



Welcome to DNAqua-Net's stakeholder workshop series

March 12th 2021

Prof. Dr. Florian Leese

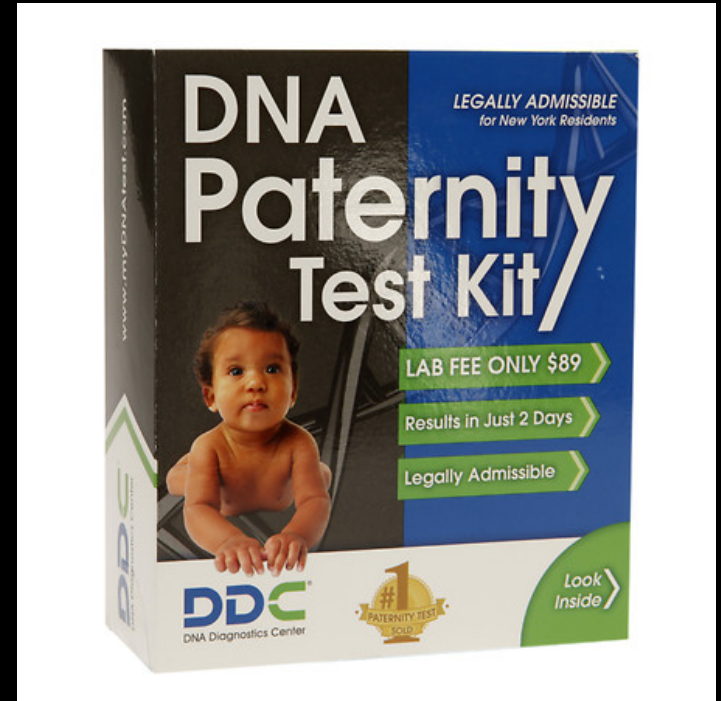
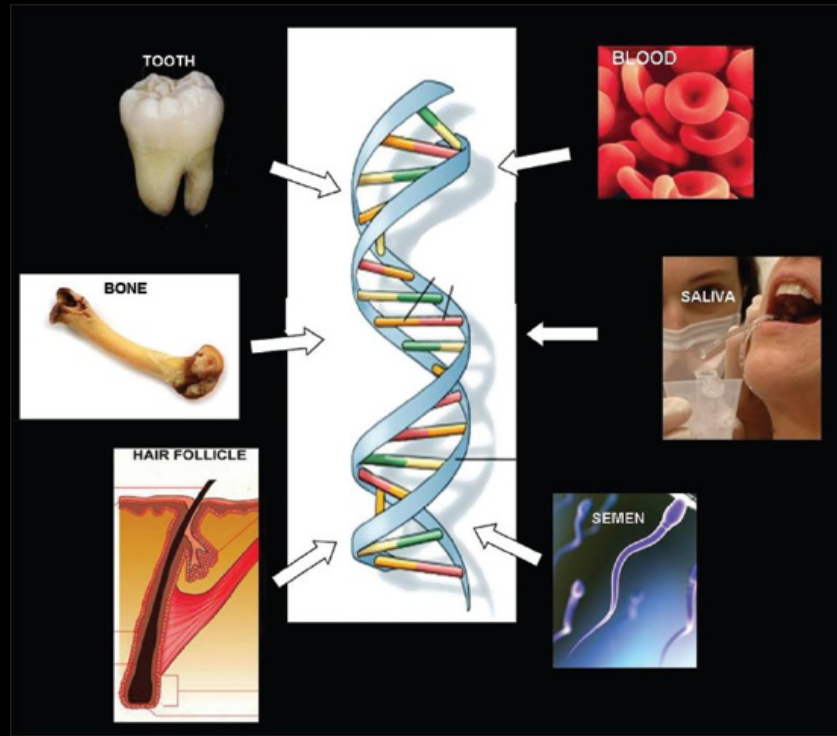
University of Duisburg-Essen

Chair of COST Action DNAqua-Net

@dnaquanet



We trust genetic methods in various sectors of our life



What about biodiversity
assessment and
biomonitoring?

