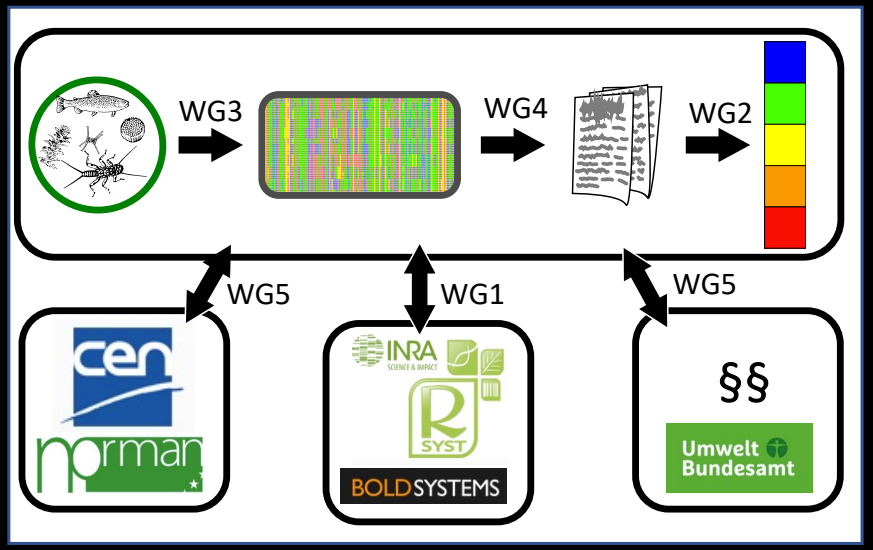
(e)DNA-based approaches for aquatic bioassessment & monitoring: chances & challenges

Florian Leese, University of Duisburg-Essen, Germany
COST Action DNAqua-Net (CA15219)

@leeselab @dnaquanet

”eDNA & other molecular methods in env. monitoring”
Nov. 12th 2021
SYKE, FI

DNAqua-Net (2016-2021)



- ~600 members
- 49 countries
- >100 publications and stakeholder reports
- connects labs, countries, disciplines

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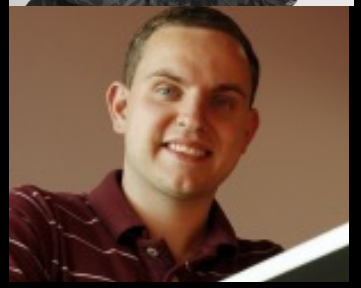

Agnès Bouchez &
Alexander Weigand

We need reliable monitoring data!

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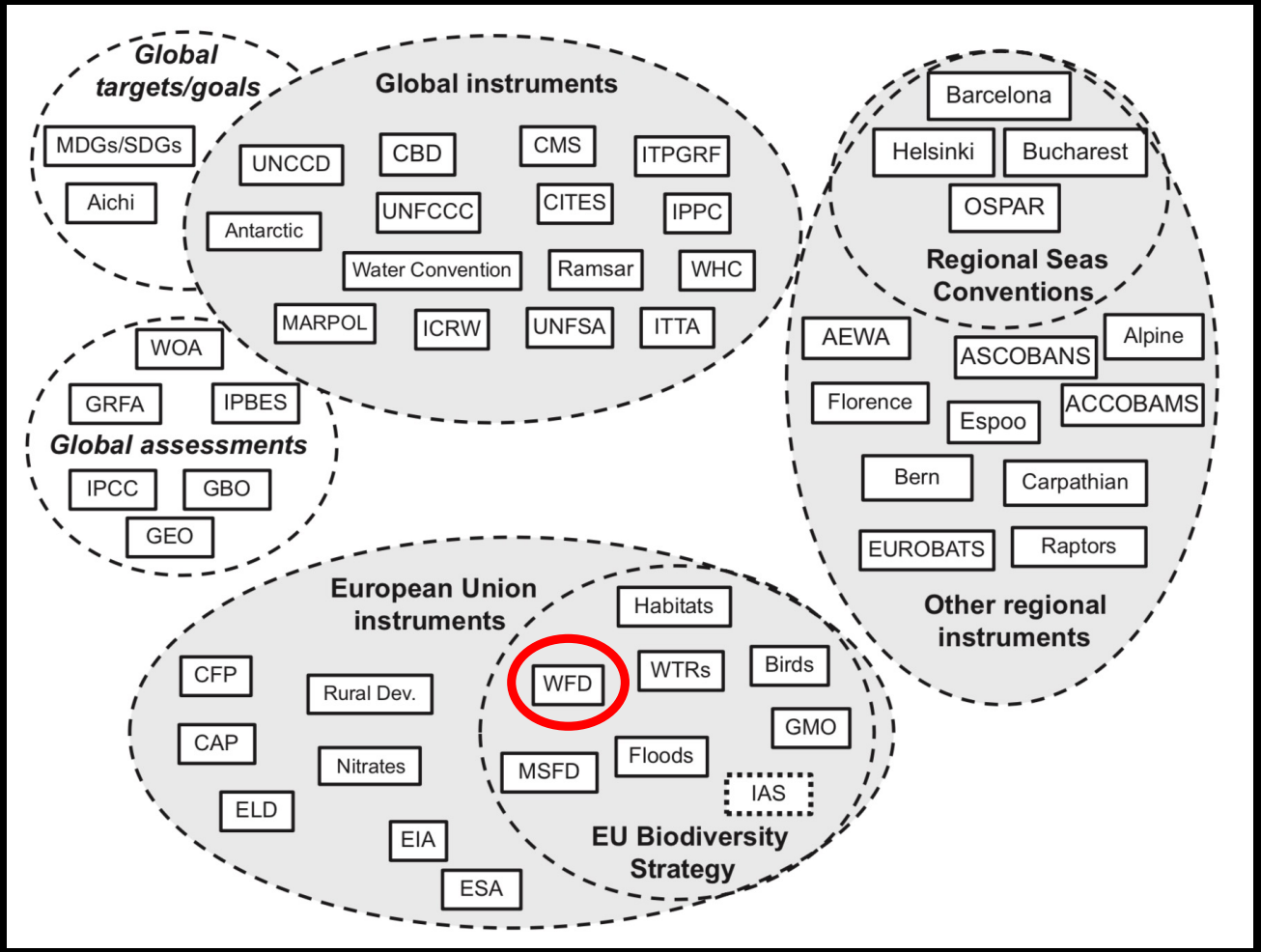
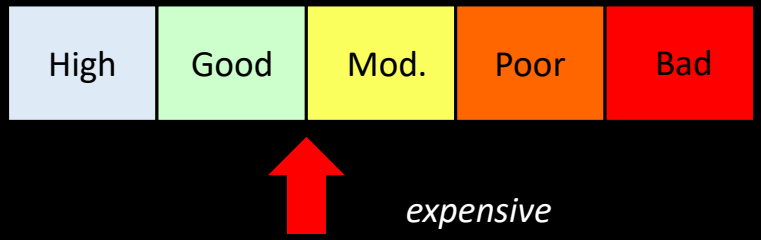


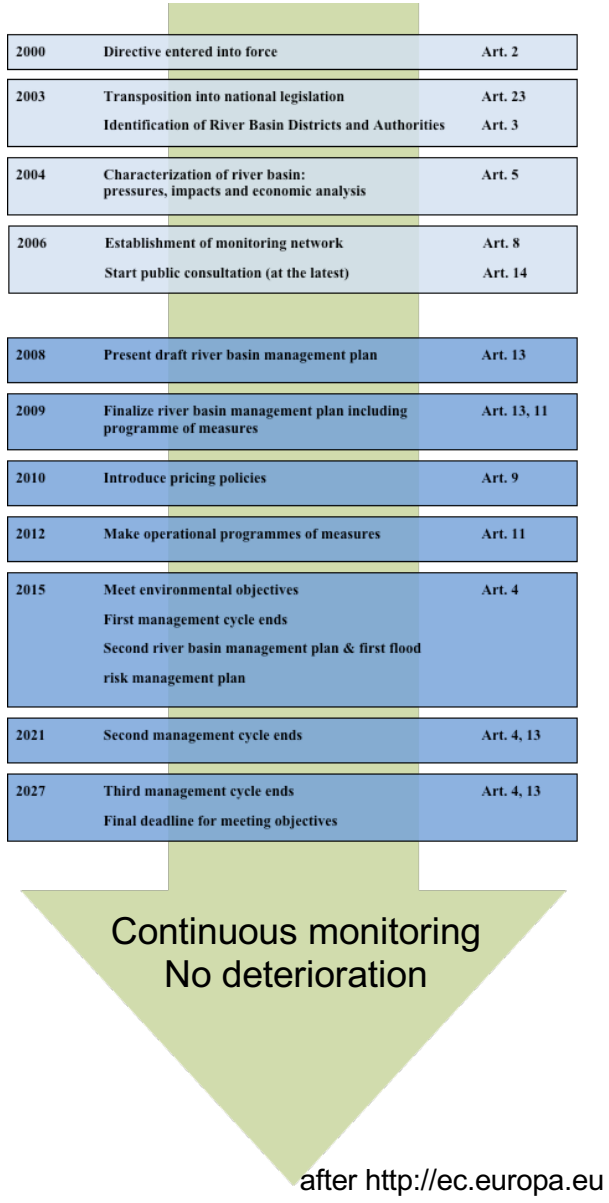
Focus today:
Community assessment!

