# Environmental DNA:
## from presence/absence to a measure of anthropogenic pressure

EURASIAN OTTER WORKSHOP

26-28 February 2021

Maurizio Casiraghi,
ZooPlantLab,
University of Milan-Bicocca

# *My long journey*

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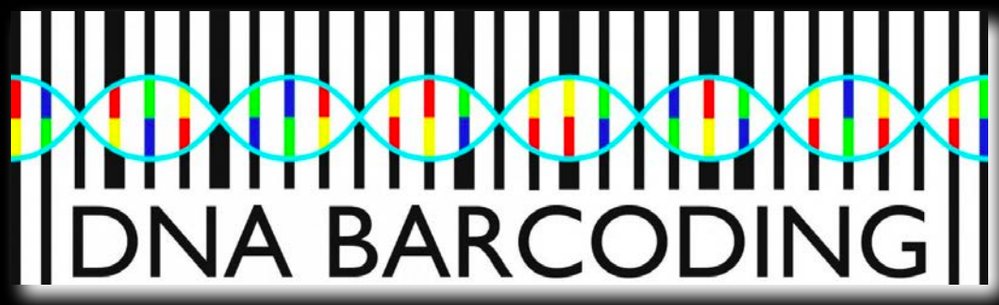
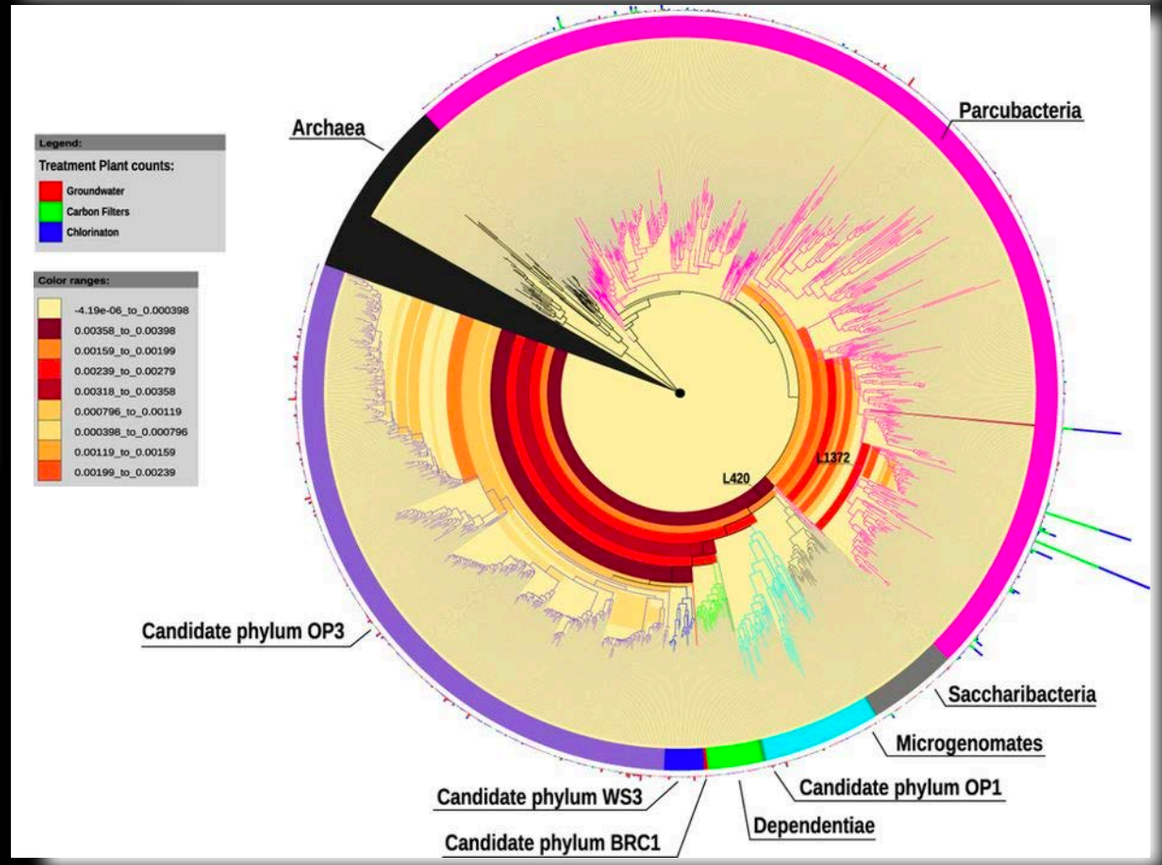
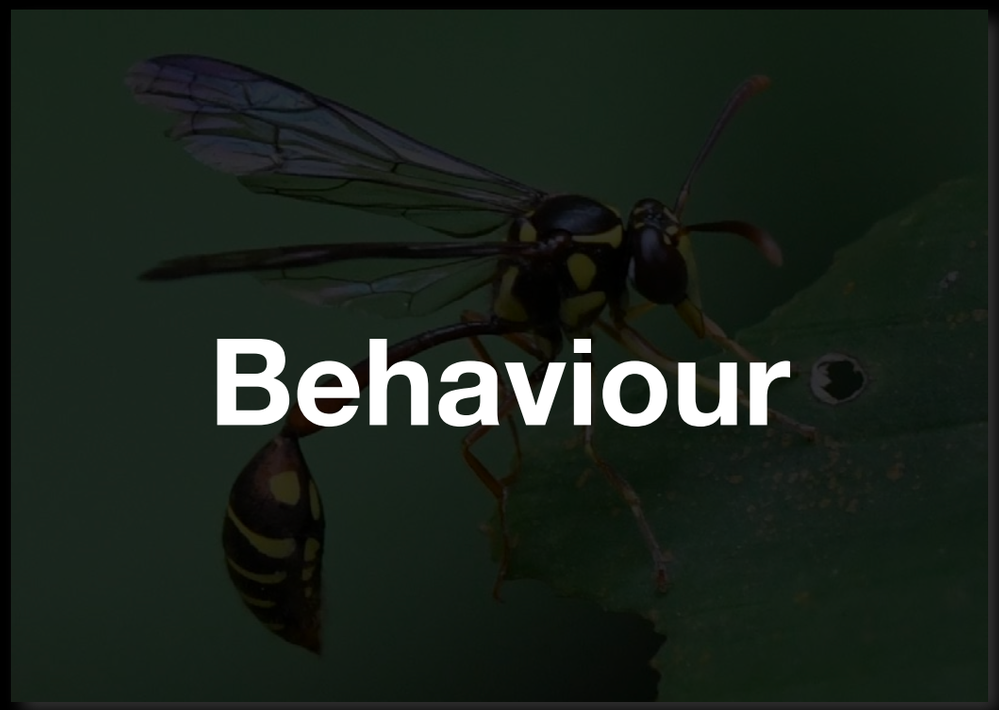


# *My long journey*

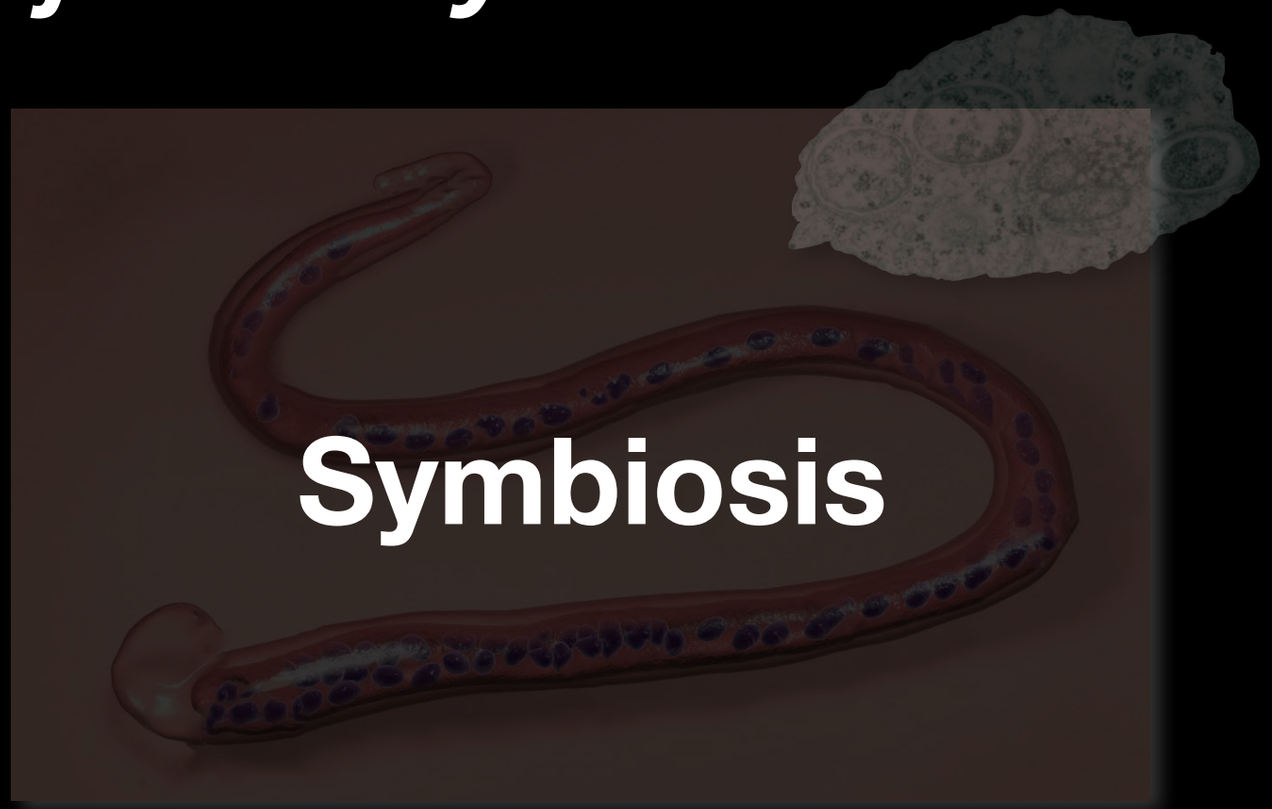
DNA BARCODING



# *My long journey*

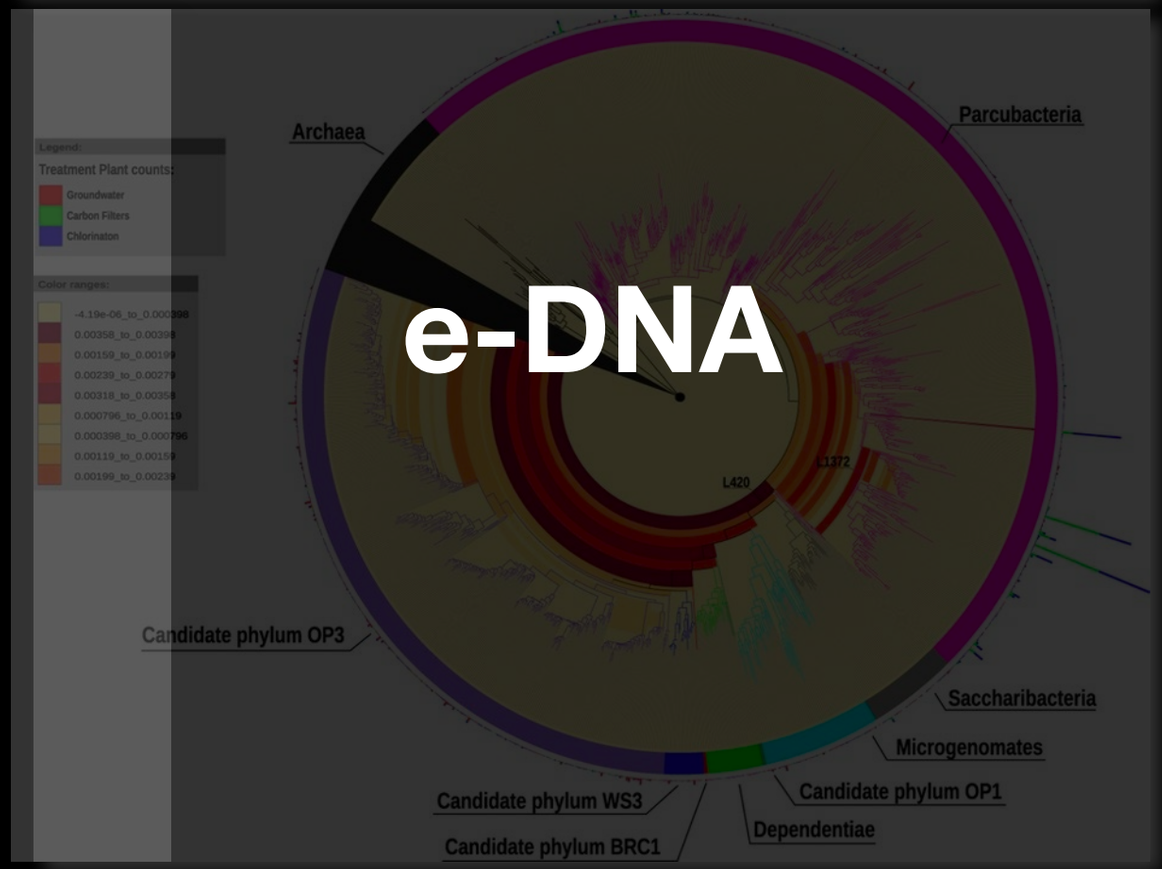


**Behaviour**



**Symbiosis**

**DNA**



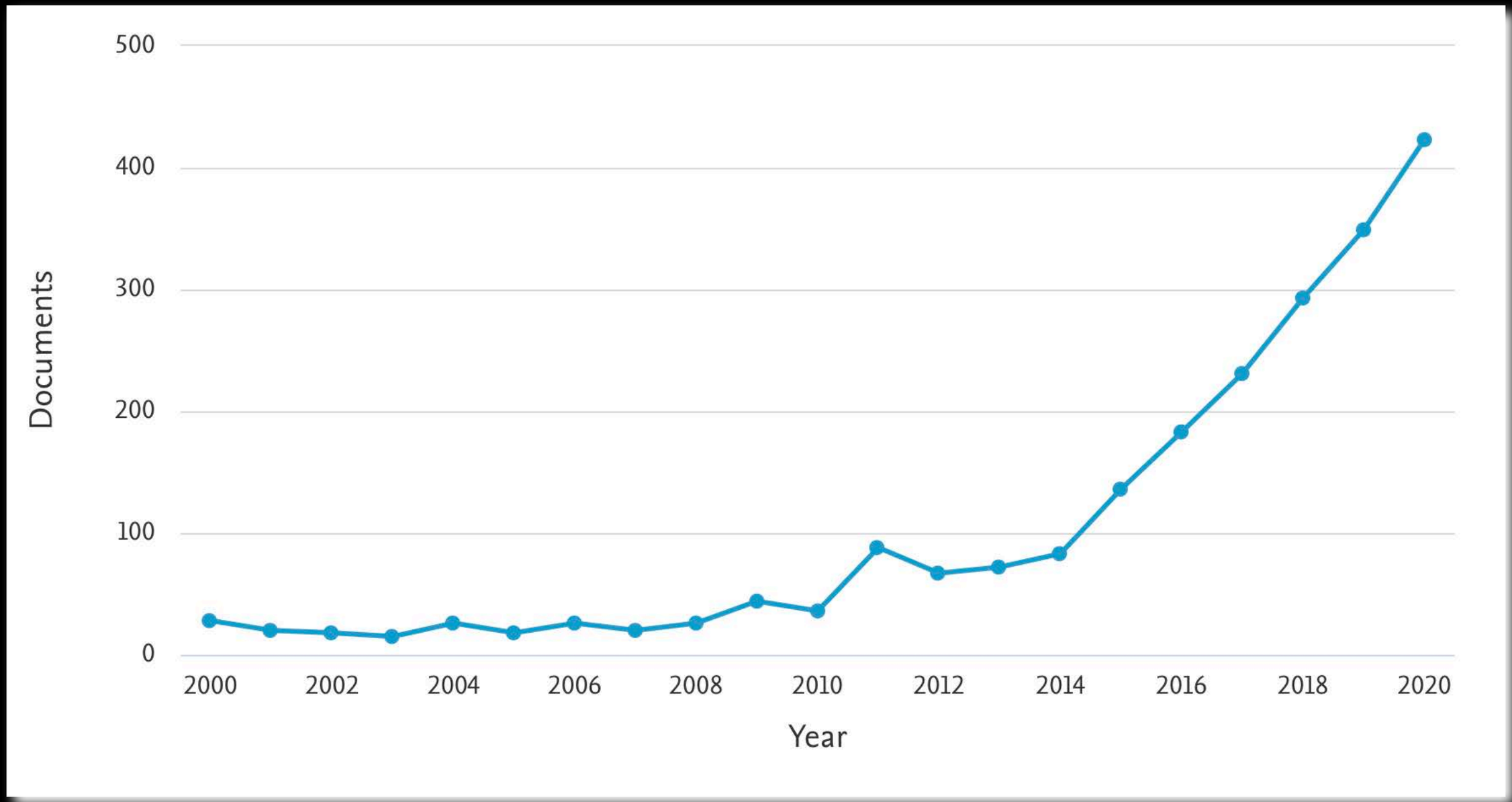

**e-DNA**

I think these are the
“right words” here today

I think these are the
“right words” here today

e-DNA

# eDNA in Scopus




www.scopus.com

# Six points tour into eDNA world

# 1. eDNA is not a tool by itself

Received: 8 June 2019 | Accepted: 22 October 2019

DOI: 10.1111/jfb.14177

FSBI SYMPOSIUM SPECIAL ISSUE REVIEW PAPER

JOURNAL OF FISH BIOLOGY fsbi

## Environmental DNA is not the tool by itself

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[2]Life Sciences, Natural History Museum, London, UK

