

TGCATTTAGCT

TATCC

AATATTCAAGGATAAAC

UNIVERSITÄT

D U I S B U R G  
E S S E N

CACCCCTTACT

AAGAT

TCGGATTCTTAT

TATAGCCC

GGATA

C

GTCGGT

TCC

AACCCCCCA

CATTCTTT

TCT

TTACCC

TAA

ACAGATT

GGTAGG

TCA

GAGAGTGCAG

AGGGTTGATTGC

TGGTA GTGTTAGC

CAAAAGAGCTCAAATT



# The future of stream monitoring: DNA Metabarcoding!

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3<sup>rd</sup> Science for the Environment conference - 2. October 2015

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GeneStream

# Classic water assessment

- Water assessment is based on the morphological identification of macroinvertebrates

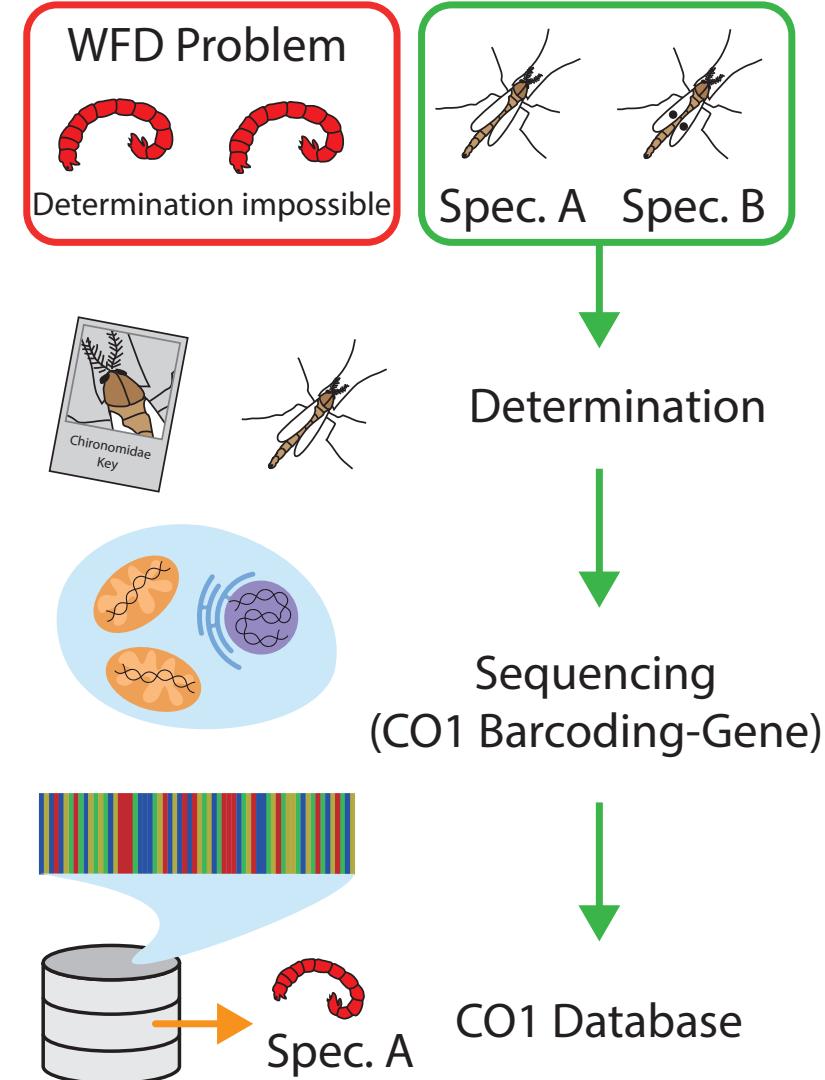
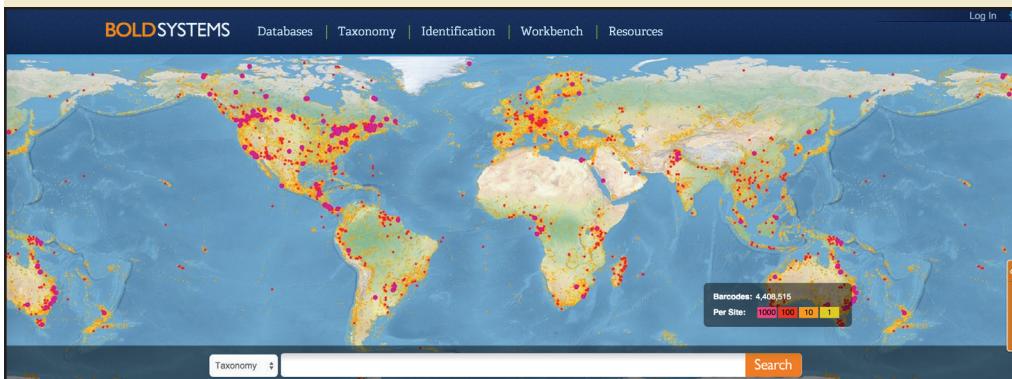
	Morphology
Missidentification	occurs!
Taxonomic resolution	— / + taxon dependent
Data comparability	—
Speed	—
Abundance	+
Cost	+