Wetzel et al. (2015)

EU Water Framework Directive

- Very advanced piece of environmental legislation (Directive 2000/60/EC)
- Aim: 2027 surface waters good status
- **Ecological** status as primary determinant of management needs
- >100,000 water bodies monitored - **long-term data**
- Decades of **intercalibration** (>400 intercalibrated methods)
- **Published CEN/ISO standards** for sampling / analysis
- But: **Few BQEs, slow, errors, limited tax. resolution**





after http://ec.europa.eu

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Proposition:

„For better monitoring, forecasting and management we have to scale-up environmental monitoring. (e)DNA-based methods will play a central role here“

Strength is obvious

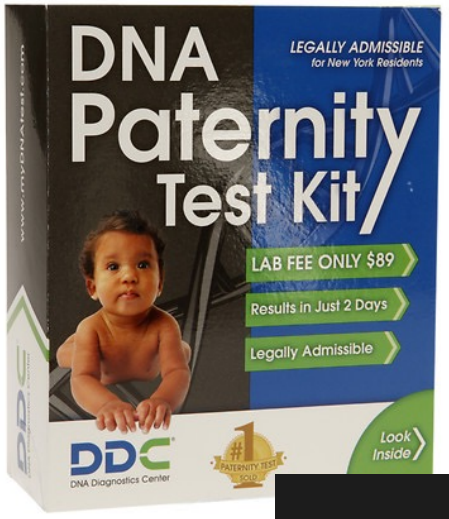
Ann. Rev. Microbiol. 1986. 40:337–65

MICROBIAL ECOLOGY AND EVOLUTION: A RIBOSOMAL RNA APPROACH

Gary J. Olsen, David J. Lane, Stephen J. G. Norman R. Pace

Department of Biology and Institute for Molecular and Cellular B Indiana, Bloomington, Indiana 47405

© Keystone (Tagesanzeiger.ch)



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BOLD SYSTEMS

9,695K	729K	232K	70K	24K
Barcodes	BINs	Animal Species	Plant Species	Fungi & Other Species

DNA differs even when morphology does not

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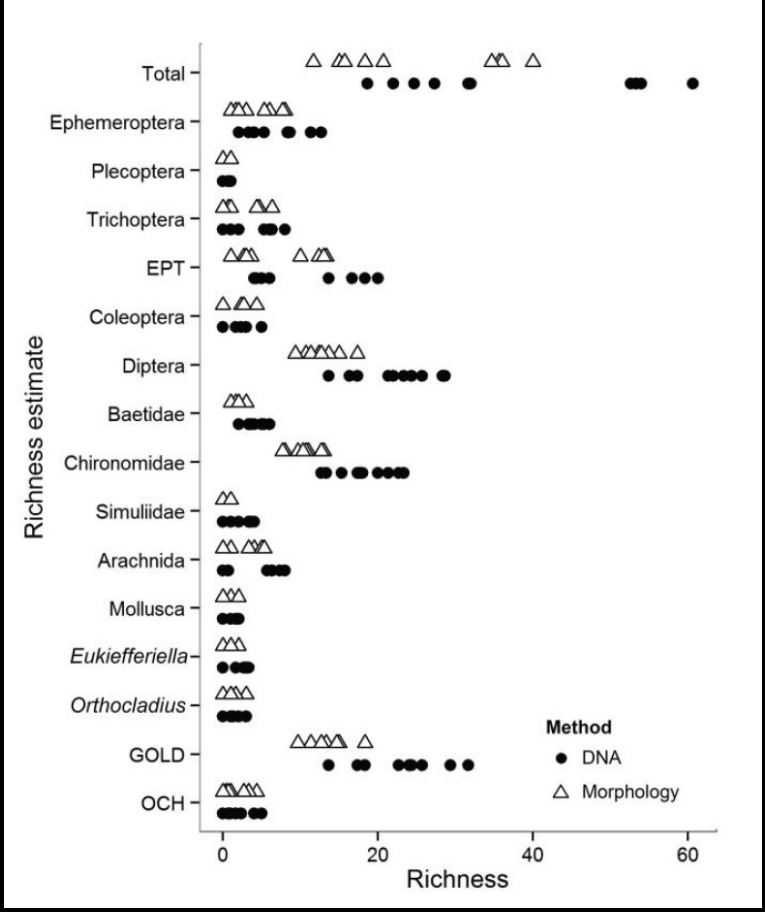
MOLECULAR APPROACHES IN FRESHWATER ECOLOGY

Does DNA barcoding improve performance of traditional stream bioassessment metrics?

Eric D. Stein[1,5], Bryan P. White[1,6], Raphael D. Mazor[1,7], John K. Jackson[2,8], Juliann M. Battle[2,9], Peter E. Miller[3,10], Erik M. Pilgrim[4,11], and Bernard W. Sweeney[2,12]

[1] Southern California Coastal Water Research Project, Costa Mesa, California 92626 USA
[2] Stroud Water Research Center, Avondale, Pennsylvania 19311 USA
[3] Canadian Centre for DNA Barcoding, Biodiversity Institute of Ontario, University of Guelph, Guelph, Ontario, Canada N1G 2W1
[4] National Exposure Research Laboratory, US Environmental Protection Agency, Cincinnati, Ohio 45268 USA



Relevant for bioassessment!

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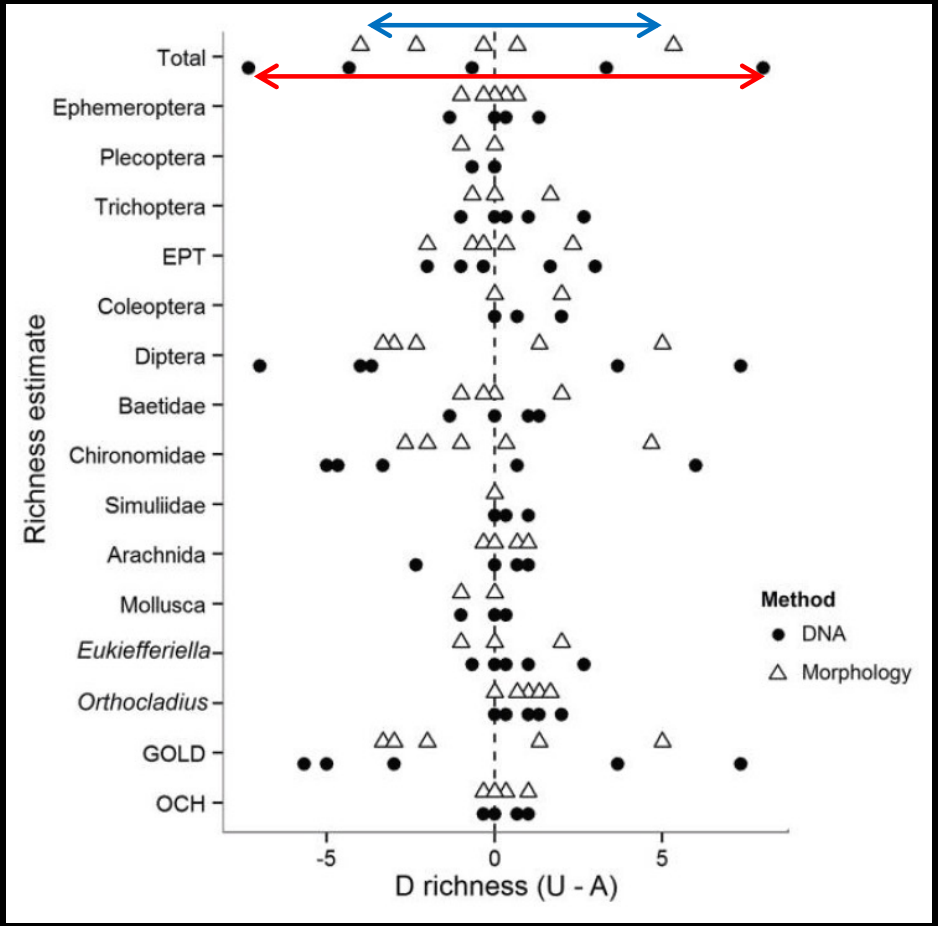