**Correspondence**
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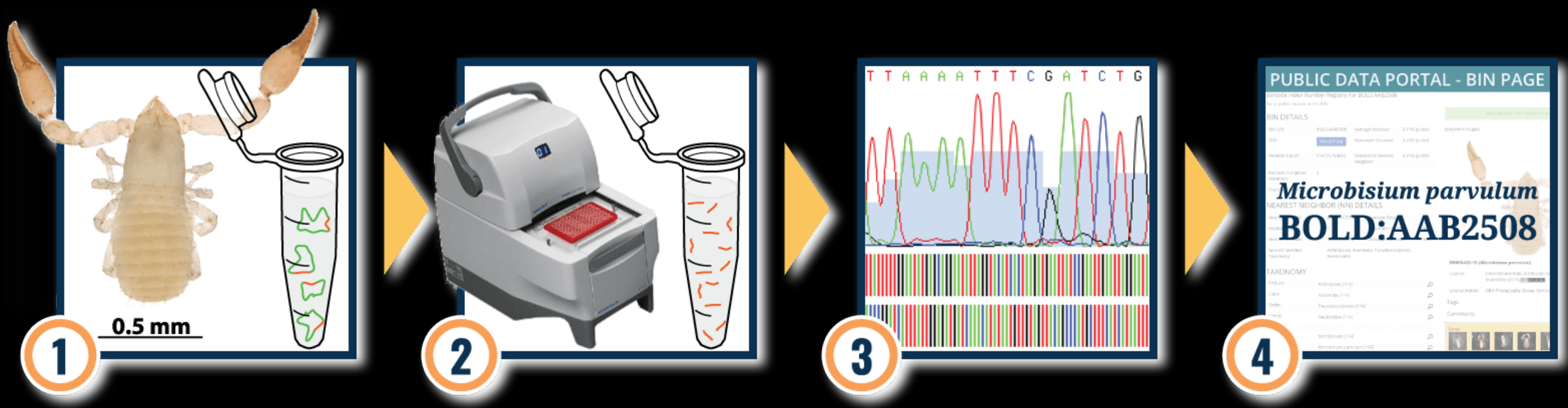
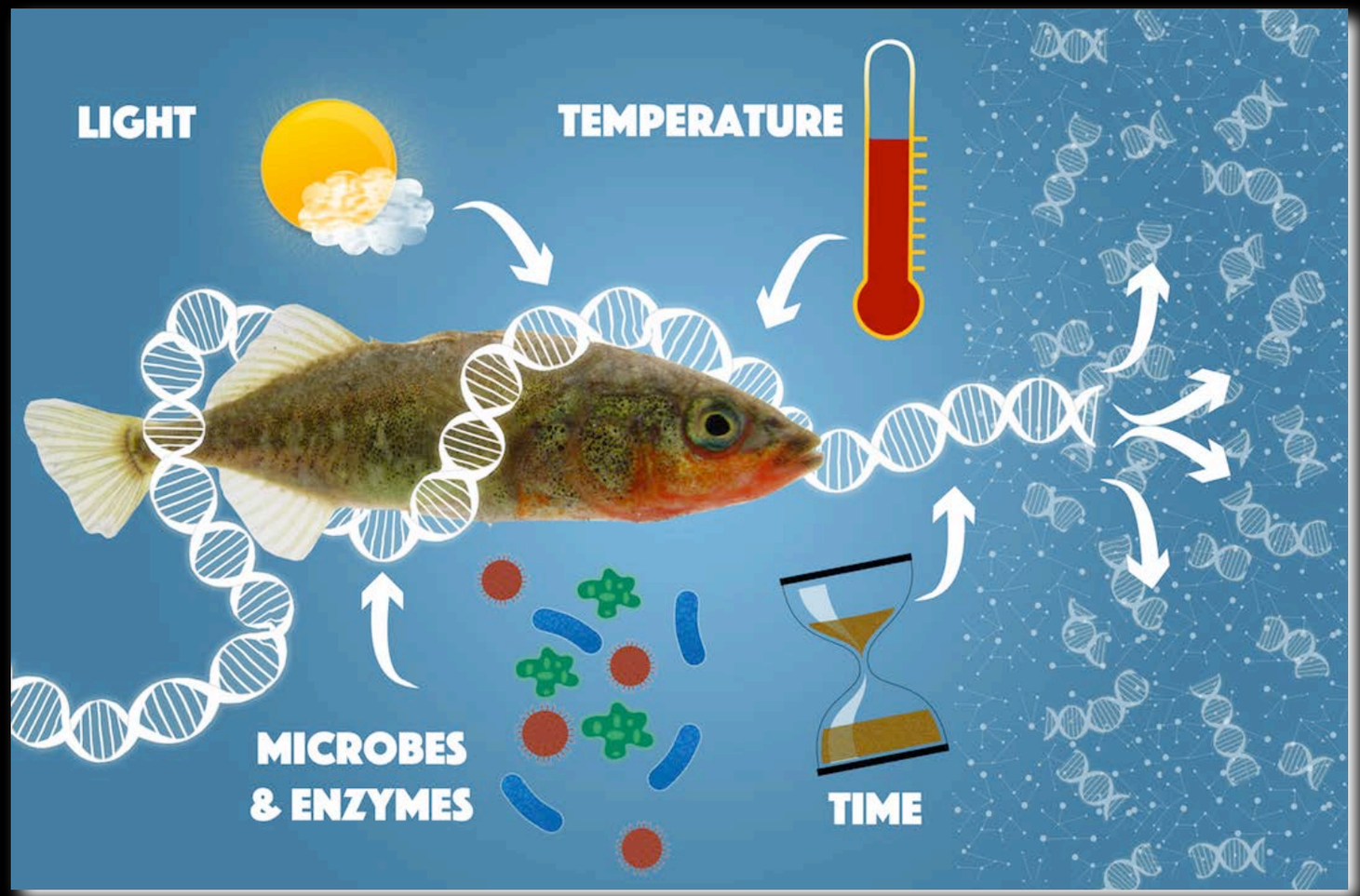
Kristy Deiner, Life Sciences, Natural History Museum, London, UK.
Email: alpinedna@gmail.com

*J Fish Biol.* 2019;1–4.

# At the beginning there was DNA barcoding



# eDNA is just DNA…




**eDNA:**
"DNA that can be extracted from environmental samples (such as soil, water or air), without first isolating any target organisms."

Molecular Ecology (2012) **21**, 1789–1793

## Environmental DNA

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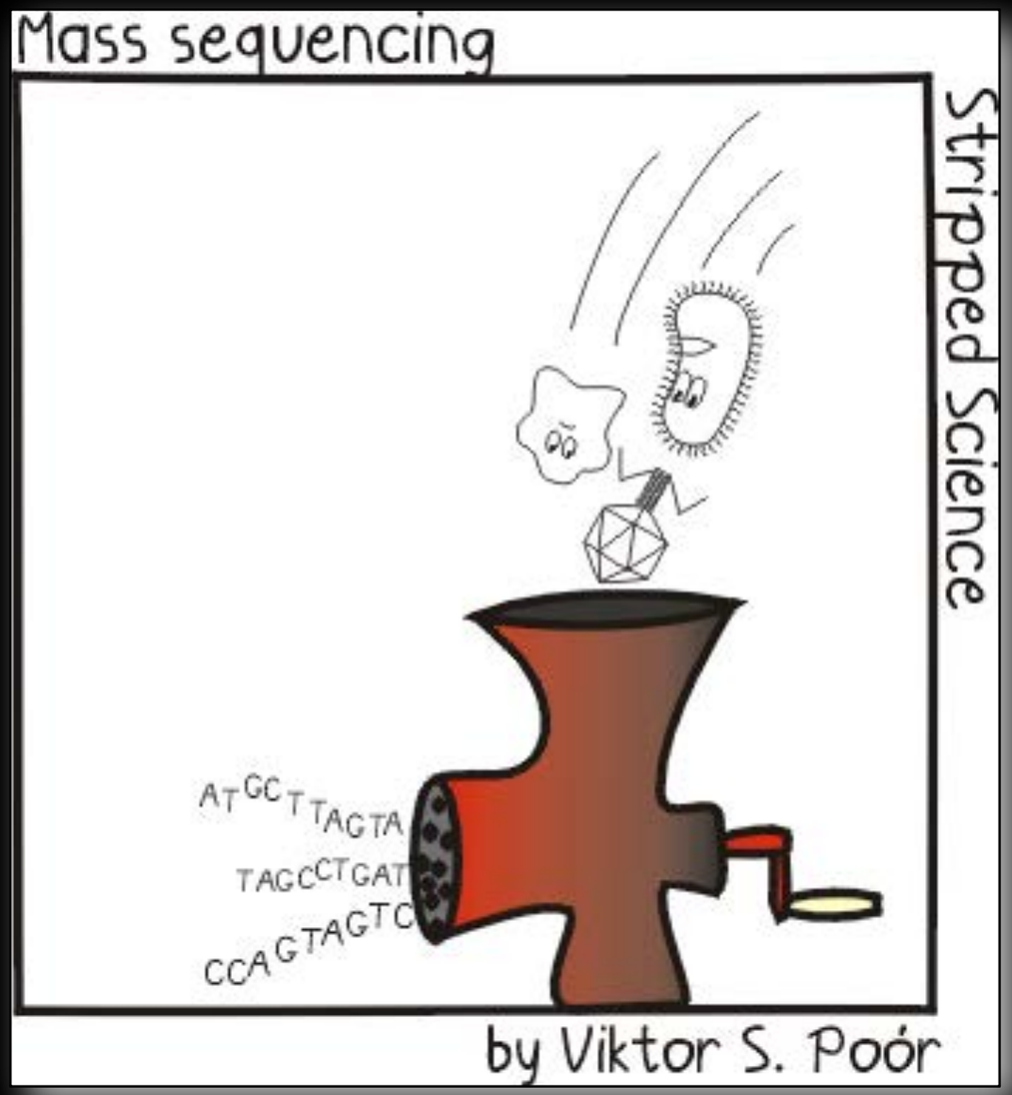
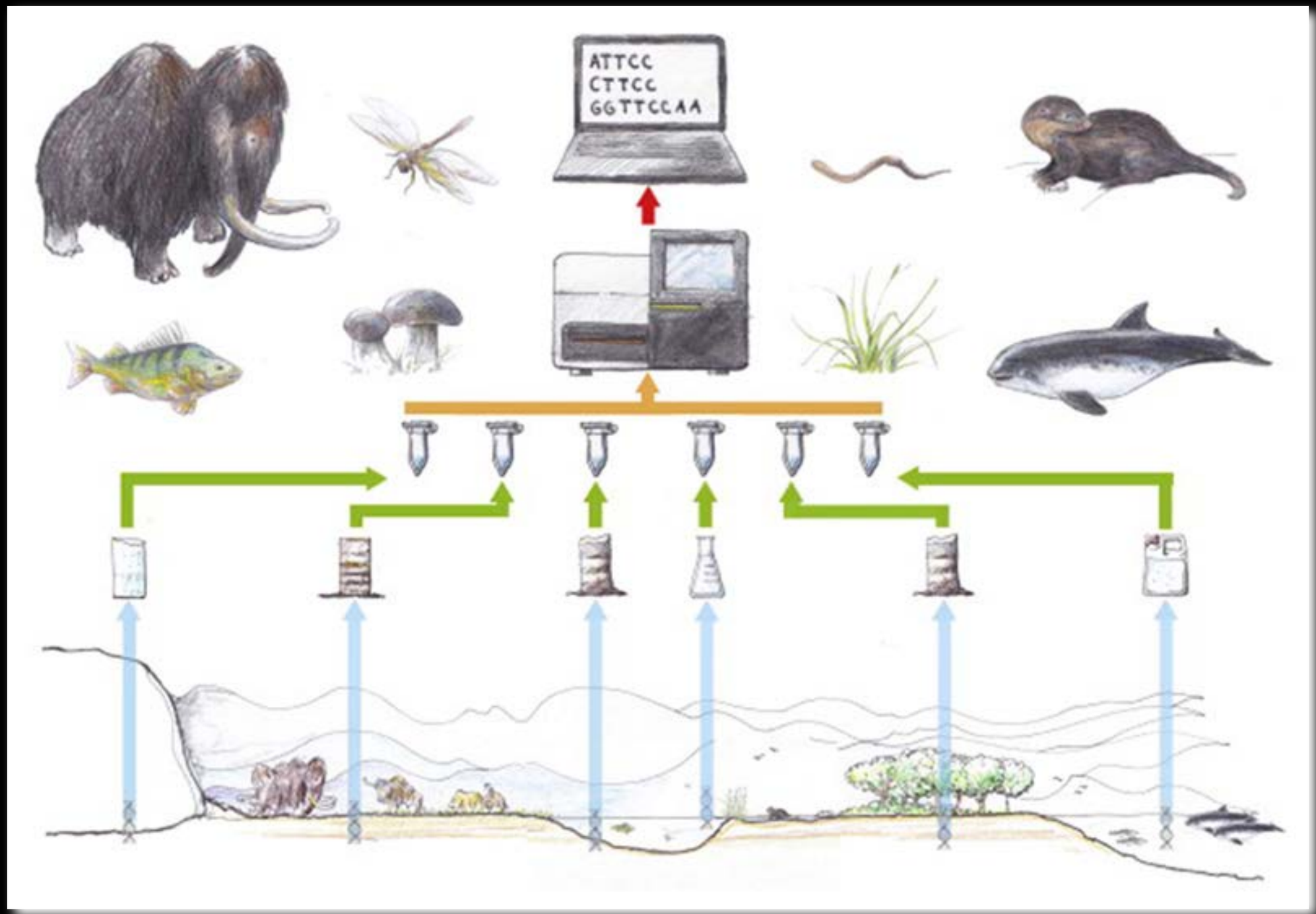
*Keywords*: DNA metabarcoding, environmental DNA (eDNA), next-generation sequencing

*Received 19 February 2012; revision accepted 19 February 2012*

# eDNA is just DNA…



# The analysis of eDNA is a plethora of methods, techniques, approaches

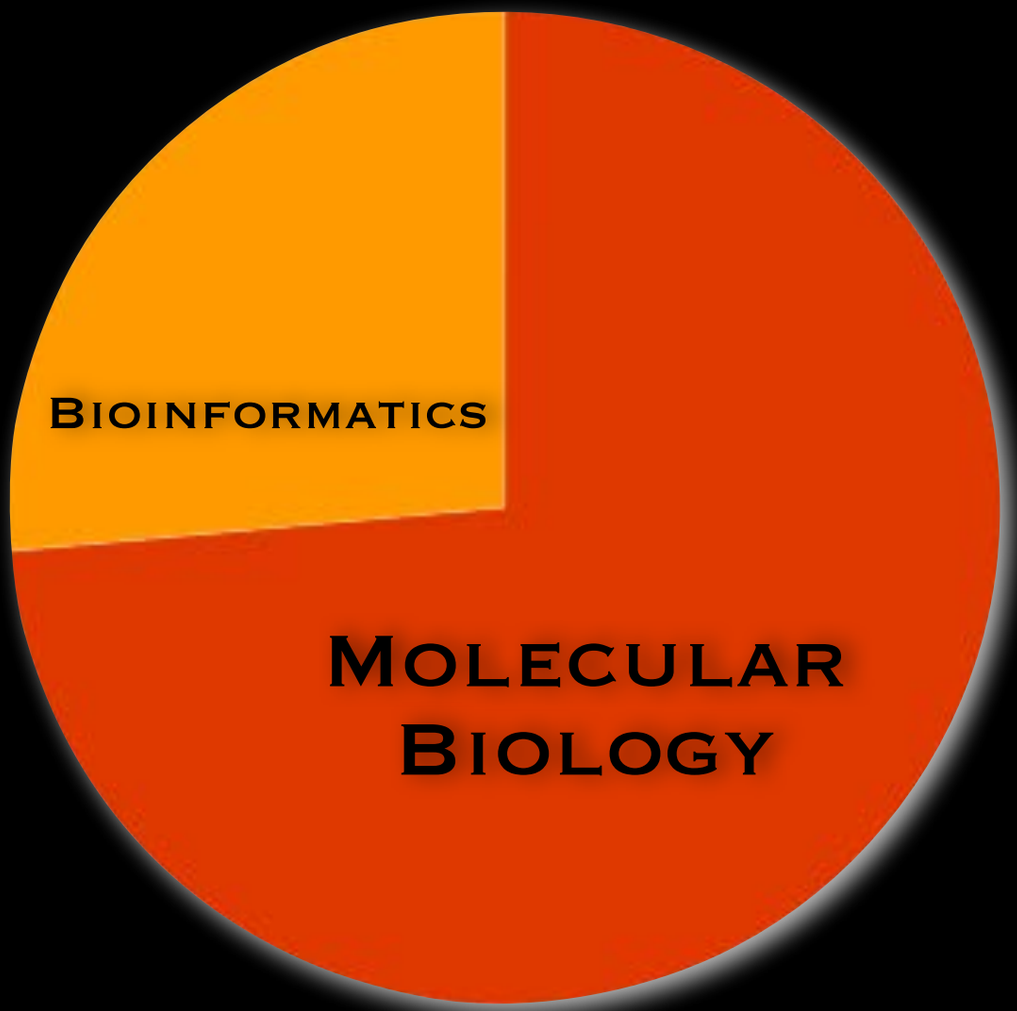



NGS: Next Generation DNA Sequencing

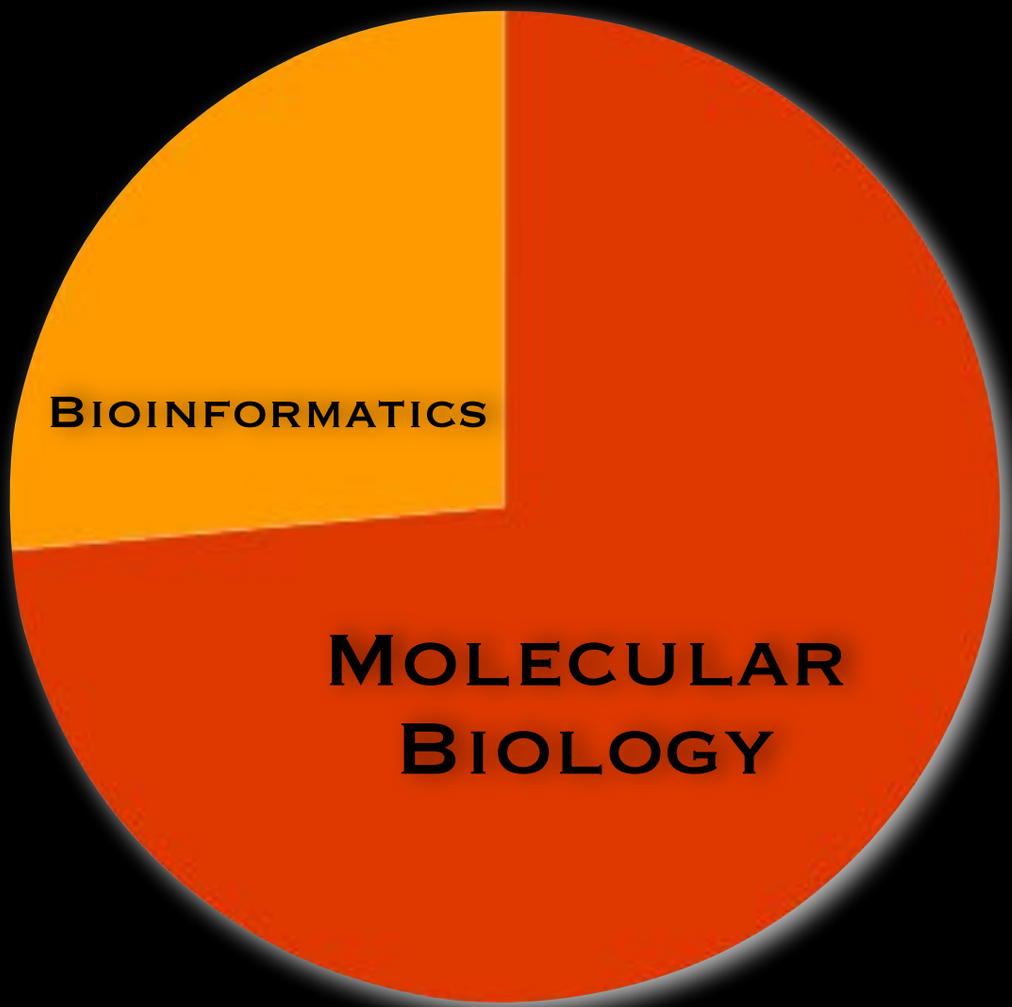
HTS: High Throughput DNA Sequencing

…these are the illumina®
DNA sequencers only!