The European version of the obvious back in 2015/2016

MoU: „Advance the application of DNA-based tools for biodiversity assessments & develop a roadmap to include these in standardized bioassessments of aquatic ecosystems in Europe and beyond.“



EU COST Action DNAqua-Net (2016 – 2021)

The European version of the obvious back in 2015/2016

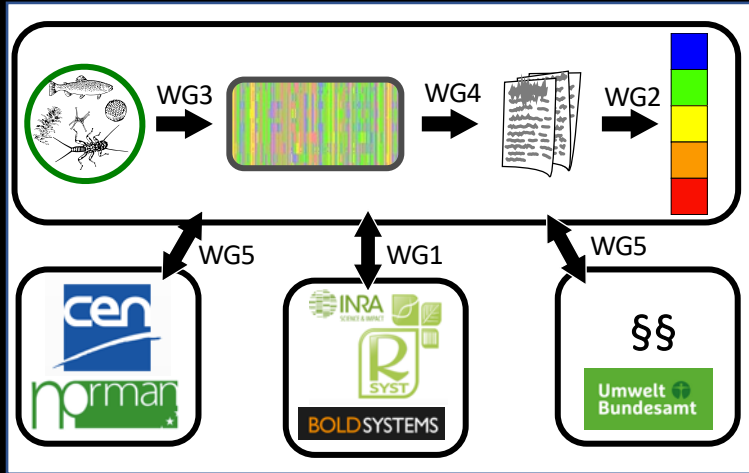
MoU: „Advance the application of DNA-based tools for biodiversity assessments & develop a roadmap to include these in standardized bioassessments of aquatic ecosystems in Europe **and beyond.**“

**THIS IS NOT ONLY A RESEARCH QUESTION;
COLLABORATION BETWEEN ACADEMIA &
APPLICATION EXTREMELY IMPORTANT!**



EU COST Action DNAqua-Net (2016 – 2021)