[But here: 1.7-3.4x more expensive]

DNA-based methods are needed to complement global biodiversity surveys

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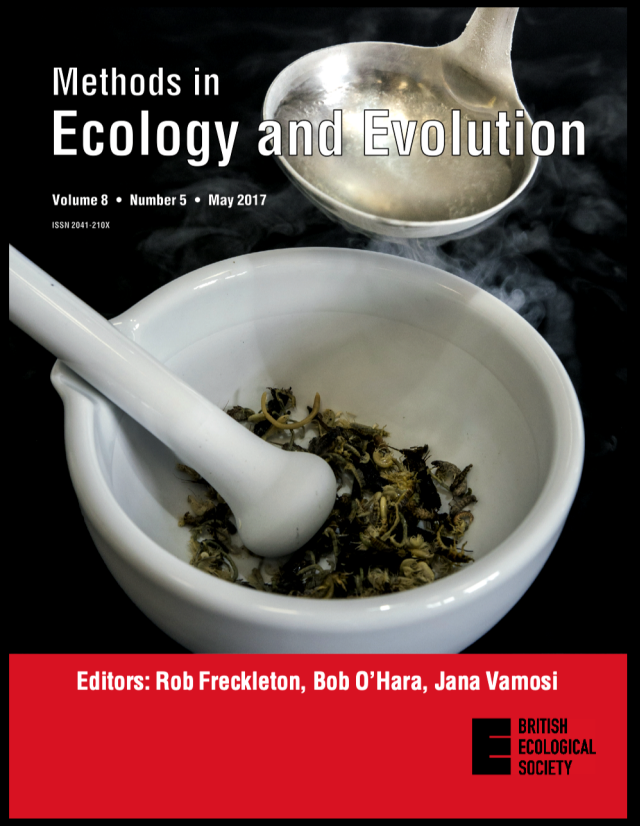
Offen im Denken



Elbrecht & Leese (2017)

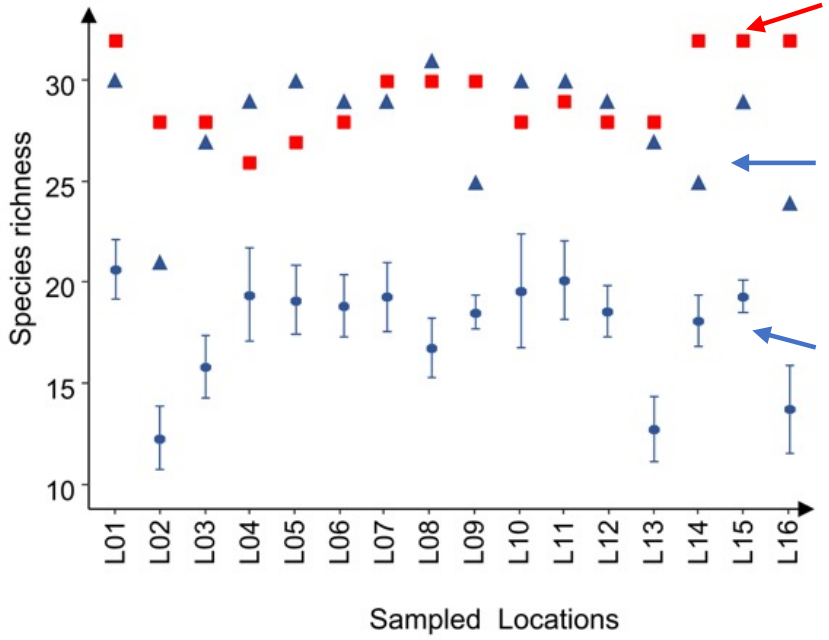
„More with less“ actually works for some BQEs

- eDNA analysis in a French stream (Rhône) shows great performance of eDNA for fish biodiversity assessments
- **Many such studies reported from many different countries!**

Environmental DNA reveals quantitative patterns of fish biodiversity in large rivers despite its downstream transportation

Didier Pont[1,2,3], Mathieu Rocle[4], Alice Valentini[1,2], Raphaël Civade[2], Pauline Jean[1], Anthony Maire[5], Nicolas Roset[6], Michael Schabuss[7], Horst Zornig[7] & Tony Dejean[1,2]

DNAqua-Net EU COST ACTION



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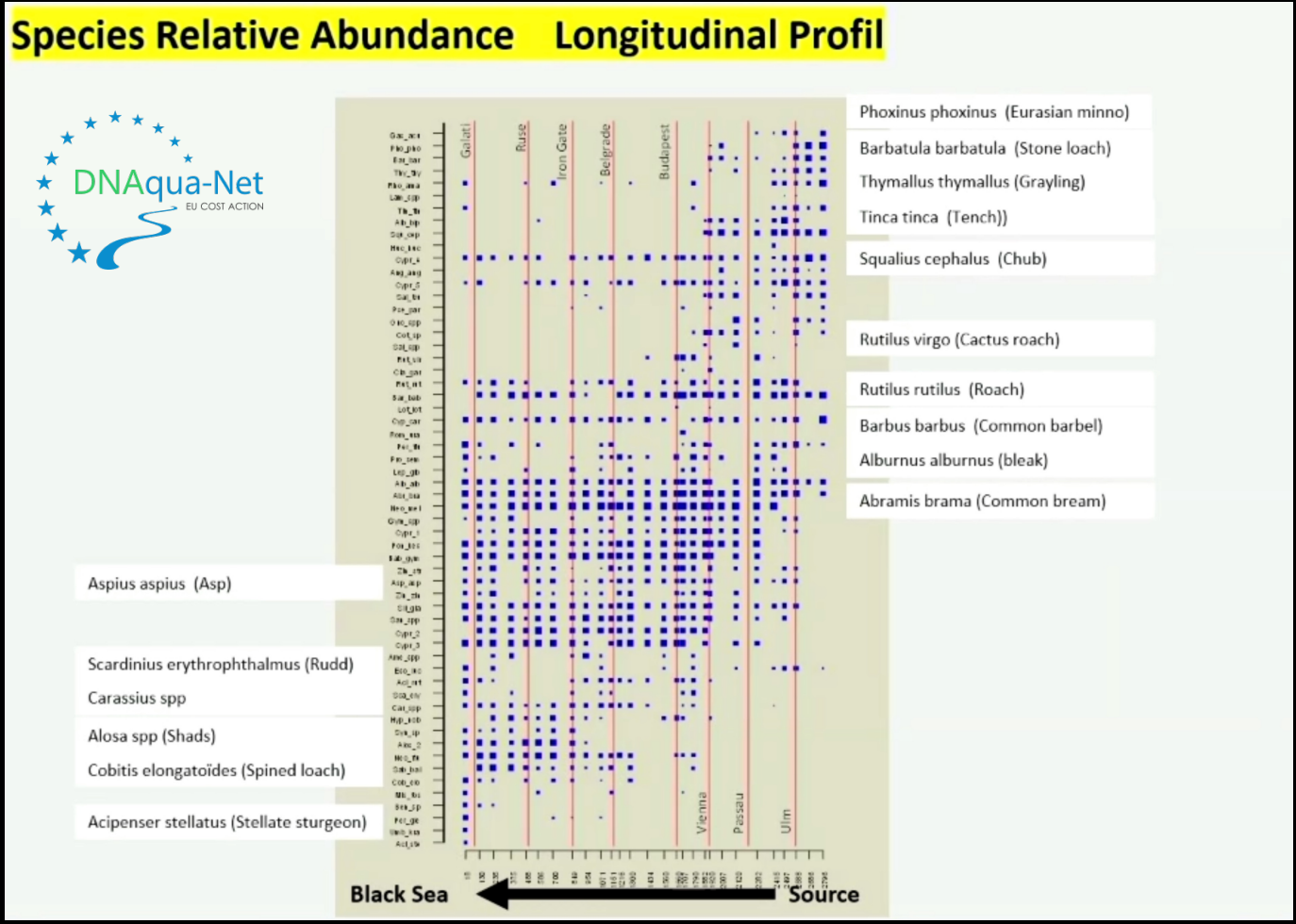
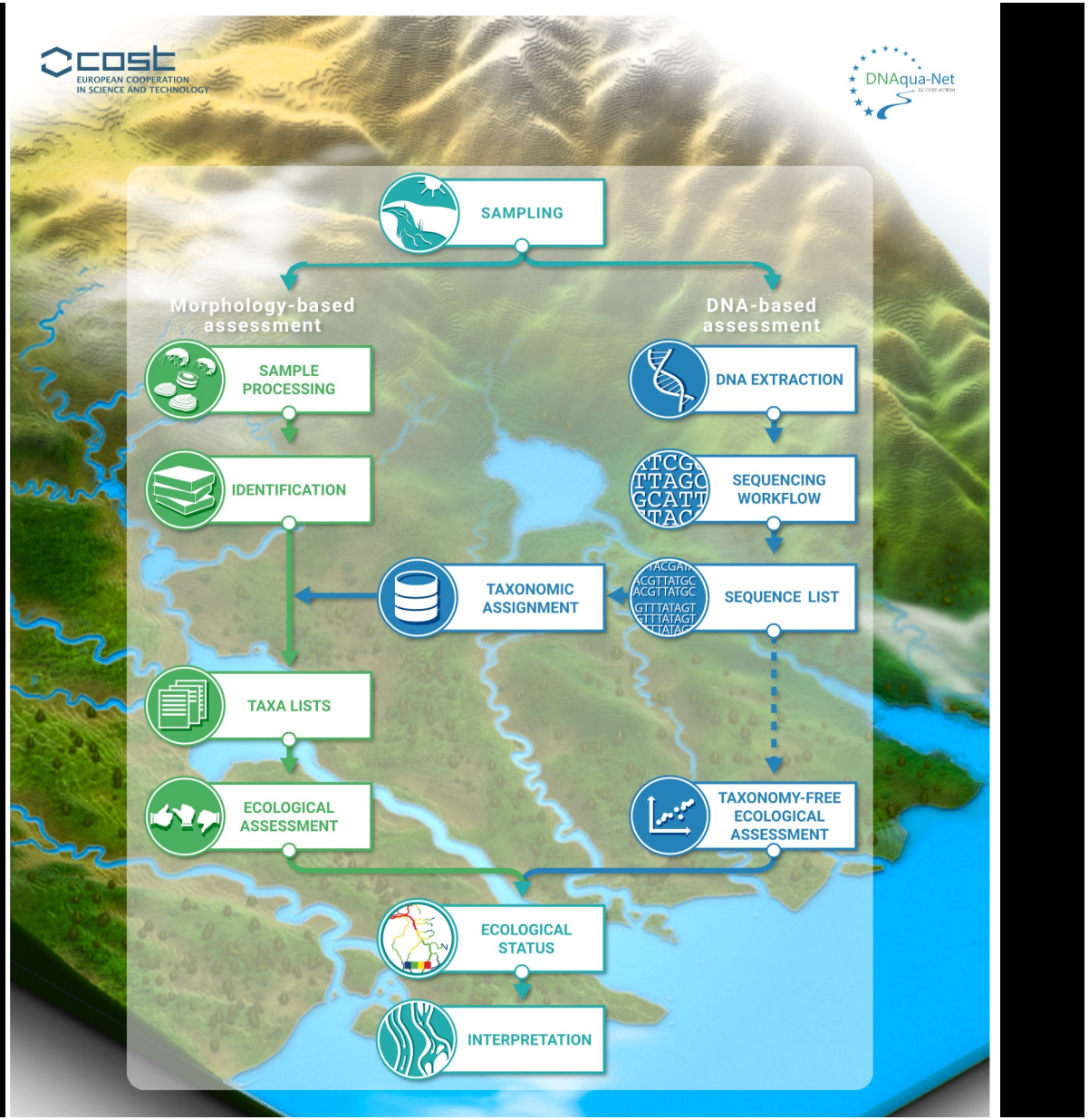