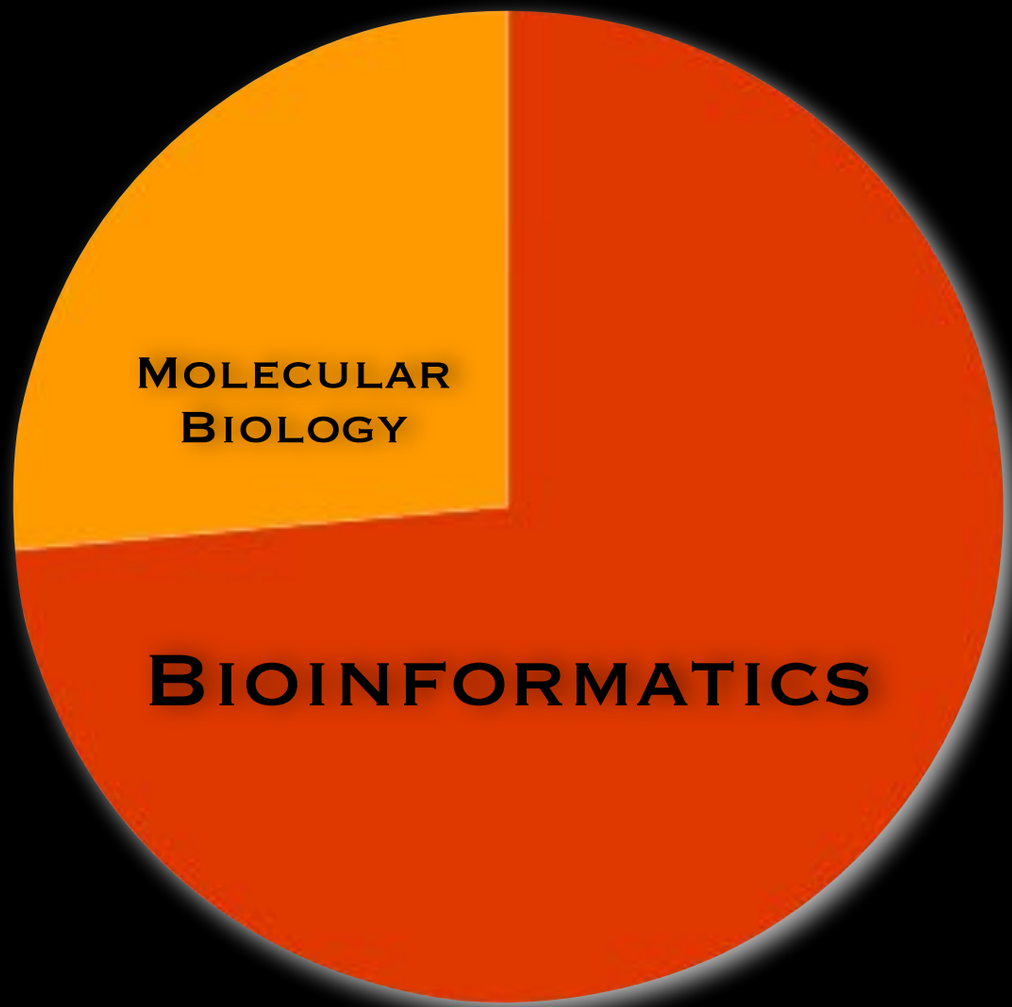

Nice...
We have so many options!
- Deep whole genome
- Low pass whole genome
- Deep whole exome
-Genomewide array
- Exome array
How would you like to be sequenced?
Genomic
Core
Facility
www.biocomicals.com




# Before



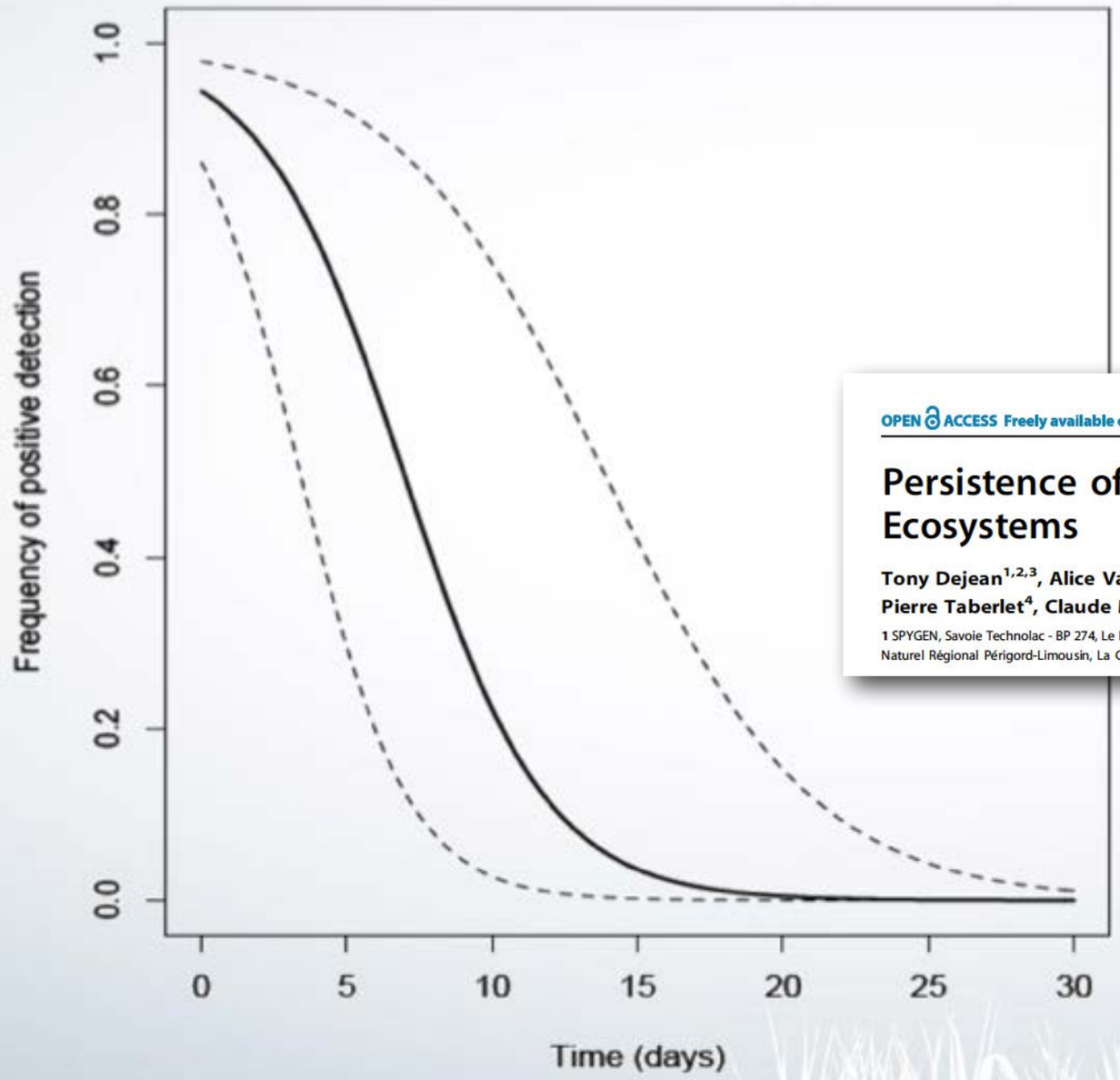

Before




Nowadays

# 2. What can I see in eDNA?

## How long does a DNA molecule persist in water?




OPEN ACCESS Freely available online

PLoS one

### Persistence of Environmental DNA in Freshwater Ecosystems

Tony Dejean[1,2,3], Alice Valentini[1,2], Antoine Duparc[2], Stéphanie Pellier-Cuit[4], François Pompanon[4], Pierre Taberlet[4], Claude Miaud[2]*

1 SPYGEN, Savoie Technolac - BP 274, Le Bourget-du-Lac, France, 2 Laboratoire d'Ecologie Alpine, UMR CNRS 5553, Université de Savoie, Le Bourget-du-Lac, France, 3 Parc Naturel Régional Périgord-Limousin, La Coquille, France, 4 Laboratoire d'Ecologie Alpine, UMR CNRS 5553, Université Grenoble I, Grenoble, France

Dejean et al. 2012

# 2. What can I see in eDNA?

**How long does a DNA molecule persist in soil?**

MOLECULAR ECOLOGY

Molecular Ecology (2012) **21**, 3647–3655 doi: 10.1111/j.1365-294X.2012.05545.x

FROM THE COVER

## DNA from soil mirrors plant taxonomic and growth form diversity

N. G. YOCCOZ,* K. A. BRÅTHEN,* L. GIELLY,† J. HAILE,‡§ M. E. EDWARDS,¶ T. GOSLAR,** H. VON STEDINGK,¶ A. K. BRYSTING,†† E. COISSAC,† F. POMPANON,† J. H. SØNSTEBØ,†† C. MIQUEL,† A. VALENTINI,† F. DE BELLO,†,‡‡ J. CHAVE,§§ W. THUILLER,† P. WINCKER,¶¶ C. CRUAUD,¶¶ F. GAVORY,¶¶ M. RASMUSSEN,‡ M. T. P. GILBERT,‡ L. ORLANDO‡ C. BROCHMANN,††[1] E. WILLERSLEV,‡[1] and P. TABERLET,†[1]

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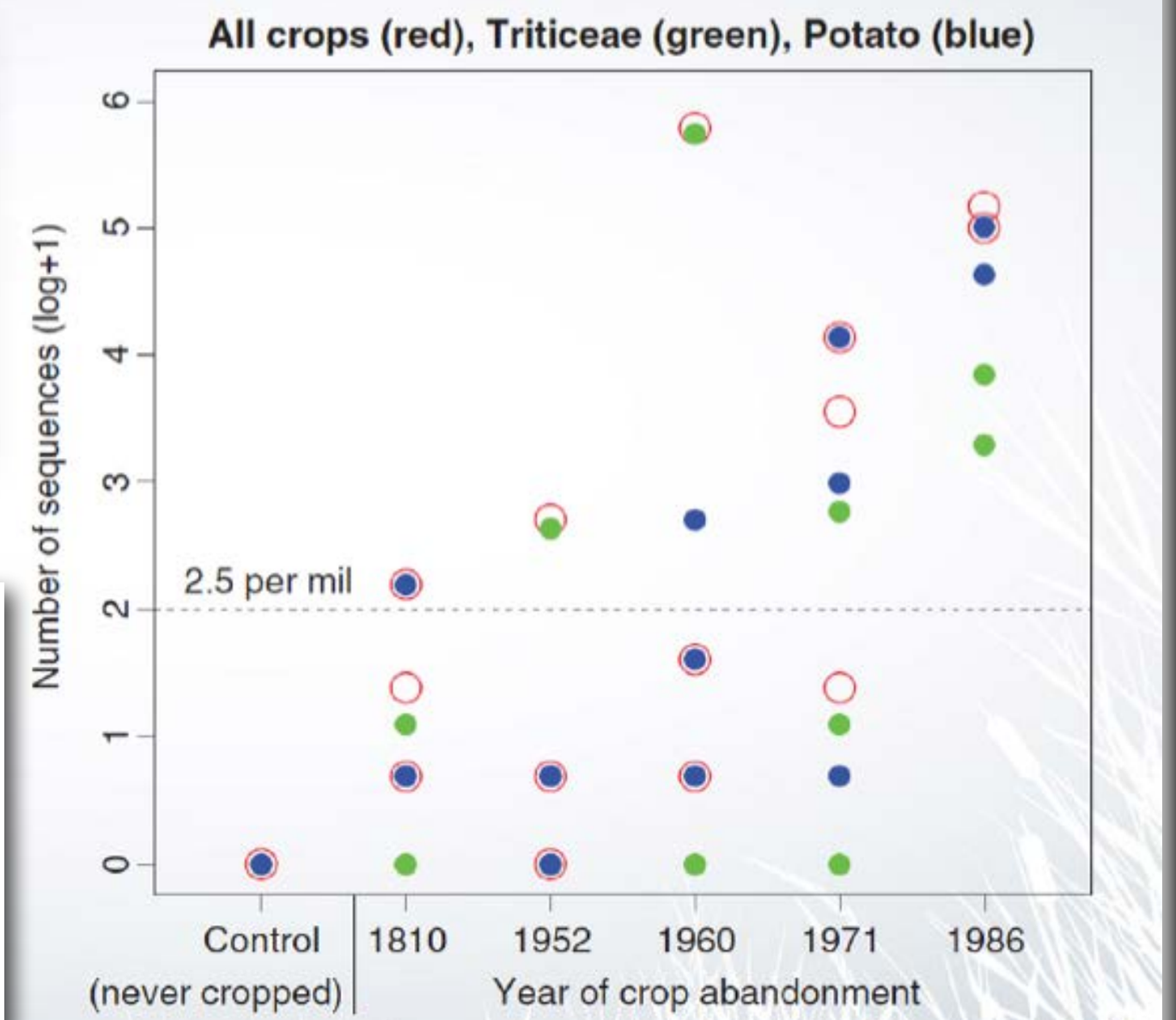


So there is a warning:
You are working on DNA,
not with organisms.
Be very careful before drawing
your conclusions

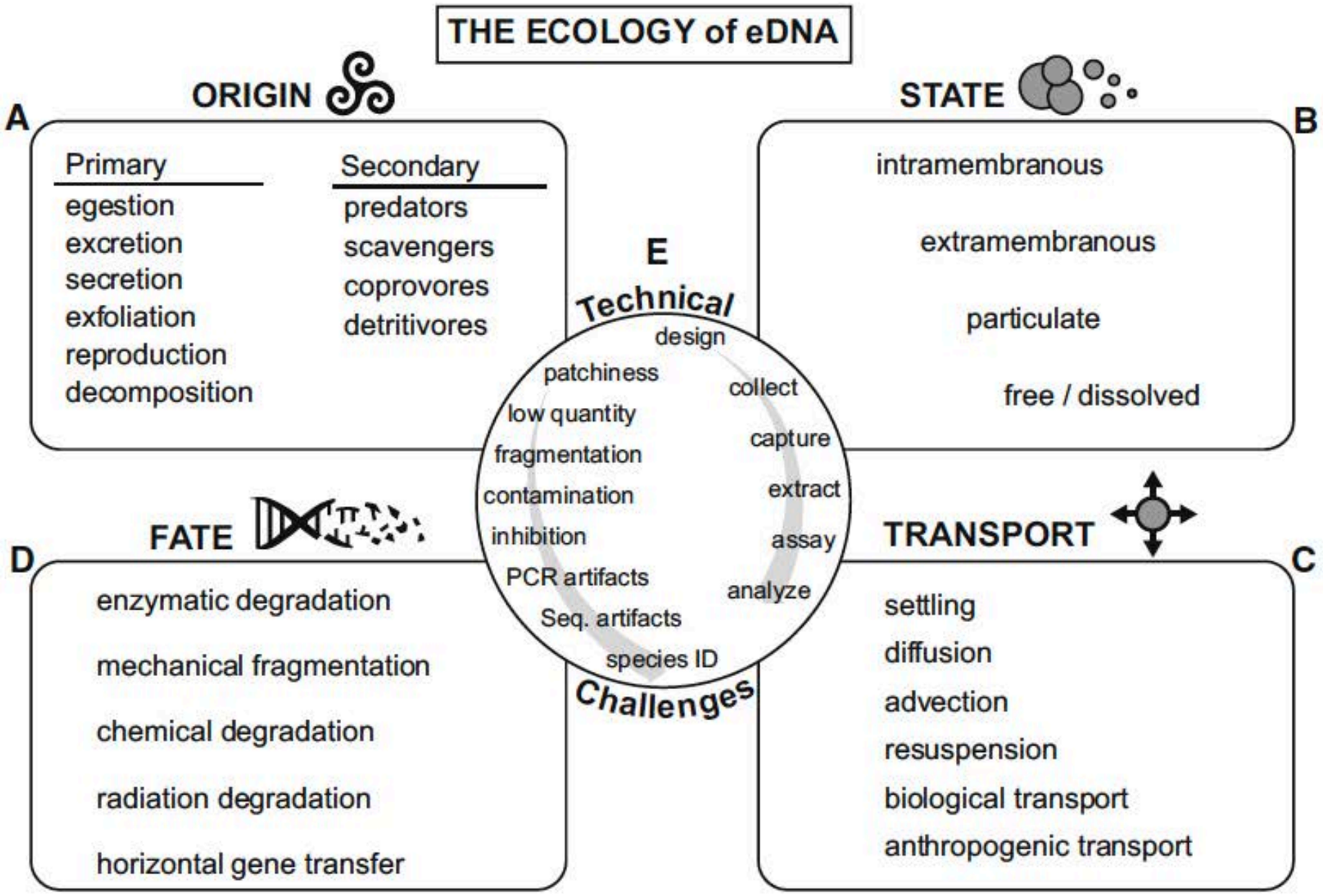



**Fig. 1** Processes and properties within four domains of eDNA ecology (**a**–**d**) and key technical challenges (**e**) can guide eDNA conservation and research applications

