

Contamination in metabarcoding studies - case: airborne eDNA

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Intended learning outcomes

After this session you will be able to:

- Identify different sources of contamination – where it can arise, how to detect it and how to avoid it
- Describe how PCR replicates can be used during data processing to balance error removal with detection of diversity
- Formulate a strategy for the lab set-up of your metabarcoding study to account for or avoid different sources of contamination and facilitate the ability to balance error removal with diversity detection during data processing

What scares you about contamination in
your metabarcoding study?

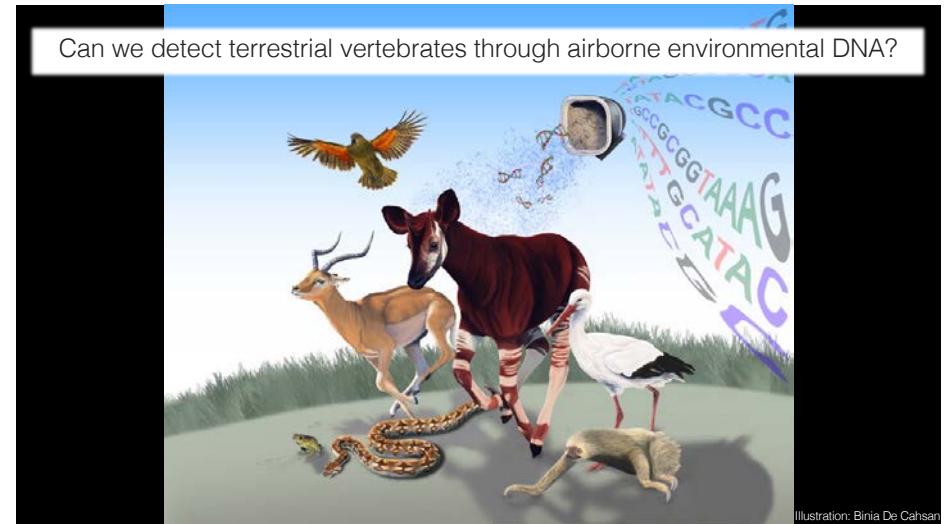
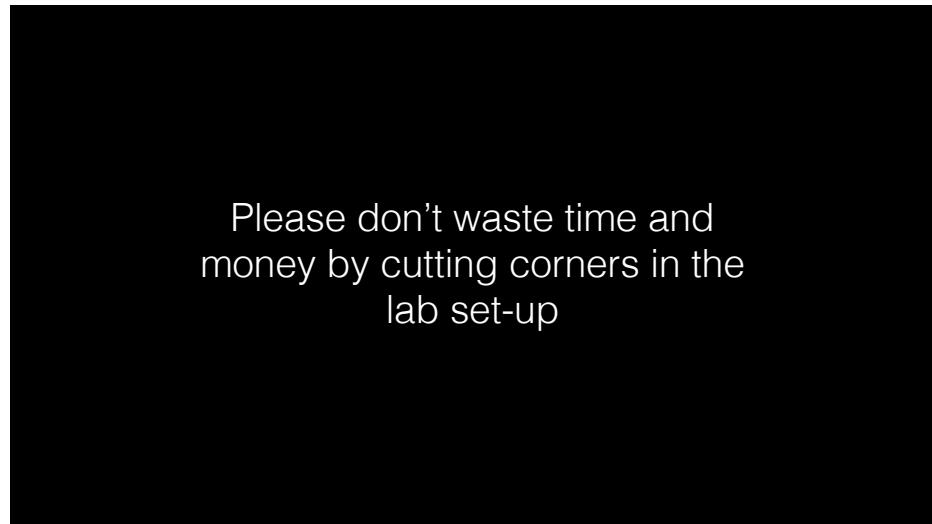


Illustration: Biria De Cahsan

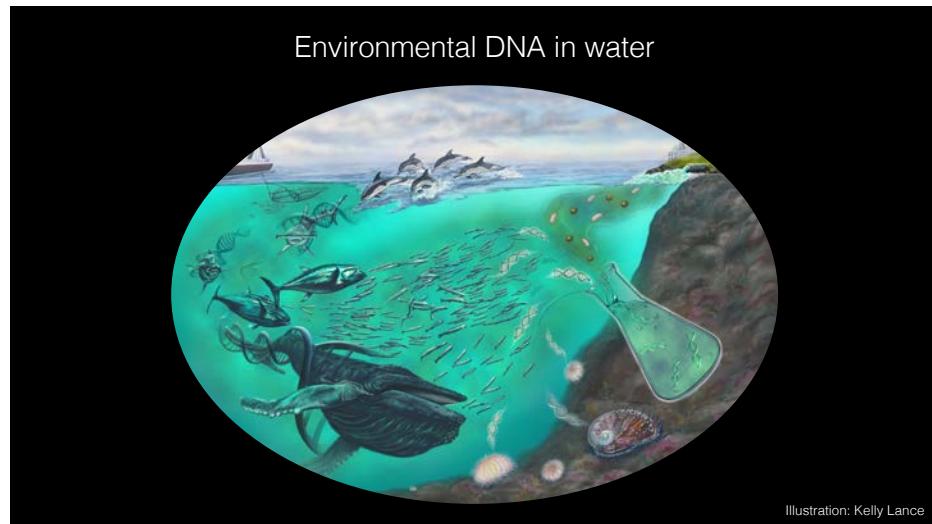
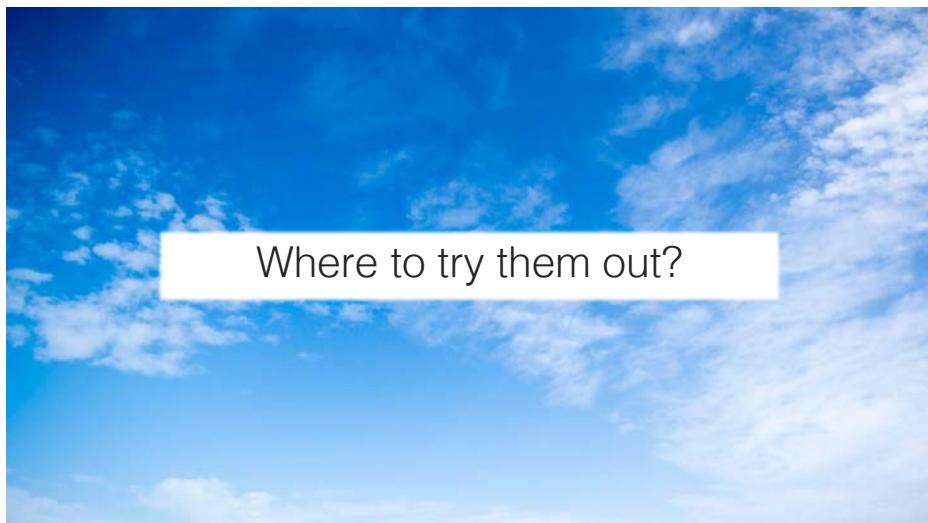
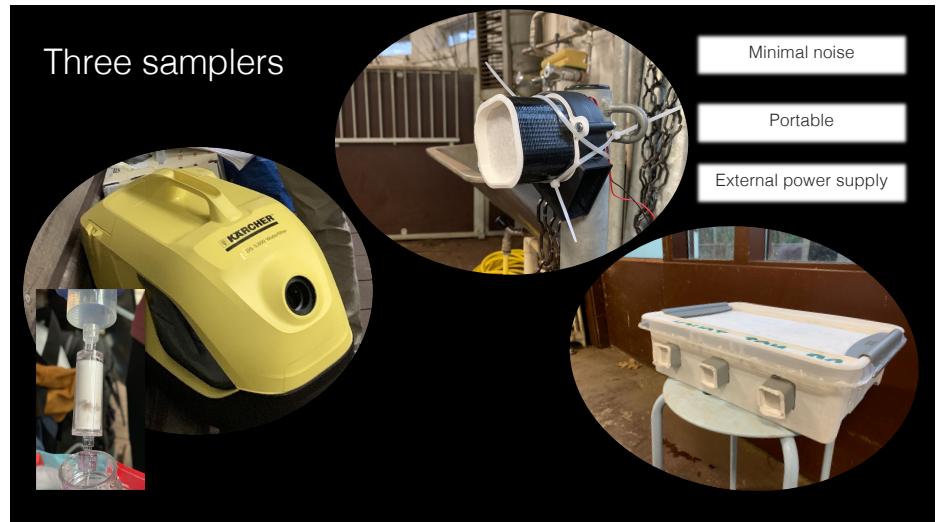


Illustration: Kelly Lance



Photos: Kristine Bohmann





3 sampling sites:

1) Okapi- and duiker stable



Lynggaard et al. 2022, Current Biology

3 sampling sites:

2) Rainforest House



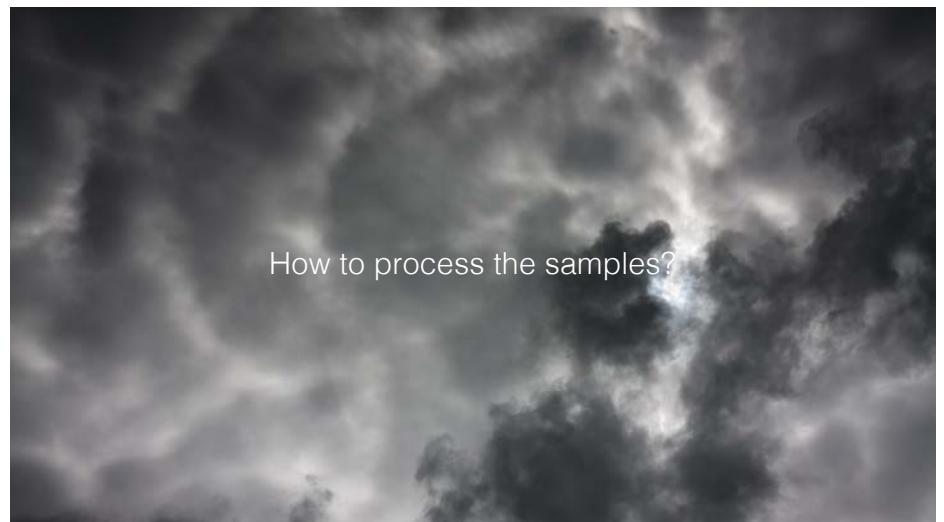
Lynggaard et al. 2022, Current Biology

3 sampling sites:

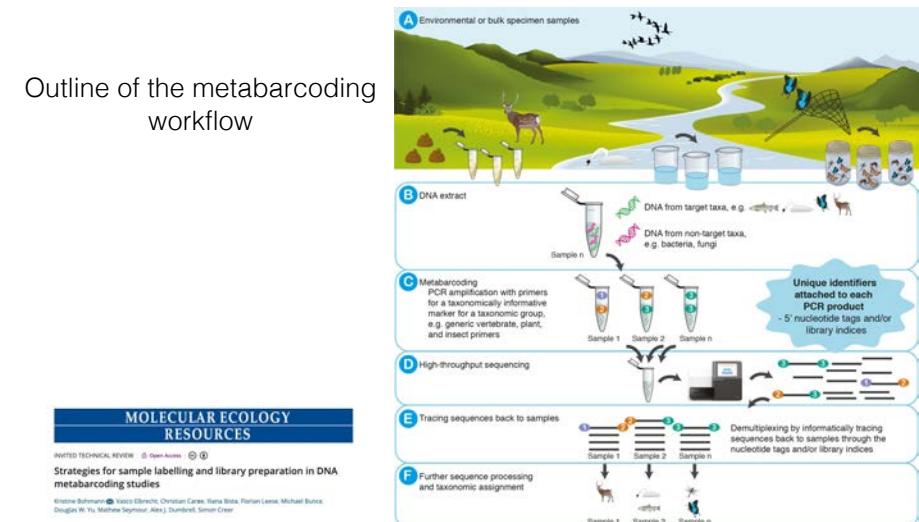
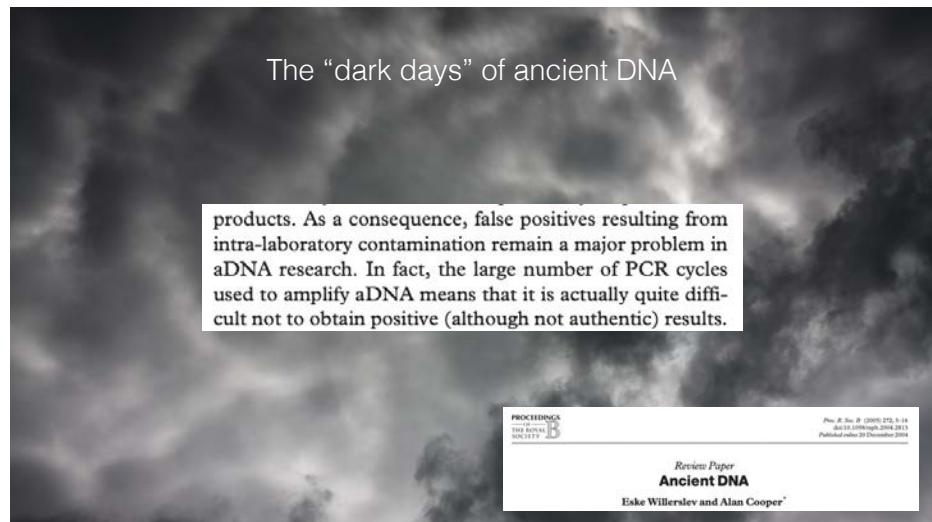
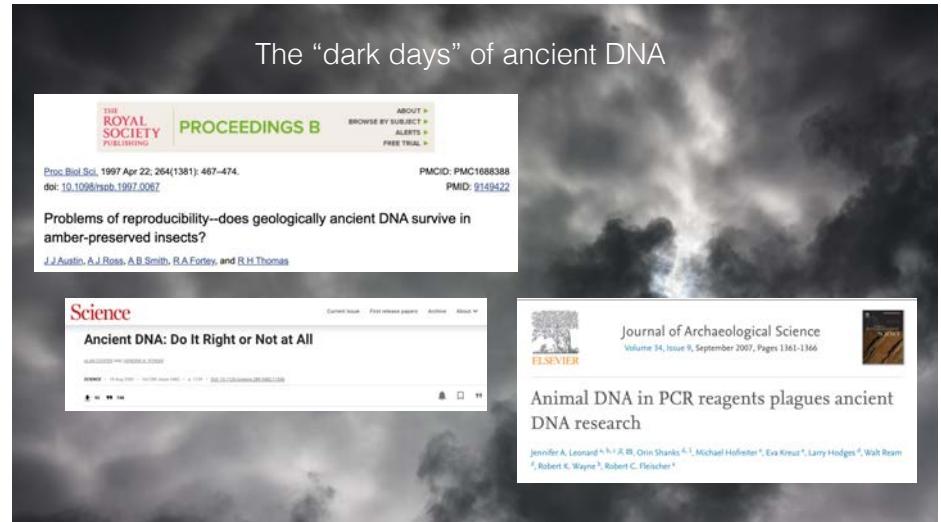
3) Outside



Lynggaard et al. 2022, Current Biology



How to process the samples?



Where can contamination arise in metabarcoding studies?

From sample collection to sequencing

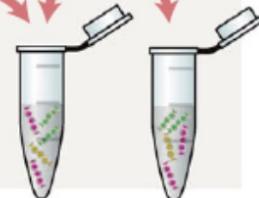
All steps where there is no label on sequences (cross-contamination between samples)

From the environment, reagents and tools

Contamination

Cross-contamination between samples

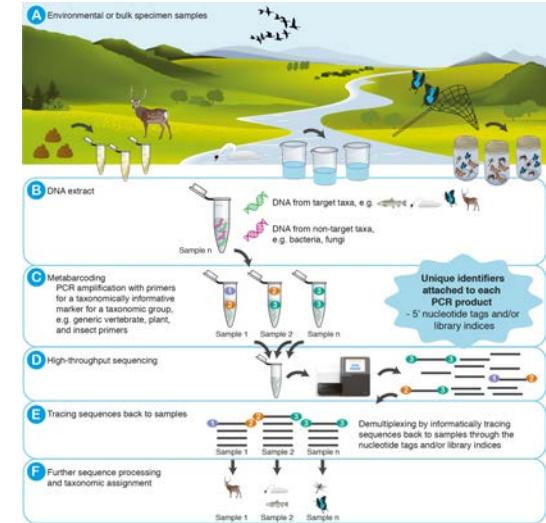
External contamination in the field and the laboratory



INVITED TECHNICAL REVIEW
Promises and pitfalls of using high-throughput sequencing for diet analysis
Antti Alberdi¹ | Ostalika Alzupur¹ | Kristine Bohmann^{1,2} | Shyam Gopalakrishnan³ | Christina Lynggaard⁴ | Martin Nielsen¹ | Marcus Thomas plus Gilbert^{1,2}

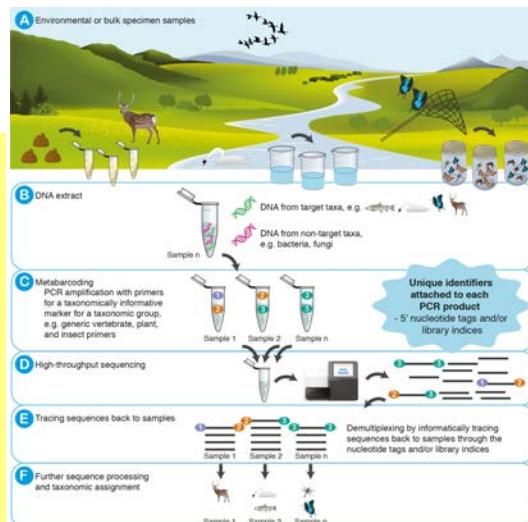
What can you do to **avoid** contamination?