JDS4 scientific report (2021)
Data: Didier Pont

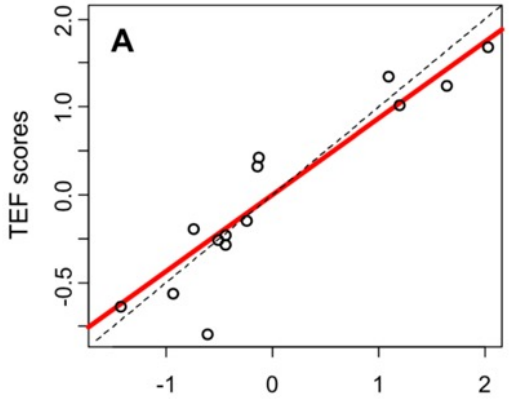
The idea is simple; in principle:

„Speed up sampling, replace microscopes by sequencers, recalibrate and move on“

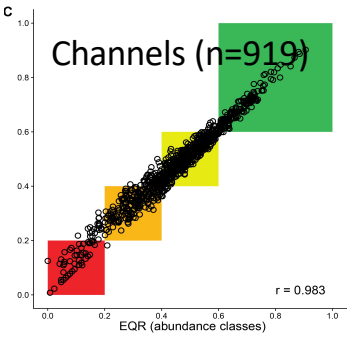
(…and maybe use more of the amazing new data you get…)



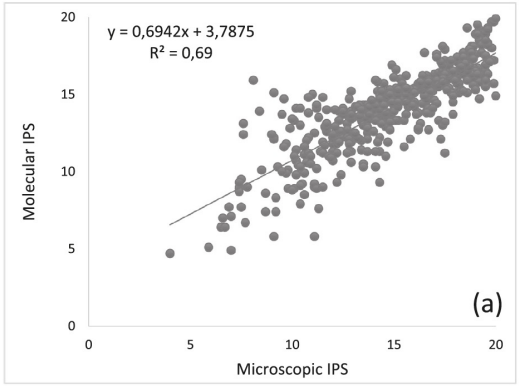
Classical indices can be compatible with metabarcoding data



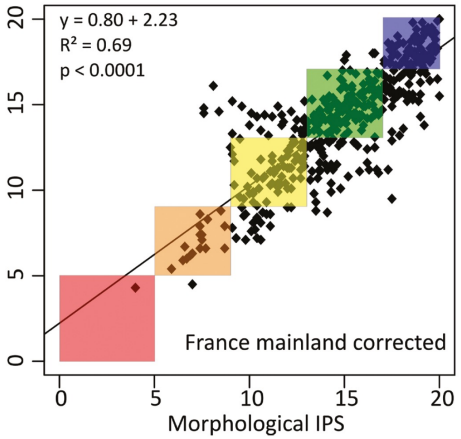
Pont et al. 2018



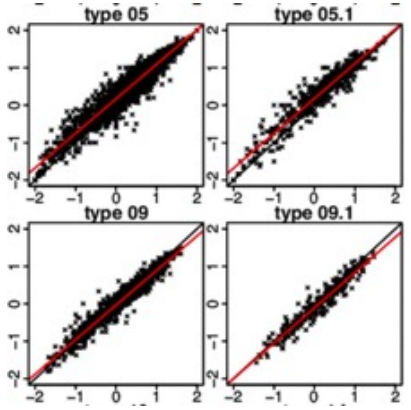
Beentjes et al. (2018)



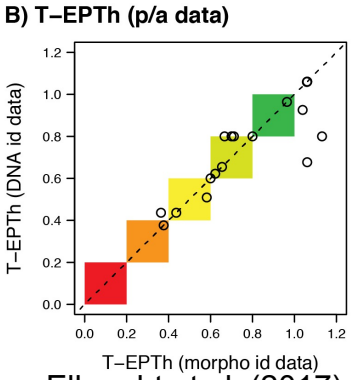
Rivera et al. (2020)



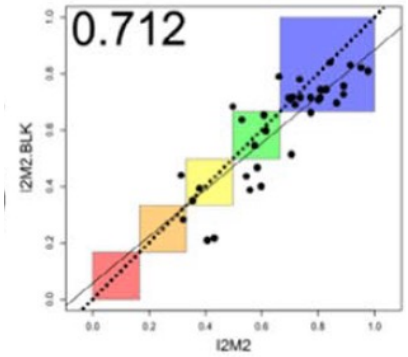
Vasselon et al. (2019)



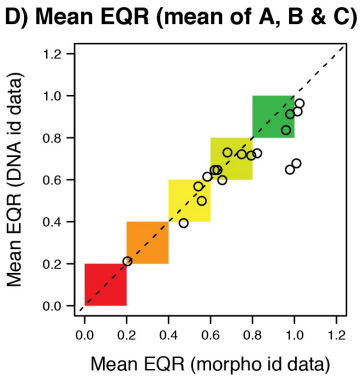
Buchner et al. (2019)