- Misidentification -> problem for management



# DNA Barcoding

- DNA based identification of single specimens (expensive & time consuming)
- Morphological identification of imagines  
-> feed barcode into data base
- BOLD reference database  
-> 5 million sequences

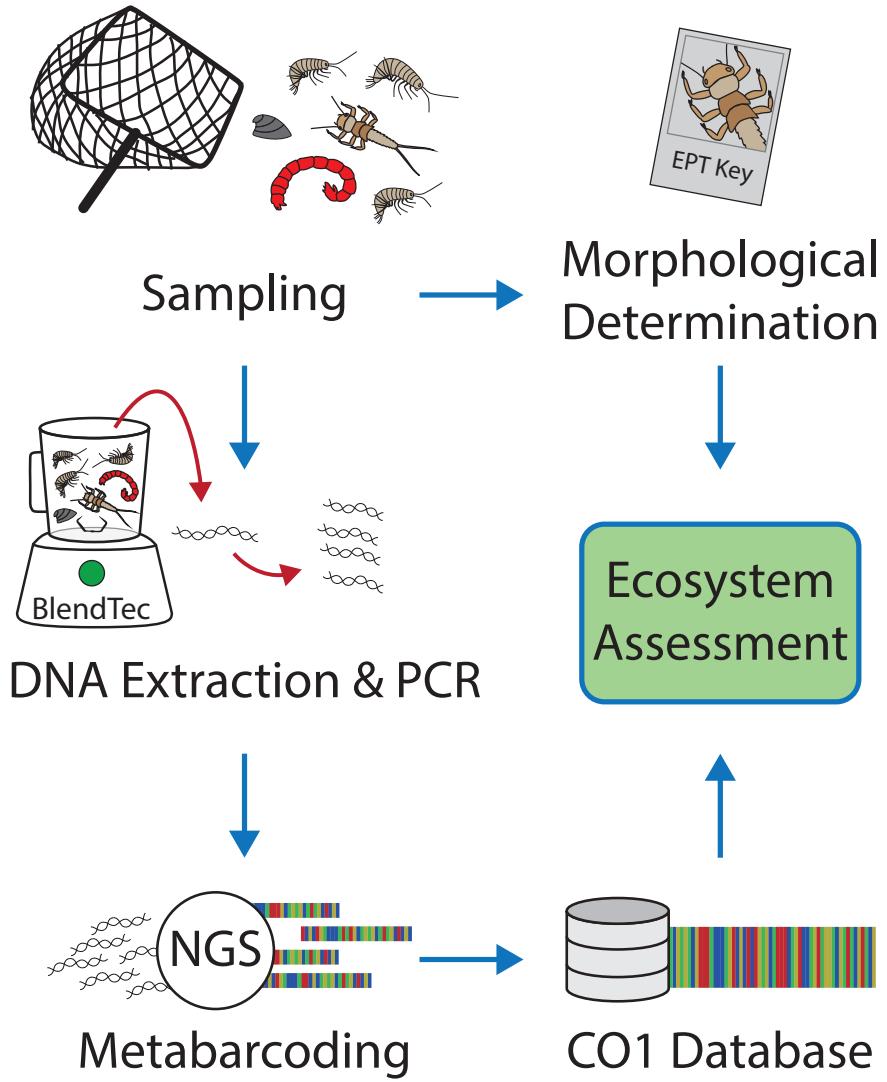


# Metabarcoding

- **Next Generation Sequencing**
- Generates millions of sequences

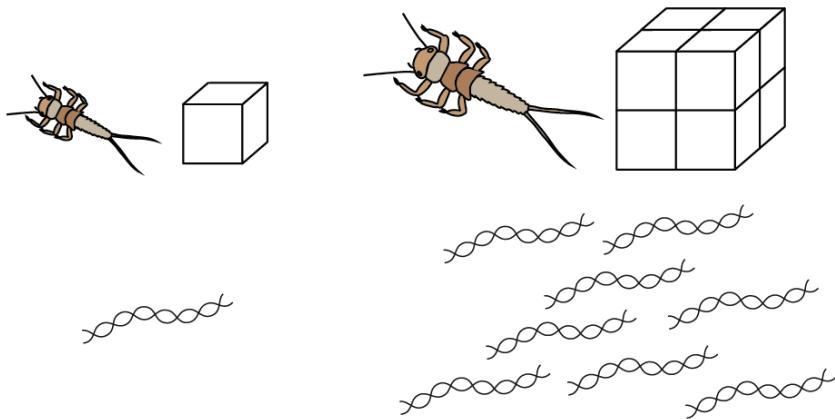


From sampling to the species level  
taxa list in less than a week!