DNAqua-Net's vision

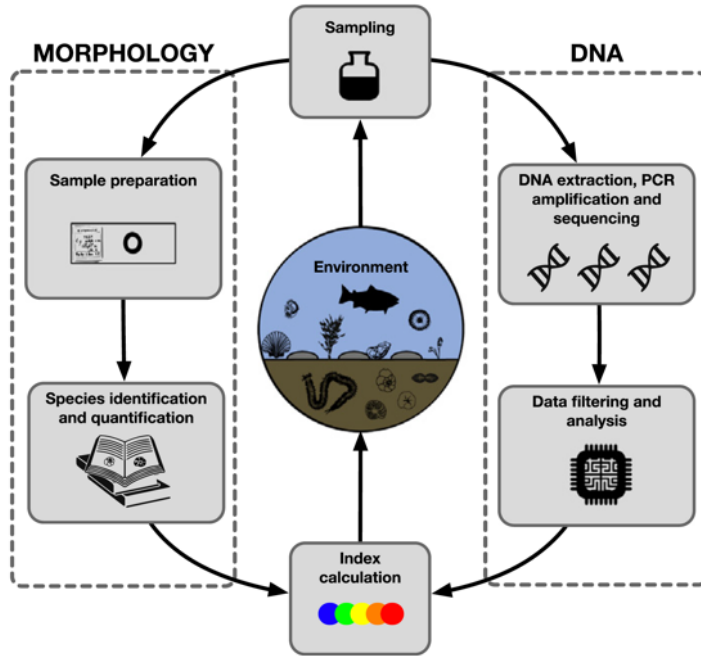


From research to application

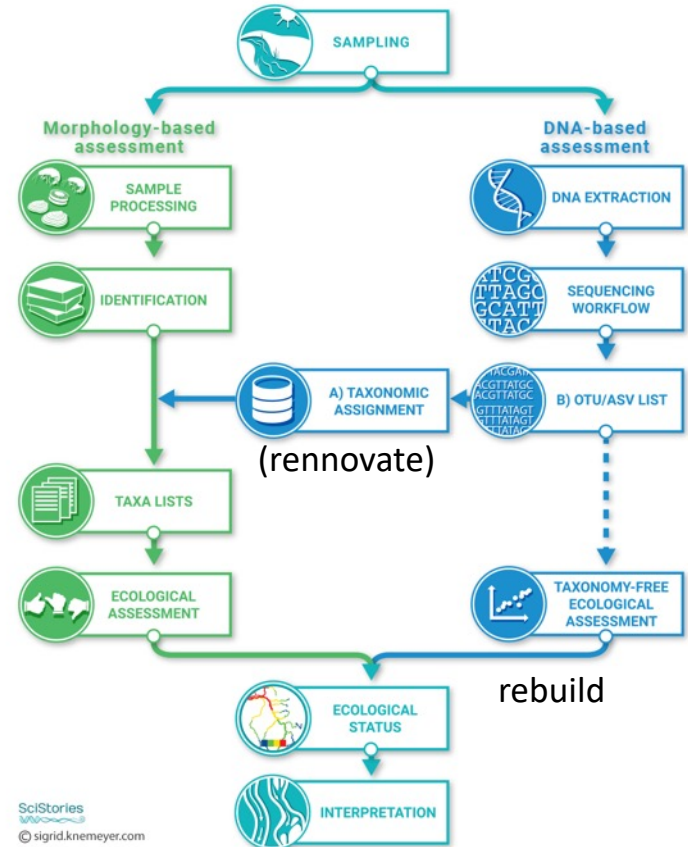
- Between 400 & 600 members
- 49 countries
- >100 publications & stakeholder reports
- >50 exchanges, ~50 meetings / round tables



General Options



Pawlowski et al. (2018)



Where are the general/specific challenges?