Discuss with the people sitting close to you



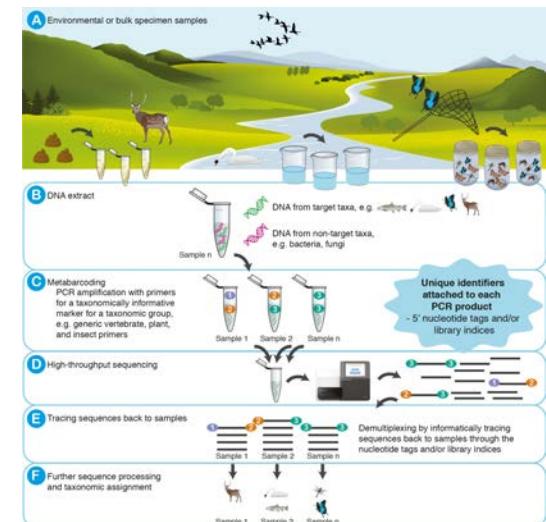
What can you do to **avoid** contamination?

- Careful handling
- Aliquoting extracts
- Use of sterile materials, filter tips, flow hoods, cleaning with hypochlorite
- Spatial division between pre-PCR and post-PCR procedures
- Tools and reagents used for processing the samples also need to be considered as putative source of contamination mostly those derived from living organisms, such as BSA (from cattle)
- Cross-contamination between samples: keep batches of processed samples low and reduce the spatial separation between tubes containing different samples and having multiple tubes open at the same time.



What can you do to enable **detection** of contamination?

Discuss with the people sitting close to you



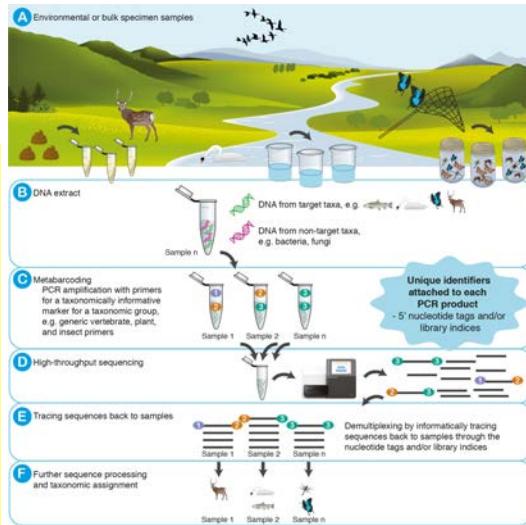
What can you do to **detect** contamination?

Including (real!) negative controls at all steps of the lab workflow

Assessment prior to sequencing: checking neg controls during quality control - e.g. qPCR, agarose gel, BioAnalyzer/TapeStation

Include positive controls in metabarcoding and sequencing

Assessment of contamination after sequencing – use both positive and negative controls



Sensitivity of PCR, contamination & other sources of artefacts in ancient and environmental DNA studies

...what about airborne eDNA?

New eDNA lab for airborne eDNA



Lyngaard et al. 2022, Current Biology

Three main approaches to label amplicons in metabarcoding studies

One-step PCR with fusion primers

Two-step PCR - first PCR with metabarcoding primers, second PCR with fusion primers

Tagged PCR with library build on amplicon pools

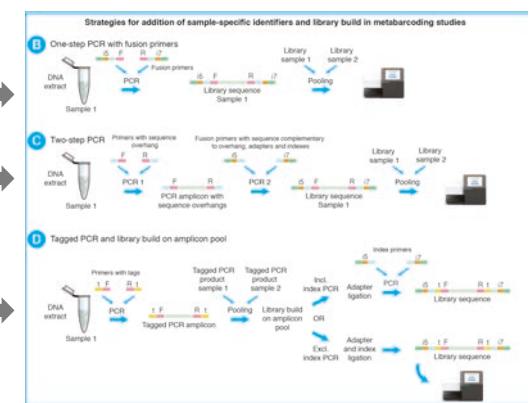
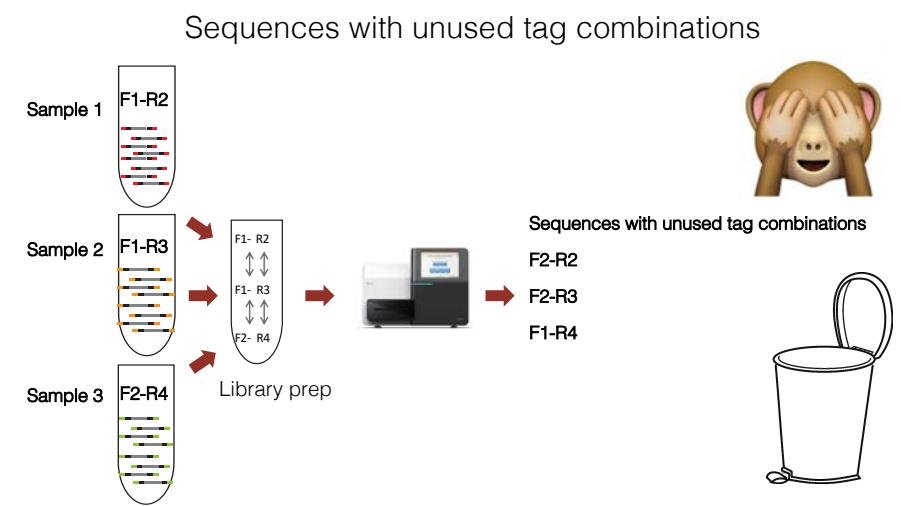
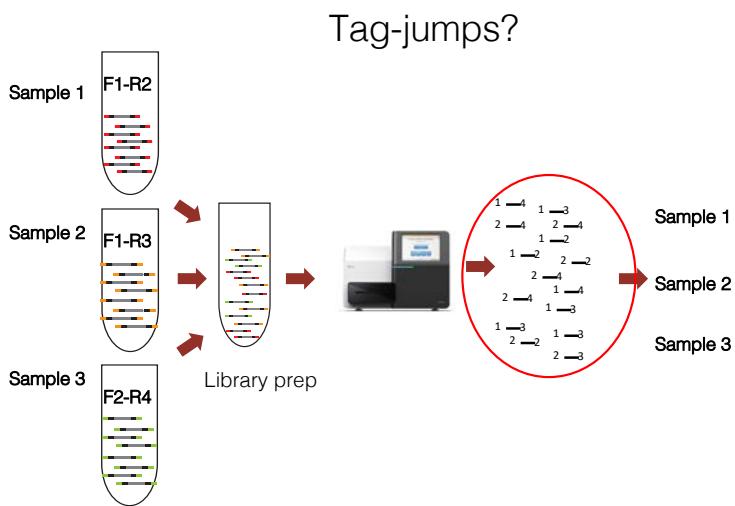
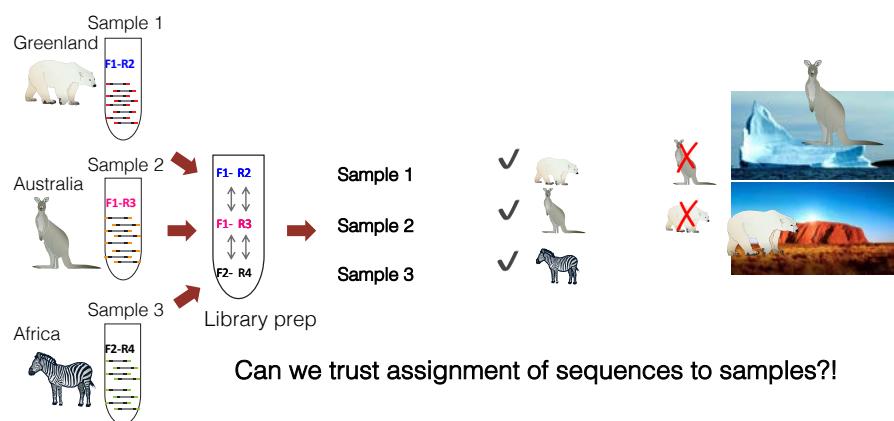


