0.107834414
application["environmental status assessment"]	-0.081755897	0.168393753	-0.485504332	0.627580529
application["invasive, harmful and pathogen species"]	-0.142124587	0.168175448	-0.845097121	0.398554352
group["bacteria and fungi"]	0.084198146	0.183020238	0.460048283	0.645727836
group["fish"]	0.436102297	0.130848478	3.332880178	0.000938367
group["invertebrates"]	0.25860505	0.130657234	1.979263161	0.048461506
group["other vertebrates"]	0.153998182	0.156359838	0.984896022	0.325261709
group["plants, algae, phytoplankton"]	0.215888012	0.175005872	1.233604391	0.218064029



299 Figure 5. Technology readiness level for different combinations of organism group and
 300 sample type for a) metabarcoding and b) PCR-based methods. Circle size represents the
 301 number of studies within each category and circle color the maximum technology readiness
 302 level reached among those studies.

304 Metabarcoding based approaches were used in 338 studies and PCR-based methods, such
 305 as quantitative PCR (qPCR) or Droplet Digital PCR (ddPCR) 80 studies. Several research
 306 papers used both approaches. In Fig. 5 all research papers that used metabarcoding
 307 approaches were classified into “metabarcoding” regardless of the possible use of additional
 308 PCR based approaches.
 309



310
 311
 312 Figure 6. Distribution of TRL classes across years and freshwater (light blue), marine
 313 (turquoise), and terrestrial (green) environments.
 314

315 The distribution of TRL classes across different environments and years (Fig 6) shows only
 316 minor changes in the interannual distribution of TRL for original papers across years. The
 317 most significant change in TRL class is observed in marine environments.
 318

319 In the reviewed papers, lack of standardization was often mentioned as one of the key
 320 restricting factors for the larger implementation of molecular monitoring methods (see eg.
 321 Agersnap et al. 2017; Baldigo et al. 2017; Gargan et al. 2017; Vasselon et al. 2017;
 322 Minerovic et al. 2020; Suter et al. 2021;). Need for further method optimization, improved
 323 quantitative estimates, and development of reference libraries are also often mentioned as
 324 restricting factors for the larger implementation of molecular monitoring methods (see eg.
 325 Vasselon et al. 2017; Schnell et al. 2018; White et al. 2020).
 326
 327

328 Discussion

329
 330 The increased demand for more accurate and timely information on the state of the
 331 ecosystem overburden current traditional methodologies and has created the demand to
 332 look for novel monitoring and analytic solutions. Molecular identification techniques have
 333 great potential to improve and extend current biological monitoring in all types of habitats.
 334 Particularly, currently unmonitored changes in patterns of biodiversity in response to global
 335 megatrends (e.g., climate change, urbanization, invasive alien species, increasing chemical
 336 stress on soils and groundwaters) stand to benefit from the speedy uptake of these methods
 337 (Thomsen & Willerslev 2015).
 338

339 Further, their application combined with traditional monitoring and assessments could
 340 greatly improve the accuracy of monitoring results and ensure that appropriate management
 341 actions are taken and potentially increase spatial coverage. MoM can produce objective,
 342 easily comparable and reproducible species identification, and can be used in large-scale
 343 monitoring (Bohmann et al. 2014). They can reliably detect and be used to monitor currently
 344 hard-to detect and poorly known groups of organisms (e.g., aquatic and soil microbes, fungi,
 345 certain groups of insects) that are currently excluded from monitoring based on the
 346 shortcomings of traditional taxonomic identification methods (e.g. Abrego et al. 2018;
 347 Frøslev et al. 2019). Some MoM already represent the better choices, compared to
 348 traditional methods, for reliable mapping of intraspecific genetic diversity, the conservation

349 of which is increasingly acknowledged internationally (Convention on Biological Diversity
350 2020, Hoban et al. 2020).

351
352 To produce meaningful results and to attain high TRL, MoMs need reliable reference
353 databases and reliable, specific genetic tools for a broad range of organisms and commonly
354 agreed upon minimum criteria for methodological and analytical pipelines. Several
355 commonly used methods (e.g., metabarcoding) have evolved from the prototype stage (TRL
356 3-4) to TRL level 6-8 where the technical operation has been demonstrated in relevant
357 settings (e.g. Meissner et al. 2019).