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



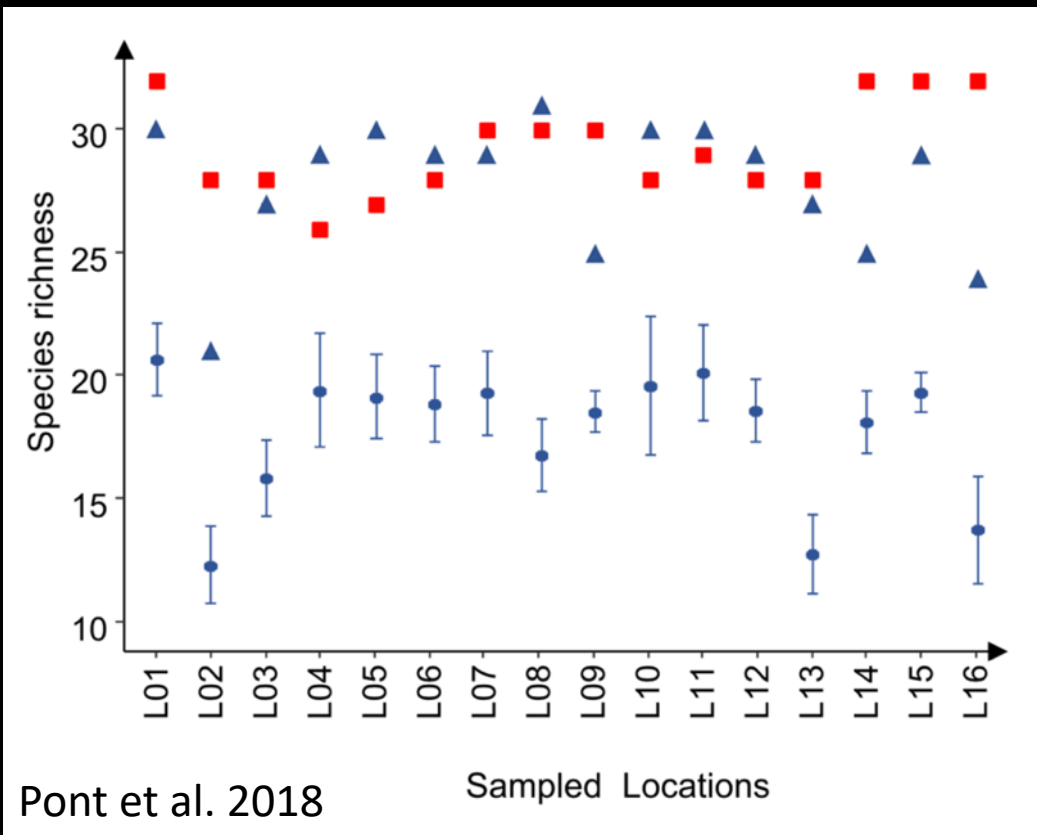
Economic & legal framework

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

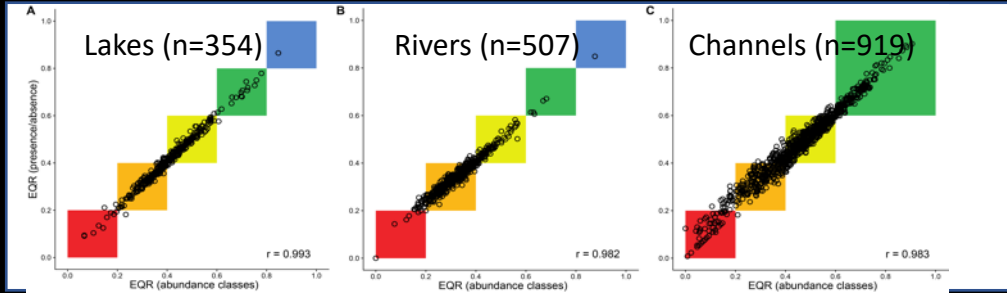


Comparability?

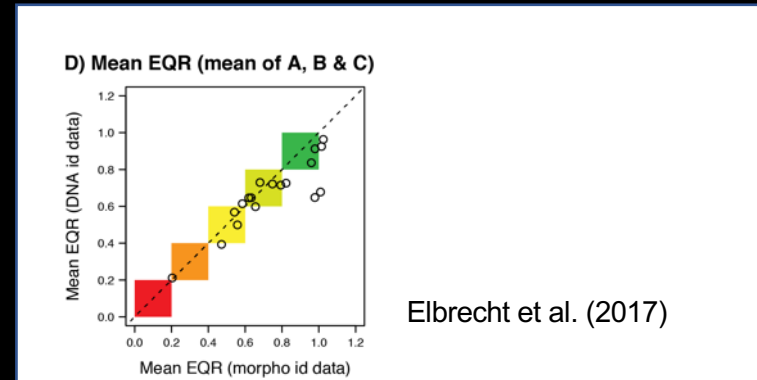
- Especially for deep rivers and lakes – better species representation using eDNA
- In particular benthic fish



Abundance sometimes not of prime importance



Beentjes et al. (2018) MBMG



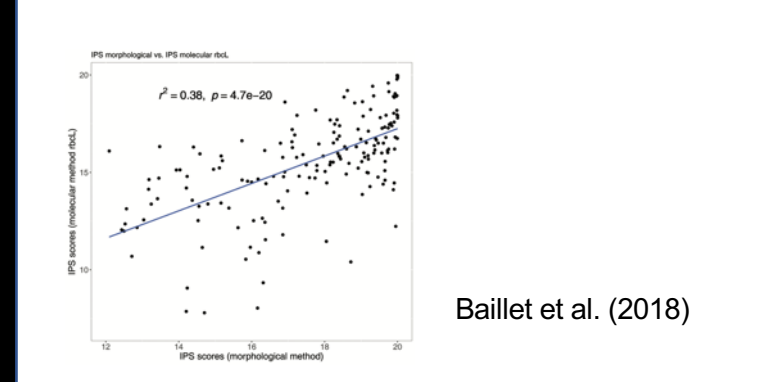
Elbrecht et al. (2017)

PLOS ONE

RESEARCH ARTICLE

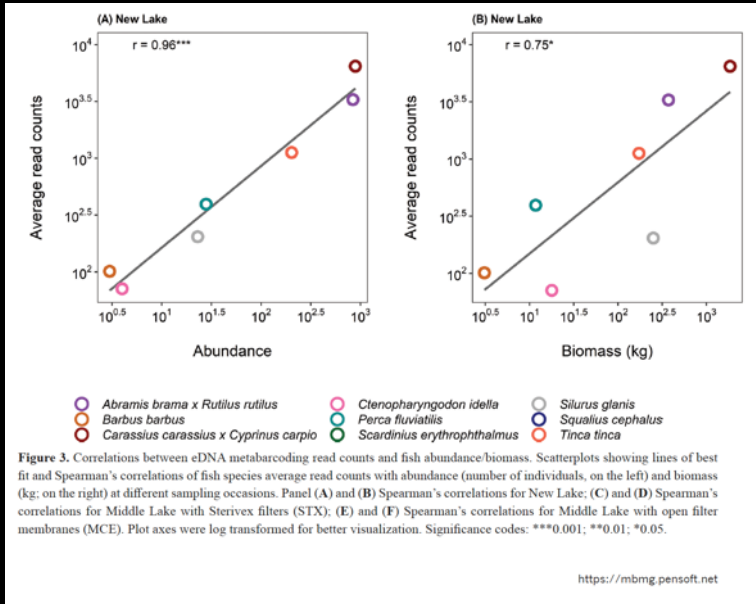
Analysis of 13,312 benthic invertebrate samples from German streams reveals minor deviations in ecological status class between abundance and presence/absence data