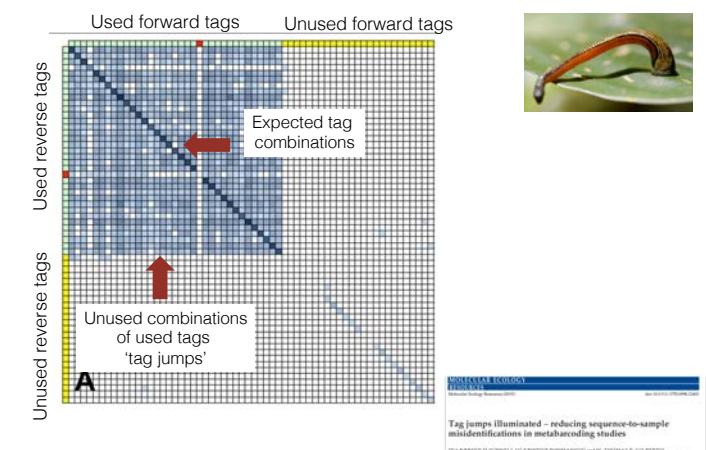
Figure from Bohmann, Elbrecht, Leese, Bunce, ... Creer, et al



Tag-jumps creating sequences with used tag combinations can make taxa spillover between samples



Documented tag-jumps on Illumina platform



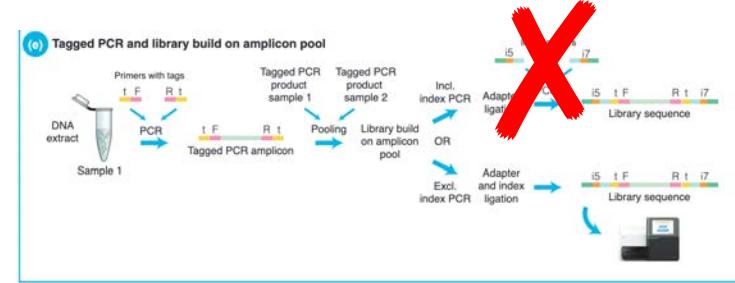


Commentary

Don't make a mista(g)ke: is tag switching an overlooked source of error in amplicon pyrosequencing studies?

Tor CARLSEN^{a,*}, Anders Bjørnsgaard AAS^a,
Daniel LINDNER^b, Trude VRÅLSTAD^a,
Trond SCHUMACHER^a, Håvard KAUSERUD^a

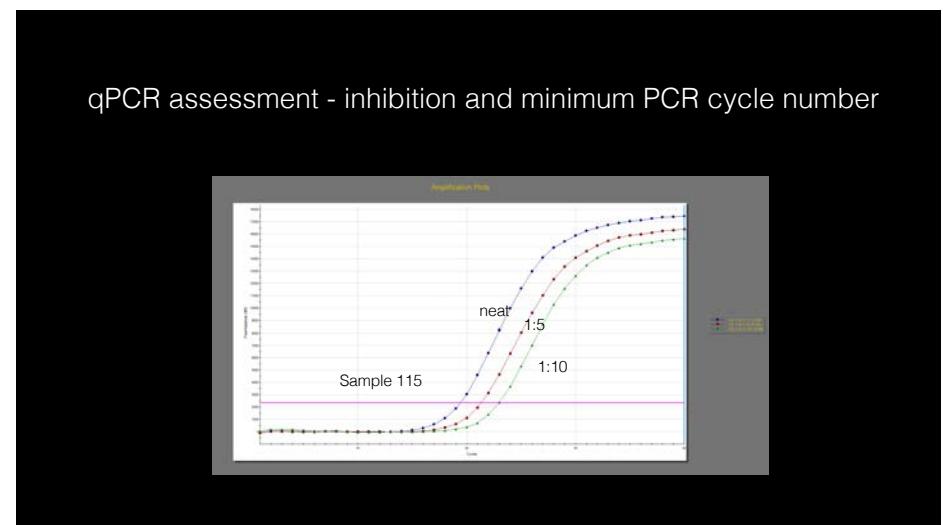
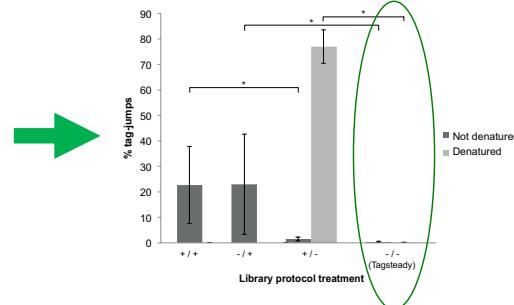
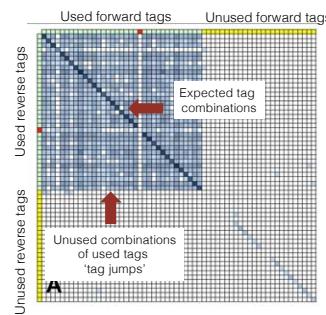
Tagged PCR approach and “Tagsteady” library build protocol



INVITED TECHNICAL REVIEW

Strategies for sample labelling and library preparation in DNA metabarcoding studies

Kristine Bohmann¹ | Vasco Elbrecht² | Christian Caree³ | Ilana Bista^{3,4} |
Florian Leese⁵ | Michael Bunce⁶ | Douglas W. Yu^{3,5} | Mathew Seymour^{1,2} |
Alex J. Dumbrell^{1,2} | Simon Creer²



Use of PCR replicates to balance error removal with detection of diversity

Methods in Ecology and Evolution

Research Article
Scrutinizing key steps for reliable metabarcoding of environmental samples

Antton Alberdi, Ostalizka Alzpirua, M Thomas P Gilbert, Kristine Bohmann

Taylor Wilcox @taylormwilcox · 22m

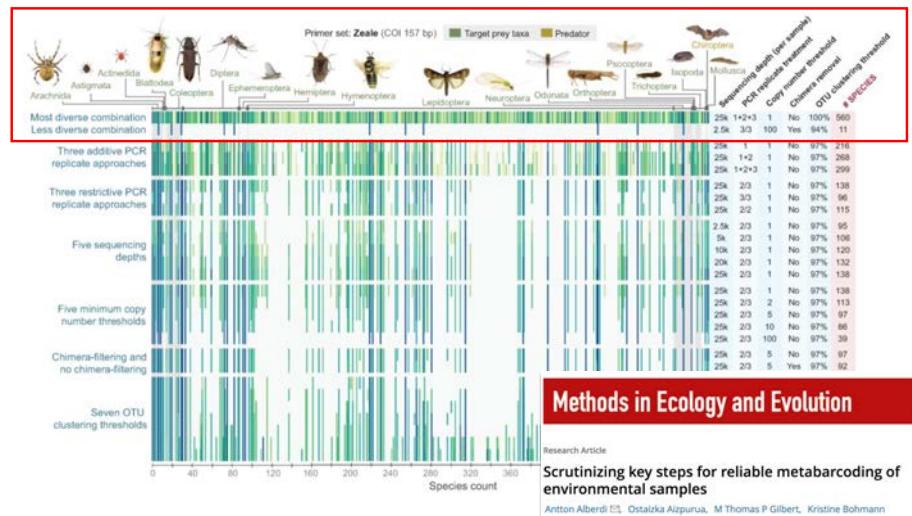
Useful technical paper. I'd recommend a cup of coffee and a pad of paper for sketching out the study design...



MethodsEcolEvol @MethodsEcolEvol

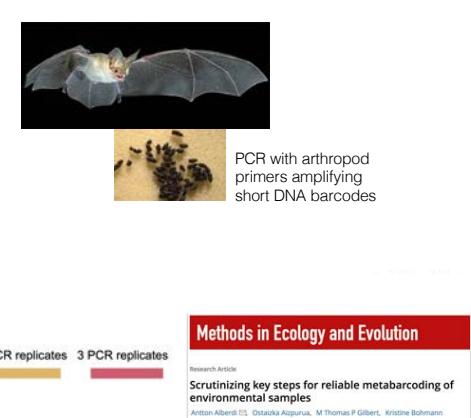
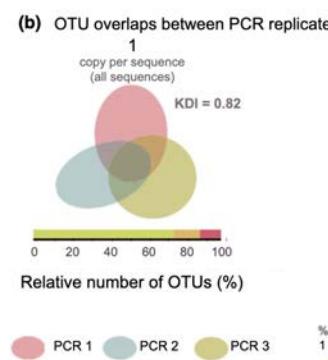
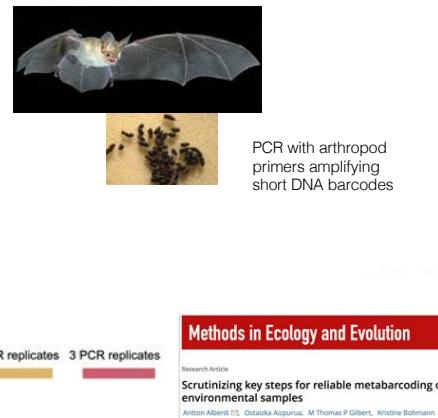
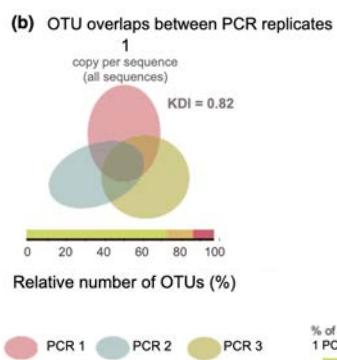
New study explores how the decisions that must be made during a #Metabarcoding workflow affect the final results
bit.ly/2exY0N4