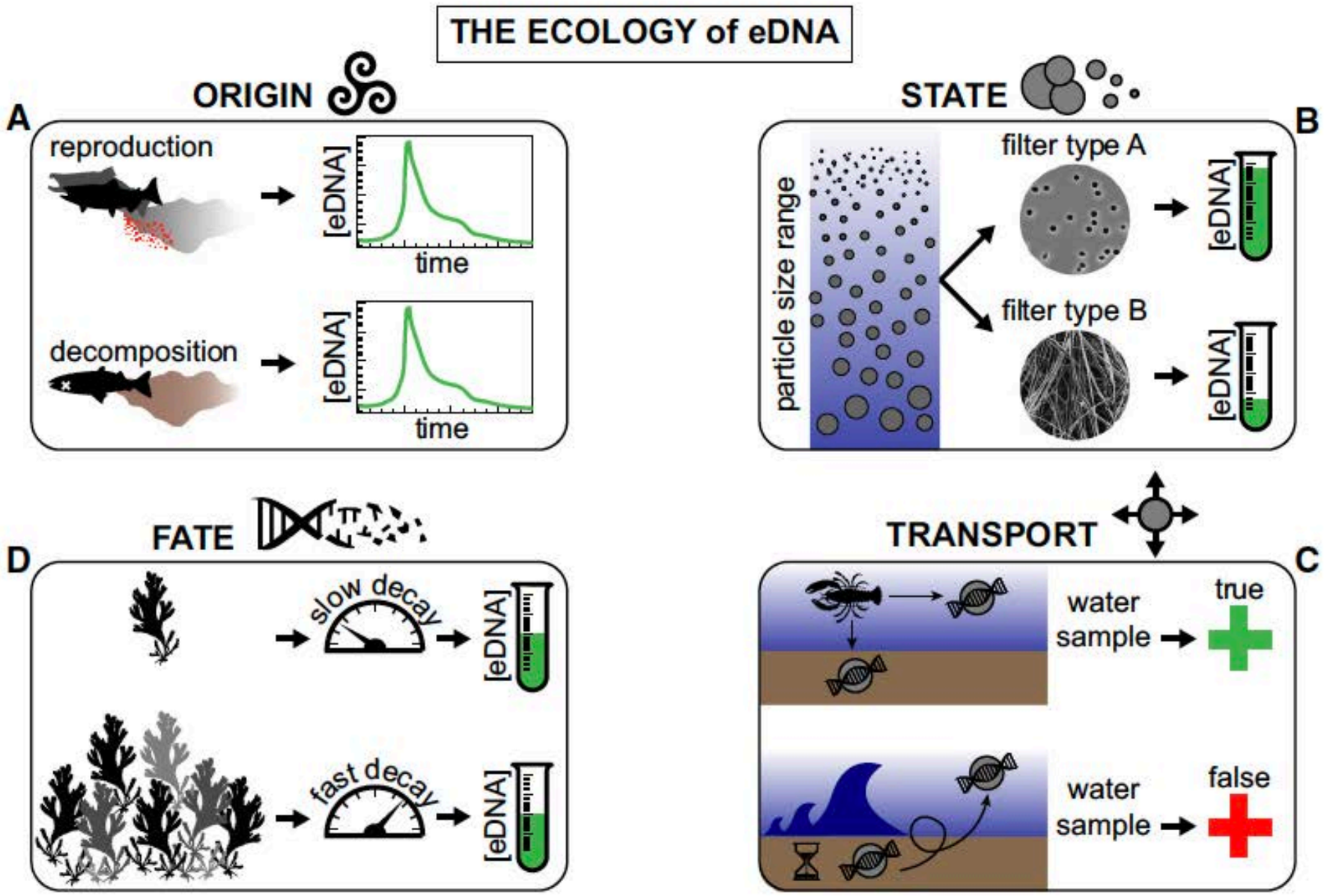



**Fig. 2** eDNA ecology affects population inferences. **a** eDNA from reproduction and decomposition could produce similar temporal patterns despite different origins. **b** Different filter types could yield different eDNA concentrations that reflect particle size classes rather than population size differences. **c** Resuspension of old sedimentary eDNA could produce false inferences of presence after organisms are gone. **d** Different environmentally-mediated eDNA decay rates could confound inferences about population size or biomass from eDNA concentration

# 3. Plan researches “like an engineer”


~2009


*“We wanna do some eDNA work…”*

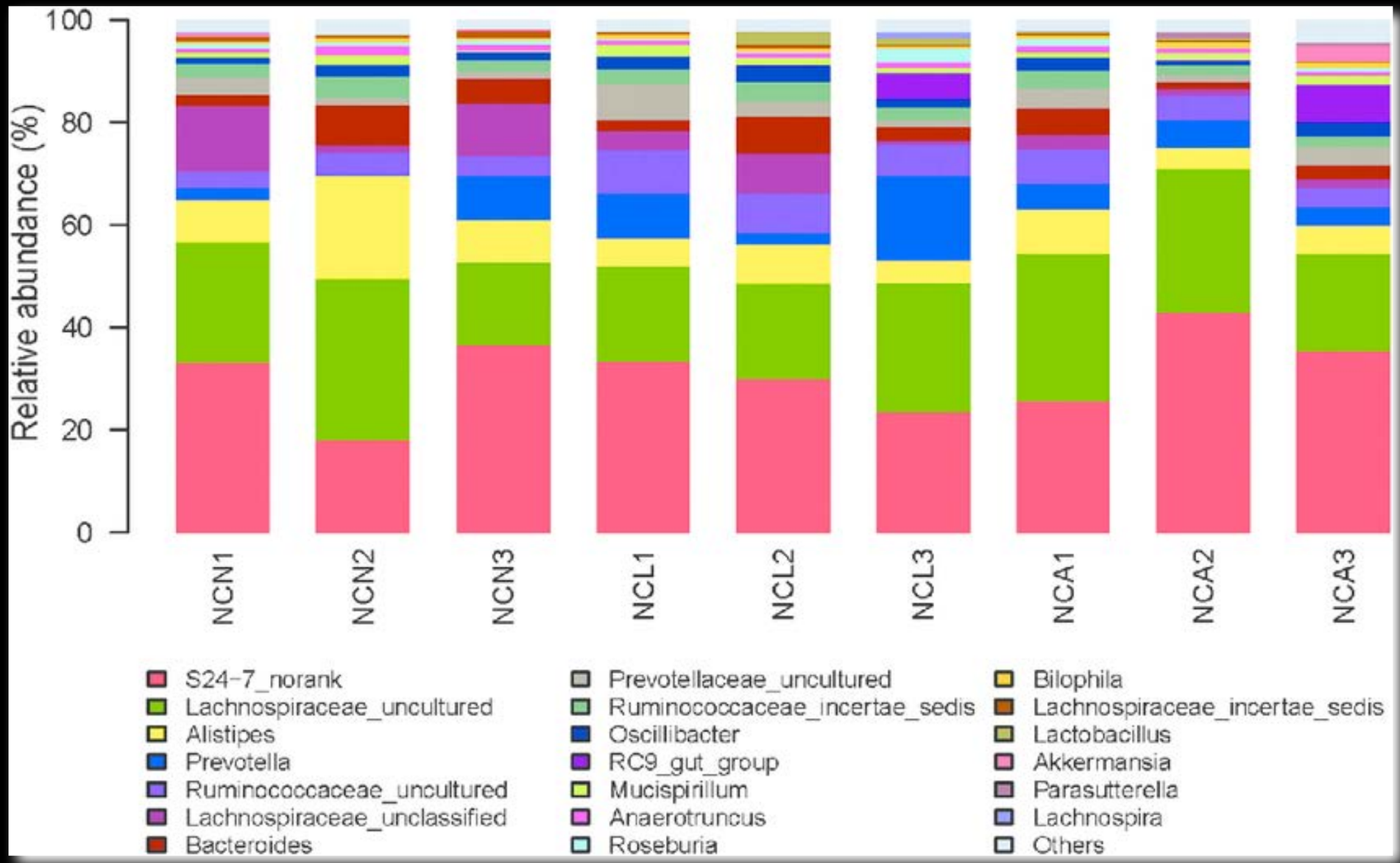
# ~2010


milk

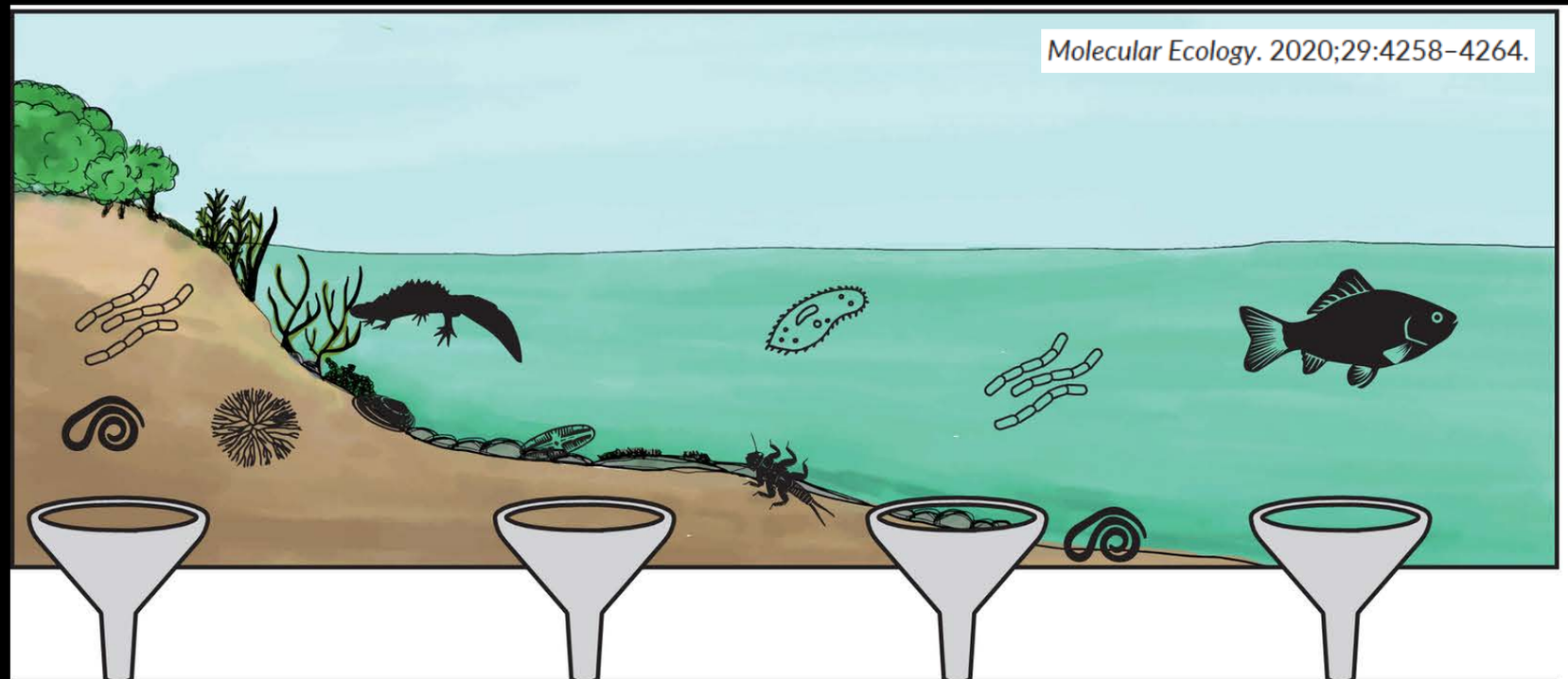
process

maturation


GRANA
PADANO
G
P


Relative abundance (%)
100
80
60
40
20
0
NCN1
NCN2
NCN3
NCL1
NCL2
NCL3
NCA1
NCA2
NCA3
S24−7_norank
Lachnospiraceae_uncultured
Alistipes
Prevotella
Ruminococcaceae_uncultured
Lachnospiraceae_unclassified
Bacteroides
Prevotellaceae_uncultured
Ruminococcaceae_incertae_sedis
Oscillibacter
RC9_gut_group
Mucispirillum
Anaerotruncus
Roseburia
Bilophila
Lachnospiraceae_incertae_sedis
Lactobacillus
Akkermansia
Parasutterella
Lachnospira
Others

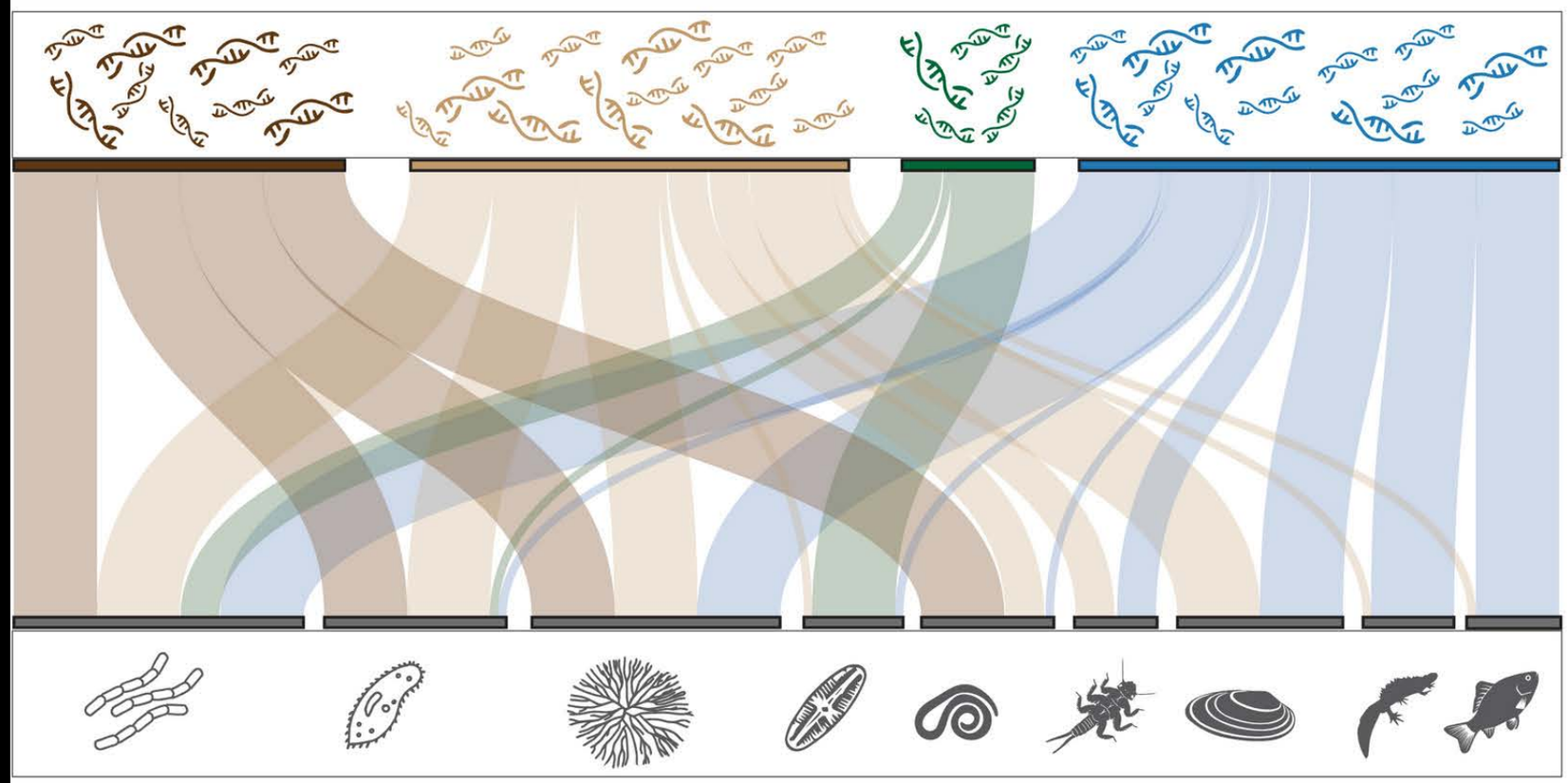
*“Too much information.*
*We do not need all of this.*
*We were interested in*
Lactobacillus *only…”*




**Soil** **Sediment** **Biofilm** **Water**

**Target environments**
*e.g. «Water eDNA»*

**Level 1**



**DNA Extraction**

**PCR**

**Target taxa**
*e.g. «Fish eDNA»*

**Level 2**

# 4. At the beginning it was presence/absence on bacteria…



*Molecular Ecology*. 2020;29:4258–4264.