Dominik Buchner¹, Arne J. Beermann^{1,2}, Alex Laini³, Peter Rolaufts⁴, Simon Vitecek^{5,6}, Daniel Hering^{2,4}, Florian Leese^{1,2,*}



Baillet et al. (2018)

eDNA can reflect fish abundance / biomass in ponds!

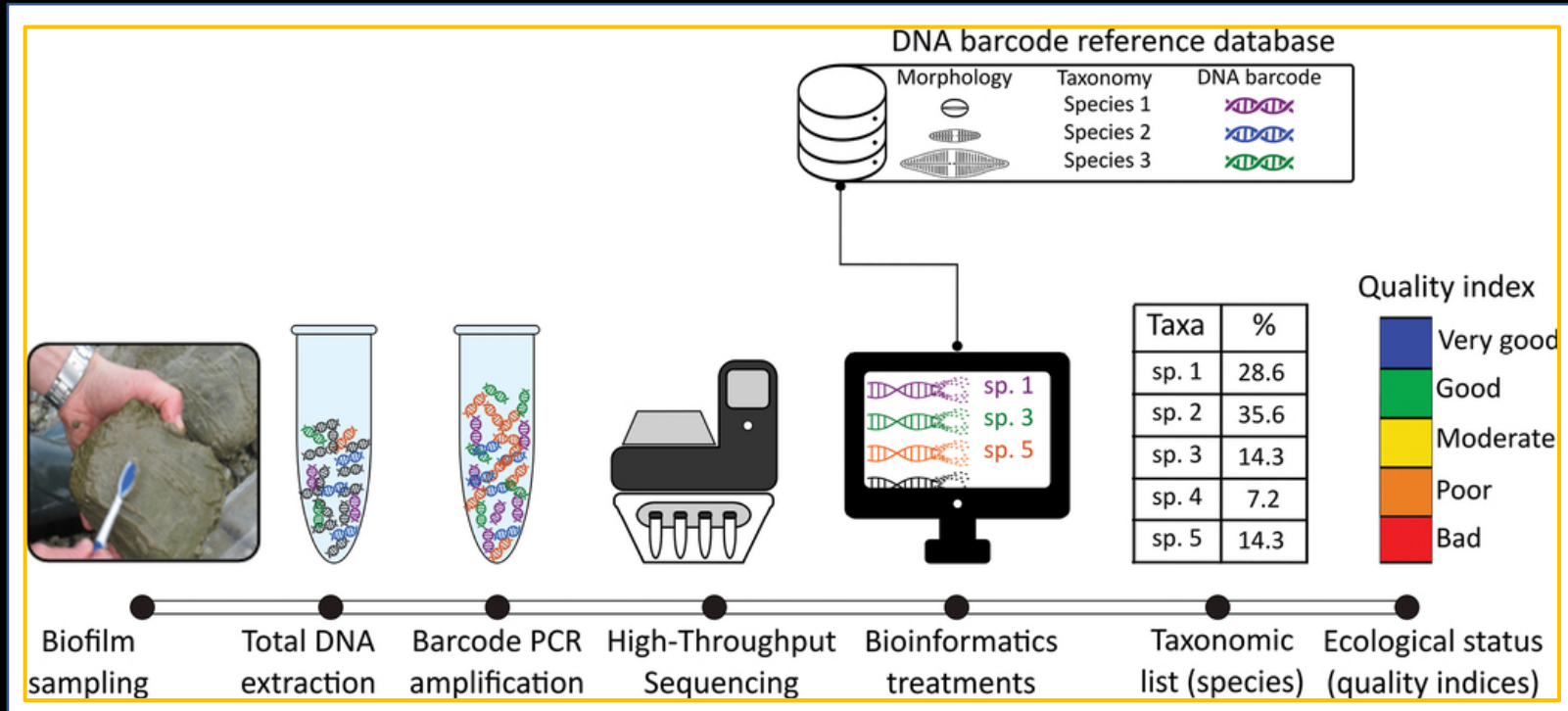


Read counts from environmental DNA (eDNA) metabarcoding reflect fish abundance and biomass in drained ponds