Something good came out of it – including a scary figure

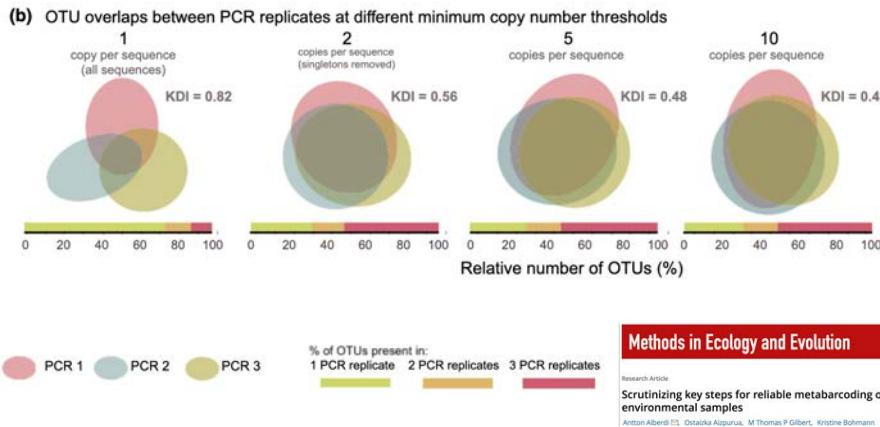


Positive and negative controls and PCR replicates can aid in balancing false positives and negatives and greatly aid your flexibility during data processing

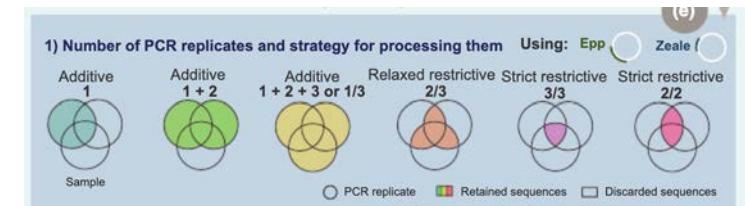
Use of PCR replicates to balance error removal with detection of diversity



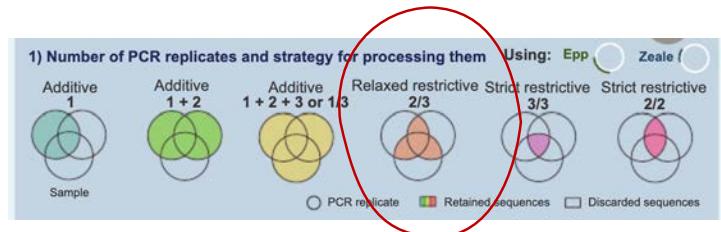
What can cause PCR replicates to not contain the same OTUs?



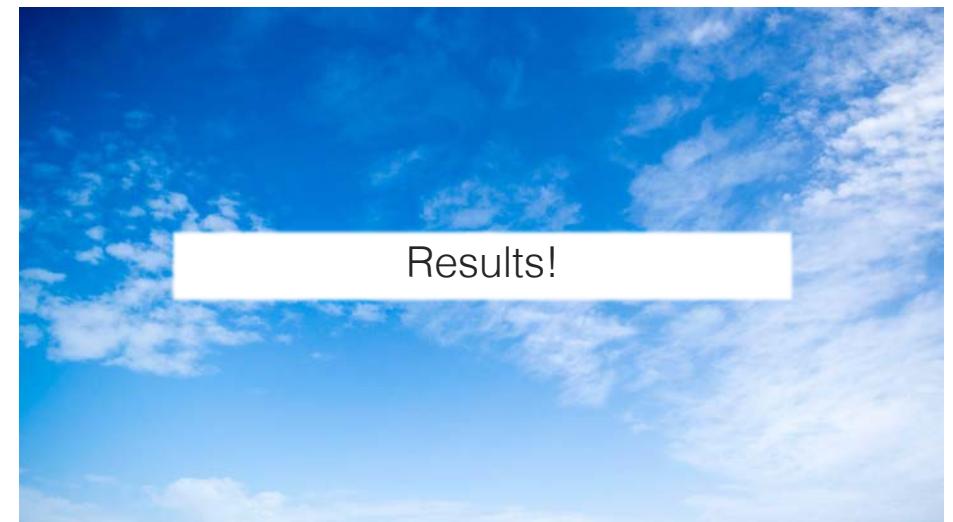
Different ways to use PCR replicates during data processing



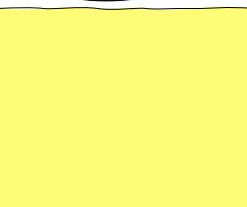
- Min 3 individually labelled PCR replicates per sample to facilitate balancing error removal with diversity detection (and account for human error)
- Study dependent how to use the PCR replicates
- Incl pos and neg controls and use of copy number thresholds, etc.



- 4 PCR replicates to balance error removal with diversity detection
- Retained sequences found in min. 3 of the 4 PCR replicates
- Used positive controls not expected in results
- Negative controls
- ...and more



Okapi and duiker stable – 12 samples, 2 metabarcoding markers



Lynggaard et al. 2022, Current Biology

Okapi and duiker stable – 12 samples, 2 metabarcoding markers



Lynggaard et al. 2022, Current Biology

Okapi and duiker stable – 12 samples, 2 metabarcoding markers



Okapi and duiker!
...and 20 other species!

Lynggaard et al. 2022, Current Biology

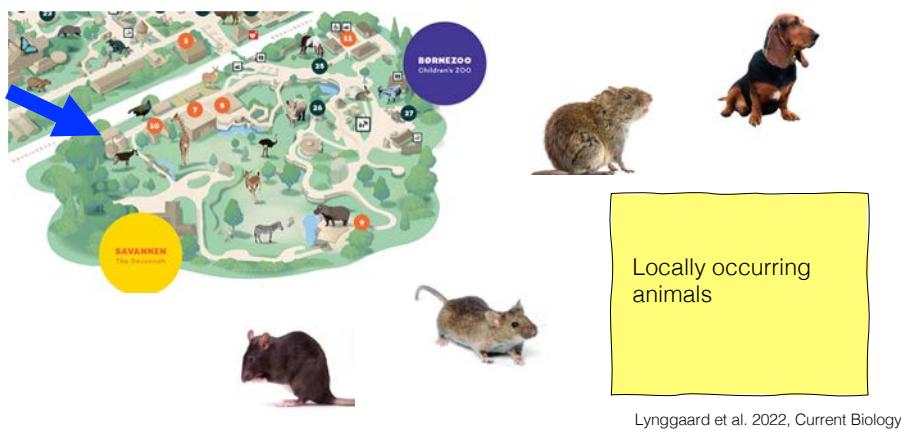
Okapi and duiker stable – 12 samples, 2 metabarcoding markers