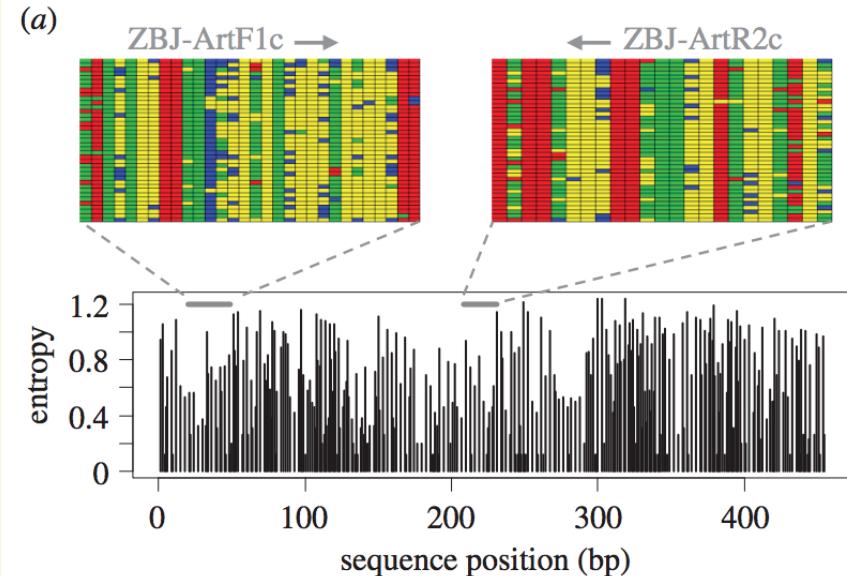
# Two challenges

## Different specimen biomass



Clear relationship between sequence abundance and biomass (within the same species)

## CO1 Primer (binding site variability)



Deagle et al. 2014, Biol Lett.

---> Taxa detection primer dependent

Can metabarcoding estimate taxon biomass?

NO!



RESEARCH ARTICLE

## Can DNA-Based Ecosystem Assessments Quantify Species Abundance? Testing Primer Bias and Biomass—Sequence Relationships with an Innovative Metabarcoding Protocol

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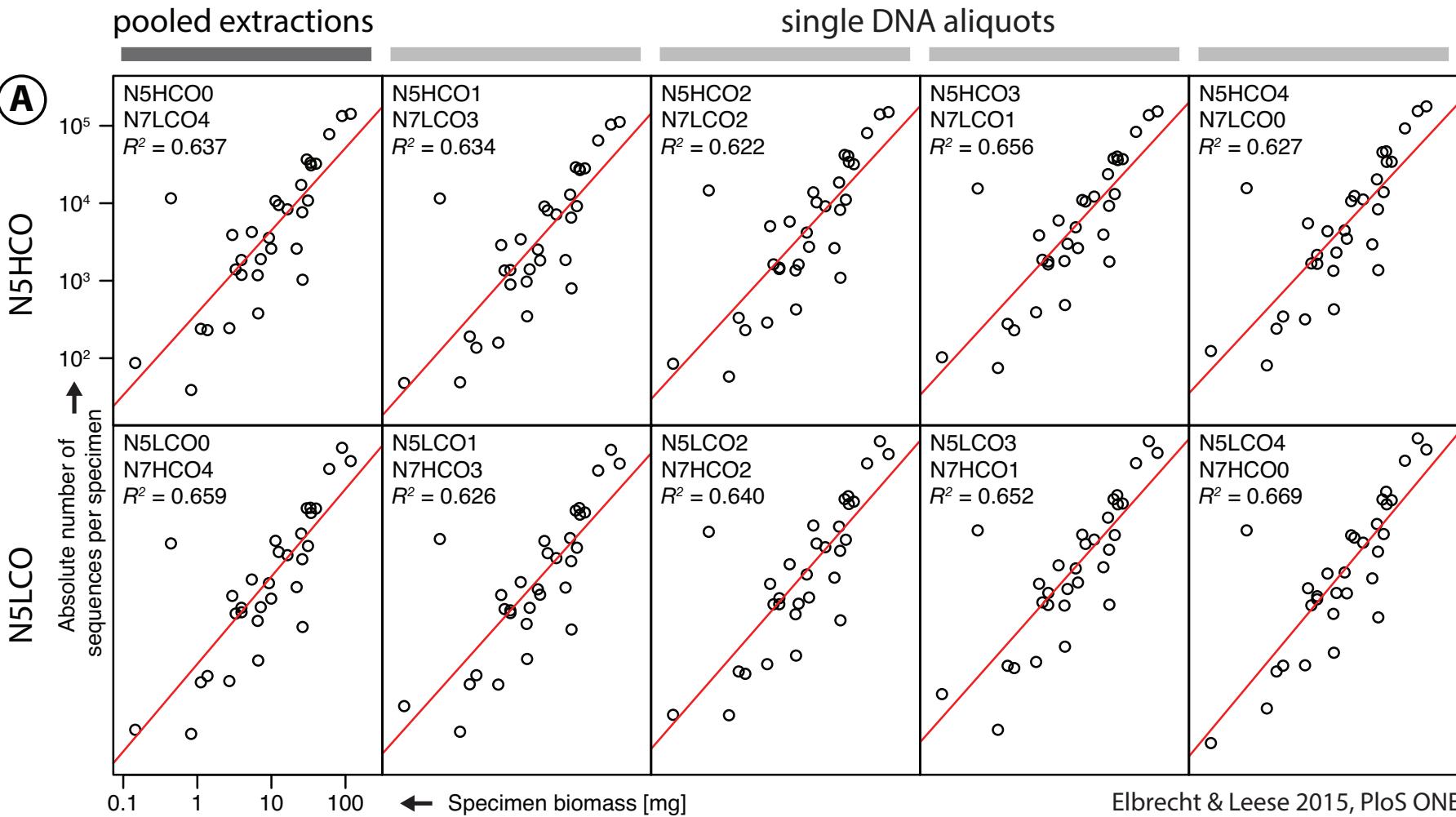
\* [florian.leese@rub.de](mailto:florian.leese@rub.de)