Cristina Di Muri¹, Lori Lawson Handley¹, Colin W. Bean², Jianlong Li^{3,4}, Graeme Peirson⁵, Graham S. Sellers¹, Kerry Walsh⁶, Hayley V. Watson¹, Ian J. Winfield⁷, Bernd Hänfling¹

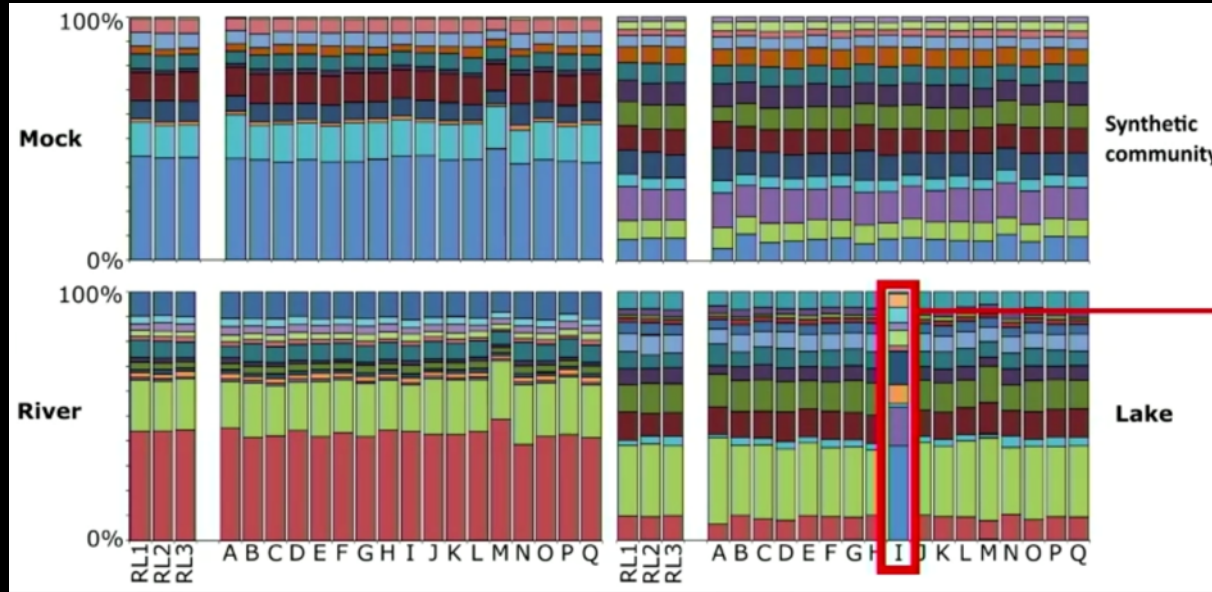
See also Ushio et al. 2019; Doi et al. (mult. ref), Li et al. (2020), Hänfling et al. (2016); Pont et al. (2018, 2020)

How comparable are the data?



How comparable are the data?

- Fast, simple, cheap in application, robust & reproducible data to in form on pressures
- Few ring tests – standardisation needed, but results promising



Same Taq in all labs!

Error, Inversion of two samples (by RL or the participant).
Removed from downstream analysis.

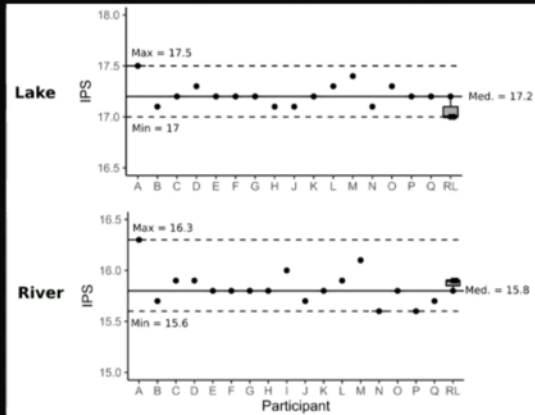
- 18 labs in 15 countries participated in diatom ring test

How reliable are the data?