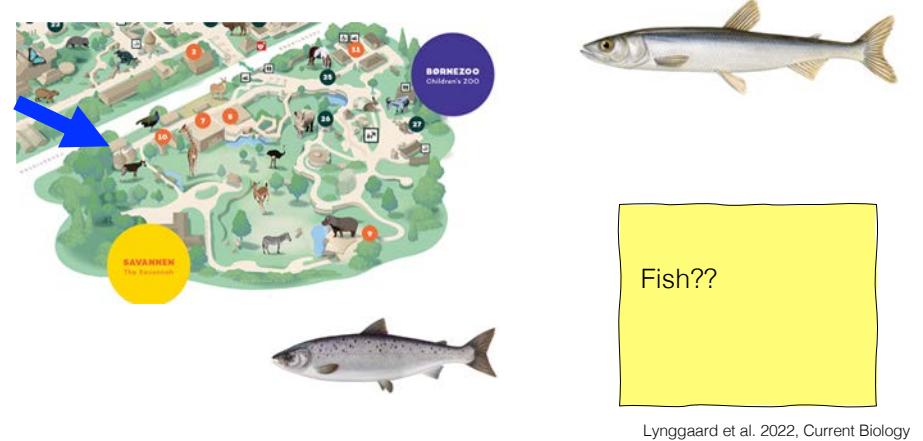
Zoo species found
outside the stable

Lynggaard et al. 2022, Current Biology

Okapi and duiker stable – 12 samples, 2 metabarcoding markers



Okapi and duiker stable – 12 samples, 2 metabarcoding markers



Rainforest House – 12 air samples, 2 metabarcoding markers



Rainforest House – 12 air samples

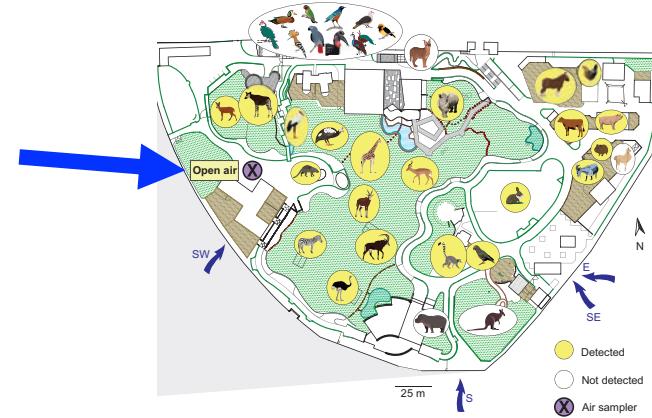


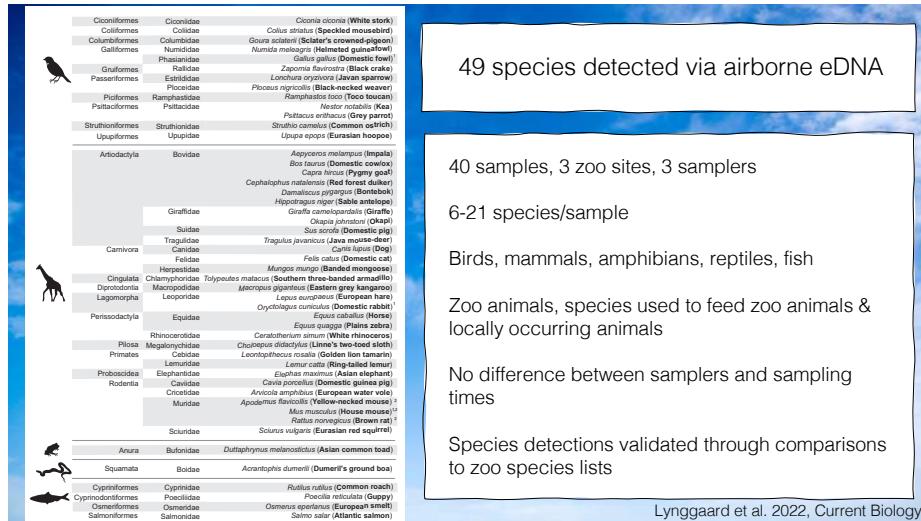


Open air – 16 samples, 2 metabarcoding markers



Outdoor sampling: 21 of the 35 species with access to outdoor enclosure





bioRxiv
THE PREPRINT SERVER FOR BIOLOGY

bioRxiv posts many COVID-19-related papers. A reminder: they have not been formally peer-reviewed and should not guide health-related behavior or be reported in the press as conclusive.

New Results [Follow this preprint](#)

Measuring biodiversity from DNA in the air

Elizabeth L. Clare,¹ Chloe K. Economou,¹ Frances J. Bennett,¹ Caitlin E. Dyer,¹ Katherine Adams,¹ Benjamin McRobie,¹ Rosie Drinkwater,¹ Joanne E. Littlefair¹
doi: <https://doi.org/10.1101/2021.07.15.452392>

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Current Biology
Volume 32 Number 3 February 7, 2020

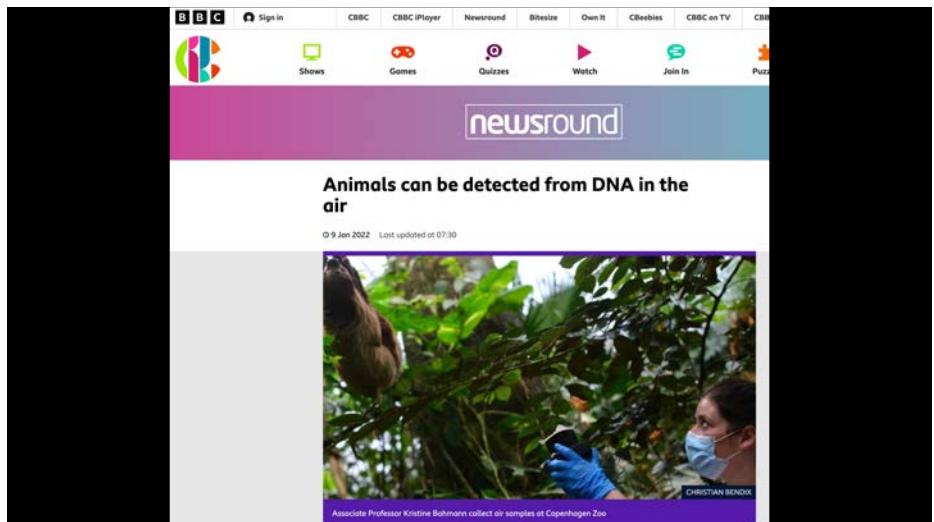
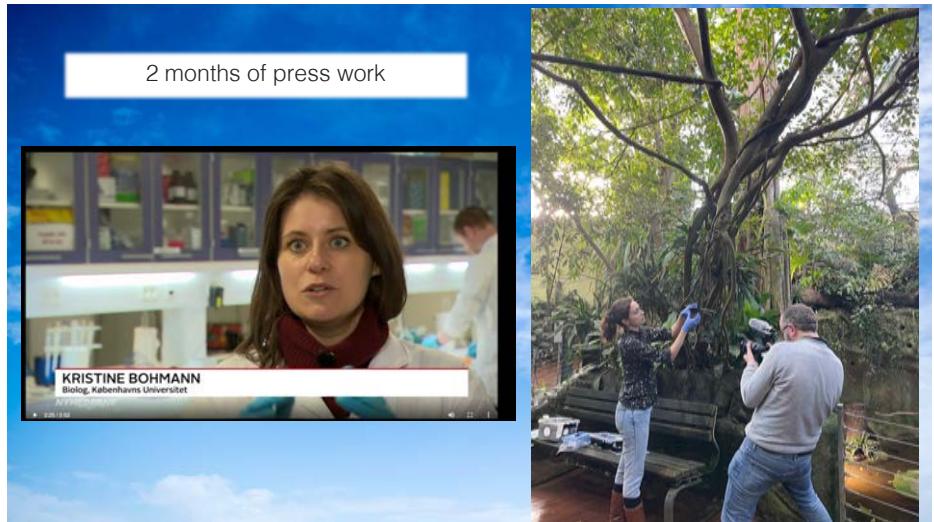
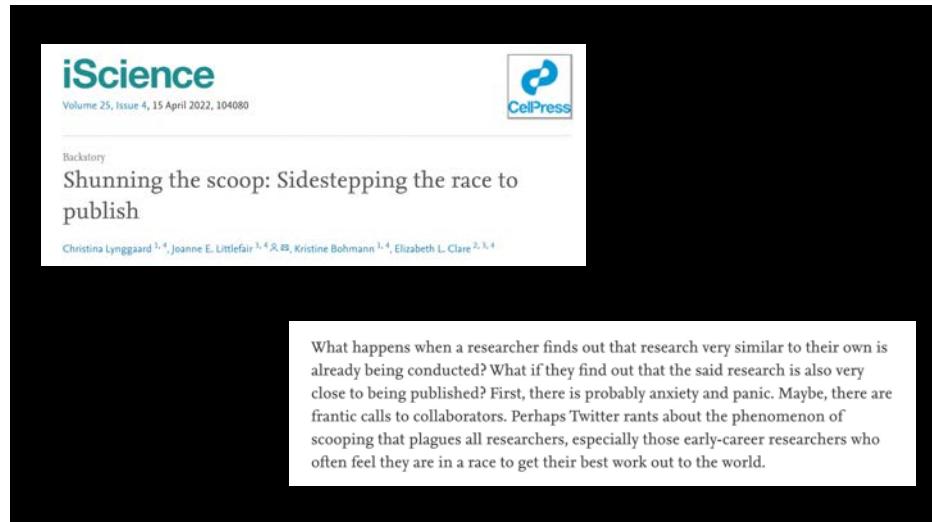
Report
Airborne environmental DNA for terrestrial vertebrate community monitoring