358
359 Despite their demonstrated success and benefits, applications of molecular identification
360 methods have mainly been limited to proof of concept or validation projects as is reflected
361 by our data which ranked the bulk of studies using MoMs below TRL 8. Making the transition
362 from TRL level 7 to TRL 8 or TRL 9 i.e. Authors of papers often state that MoMs method
363 uptake into routine use is rarely to do with the inability of the MoMs to be used at larger
364 scales or in routine use but point the other roadblocks related to “legitimization” and
365 “legalization” i.e. acceptance and regulatory readiness levels (sensu Vik et al. 2021) such
366 as the lack of standardization.

367
368 To increase acceptance and regulatory readiness levels further there is an urgent need for
369 both national and international cooperation to expedite the routine implementation of these
370 methods. More generally three modes of action with respect to the implementation of MoMs
371 into routine use exist; i) focus on national or regional cooperation to produce guidelines, ii)
372 the “wait and see” option and iii) strengthening of international cooperation to develop
373 common standards. We will briefly discuss each one in the following.

374
375 Strengthening regional or national cooperation has several short-term advantages but also
376 entails historically proven drawbacks. National guidelines often are much easier to develop
377 than international ones since the number of stakeholders that are needed to be engaged is
378 often more limited. Thus, time spent on efforts to develop and reach consensus on national
379 or regional guidelines and to implement methods may be reduced. It is important to
380 recognize that this initial timesaving aspect only prevails if the endpoint of the analysis is
381 indeed only national or regional. However, for biodiversity monitoring or in bioassessments,
382 e.g., of the status of water bodies national assessments are just one goal. In Europe and
383 globally, methods and data collected by them often need to answer more than single national
384 scale questions on patterns of biodiversity or the state of the environment. Producing
385 accurate and comparable data to such multifaceted questions requires international
386 cooperation.

387
388 Cooperation on international method or method standard development is often slower to
389 begin with as the identification of stakeholders and the definition of an efficient engagement
390 process of the relevant stakeholders takes more time to set up. However, choosing a
391 national approach to attain short-term time savings over an international one has multiple
392 significant consequences when the ultimate goal is a global-level endpoint. The ability to
393 directly compare results from one nation to another and to meaningfully combine them is
394 decreasing with method complexity as independently developed national guidelines will
395 have facets that will differ and create different end results. To be able to make international
396 inferences on general patterns that several different national methods describe often
397 requires intercalibration of results from these national methods. This is a far from trivial task,

398 as the implementation of existing national methods to assess water quality in the EU aptly
399 demonstrated. In the intercalibration of methods for the WFD, a total of over 300 national
400 methods existed that required intercalibration which took over 20 years to complete and still
401 resulted in often less than optimal comparability (Birk et al 2012).

402
403 For MoMs, a future of similarly laborious intercalibration can and should be effectively
404 averted. The solution to both attaining higher TRLs and thus routine use involves
405 international cooperation on defining minimum criteria for high TRL level MoMs application
406 intended for use in routine biodiversity monitoring and bioassessment.

407
408 Currently, there is little coordination between national research organizations and other end-
409 users, both for MoMs specifically and for new environmental monitoring methods in general.
410 Some prominent examples of national roadmaps for the implementation of MoMs exist (e.g.,
411 Norros et al. 2022; De Brauwer et al. 2023; Kelly et al. 2023; The National Aquatic eDNA
412 Strategy 2024), which could be extended to other countries and regions. If these general
413 roadmaps were combined with strategic implementation plans for specific methods that
414 accounted for and integrated the pros and cons of MoMs across various taxa, these novel
415 methods could support and expedite transnational biodiversity monitoring schemes.

416
417 While national coordination around MoMs is building up rapidly in many countries, the
418 number of national key stakeholders is currently still relatively low. The fact that MoMs
419 intended for routine use provide data that is not only necessarily directed only towards
420 national endpoints creates a window of opportunity to choose international standardization
421 of MoMs minimum requirements as a common starting point.

422
423 Contrary, focusing development on national guidelines without concurrent international
424 coordination of efforts entails a high risk of duplication of work, creating internationally
425 incompatible solutions in a quickly evolving field and sidelining inputs of stakeholders from
426 less advanced regions like the Global South, which are the regions holding most of the
427 threatened biodiversity we globally seek to protect.