### Abstract

Metabarcoding is an emerging genetic tool to rapidly assess biodiversity in ecosystems. It



# Experiment I: Biomass



# Experiment II: Primer bias



Ephemeroptera:	8
Plecoptera:	4
Trichoptera:	15
Diptera:	8
Others:	17
<b>Σ Taxa:</b>	<b>52</b>

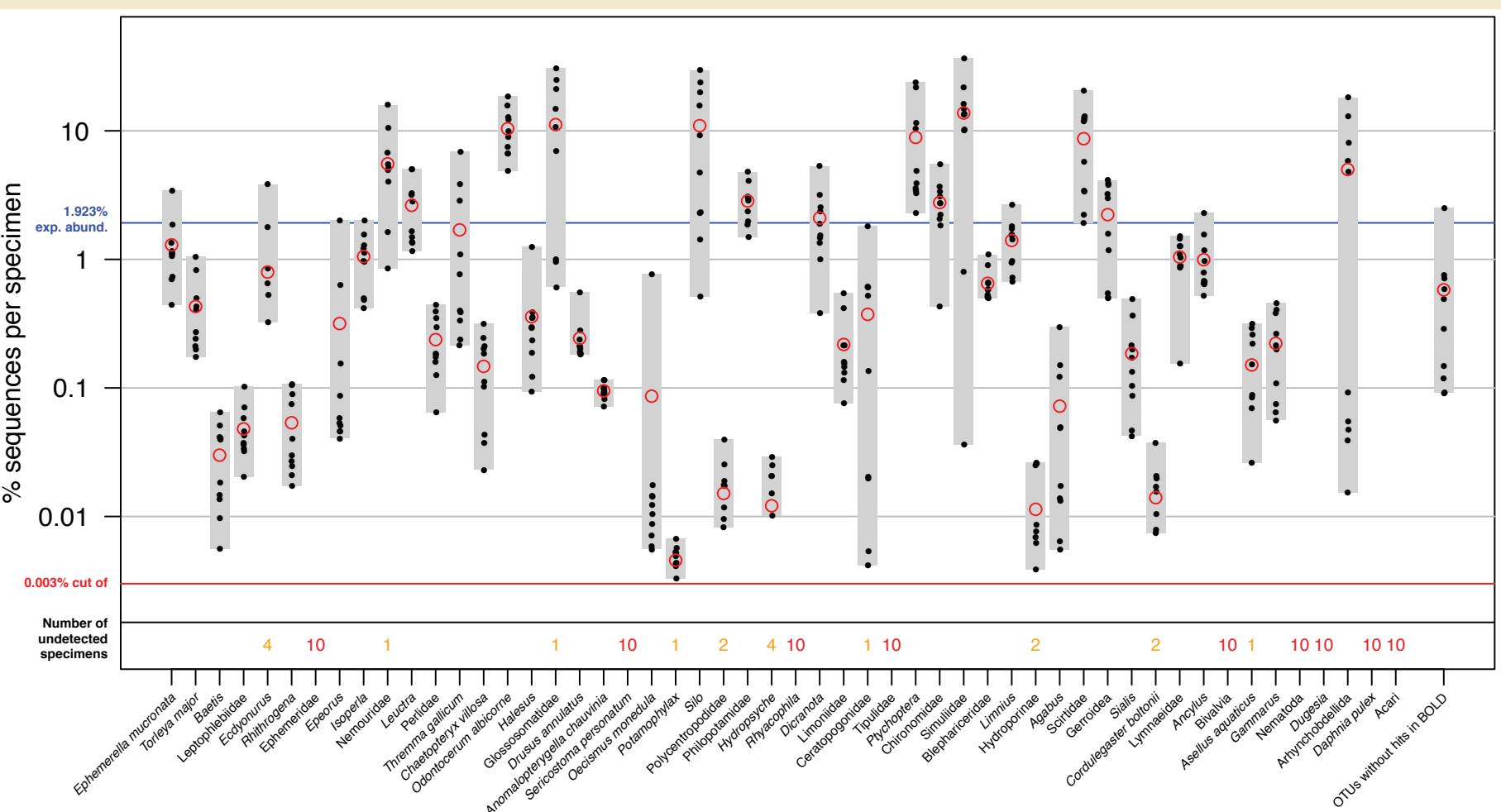


**10 replicates**

**Same amount of tissue  
of each taxon**

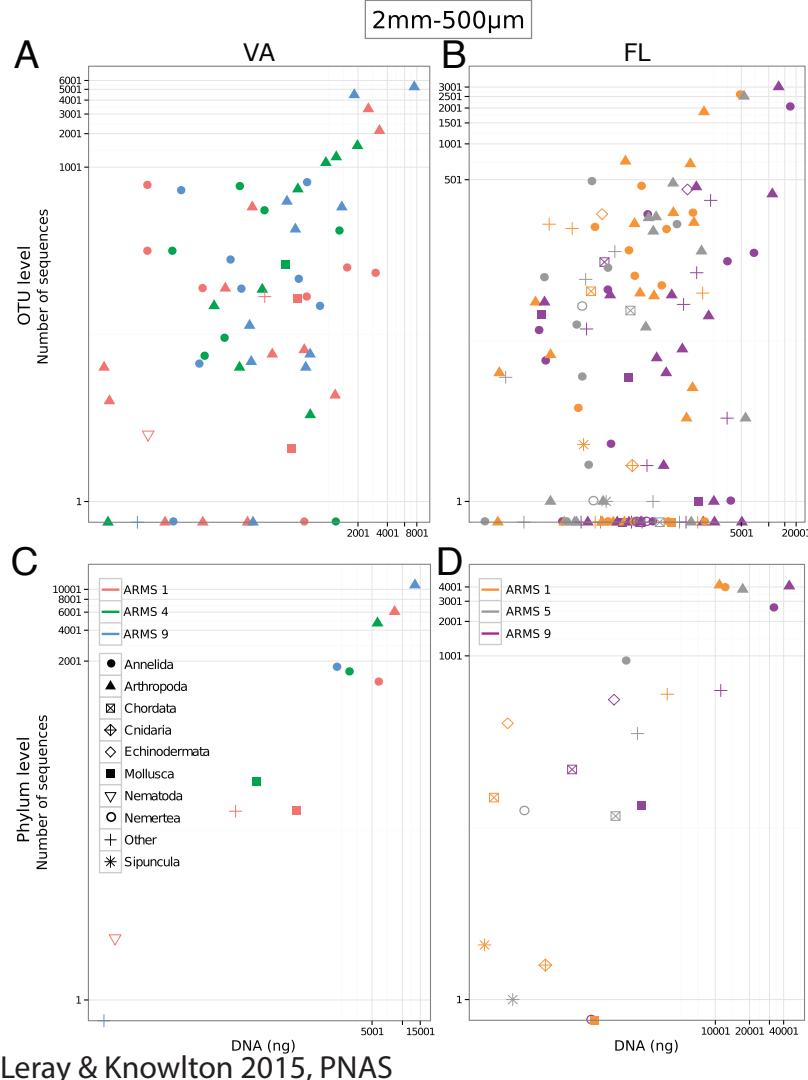
Elbrecht & Leese 2015, PloS ONE