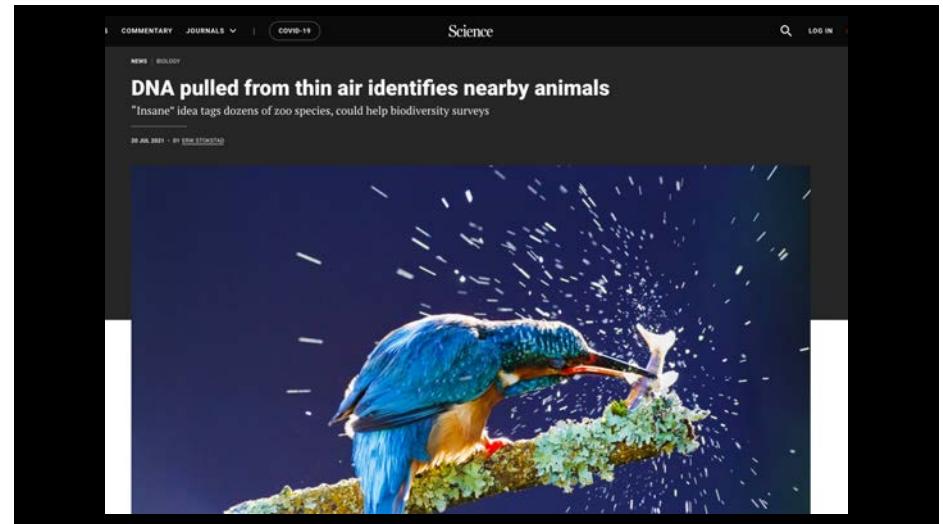
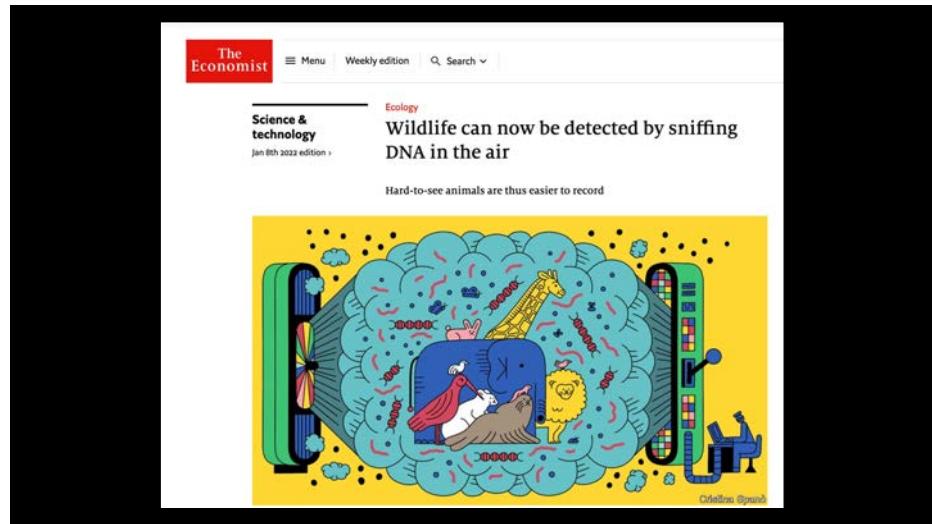
Christina Lynggaard,^{1,2}* Mads Frost Bertelsen,² Casper V. Jensen,² Matthew S. Johnson,^{2,4} Tobias Guldberg Freslev,³ Morten Tange Olsen,¹ and Kristine Bohmann^{1,2,*}

Current Biology
Report
Measuring biodiversity from DNA in the air

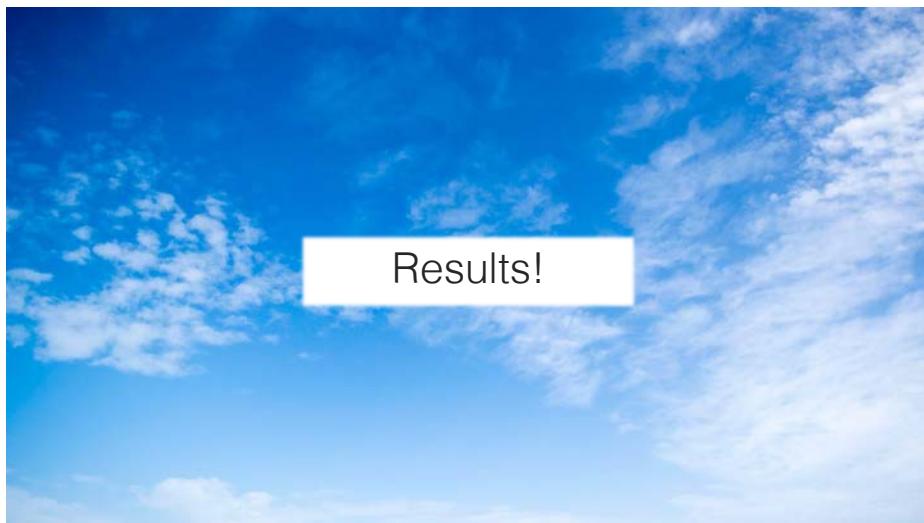
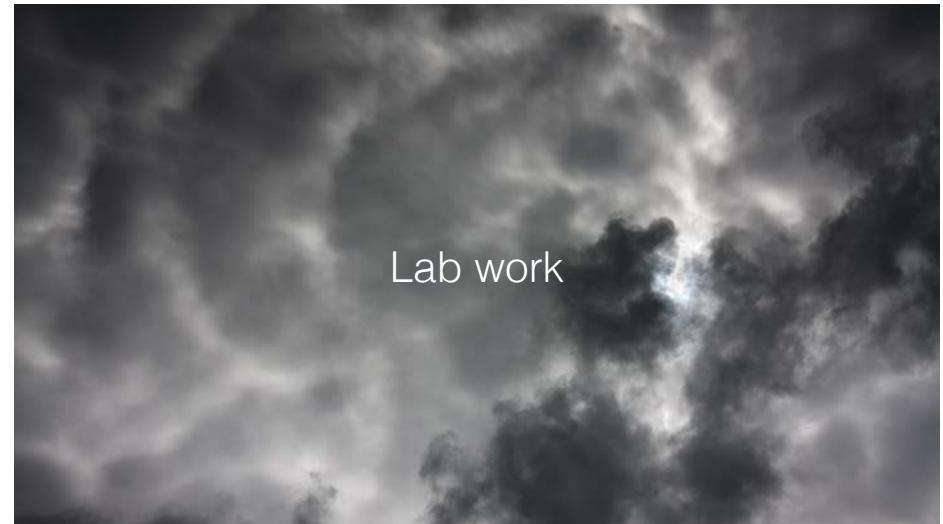
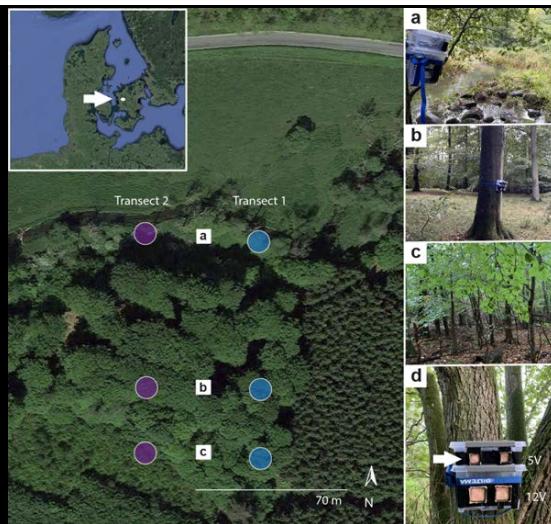
Elizabeth L. Clare,^{1,2,4,5*} Chloe K. Economou,¹ Frances J. Bennett,¹ Caitlin E. Dyer,¹ Katherine Adams,¹ Benjamin McRobie,¹ Rosie Drinkwater,¹ and Joanne E. Littlefair,^{1,5}

CellPress OPEN ACCESS

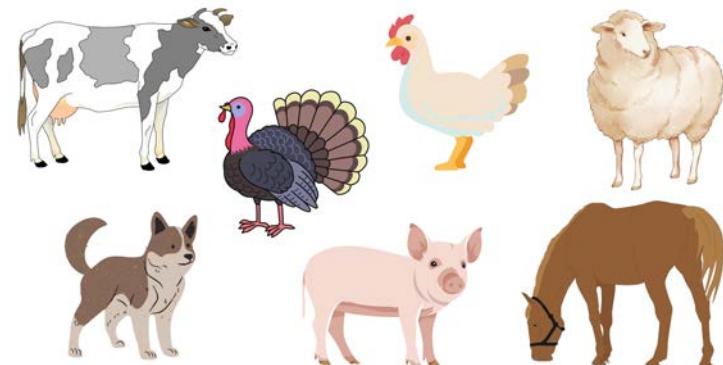




6 trees
2 field replicates for 5V samplers
2 field replicates for 12V samplers



64 vertebrate taxa – domestic animals!