428
429 Several central European nations i.e., Germany, France and in particular Finland have taken
430 an active role to advance the international standardization of forerunner MoMs for routine
431 biological monitoring. In the past few years, these efforts have spawned European work to
432 standardize sampling of eDNA from water and progressed to the creation of a dedicated
433 working group in 2018 as well as the development of its first CEN standard (EN 17805:2023).
434 The European decision to focus further standardization efforts on periphytic diatoms and
435 aquatic macroinvertebrate metabarcoding is strongly mirrored by our data.

436
437 After the ratification of the Kunming-Montreal protocol, the need for an international platform
438 to advance the implementation of the global biodiversity Framework (GBF) was realized and
439 met by establishing a dedicated Technical Committee (TC) for Biodiversity under ISO (i.e.,
440 TC 331) to develop international standards. However, this TC's scope did not specifically
441 advance the minimum method requirements of MoMs needed for routine implementation in
442 biodiversity monitoring. Mirroring both our study results that highest TRL of MoMs are found
443 in aquatic environments and the desire to create international comparability to counteract
444 skepticism about the reliability and reproducibility of environmental genomics metrics led to
445 the establishment of a new working group under ISO TC 147 "Water quality" in 2023. The

446 new working group is specifically dedicated to MoMs international method minimum
447 requirement standardization for MoMs use in routine bioassessment.

448
449 To further facilitate inclusive access to method standard development for Global South
450 stakeholders and to expedite the formulation of seed documents for introduction into ISO
451 standardization, the International eDNA Standardization Task Force iESTF
452 (<https://iestf.global>) was established in 2023. iESTF offers an inclusive platform that
453 cooperates closely with the international research community and various other key
454 stakeholders and transparently works on the creation of seed documents for specific steps
455 of the MoMs process.

456
457 These recent developments partly reflect the growing level of international interest and
458 commitment of countries to the future routine and comparable implementation of these
459 methods. However, both our data and current developments in the field of standard creation
460 for marine and terrestrial environments lags behind. A clear danger is that these
461 developments in these environments will turn to national guideline creation at the expense
462 of international standardization which will delay the creation of internationally comparable
463 biodiversity data. Clearly, further coordinated work within and between different
464 environments is needed to ensure unified application and interpretation of MoMs in future
465 global and national legislative monitoring implementation.

466
467 Molecular monitoring methods have advanced to a stage where methods to target new
468 target taxonomic groups are continually emerging. These new developments often begin
469 with lower TRL-level activities and may in part. This could partly explain the observed
470 distribution of TRL classes across different environments (Fig 6).

471 472 **Conclusion**

473
474 Molecular monitoring methods have reached critical maturity and their implementation has
475 started worldwide. MoMs have great potential to benefit, improve and extend current
476 biological monitoring in all types of habitats. However, the research field is fragmented
477 causing a risk of unnecessary duplication of efforts, method pluralism and resulting
478 incompatibility of the end results.

479
480 Based on the 420 paper assessed in this review, last years of international research on
481 MoMs has predominantly focused on aquatic environments, with equal emphasis on
482 freshwater and marine studies at Technology Readiness Levels (TRL) 6 and 7. High TRLs
483 are mainly seen in freshwater studies, including the highest TRL 9 applications for invasive
484 fish species and benthic invertebrates. TRL classes 5-9 roughly followed a normal
485 distribution, with most of the studies falling into categories 6 and 7, indicating that while the
486 methods are broadly validated in small-scale field studies, systematic large-scale
487 demonstrations are still scarce.

488
489 Restricting factors described in the reviewed papers were lack of standardization,
490 methodological optimization, and comprehensive reference libraries. National and
491 international cooperation is crucial to establish common standards and ensure consistent,
492 reliable, and comparable results. Recent international efforts and the establishment of
493 international working groups indicate progress, but further coordinated action is necessary
494 to achieve unified application and interpretation of MoMs globally.

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502

503 **References**

504

505 Agersnap S, Larsen WB, Knudsen SW, Strand D, Thomsen PF, Hesselsøe M, Bondgaard
506 Mortensen P, Vrålstad T, Møller PR (2017) Monitoring of noble, signal and narrow-clawed
507 crayfish using environmental DNA from freshwater samples. *PLoS One*, 12(6), e0179261.