- Fast, simple, cheap in application, robust & reproducible data to in form on pressures

Ecological assessment using IPS diatom index

- IPS quality scores calculated using OMNIDIA 6



Q3 - Variability observed on IPS score is satisfactory (max diff. = 0.7 point)

Calculation of z-score using Lake and River IPS scores

- calculated for each participant : $z = (x - \mu) / \sigma$
- evaluates how far a participant result is from the mean

	Z-score	
	IPS_Lake	IPS_River
RL	-1,29	0,17
A	2,46	2,70
B	-1,00	-0,80
C	-0,14	0,37
D	0,73	0,37
E	-0,14	-0,22
F	-0,14	-0,22
G	-0,14	-0,22
H	-1,00	-0,22
I		0,95
J	-1,00	-0,80
K	-0,14	-0,22
L	0,73	0,37
M	1,60	1,53
N	-1,00	-1,38
O	0,73	-0,22
P	-0,14	-1,38
Q	-0,14	-0,80

$|z\text{-score}| \leq 2.0$ satisfactory
 $2.0 < |z\text{-score}| < 3.0$ questionable
 $|z\text{-score}| \geq 3.0$ unsatisfactory

Q3 - Ring test successful for PCR
no participant considered unsatisfactory

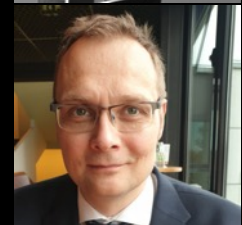
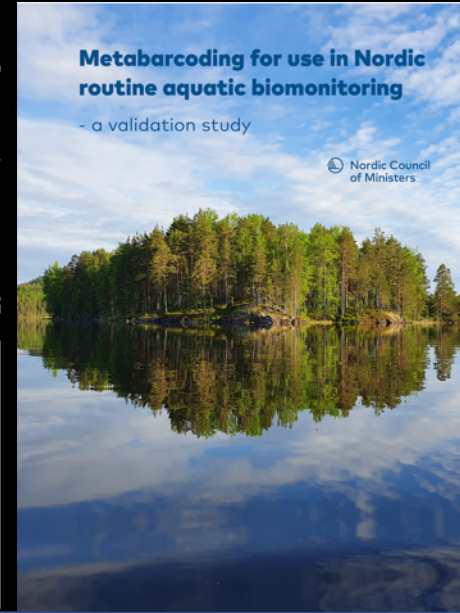
How reliable are the data?



	<i>Rutilus rutilus</i>	<i>Percis fluviatilis</i>	<i>Platichthys flesus</i>	<i>Esoc lucius</i>	<i>Gasterosteus aculeatus</i>	<i>Cottus gobio</i>	<i>Anguilla anguilla</i>	<i>Salmo trutta</i>	<i>Abramis brama</i>	<i>Barbus haasi</i>	<i>Leuciscus leuciscus</i>	<i>Gobio gobio</i>	<i>Pungitius pungitius</i>	<i>Blecca bleekera</i>	<i>Alburnus alburnus</i>
Workflow 1	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Workflow 2	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Workflow 3	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Workflow 4	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Workflow 5	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Workflow 6	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●

- Fish eDNA metabarcoding comparison
- 6 very different workflows and labs
- Very consistent results

Co-designed validation studies are important now!



Financed by
Nordic Council of
Ministers



EDNA-
VALIDATION.
COM

Infrastructure – DIY, or rely on public infrastructure

- Data storage
- Data processing
- Taxonomic assignment
- Ecological analysis / index calculation



DIAT.BARCODE



BARCODE OF LIFE DATA SYSTEM v4

Advancing biodiversity science through DNA-based species identification.

EXPLORE THE DATA

www.freshwaterecology.info The logo for the Freshwater Ecology Database, featuring a blue wavy line representing water with various freshwater organisms like a fish, a frog, a dragonfly, and a starfish.