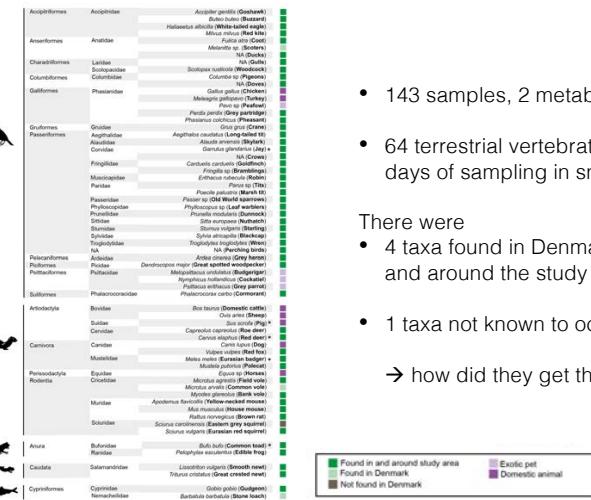


(143 samples, 12S vertebrate and 16S mammal primers)

64 vertebrate taxa – 57 “wild”! ...some of them:



64 vertebrate taxa – 57 “wild”! ...some of them:



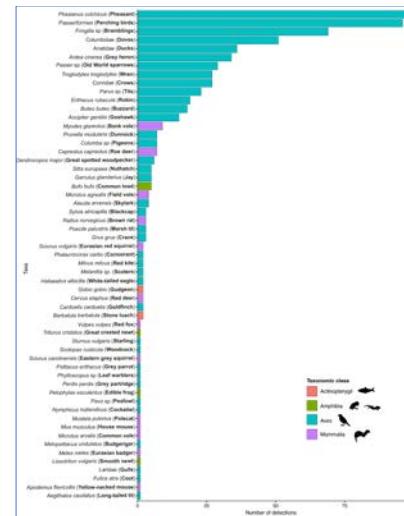
- 143 samples, 2 metabarcoding markers
- 64 terrestrial vertebrate taxa detected in 3 days of sampling in small sampling area

There were

- 4 taxa found in Denmark, but not observed in and around the study area
- 1 taxa not known to occur in Denmark
- how did they get there?

PREPRINT

Lynggaard et al. 2022, biorxiv

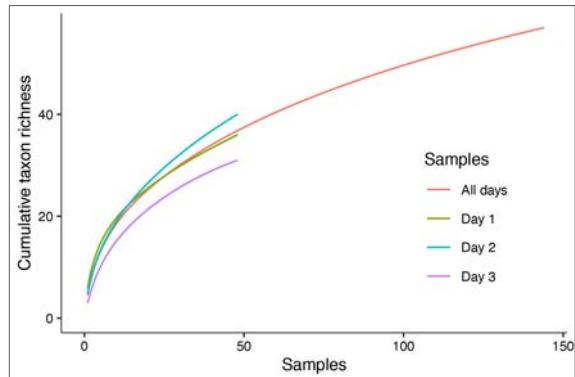


The 57 “wild” vertebrate taxa

- Most taxa were rarely detected in samples
- Vertebrate detections in ca. 90% of samples
- 1-12 taxa detected per sample

PREPRINT

Lynggaard et al. 2022, biorxiv

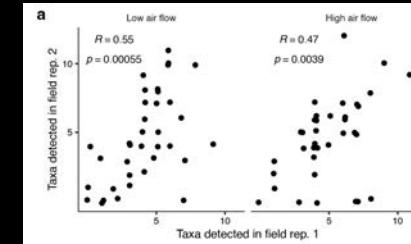


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Field replicates



Moderate correlation between number of detections in field replicates

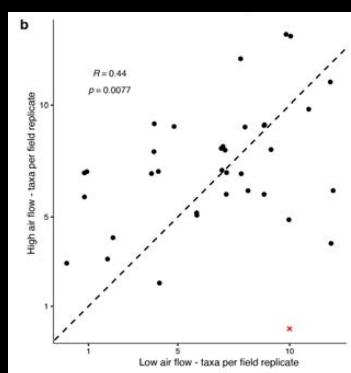
No pair of field replicates detected the exact same community

PREPRINT

Lynggaard et al. 2022, biorxiv



Low vs high air flow



Moderate correlation between number of detections in 5 V and 12 V samples

Sampling depends more on position and air currents than volume air sampled?

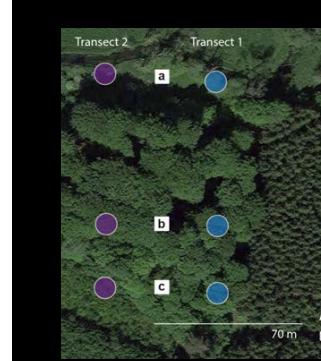
However, more shared taxa between field replicates with 12 V sampler

Patchy and scarce distribution of airborne eDNA

Need to incorporate field replicates

PREPRINT

Lynggaard et al. 2022, biorxiv



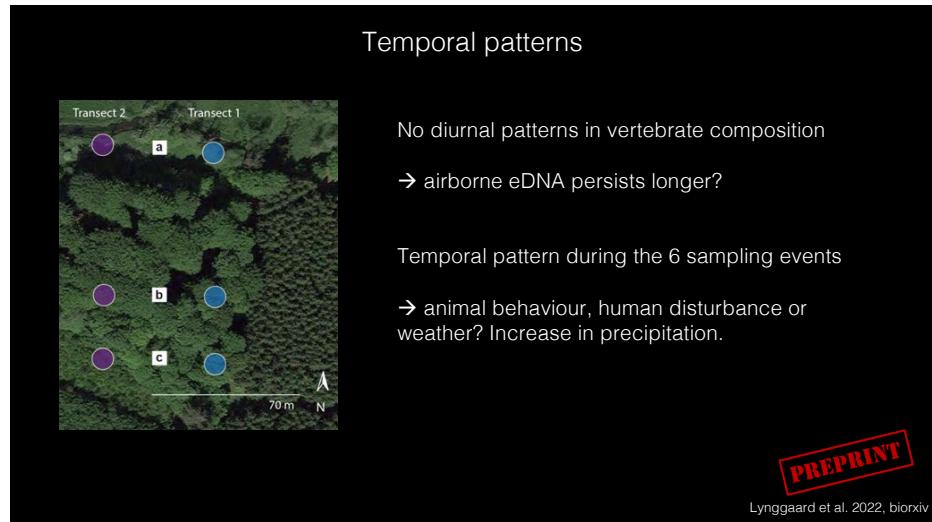
No spatial pattern

No difference in vertebrate composition between sampling sites

-> random distribution of vertebrate airborne eDNA at least in this limited site

PREPRINT

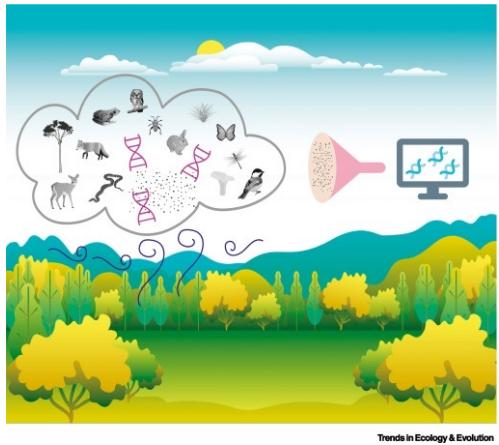
Lynggaard et al. 2022, biorxiv



Intended learning outcomes

After this session you will be able to:

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- Formulate a strategy for the lab set-up of your metabarcoding study to account for or avoid different sources of contamination and facilitate the ability to balance error removal with diversity detection during data processing



Trends in Ecology & Evolution

Forum

Transforming terrestrial biodiversity surveys using airborne eDNA

Kristine Bohmann ^{1,*} and Christina Lynggaard ^{1,†}

extracted primers design informative organisms, labelling of high-through metabarcoding cost-effect

Trends in Ecology & Evolution



Christina Lynggaard

Thank you

Mads Frost Bertelsen
Casper V. Jensen
Matthew S. Johnson
Tobias Frøslev
Morten Tange Olsen
Sarah Mak
Lasse Vinner
Jacob Agerbo Rasmussen
Christian Carøe
Tina Brand
Pernille Selmer Olsen
Kurt Kjær
Tom Gilbert
... & many more

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Questions?