508

509 Aylagas E, Borja Á, Muxika I, Rodríguez-Ezpeleta N (2018) Adapting metabarcoding-
510 based benthic biomonitoring into routine marine ecological status assessment networks.
511 *Ecological Indicators*, 95, 194-202.

512

513 European Association of Research & Technology Organisations (2014) The TRL Scale as
514 a Research & Innovation Policy Tool, EARTO Recommendations.

515

516 Baird DJ, Hajibabaei M (2012) Biomonitoring 2.0: a new paradigm in ecosystem
517 assessment made possible by next-generation DNA sequencing.

518

519 Baldigo BP, Sporn LA, George SD, Ball JA (2017) Efficacy of environmental DNA to detect
520 and quantify brook trout populations in headwater streams of the Adirondack Mountains,
521 New York. *Transactions of the American Fisheries Society*, 146(1), 99-111.

522

523 Biggs J, Ewald N, Valentini A, Gaboriaud C, Dejean T, Griffiths RA, Foster J, Wilkinson
524 JW, Arnell A, Brotherton P, Penny Williams P, Dunn F (2015) Using eDNA to develop a
525 national citizen science-based monitoring programme for the great crested newt (*Triturus*
526 *cristatus*). *Biological Conservation*, 183, 19-28.

527

528 Birk S, Bonne W, Borja A, Brucet S, Courrat A, Poikane S, Solimini A, van de Bund W,
529 Zampoukas N, Hering D (2012) Three hundred ways to assess Europe's surface waters:
530 an almost complete overview of biological methods to implement the Water Framework
531 Directive. *Ecological Indicators*, 18, 31-41.

532

533 Bohmann K, Evans A, Gilbert MTP, Carvalho GR, Creer S, Knapp M, Yu DW, De Bruyn M
534 (2014). Environmental DNA for wildlife biology and biodiversity monitoring. *Trends in*
535 *ecology & evolution*, 29(6), 358-367.

536

537 Carim KJ, Bean NJ, Connor JM, Baker WP, Jaeger M, Ruggles MP, McKelvey KS,
538 Franklin TW, Young MK, Schwartz MK (2020) Environmental DNA Sampling Informs Fish
539 Eradication Efforts: Case Studies and Lessons Learned. *North American Journal of*
540 *Fisheries Management*, 40, 488-508.

541

542 De Brauwere M, Deagle B, Dunstan P, Berry O (2023) Integrating environmental DNA
543 science into Australia's marine parks: a roadmap. CSIRO, Hobart.

544

545 Frøslev TG, Kjøller R, Bruun HH, Ejrnæs R, Hansen AJ, Læssøe T, Heilmann-Clausen J
546 (2019) Man against machine: Do fungal fruitbodies and eDNA give similar biodiversity