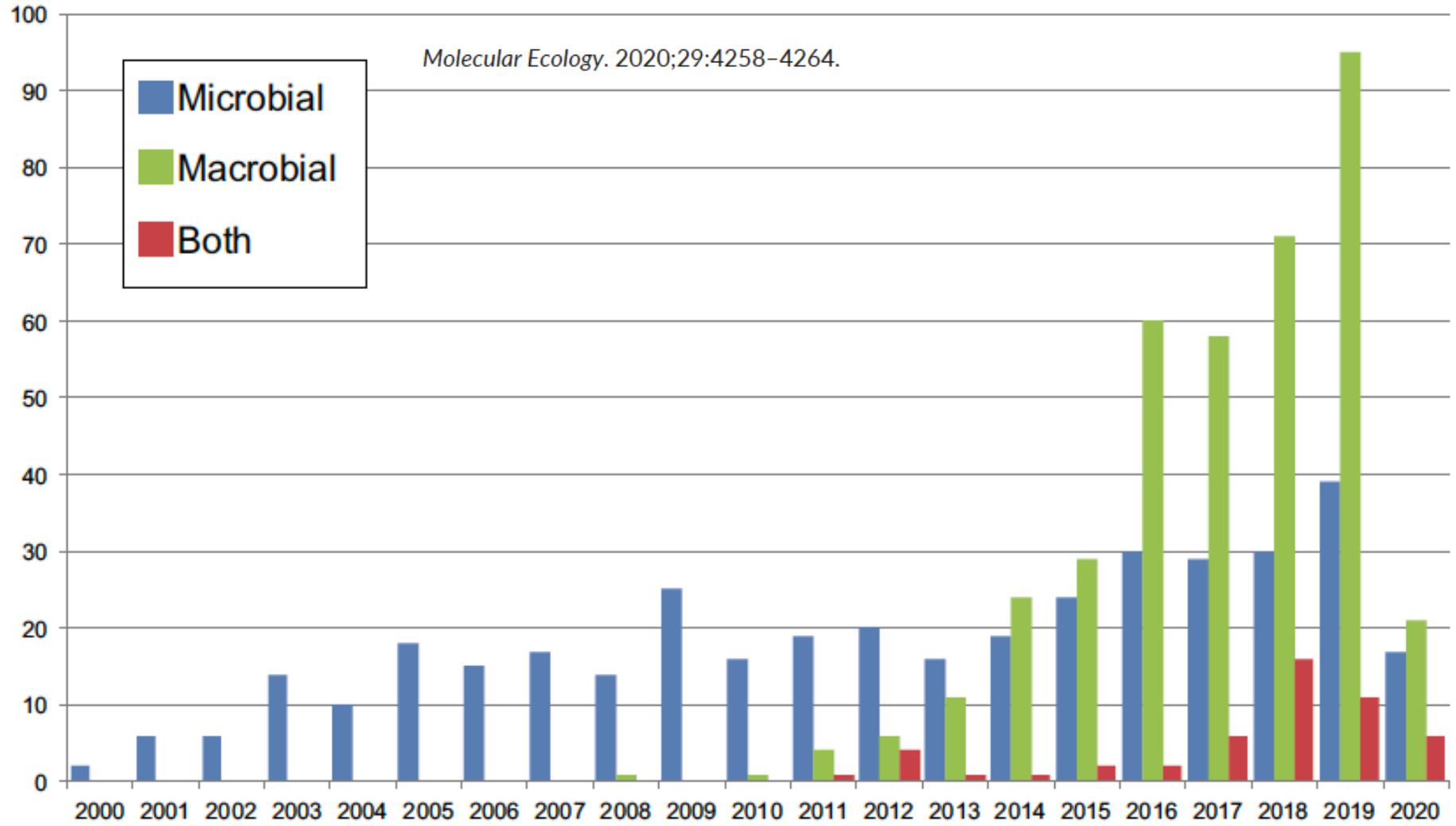



**FIGURE 1** The number of publications by years referring to environmental DNA studies targeting microbial diversity, macrobial diversity or both. Microbial diversity encompasses bacterial and viral diversity as well as eukaryotic micro- and meiofauna. The figure is based on a PubMed NCBI search (on May 5, 2020) of titles and abstract containing the term "Environmental DNA," excluding studies containing "medical" or "cancer." This resulted in 1,009 papers. After manual inspection, 192 papers were removed from this list because they clearly were outside a biodiversity context. The full list of all papers considered is available upon request [Colour figure can be viewed at wileyonlinelibrary.com]

# …then the macrobial life

biology letters

*Biol. Lett.* (2008) 4, 423–425
doi:10.1098/rsbl.2008.0118
*Published online* 9 April 2008

Population genetics

## Species detection using environmental DNA from water samples

**Gentile Francesco Ficetola[1,2,*], Claude Miaud[2], François Pompanon[1] and Pierre Taberlet[1]**

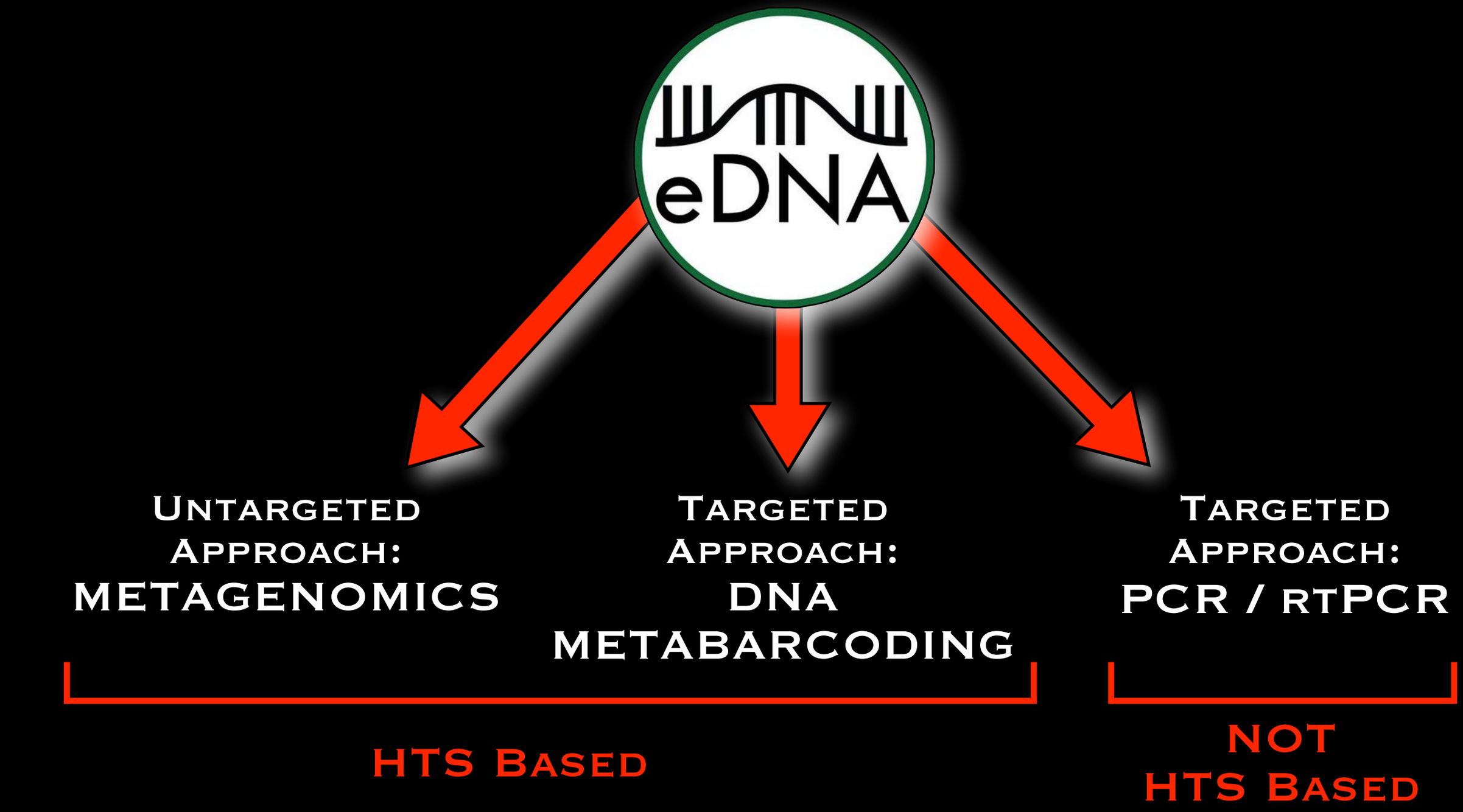
[1]*Laboratoire d'Ecologie Alpine, CNRS-UMR 5553, Université Joseph Fourier, BP 53, 38041 Grenoble Cedex 09, France*
[2]*Laboratoire d'Ecologie Alpine, CNRS-UMR 5553, Université de Savoie, 73376 Le Bourget du Lac Cedex, France*
**Author and address for correspondence: Dipartimento di Scienze dell'Ambiente e del Territorio, Università Milano Bicocca, Piazza della Scienza 1, 20126 Milano, Italy (francesco.ficetola@unimi.it).*

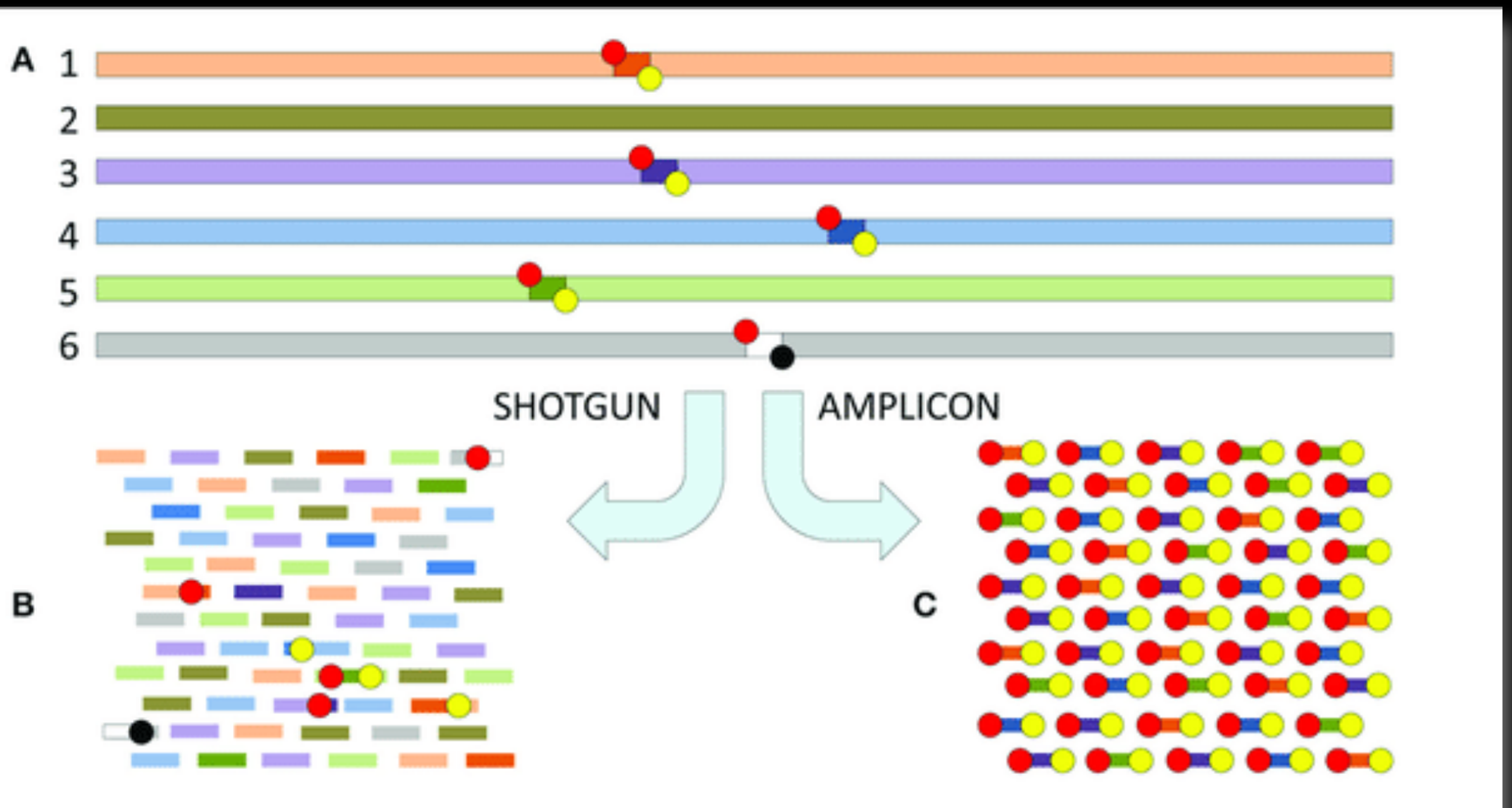
American bullfrog (*Lithobates catesbeianus*)

<u>But there is a caveat:</u>

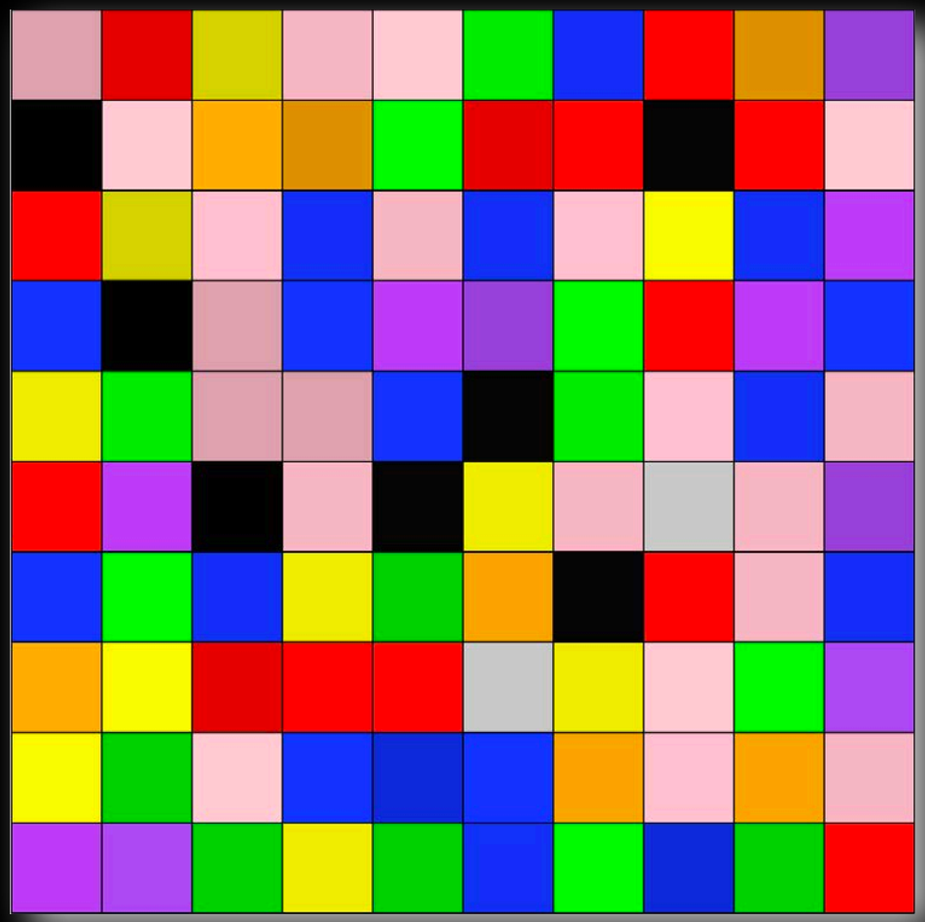
Working on macrobial leads to some limitations to the possible eDNA approaches

# 5. DNA metabarcoding *vs*. metagenomics



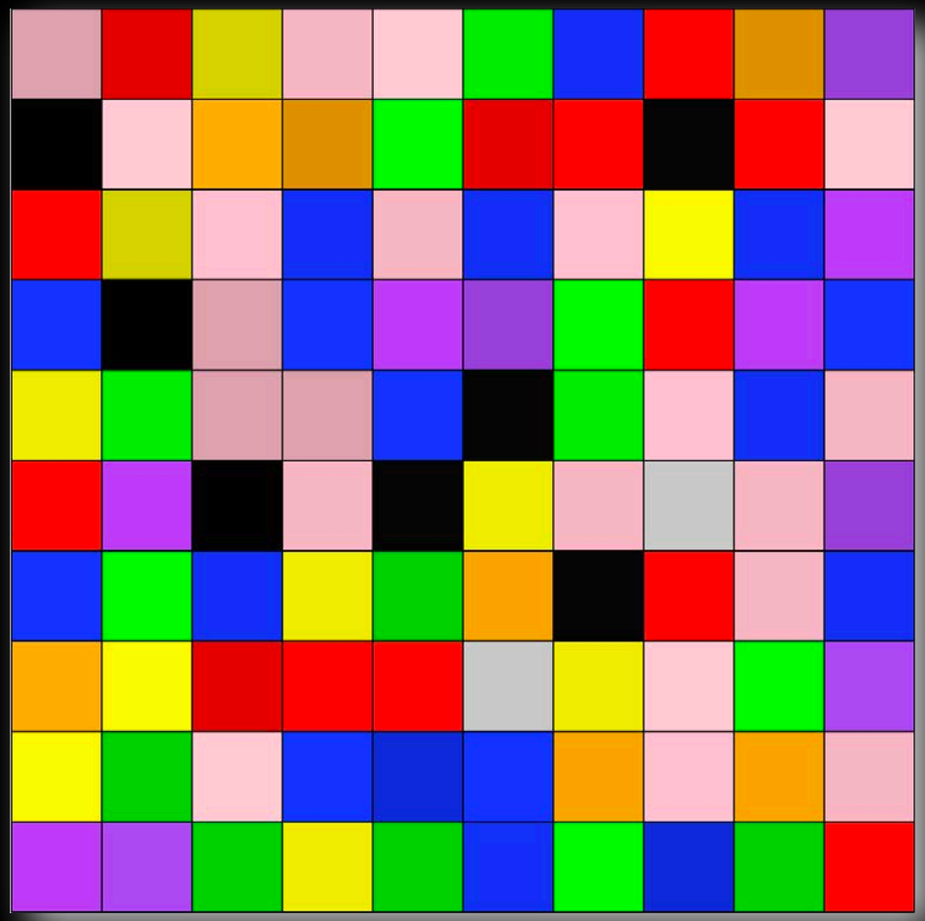



The shotgun approaches targeting macrobial life is much more complicated (and expensive)