# Experiment II: Primer bias

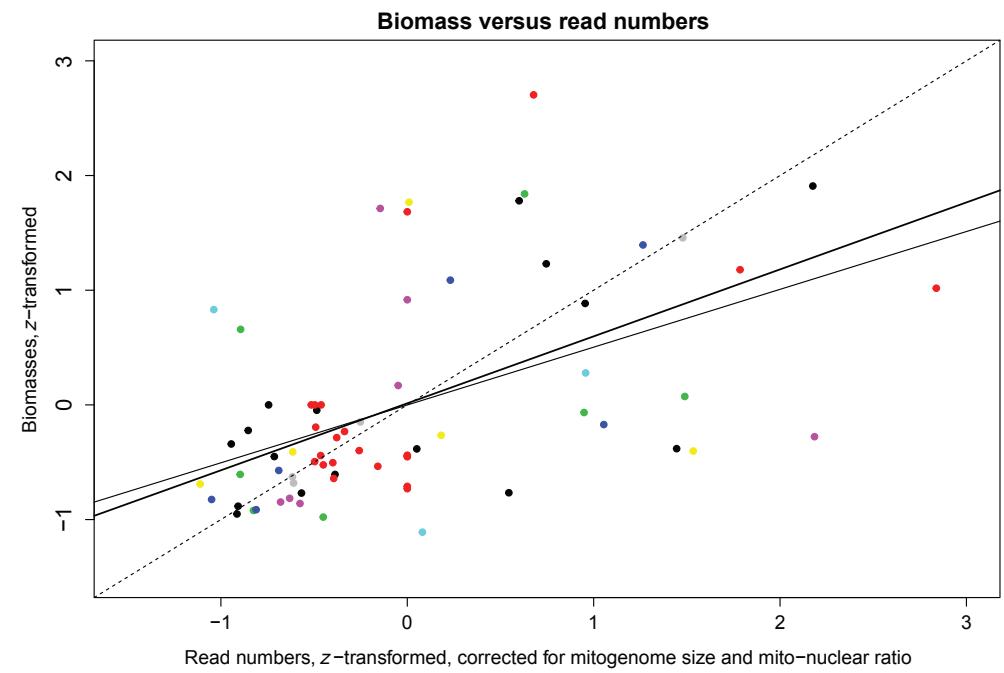


Elbrecht & Leese 2015, PloS ONE

# Results confirmed by other studies

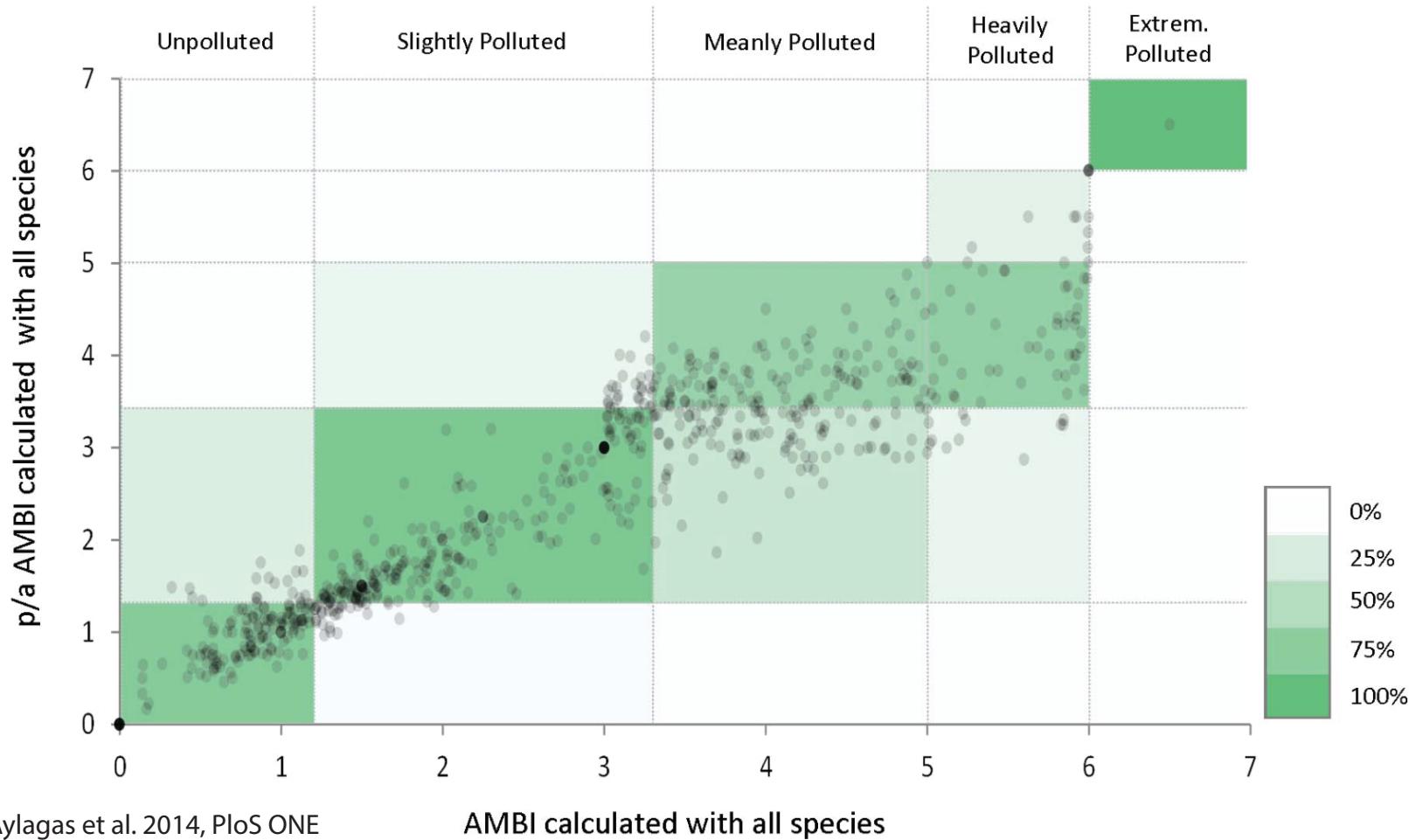


◀ Marine benthos  
▼ Bees (metagenomics, PCR free!)