- 547 assessments across broad environmental gradients? *Biological Conservation*, 233, 201-
548 212.
549
- 550 Gargan LM, Morato T, Pham CK, Finarelli JA, Carlsson JE, Carlsson J (2017)
551 Development of a sensitive detection method to survey pelagic biodiversity using eDNA
552 and quantitative PCR: a case study of devil ray at seamounts. *Marine Biology*, 164, 1-9.
553
- 554 Héder M (2017) From NASA to EU: the evolution of the TRL scale in Public Sector
555 Innovation. *The Innovation Journal*, 22(2), 1-23.
556
- 557 Hering D, Borja A, Jones JI, Pont D, Boets P, Bouchez A, Bruce K, Drakare S, Hänfling B,
558 Kahlert M, Leese F, Meissner K, Mergen P, Reyjol Y, Segurado P, Vogler A, Kelly M
559 (2018) Implementation options for DNA-based identification into ecological status
560 assessment under the European Water Framework Directive. *Water Research*, 138, 192-
561 205.
562
- 563 Hoban S, Bruford M, Jackson JD, Lopes-Fernandes M, Heuertz M, Hohenlohe PA, Laikre
564 L (2020) Genetic diversity targets and indicators in the CBD post-2020 Global Biodiversity
565 Framework must be improved. *Biological Conservation*, 248, 108654.
566
- 567 IPBES (2018) Summary for policymakers of the regional assessment report on biodiversity
568 and ecosystem services for Europe and Central Asia of the Intergovernmental Science-
569 Policy Platform on Biodiversity and Ecosystem Services. Fischer M, Rounsevell M, Torre-
570 Marin Rando A, Mader A, Church A, Elbakidze M, Elias V, Hahn T, Harrison PA, Hauck J,
571 Martín-López B, Ring I, Sandström C, Sousa Pinto I, Visconti P, Zimmermann NE, Christie
572 M (eds.). IPBES secretariat, Bonn, Germany. 48 pages
573 <https://doi.org/10.5281/zenodo.3237428>.
574
- 575 Kareiva P, Marvier M (2012) What is conservation science? *BioScience*, 62(11), 962-969.
576
- 577 Kelly RP, Lodge DM, Lee KN, Theroux S, Sepulveda AJ, Scholin CA, Weisberg SB (2024)
578 Toward a national eDNA strategy for the United States. *Environmental DNA*, 6(1), e432.
579
- 580 Kohl C, McIntosh EJ, Unger S, Haddaway NR, Kecke S, Schiemann J, Wilhelm R (2018)
581 Online tools supporting the conduct and reporting of systematic reviews and systematic
582 maps: a case study on CADIMA and review of existing tools. *Environmental Evidence*, 7,
583 1-17.
584
- 585 Meissner K, Aroviita J, Majaneva M, Ekrem T, Schartau AK, Friberg N, Ólafsson J,
586 Johnson RK, Baattrup-Pedersen A, Leese F, Elbrecht V (2019) SCANDNAnet-Validating
587 and intercalibrating metabarcoding for routine use in Nordic freshwater biomonitoring.
588
- 589 Minerovic AD, Potapova MG, Sales CM, Price JR, Enache MD (2020) 18S-V9 DNA
590 metabarcoding detects the effect of water-quality impairment on stream biofilm eukaryotic
591 assemblages. *Ecological Indicators*, 113, 106225.
592
- 593 Norros V, Laamanen T, Meissner K, Iso-Touru T, Kahilainen A, Lehtinen S, Lohtander-
594 Buckbee K, Nygård H, Pennanen T, Ruohonen-Lehto M, Sirkiä P, Suikkanen S, Tolkinen
595 M, Vainio E, Velmala S, Vuorio K, Vihervaara P (2022) Roadmap for implementing
596 environmental DNA (eDNA) and other molecular monitoring methods in Finland—Vision
597 and action plan for 2022–2025
598

- 599 Schnell IB, Bohmann K, Schultze SE, Richter SR, Murray DC, Sinding MHS, Gilbert MTP
600 (2018) Debugging diversity – a pan-continental exploration of the potential of terrestrial
601 blood-feeding leeches as a vertebrate monitoring tool. *Molecular Ecology Resources*,
602 18(6), 1282-1298.
- 603
604 Secretariat of the Convention on Biological Diversity (2020) *Global Biodiversity Outlook 5 –*
605 *Summary for Policy Makers*. Montréal.
- 606
607 Suter L, Polanowski AM, Clarke LJ, Kitchener JA, Deagle BE (2021) Capturing open
608 ocean biodiversity: Comparing environmental DNA metabarcoding to the continuous
609 plankton recorder. *Molecular Ecology*, 30(13), 3140-3157.
- 610
611 Thomsen PF, Willerslev E (2015) Environmental DNA – An emerging tool in conservation
612 for monitoring past and present biodiversity. *Biological Conservation*, 183, 4-18.
- 613
614 Vasselon V, Rimet F, Tapolczai K, Bouchez A (2017) Assessing ecological status with
615 diatoms DNA metabarcoding: Scaling-up on a WFD monitoring network (Mayotte island,
616 France). *Ecological Indicators*, 82, 1-12.
- 617
618 Vik J, Melås AM, Stræte EP, Søråa RA (2021) Balanced readiness level assessment
619 (BRLa): A tool for exploring new and emerging technologies. *Technological Forecasting*
620 *and Social Change*, 169, 120854.
- 621
622 White NE, Guzik MT, Austin AD, Moore GI, Humphreys WF, Alexander J, Bunce M (2020)
623 Detection of the rare Australian endemic blind cave eel (*Ophisternon candidum*) with
624 environmental DNA: implications for threatened species management in subterranean
625 environments. *Hydrobiologia*, 847, 3201-3211.
- 626
627 Wetterstrand KA (2023) DNA Sequencing Costs: Data from the NHGRI Genome
628 Sequencing Program (GSP) www.genome.gov/sequencingcostsdata