Elbrecht et al. (2017)

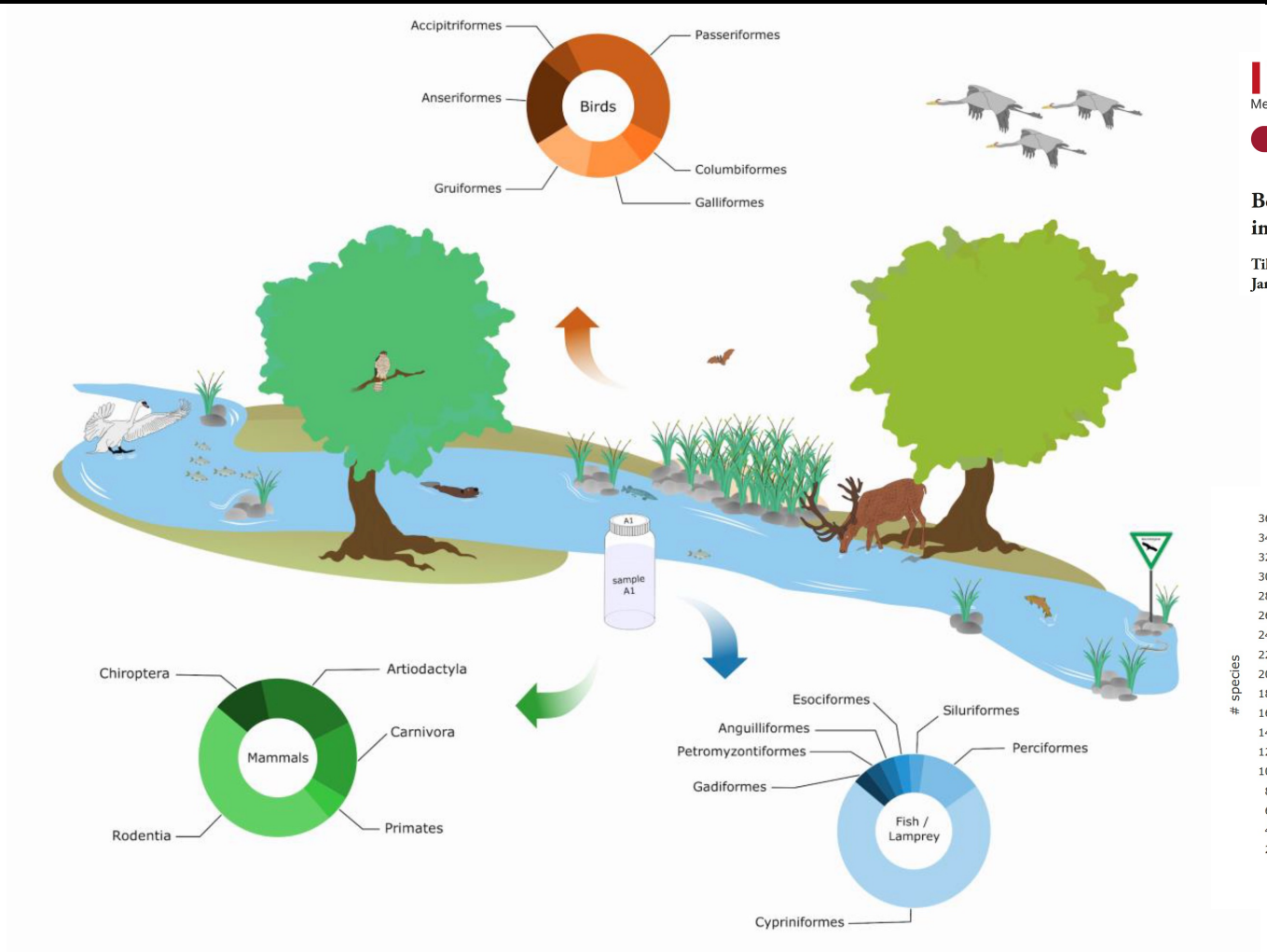


Meyer et al. (2021)



Elbrecht et al. (2017)

Beyond water



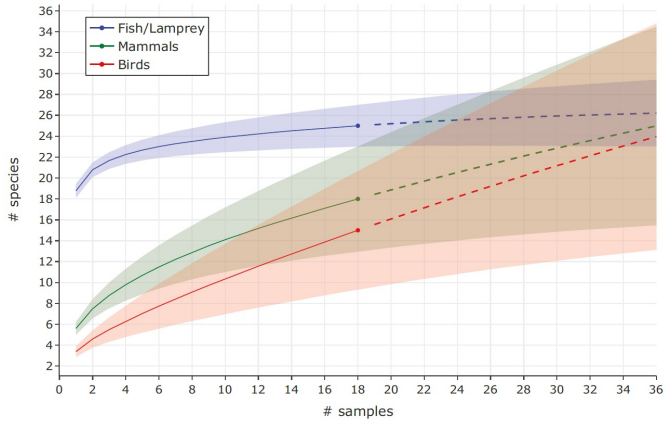
MBMG
Metabarcoding & Metagenomics

Metabarcoding and Metagenomics 5: 59–71
DOI 10.3897/mbmg.5.66557

Research Article

Beyond fish eDNA metabarcoding: Field replicates disproportionately improve the detection of stream associated vertebrate species

Till-Hendrik Macher[1], Robin Schütz[1], Jens Arle[2], Arne J. Beermann[1,3], Jan Koschorreck[2], Florian Leese[1,3]



Automation/throughput possible

Contents lists available at ScienceDirect

Environmental Science and Ecotechnology

journal homepage: www.journals.elsevier.com/environmental-science-and-ecotechnology/

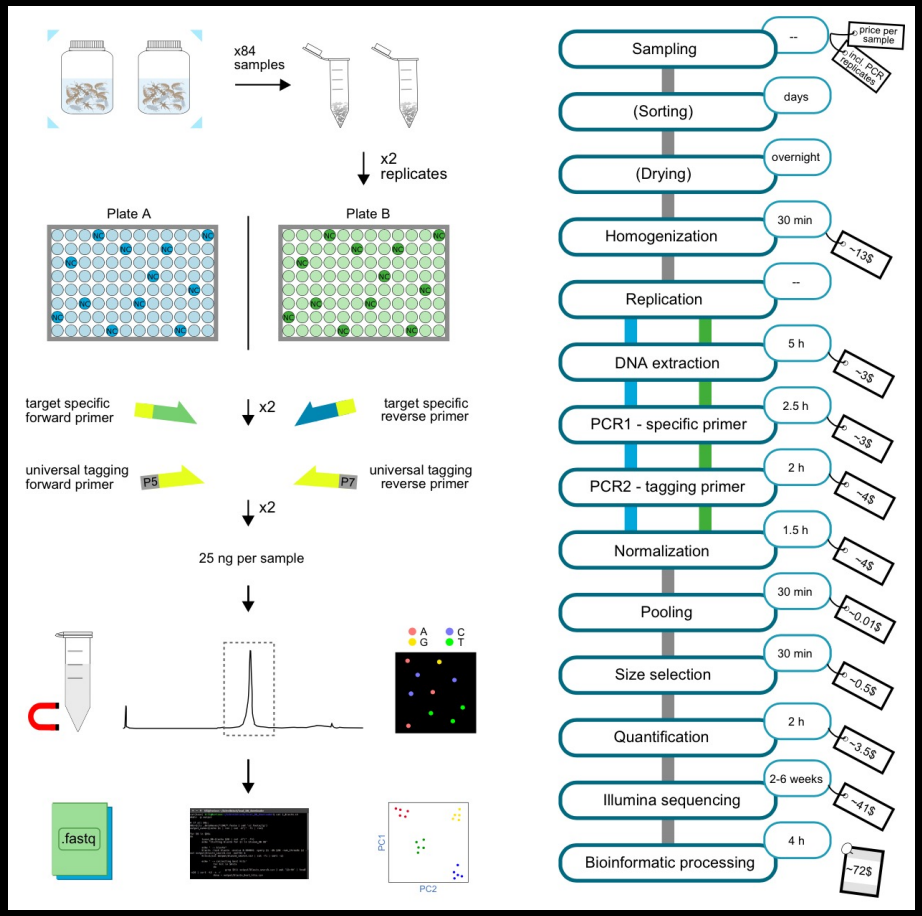
Standardized high-throughput biomonitoring using DNA metabarcoding: Strategies for the adoption of automated liquid handlers

Dominik Buchner [a, 1], Till-Hendrik Macher [a, 1], Arne J. Beermann [a, b], Marie-Thérése Werner [a], Florian Leese [a, b, *]

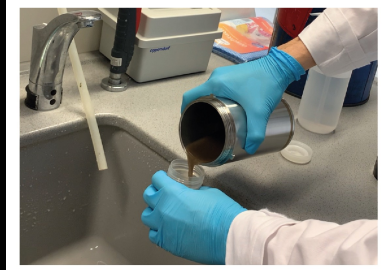
[a] University of Duisburg-Essen, Aquatic Ecosystem Research, Universitätsstr. 5, 45141, Essen, Germany
[b] University of Duisburg-Essen, Centre for Water and Environmental Research (ZWU), Universitätsstr. 3, 45141, Essen, Germany

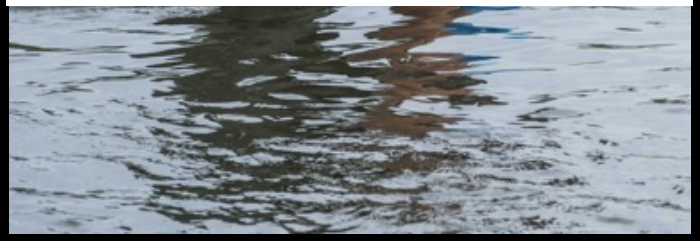
- Several hundred samples per week possible!