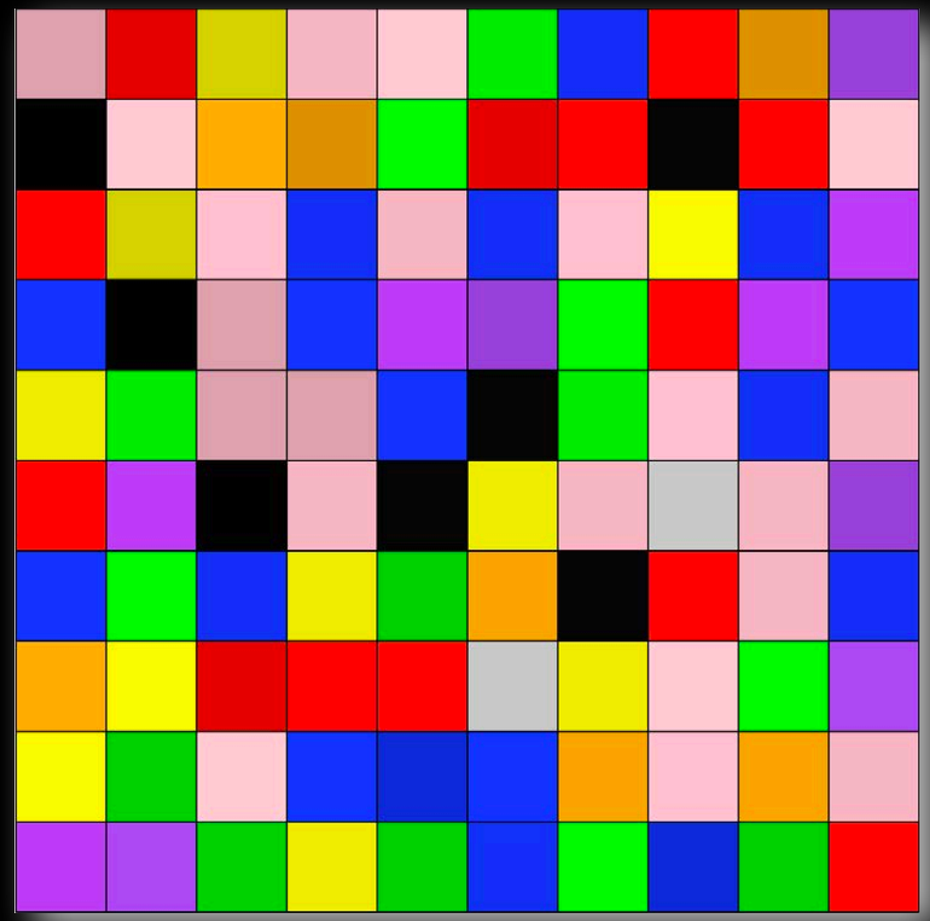


100 x 1.000 reads

1 Genome

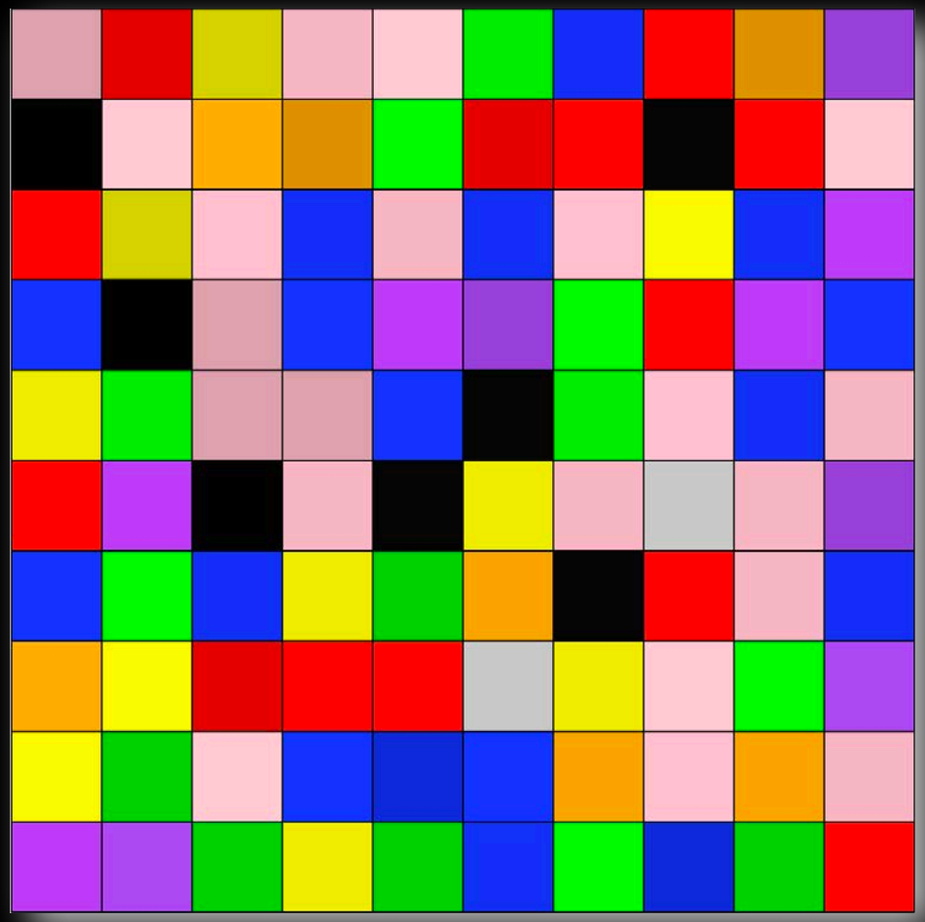


100 x 1.000 reads

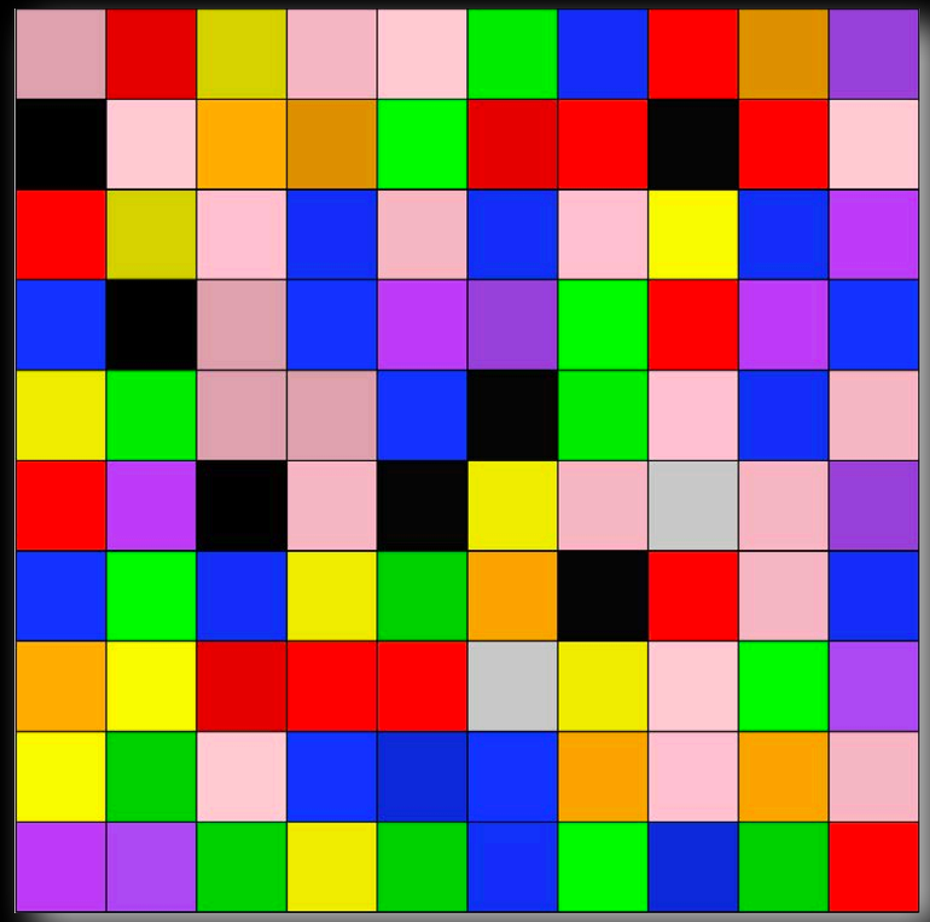


100 x 1.000 reads

1 Metagenome

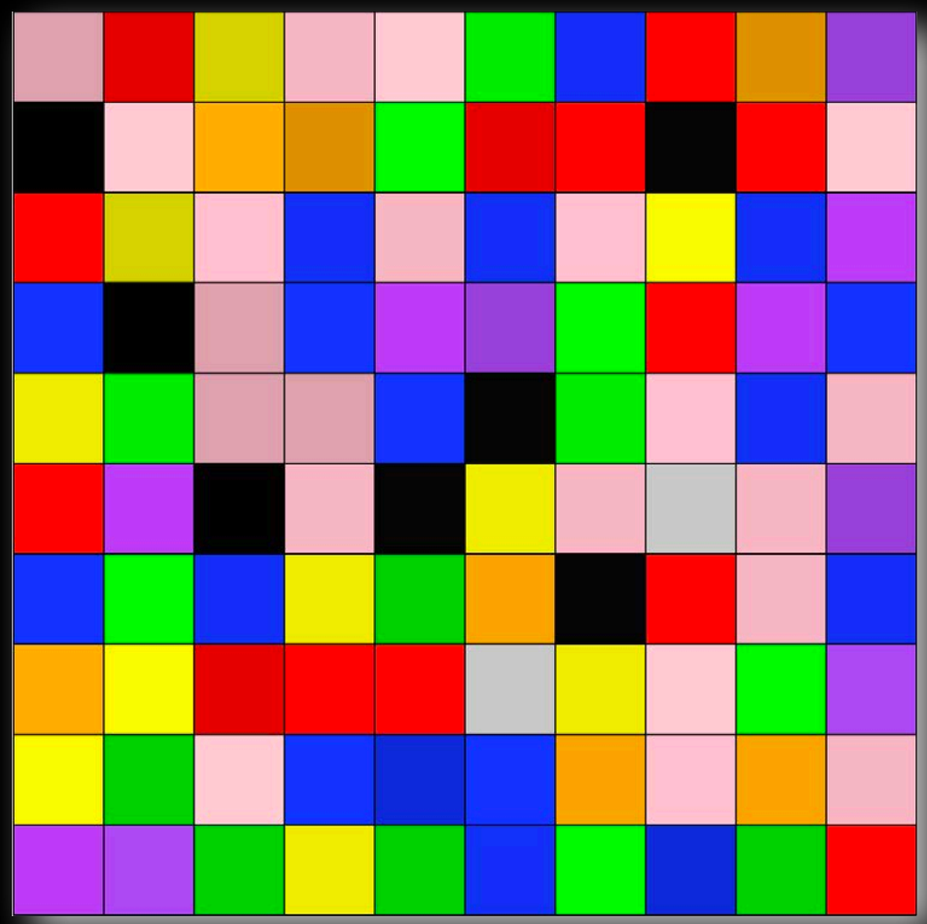


100 x 1.000 reads

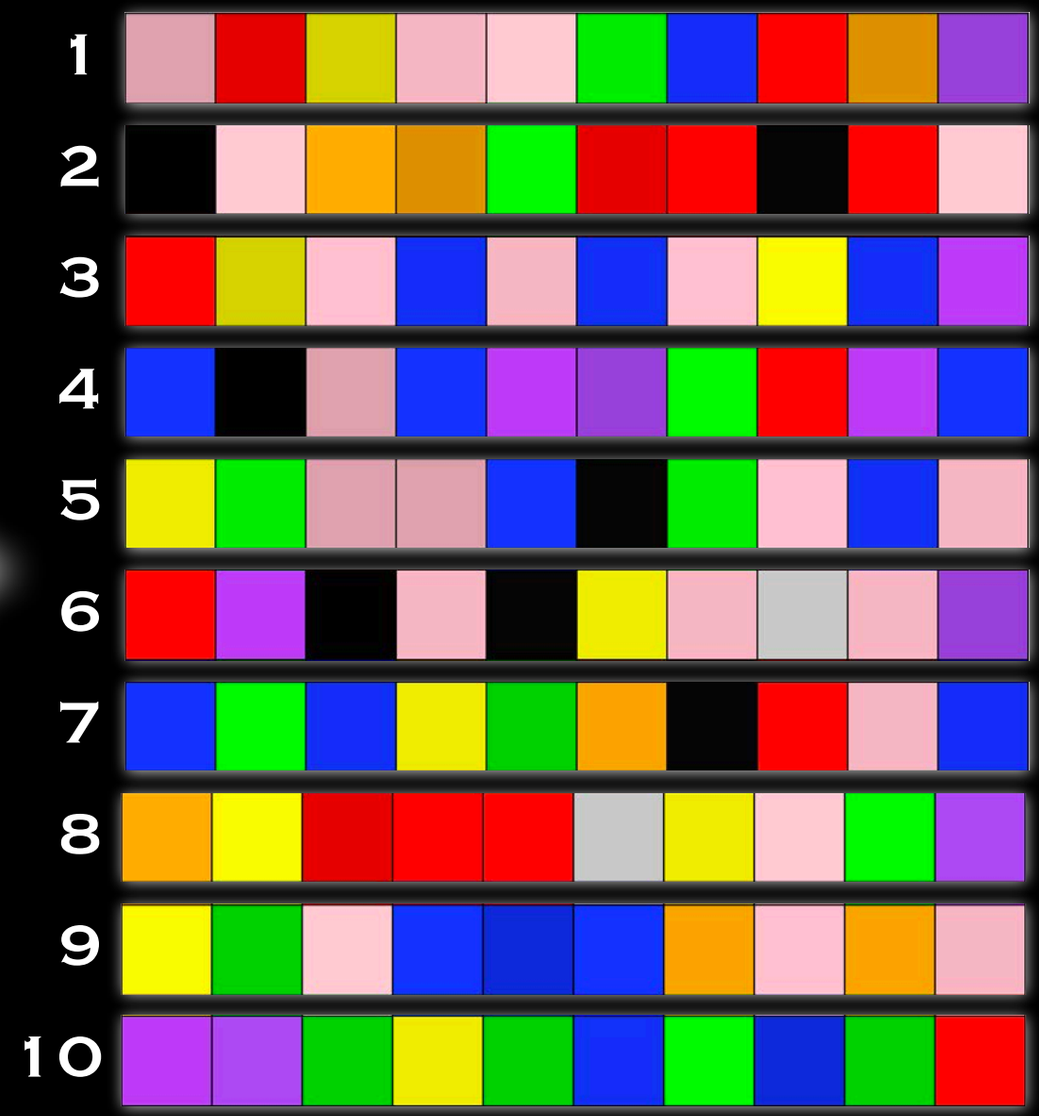


100 x 1.000 reads

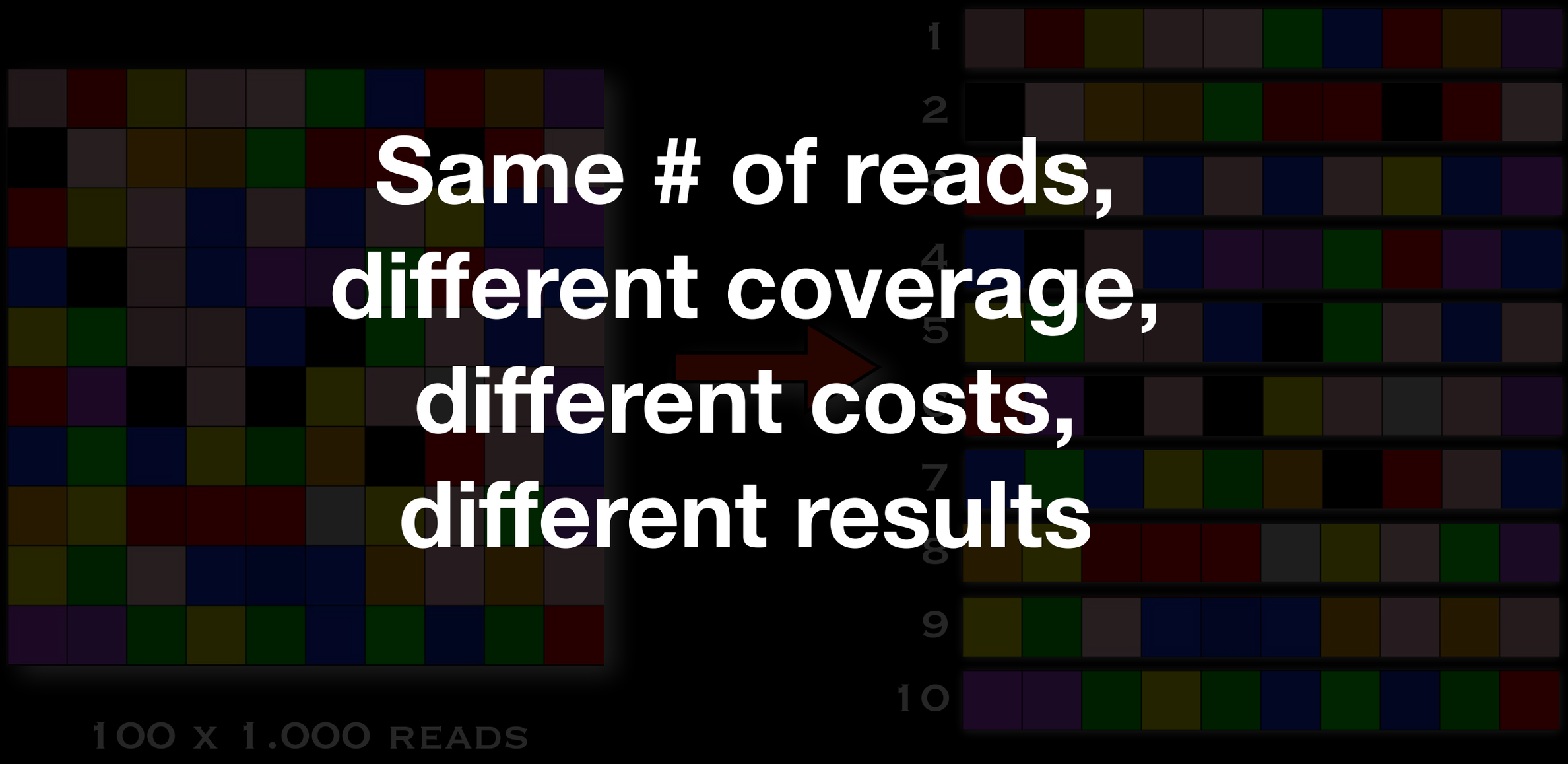
10 Genomes /
Metagenomes



100 x 1.000 reads




100 x 1.000 reads




# Same # of reads, different coverage, different costs, different results

# 6. eDNA as a barometer of anthropogenic pressure

Science of the Total Environment 637–638 (2018) 1295–1310

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]

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[e] LifeWatch, Italy and CNR-Institute of Ecosystem Study (CNR-ISE), Largo Tonolli 50, 28922 Verbania Pallanza, Italy
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[r] Department of Biological and Environmental Sciences and Technologies, University of Salento, S.P. Lecce-Monteroni, 73100 Lecce, Italy
[s] Israel Oceanographic and Limnological Research, Tel- Shikmona, Haifa 31080, Israel
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# 6. eDNA as a barometer of anthropogenic pressure

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## Environmental DNA can act as a biodiversity barometer of anthropogenic pressures in coastal ecosystems

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Loss of biodiversity from lower to upper trophic levels reduces overall productivity and stability of coastal ecosystems in our oceans, but rarely are these changes documented across both time and space. The characterisation of environmental DNA (eDNA) from sediment and seawater using metabarcoding offers a powerful molecular lens to observe marine biota and provides a series of 'snapshots' across a broad spectrum of eukaryotic organisms. Using these next-generation tools and downstream analytical innovations including machine learning sequence assignment algorithms and co-occurrence network analyses, we examined how anthropogenic pressures may have impacted marine biodiversity on subtropical coral reefs in Okinawa, Japan. Based on 18 S ribosomal RNA, but not ITS2 sequence data due to inconsistent amplification for this marker, as well as proxies for anthropogenic disturbance, we show that eukaryotic richness at the family level significantly increases with medium and high levels of disturbance. This change in richness coincides with compositional changes, a decrease in connectedness among taxa, an increase in fragmentation of taxon co-occurrence networks, and a shift in indicator taxa. Taken together, these findings demonstrate the ability of eDNA to act as a barometer of disturbance and provide an exemplar of how biotic networks and coral reefs may be impacted by anthropogenic activities.