**Abundance = does not look promising!**

# Is abundance necessary?



Aylagas et al. 2014, PLoS ONE

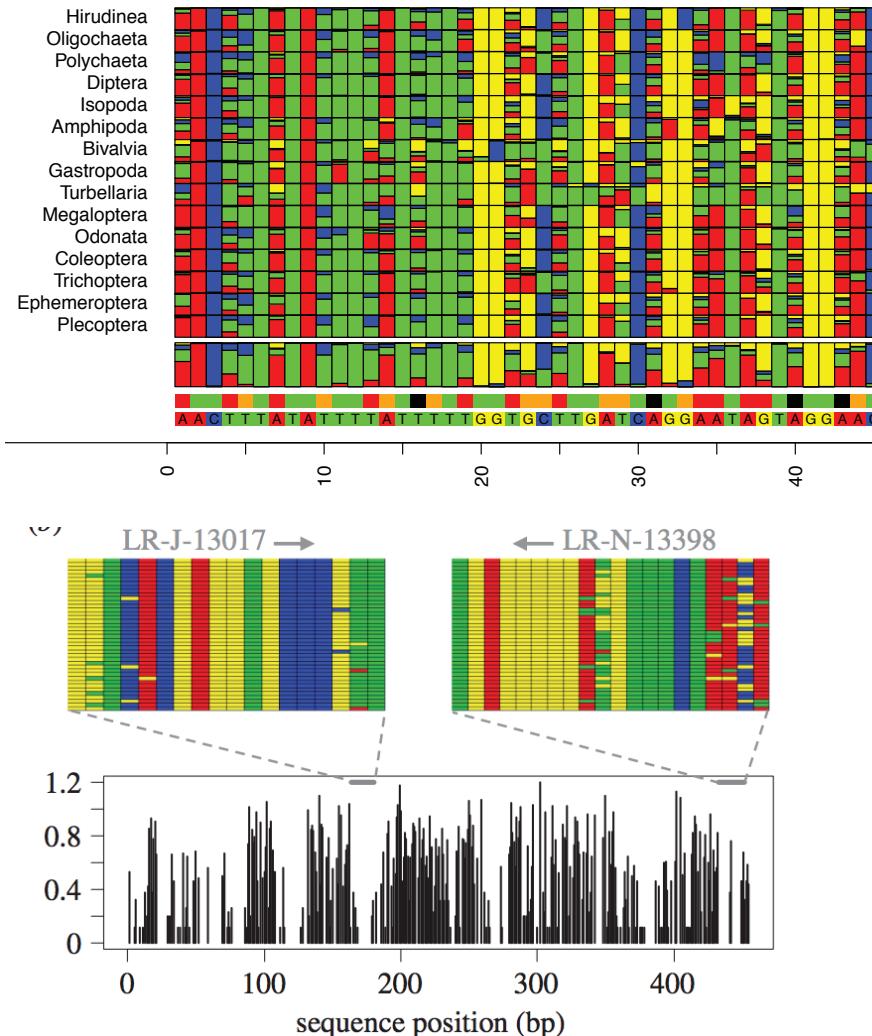
Abundance vs presence / absence = similar + species level identific. with DNA!

## Optimised COI Primer

- Software: PrimerMiner
- Group specific primer
- OpenSource on GitHub
- 4 primer pairs

## Alternative Markers

- 16S ribosomal marker
- DNA from Exp. II -> NextSeq  
140.000.000 sequences
- lack of reference sequences



Deagle et al. 2014, Biol Lett.

# Biomass problem

- Sort kick sample by specimen size



- 2 sample sites (Kleine Schmalenau)  
West Germany

- Sorting -> sequences more equally distributed

- Morphology: 52 taxa

- DNA metabarcoding: 104 taxa

( Preliminary data! )