Automation/throughput possible



(usually cheaper than listed here…)





Sampling the classical way and/or sampling environmental DNA

(semi-)automated handling

4 photos by Till Macher

What are the main roadblocks?

Revolution is not enough

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Open-Minded

Bilder: WikiCommons

The ‚four-field challenge'

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Concept

- unrepresentative sampling
- new taxonomic / community information
  (e.g. terrestrial eDNA, gut content)
- abundance / biomass / copy-number vs. presence-absence data
- new reference conditions
- new metrics

Technology

- sample / storage conditions
  (e.g. preservation liquid, inhibitors)
- primer bias / PCR stochasticity
- misidentifications
  (e.g. wrong references, shared barcodes)
- reference database development
- non-corresponding taxonomy
  (e.g. between reference list and results)

Perception

- new 'units' to quantify biodiversity
- new technical language
- more complex / integrative settings

‚human dimension'

Economic & legal framework

- costs
- knowledge transfer
- legislative requirements
  (e.g. abundance data, intercalibration)

Leese et al. (2018)

Reference databases

- For fish and macroinvertebrates the European Operational Taxalists are often well-covered (JDS4: 90%, 81%)
- Priorities defined to close gaps



Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Review

DNA barcode reference libraries for the monitoring of aquatic biota in Europe: Gap-analysis and recommendations for future work

Hannah Weigand [a], Arne J. Beermann [b], Fedor Čiampor [c], Filipe O. Costa [d,e], Zoltán Csabai [f], Sofia Duarte [d,e], Matthias F. Geiger [g], Michał Grabowski [h], Frédéric Rimet [i], Björn Rulik [g], Malin Strand [j], Nikolaus Szucsich [k], Alexander M. Weigand [a,b], Endre Willassen [l], Sofia A. Wyler [m], Agnès Bouchez [i], Angel Borja [n], Zuzana Čiamporová-Zat'ovičová [c], Sónia Ferreira [o], Klaas-Douwe B. Dijkstra [p], Ursula Eisendle [q], Jörg Freyhof [r], Piotr Gadawski [h], Wolfram Graf [s], Arne Haegerbaeumer [t], Berry B. van der Hoorn [p], Bella Japoshvili [u], Lujza Keresztes [v], Emre Keskin [w], Florian Leese [b], Jan N. Macher [p], Tomasz Mamos [h], Guy Paz [x], Vladimir Pešić [y], Daniela Maric Pfannkuchen [z], Martin Andreas Pfannkuchen [z], Benjamin W. Price [aa], Buki Rinkevich [x], Marcos A.L. Teixeira [d,e], Gábor Várbíró [ab], Torbjørn Ekrem [ac,*]

→ TRANSPARENT & OPEN DATA! Versioning, DOI…
→ QA/QC basis
→ Several countries working on this

Improve taxonomic backbone

- Improve available ref DB, link to ecological information
- Lot‘s of work, but low hanging fruits, taxonomic initatives exist; GBOL, BeBOL, SweBOL, iBOL, AquaBOL.sk, PolBOL etc.

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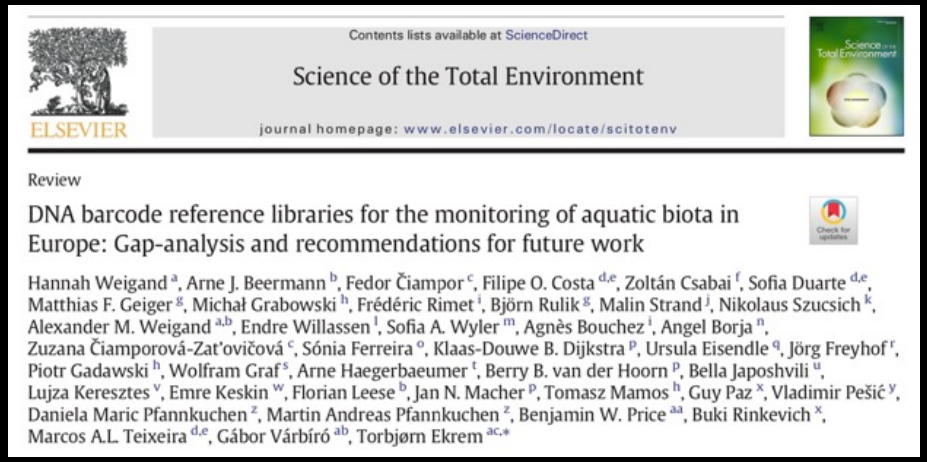
Table 1
Overall barcode coverage for selected major groups.

Taxonomic group	Barcode marker	Species in checklist	Barcode coverage [%]		Database source
			≥1 barcode	≥5 barcodes	
Marine invertebrates - ERMS	COI	16,962	22.1	9.9	BOLD
Marine invertebrates - AMBI	COI	3012	47.6	25.0	BOLD
Marine fish[a]	COI	1489	82.1	64.3	BOLD
Diatoms (marine and freshwater)	rbcL/18S	3716	[illegible]	N/A	Diat.barcode v7
Freshwater vascular plants	rbcL/matK	683	83.0	69.4	BOLD
Freshwater invertebrates	COI	4502	64.5	41.8	BOLD
Freshwater fish	COI	627	87.9	66.2	BOLD/NCBI
Freshwater fish	12S	627	36.4	-	Mitofish

[a] Actinopterygii, Elasmobranchii and Holocephali.

Improve taxonomic backbone

- Improve available ref DB, link to ecological information
- Lot‘s of work, but low hanging fruits, taxonomic initatives exist; GBOL, BeBOL, SweBOL, iBOL, AquaBOL.sk, PolBOL etc.
- THINK INTERNATIONAL!



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GeDNA

Umwelt Bundesamt

freshwaterecology.info

Member of the Freshwater Information Platform

Welcome

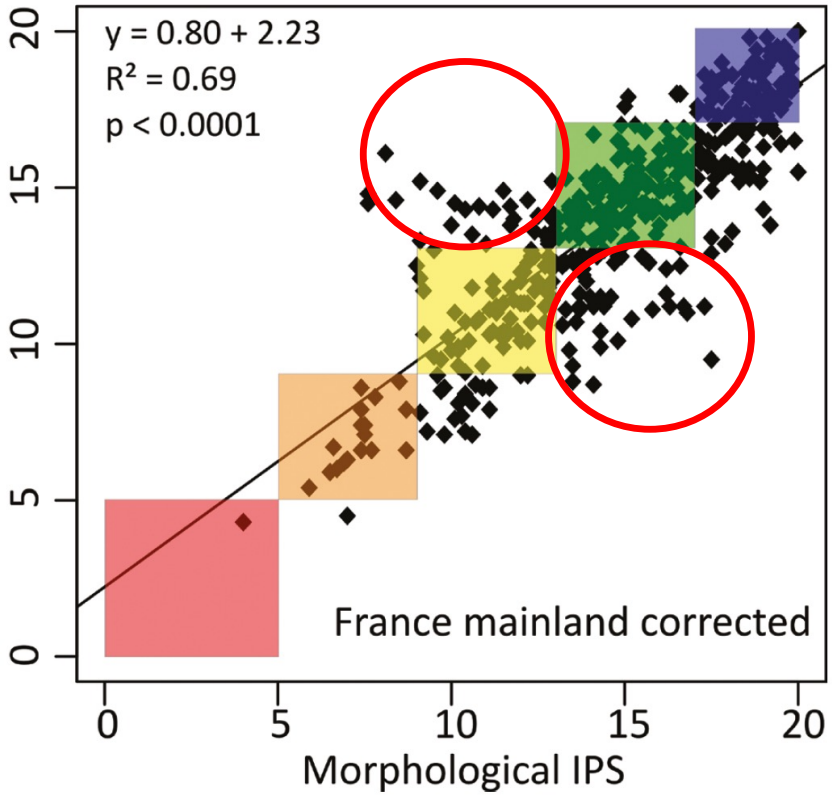
Welcome to the freshwaterecology.info database. Here you can find autecological characteristics, ecological preferences and biological traits as well as distribution patterns of more than 20,000 European freshwater organisms belonging to fish, macro-invertebrates, macrophytes, phytobenthos and phytoplankton.