The Taxa and Autecology Database for Freshwater Organisms

Knowledge Transfer – consensus building!



A practical guide to DNA-based methods for biodiversity assessment

Bruce, K., Blackman, R.C., Bourlat, S., Hellarón, M., Bakker, J., Bata, I., Bouchet, A., Bryn, R., Clark, K., Elbrecht, V., Fast, S., Fontana, V.G., Harling, B., Lewis, F., Macklin, E., Mahon, A.R., Massner, K., Pawlows, K., Pawlowski, J., Schmidt, P., Seymour, M., Thalingen, B., Traugott, M., Valentini, A., Woodcock, P., Yesselon, V. & Deiner, K.

DNA-based methods for species detection and identification have transformed our ability to monitor biodiversity in aquatic and terrestrial systems. While these approaches continue to develop, a significant level of consensus on scientific best-practice now exists in many areas, and practitioners and policy makers are now starting to integrate DNA-based methods into routine monitoring applications. Thus, emphasis now shifts to robust and efficient application of DNA-based methods for operational use at large scale.

This book aims to summarize the scientific consensus relating to every step of the field and laboratory workflows involved in the most common types of samples and analyses. The book uniquely sets the field and lab steps in the context of the practical and logistical constraints faced by environmental managers in terms of cost, logistics, safety, ease-of-use, and quality assurance, highlighting key decisions to be made and the inherent trade-offs associated with the various options. The authors hope that this will support non-experts, and those new to the field, to navigate the key considerations associated with planning or evaluating monitoring programmes using DNA-based monitoring methods. Additionally, it will aid decision makers in writing and evaluating tenders, ensuring that the methods used for a given project are fit-for-purpose and that results are correctly interpreted.



<https://dnaqua.net>
<https://dnaquahub.eu>



Environmental DNA applications in biomonitoring and bioassessment of aquatic ecosystems

Guidelines



4.2 Precautions for handling eDNA samples

Laboratory methods for the detection of eDNA are optimized to detect small traces of DNA and therefore these techniques are actively susceptible for contamination. Precautions need to be taken at every step and used to take several actions of different sampling steps to minimize the probability to contaminate the samples (Fig. 7).

Warnings

The general principle is that all material and equipment that gets in contact with the eDNA sample must be either single-use or cleaned to be DNA-free. Field practices are to use single-use disposable gloves during sampling. This will only partially contribute to reduce eDNA, which can persistently adhere and contaminate the equipment, but will also reduce cross-contamination between sampling sites.

Best practice

Best practice is to use single-use gloves and equipment that gets in contact with the eDNA sample must be either single-use or cleaned to be DNA-free. Field practices are to use single-use disposable gloves during sampling. This will only partially contribute to reduce eDNA, which can persistently adhere and contaminate the equipment, but will also reduce cross-contamination between sampling sites.

Figure 7

Measures to avoid contamination during sampling. An equipment must be cleaned or eDNA-free. Single-use gloves and tools must be used in general conditions, with their correct usage and use in the same general conditions.



5. Take Stramon filter out of sampling

6. Stramon filter only full springs

7. Take sample from the substrate. Avoid stepping into the substrate to prevent cross-contamination and stirring up sediments. Select a representative site and sample the water about 30 cm off the bank. Sample water about 5 cm below surface.

8. Flush water of regular used through filter. Don't hold any filter in order not to lose the filter in case a hole is off.

9. Stramon springs from Stramon filter.

10. Filter sample directly with water from the main lake (avoid 50%); without any of bubbles. In case there are bubbles, hold the spring upright and pump them off.

11. Repeat steps 5-10 three times in order to filter 100% water through the filter. Due to sediment particles, it may not always be possible to filter 100%, or a single filter in the case, it is important to keep the Stramon volume.

→ DNAqua-Net WG3; Appearing soon!

Pawlowski et al. 2020: Available in 3 languages!



Aims of this workshop

Aims



- Present the state-of-the-art of DNA and eDNA-based tools
- Discuss details (reference databases, quantification, taxonomic assignment, costs) for the purpose of routine monitoring
- Link actors in the field at national level

- Where do you see use of the methods in your country?
- What are main obstacles for the uptake of the methods?

→ Please perform in the survey