S	M	L	sample site 8
39 (3,6%)	5 (1,8%)		Trombidiformes
			Trichoptera
			Sericostoma sp.
			Trichoptera
			Sericostoma sp.
			Trichoptera
			Sericostoma sp.
3 (0,002%)			Trichoptera
1 (0,93%)	2 (0,7%)		Oecismus monedula
1 (0,35%)			Trichoptera
2 (0,7%)			Polycentropus irroratus
7/5 (0,65%/0,47%)	5 (1,8%)		Glycentropus flavomaculatus
2 (0,19%)	13 (4,6%)	1 (1,9%)	Cyrnus trimaculatus
			Trichoptera
			Wormaldia occipitalis
			Trichoptera
			Odontocerum albicorne
			Trichoptera
			Potamophylax cingulatus
		2 (3,7%)	
		8 (14,8%)	Trichoptera
			Halesus radiatus
	1 (0,35%)		Trichoptera
9 (0,84%)	65 (22,8%)	2 (3,7%)	Drusus annulatus
1 (0,35%)			Trichoptera
			Chaetopteryx villosa
1 (0,35%)			Trichoptera
			Ornithopterygella chauviniana
			Symplypleona
			Dicyrtomina minuta
1 (0,35%)			Rhynchocephalida
			Helobdella sp.
			Pythiales
			Phytophthora sp.
2 (0,19%)	6 (2,1%)		Pulmonata
			Ancylus fluviatilis
			Plecoptera
			Diura bicaudata
54 (5%)	6 (2,1%)		Plecoptera
1 (0,093%)			Protonemura auberti
			Plecoptera
			Nemoura leucularis
			Plecoptera
			Leuctridae
69 (6,4%)			Plecoptera
			Leuctra sp.
			Plecoptera
			Leuctra digitata
38 (3,5%)	3 (1%)		Plecoptera
1 (0,093%)			Leuctra braueri
			Plecoptera
			Leuctra aurita
			Plecoptera
1 (0,093%)			Leuctra albida
			Plecoptera
			Siphonoperla torrentium
			Lumbriculida
			Stylodrilus heringianus
			Lumbriculida
			Lumbriculus variegatus
			Lumbriculidae
			Lumbriculidae
			Myrmica rugosodis
1 (0,35%)	3 (5,6%)		Hymenoptera
			Hydrophila
			Radix labialis
1 (0,093%)			Hemiptera
8 (0,75%)			Velia caprai
			Hemiptera
			Micronecta sp.
			Haplotauxida
			Nais sp.
			Haplotauxida
			Nais alpina
			Haplotauxida
			Eiseniella tetraedra
89 (8,3%)			Ephemeroptera
14 (1,3%)			Leptophlebia marginata
			Ephemeroptera
			Habrophlebia lauta

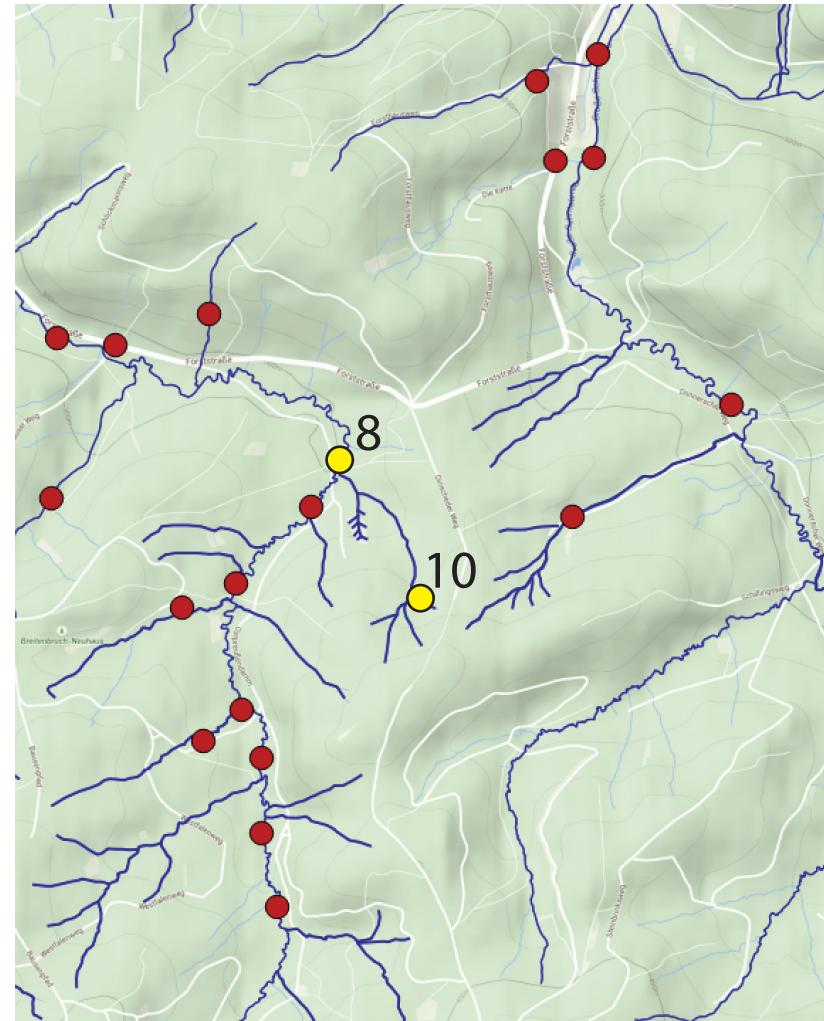
# HiSeq 2\*250 bp

# Conclusions and outlook

## Metabarcoding works!

	Morphology	Metabarcoding
Missidentification	occurs!	does not occur
Taxonomic resolution	— / + taxon dependent	+ taxon independent
Data comparability	—	+
Speed	—	+
Abundance	+	—
Cost	+	— cost is sinking

- No abundance data possible!  
presence / absence data
- Upcoming:  
metabarcoding of complete kick  
samples from 20 sampling sites



5 kick samples per site - WFD

# Help and financing

## Involved people and partners

- Jannis Neuman
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Vasco Elbrecht

Florian Leese

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