The ecology data feature occurrence related parameters (e.g. ecoregional distribution or endemism, etc.), region related parameters (e.g. stream zonation or altitudinal preference, etc.), habitat related parameters (e.g. temperature or substrate preference, etc.) or life and body related parameters (e.g. feeding type or life duration, etc.) and others. All biological traits and ecological parameters can be individually combined and queried.

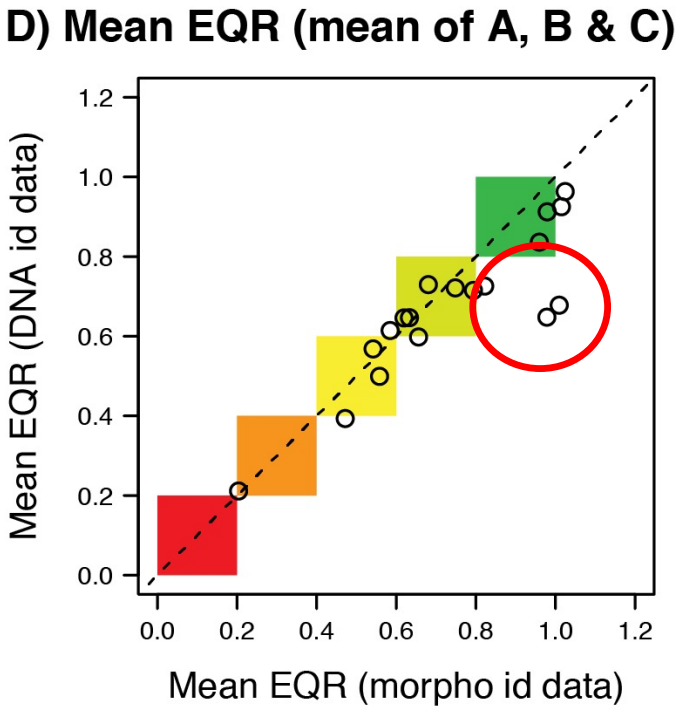
→ Definition of “Gold standard DNA barcodes” for referencing?

→ Workshop initiation 2022 by GeDNA project / UBA & UDE

But when do we accept / recject? How to harmonize?



Vasselon et al. (2019)



Elbrecht et al. (2017)

Who can do the work?

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- Over 35 commercial labs offering services (and many are good!)
- More and more state agencies have own labs
- Central analysis aspects

A constitution is needed

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Bilder: WikiCommons

We urgently need guidance & standards!(!)

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Ecological Indicators

journal homepage: www.elsevier.com/locate/ecolind

thousand

Three ~~hundred~~ ways to assess Europe's surface waters: An almost complete overview of biological methods to implement the Water Framework Directive

Sebastian Birk[a,*], Wendy Bonne[b], Angel Borja[c], Sandra Brucet[b], Anne Courrat[d], Sandra Poikane[b], Angelo Solimini[e], Wouter van de Bund[b], Nikolaos Zampoukas[b], Daniel Hering[a]

'Gamechanging' £10m environmental DNA project to map life in world's rivers

eBioAtlas programme aims to identify fish, birds, amphibians and land animals in freshwater systems from the Ganges to the Mekong

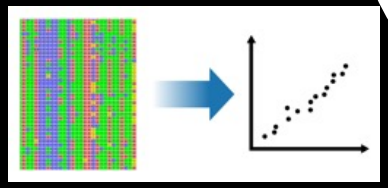
▲ A giant stingray in the Mekong River, near the Cambodian and Vietnam border. It is hoped the scheme will help identify species at risk of extinction. Photograph: Zeb Hogan/AP

- We need standards and guidance (revolution -> consitution)
- Certification / QC instances
- We need central coordination (e.g. linked to ECOSTAT)

Ease data analysis! (e.g. TTT)

Platform independent

Translate sequence data into biological information

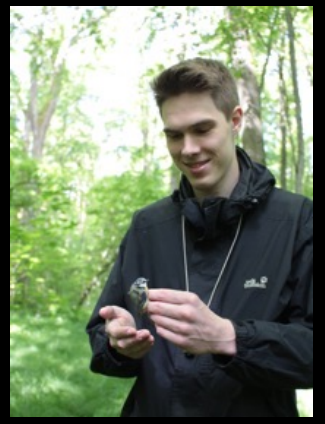


TAXON ABLE OOLS

Biologists, non-informaticians

GUI

Modular / reproducible workflow



Macher et al. (2021) Mol Ecol Res

The future of biotic indices in the ecogenomic era

Contents lists available at ScienceDirect

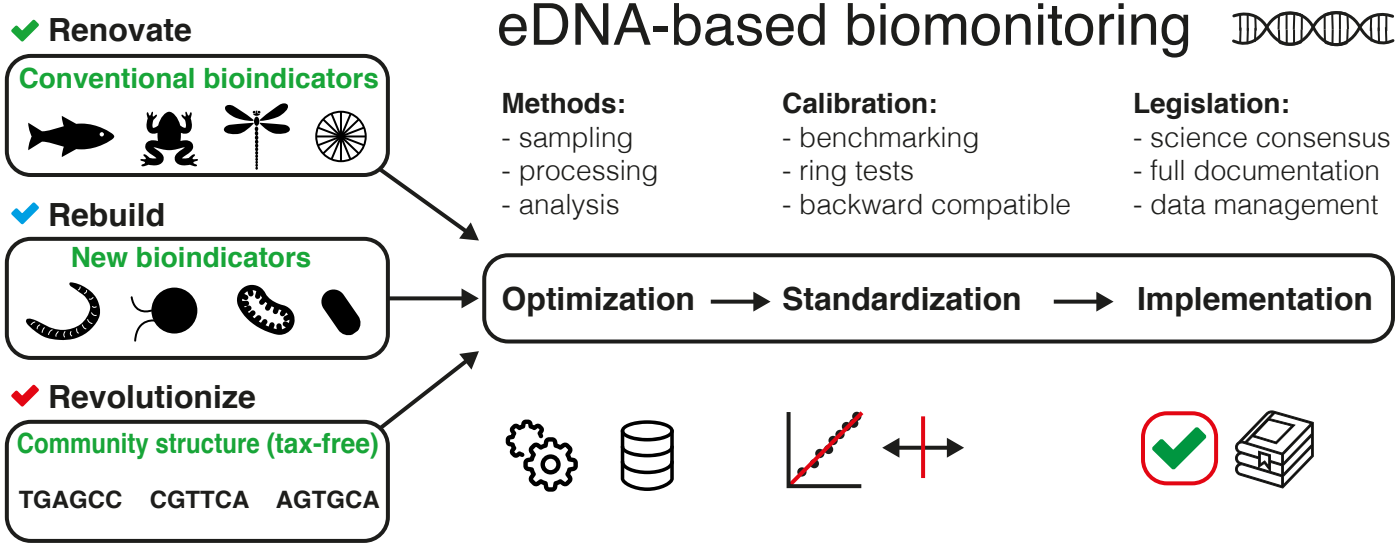
Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Review

The future of biotic indices in the ecogenomic era: Integrating (e)DNA metabarcoding in biological assessment of aquatic ecosystems

Jan Pawlowski [a,*], Mary Kelly-Quinn [b], Florian Altermatt [c], Laure Apothéloz-Perret-Gentil [a], Pedro Beja [d], Angela Boggero [e], Angel Borja [f], Agnès Bouchez [g], Tristan Cordier [a], Isabelle Domaizon [g], Maria Joao Feio [h], Ana Filipa Filipe [d], Riccardo Fornaroli [i], Wolfram Graf [j], Jelger Herder [k], Berry van der Hoorn [l], J. Iwan Jones [m], Marketa Sagova-Mareckova [n], Christian Moritz [o], Jose Barquín [p], Jeremy J. Piggott [q], Maurizio Pinna [r], Frederic Rimet [g], Buki Rinkevich [s], Carla Sousa-Santos [t], Valeria Specchia [r], Rosa Trobajo [u], Valentin Vasselon [g], Simon Vitecek [v], Jonas Zimmerman [w], Alexander Weigand [x,y], Florian Leese [x], Maria Kahlert [z]