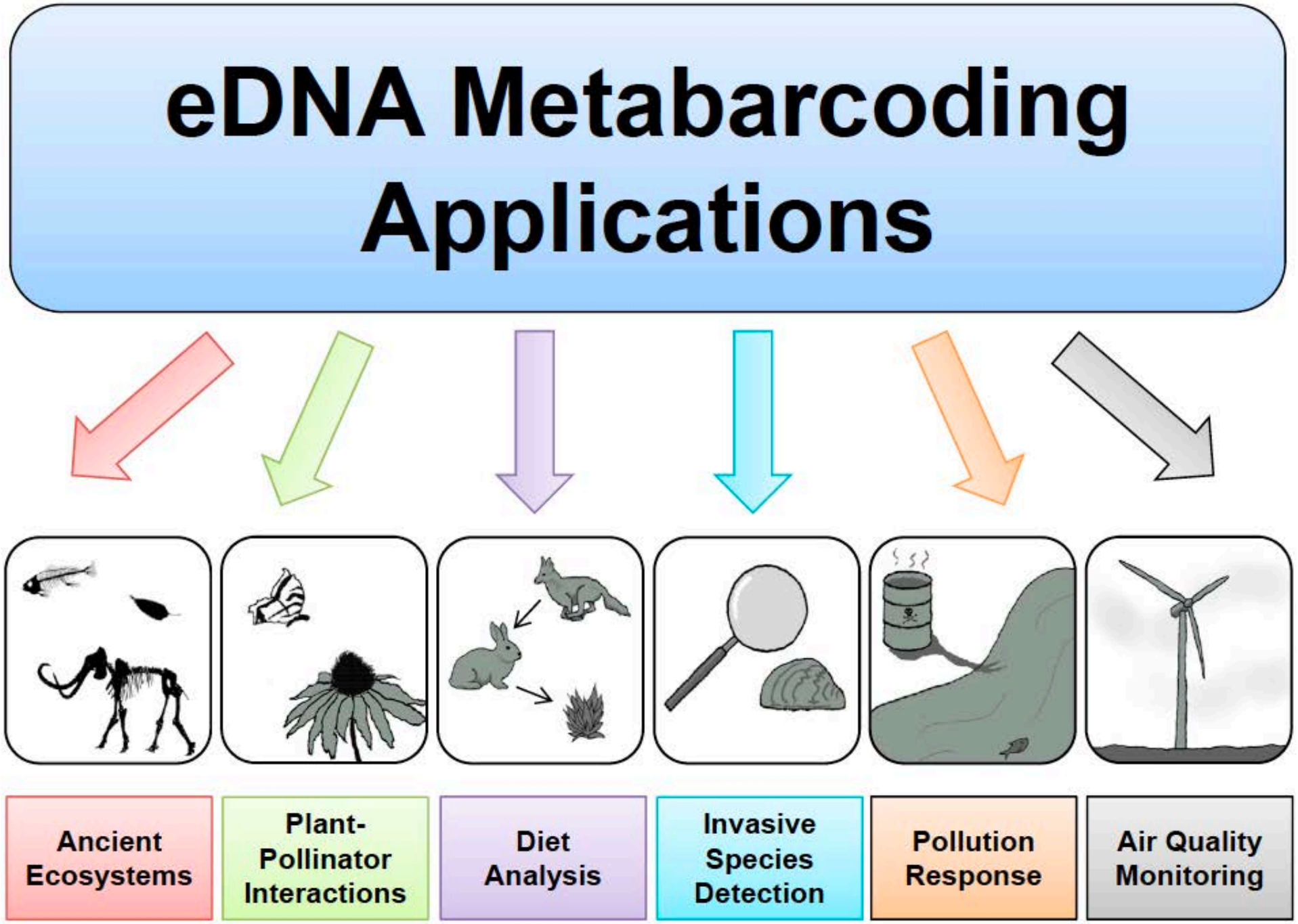



**Fig. 2.** Applications of environmental DNA metabarcoding in aquatic and terrestrial ecosystems.

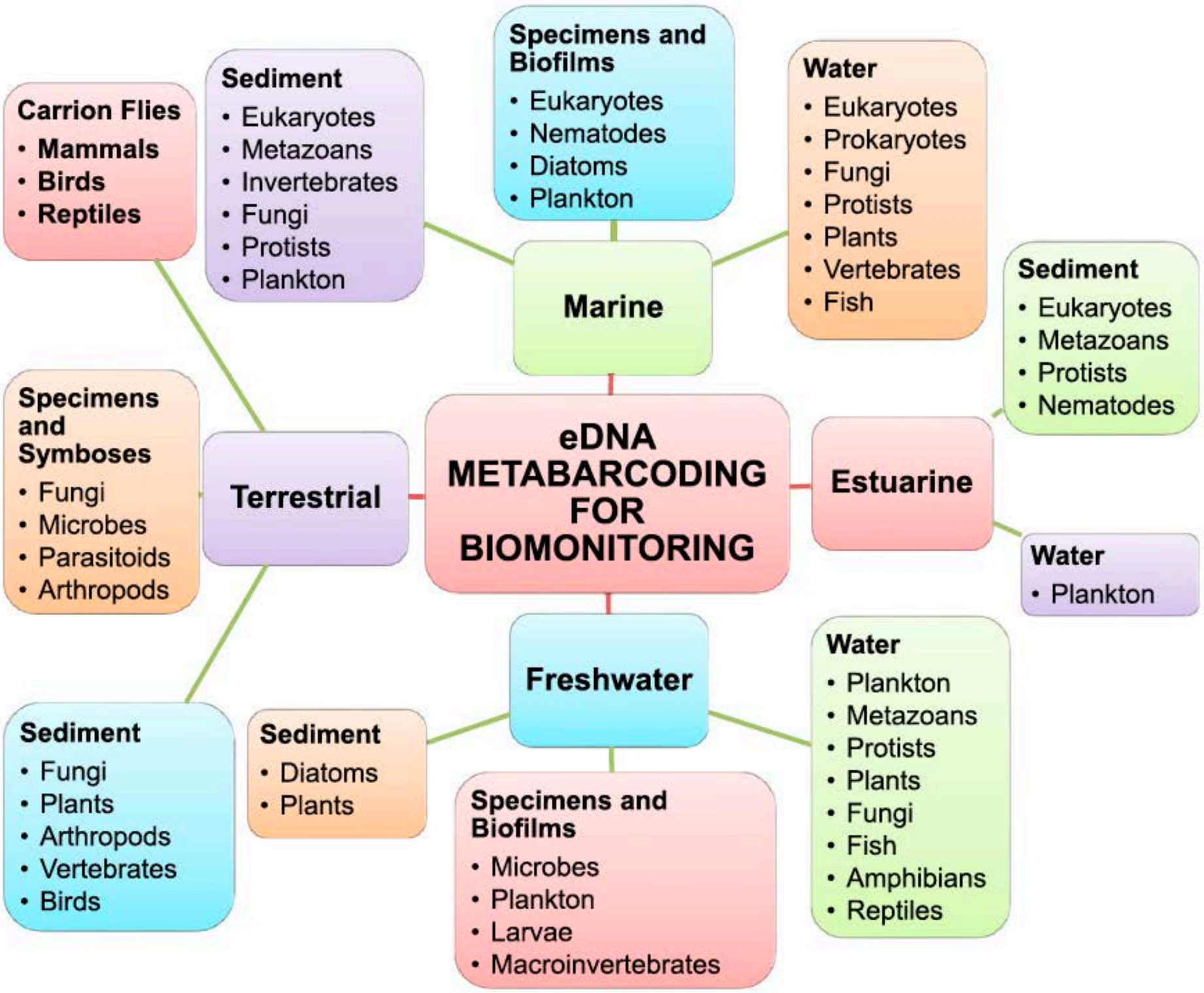



**Fig. 1.** Schematic diagram of global ecosystem and biodiversity monitoring with environmental DNA metabarcoding.

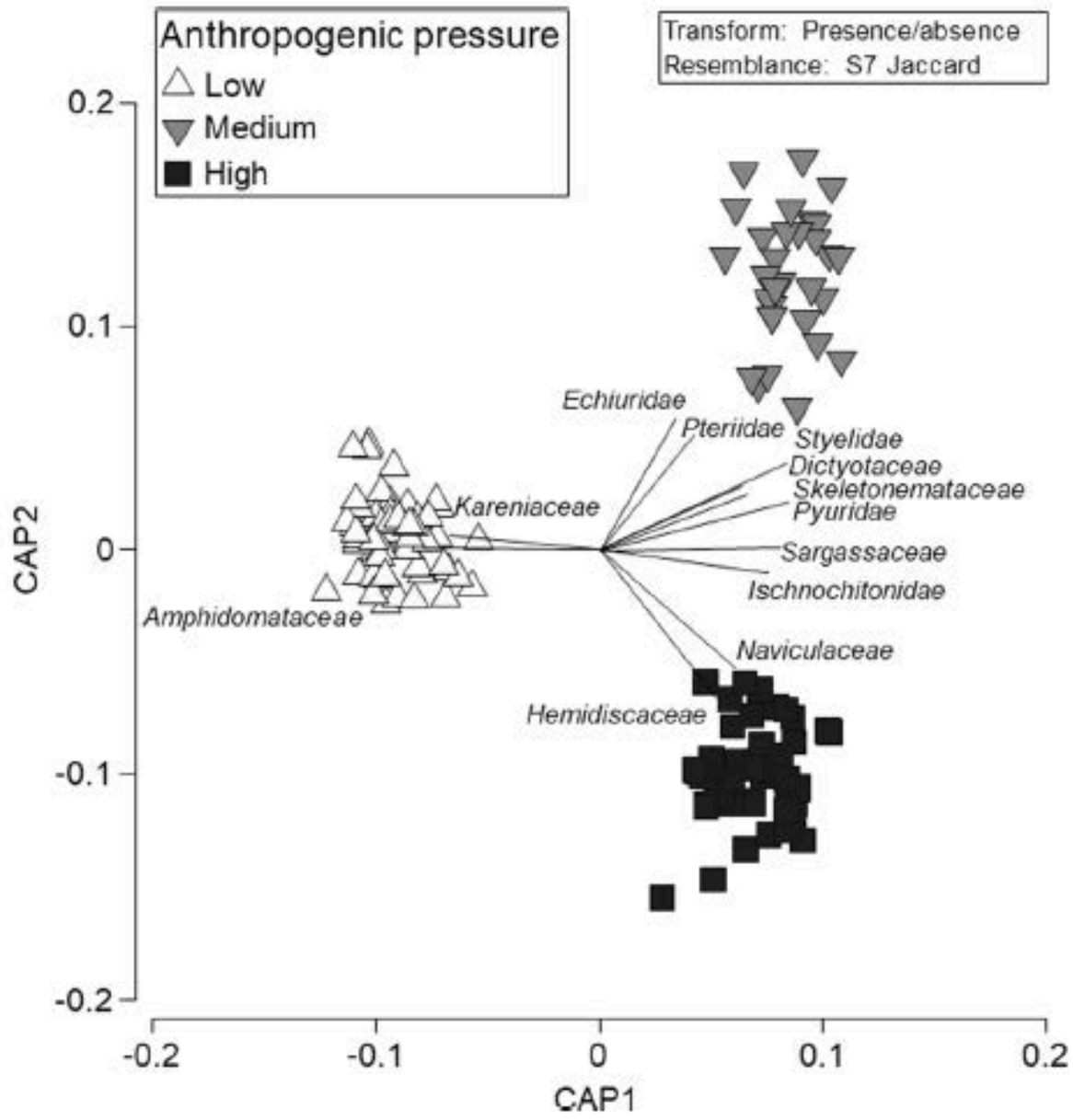



**Figure 2.** Canonical Analysis of Principle Coordinates (CAP) ordination plot of the presence/absence of eukaryotic families detected based on seawater samples collected at 14 sites in Okinawa, Japan and 18 S rRNA sequences. The relationship of eukaryotic community assemblages identified in each sample using a Jaccard's coefficient for factor "Impact" is shown. Pearson correlation vectors ($r > 0.4$) represent the eukaryotic taxa driving the relationship among samples.