Source: Pawlowski, Taberlet et al. (2021)

Realistic plans, important milestones

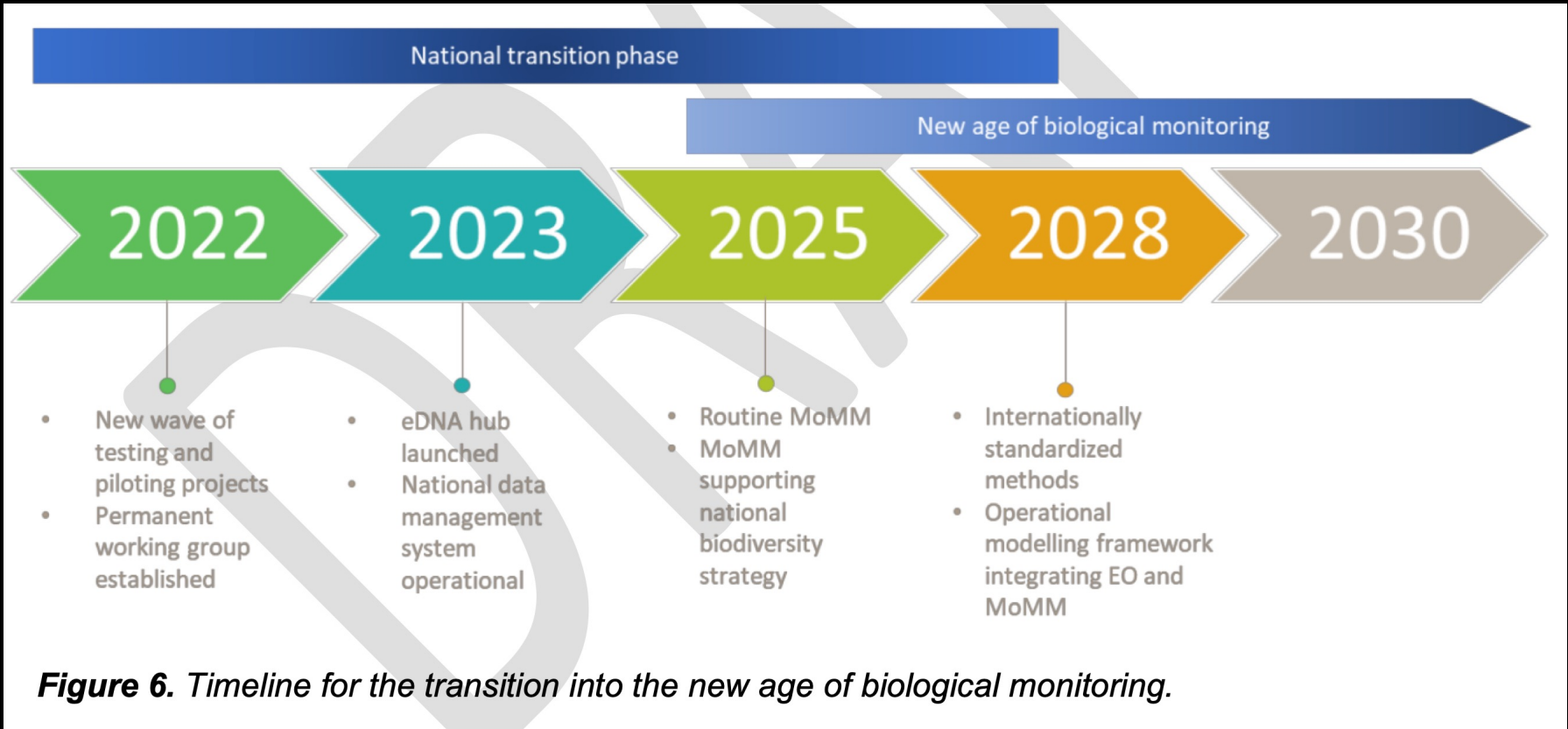
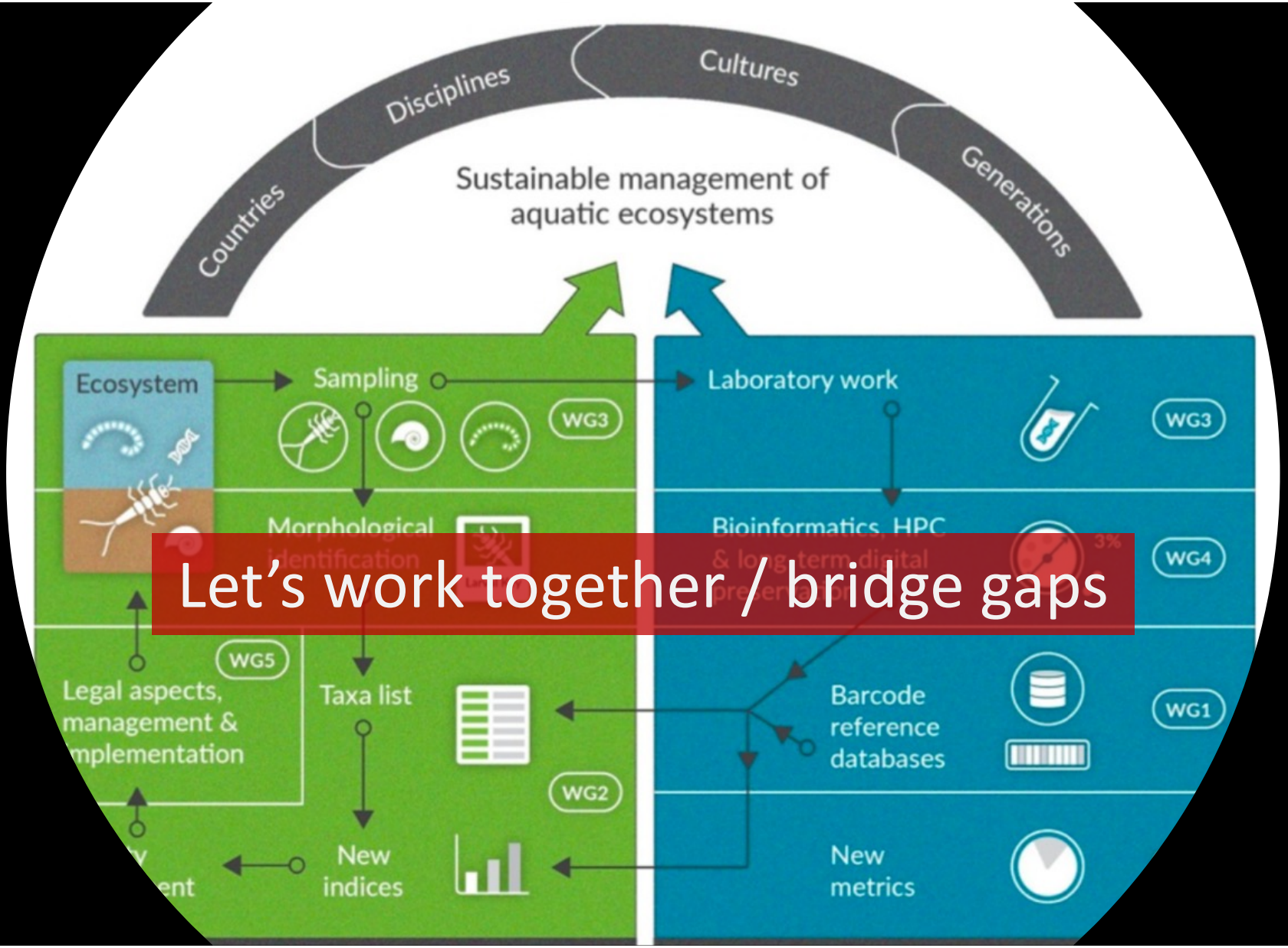


Figure 6. *Timeline for the transition into the new age of biological monitoring.*

Source: 'Stolen' from the Finnish roadmap draft today (12.1.2021)



Let's work together / bridge gaps

UNIVERSITÄT DUISBURG ESSEN

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Bundesministerium für Bildung und Forschung

DFG

HORIZON 2020

GBOL German Barcode of Life

DNAqua-Net EU COST ACTION

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Thank you!

https://dnaqua.net