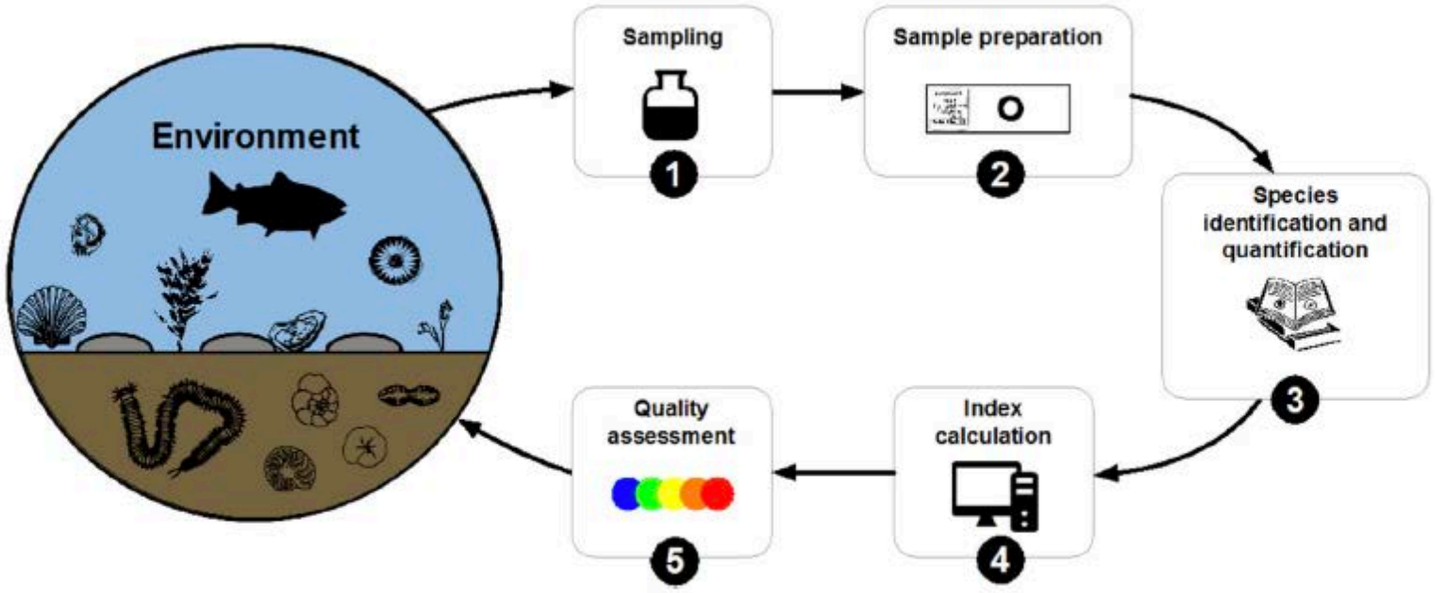



**Fig. 1.** Schema of key steps in traditional biological monitoring and assessment procedures.

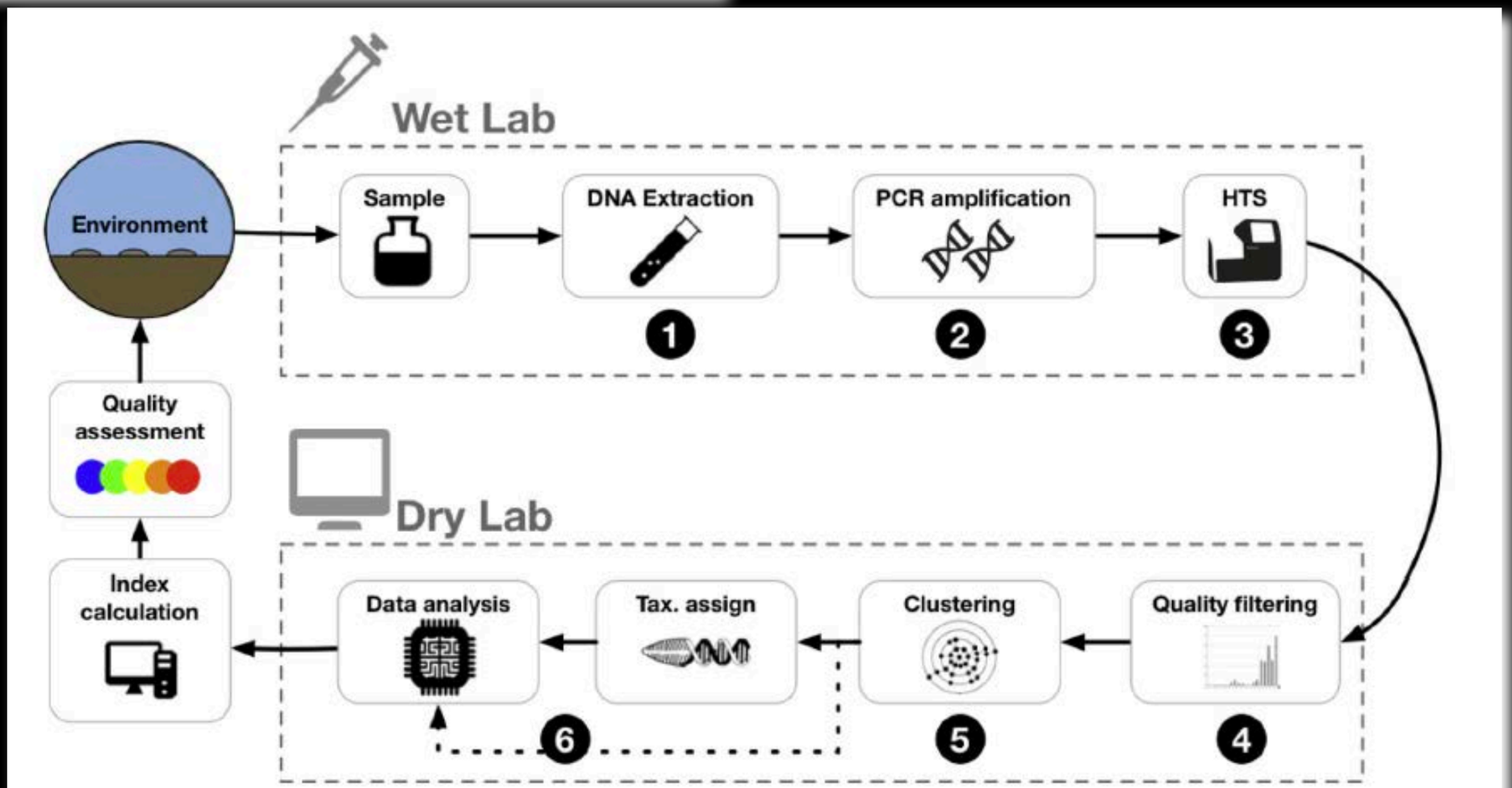
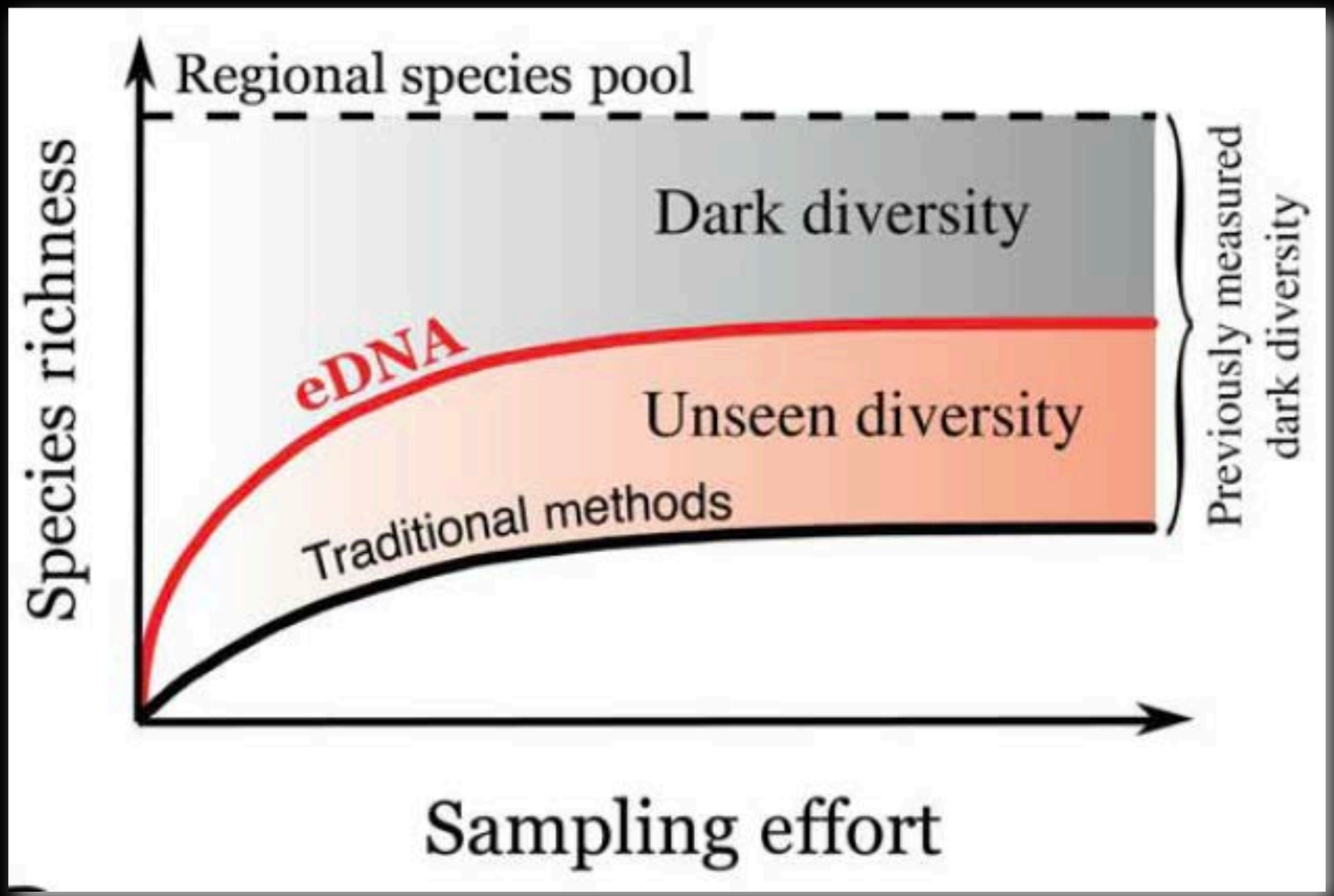
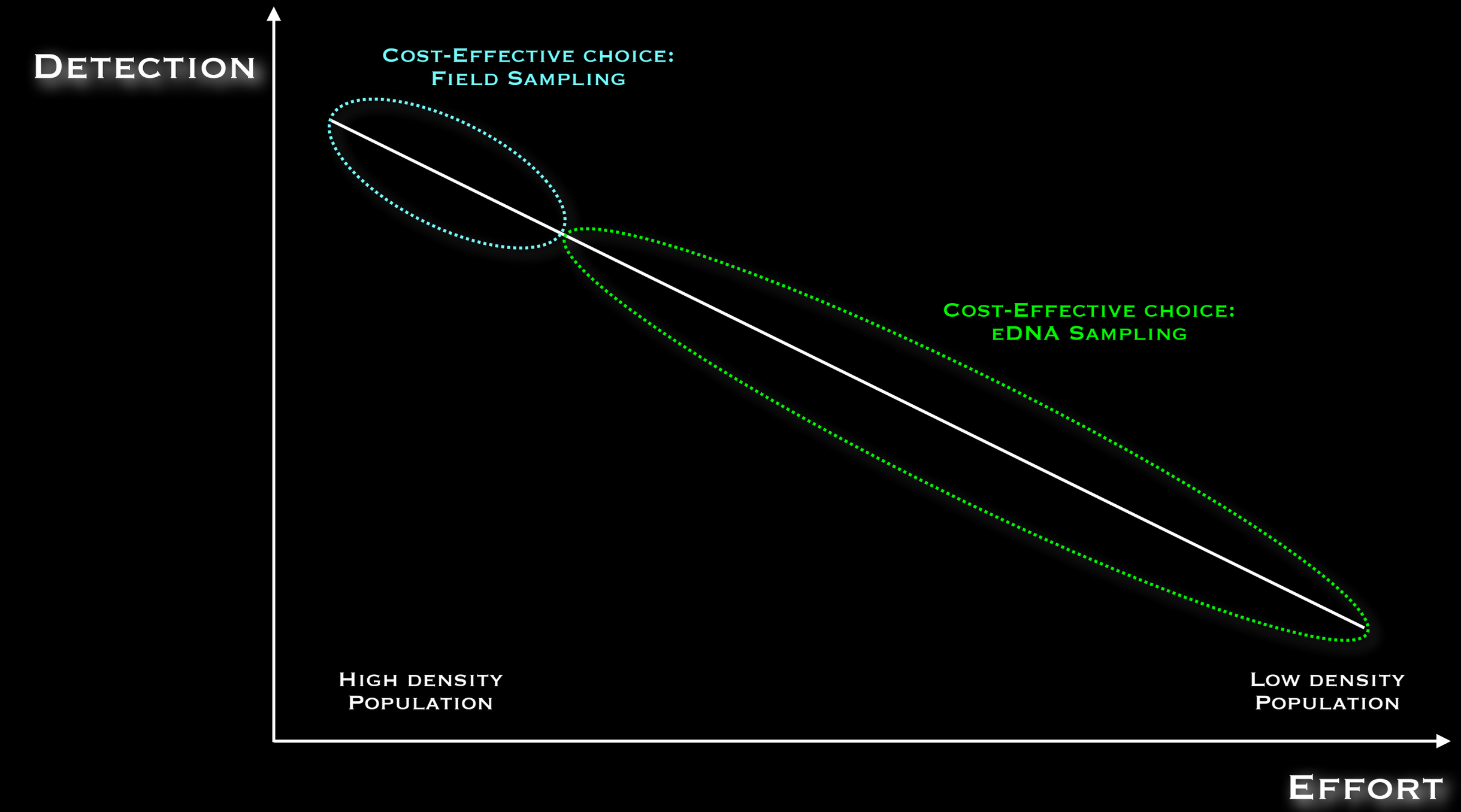



**Fig. 2.** Schema of key steps in DNA metabarcoding applied to bioassessment.

# Integrated approach




Detection
Cost-Effective choice:
Field Sampling
Cost-Effective choice:
eDNA Sampling
High density
Population
Low density
Population
Effort

# But still there are open questions…


Can we use eDNA for the species X, Y, Z?

Can we use eDNA to estimate abundance/density?

What are the chances of false positive/negative?

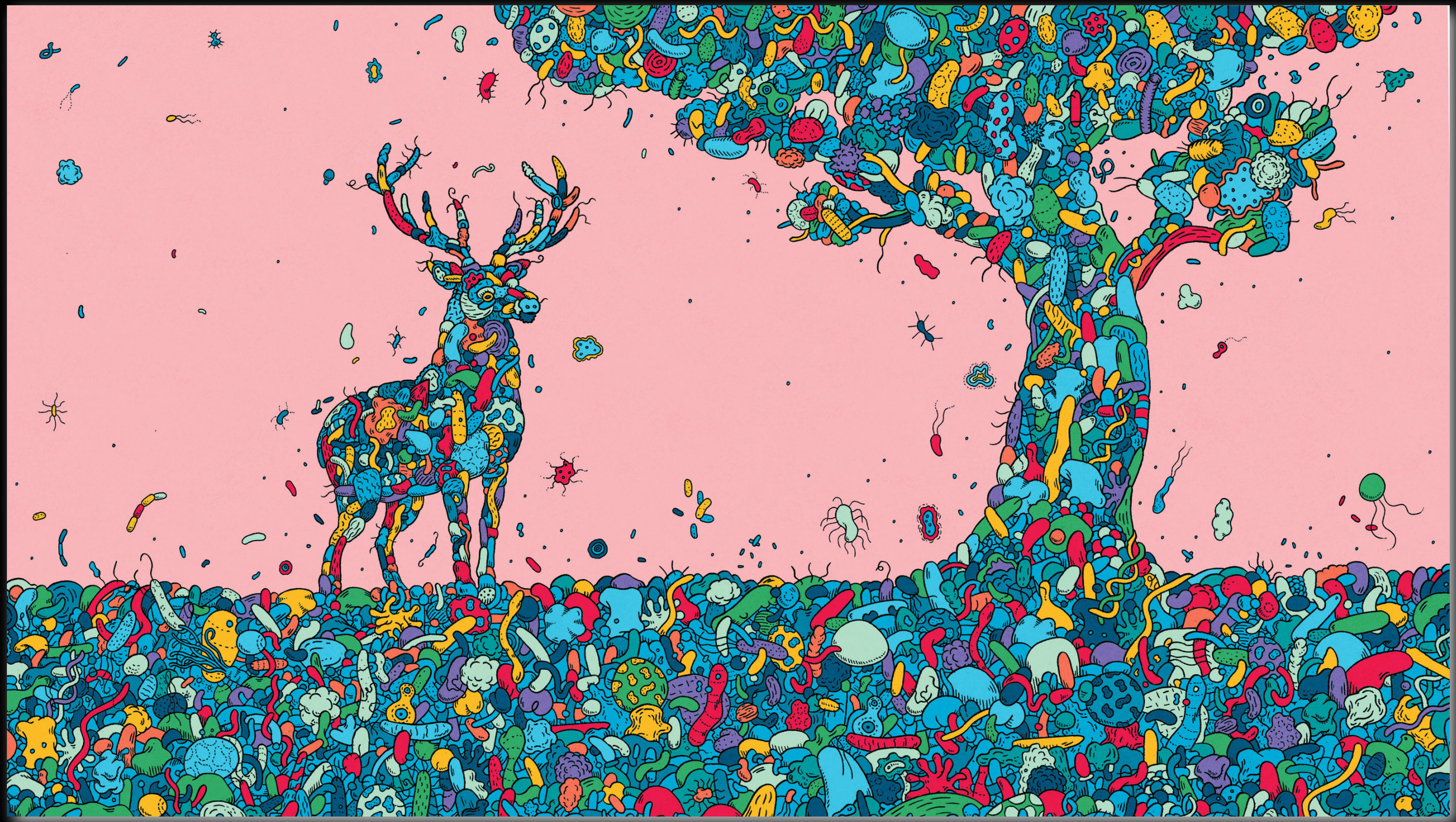
How much does it really cost?

How far downstream can eDNA be detected in a river?

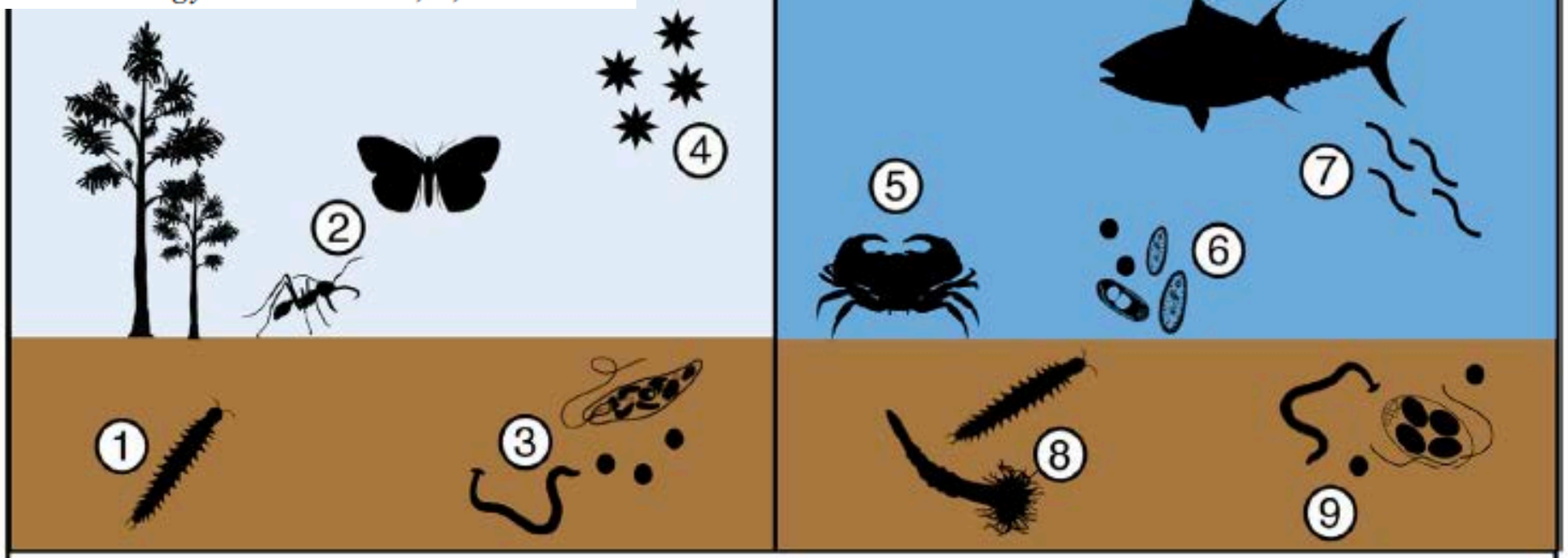
….

# Ready for questions on eDNA

Edna Marie Mode
The Incredibles



**WHAT to sample?**




**Terrestrial habitats**: 1. Soil macrofauna, 2. Aboveground macrofauna, 3. Soil microbes, meiofauna and microfauna. **Air**: 4: Spores & pollen **Aquatic habitats**: 5. Macro-fauna, 6. Suspended micro-organisms, 7. "eDNA", 8. Sediment macrofauna, 9. Sediment microbes, meiofauna and microfauna.

**HOW to sample?**

"Conventional" macro-invertebrate sampling, e.g. kick sampling, malaise/pitfall traps

- Subsampling taxa/ parts e.g. **1, 2, 5, 8**
- Homogenisation e.g. **1, 2, 5, 8**

Filtration of water/air samples

e.g. **4, 6, 7**

Soil or sediment cores

- Organismal extraction e.g. **1, 3, 8, 9**
- Homogenisation e.g. **3, 9**

**DNA extraction**
Sample-specific extraction methods

Technique based on research question

- Metatranscriptomics
- Metabarcoding
- Single-cell Genomics
- Metagenomics

Data analysis, e.g.:

- Assembly
- Comparison to reference databases (taxonomic and functional)
- OTU-clustering