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Primer Validation for Metabarcoding

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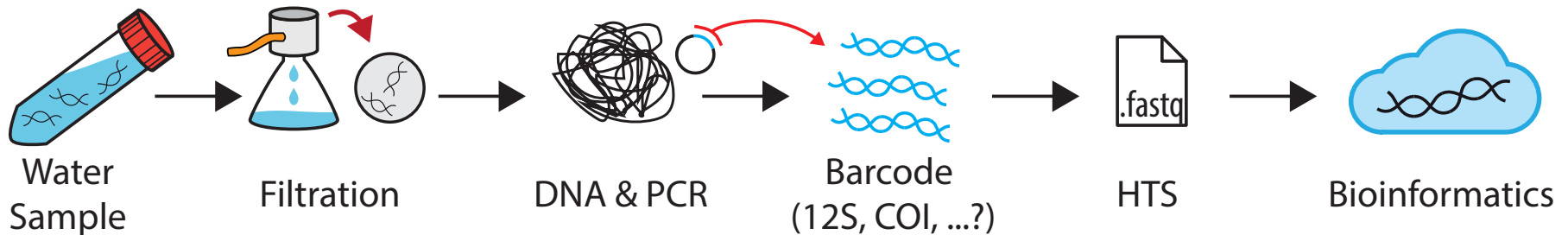


@VascoElbrecht

eDNA metabarcoding: Sounds too good to be true?

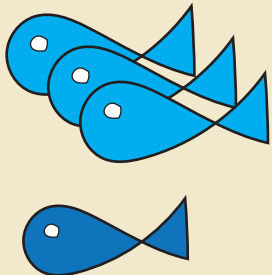


“just” collect a water sample - Capture **all** the biodiversity!?



Traditional surveys

- Lot's of work
- Count data (Abundance)



3 x Fish **A**
1 x Fish **B**

eDNA surveys

- What count data do we get?
- Biomass, relative abundance, p/a!?



1000 reads Fish **A**
0 reads Fish **B**
300 reads Fish **C**

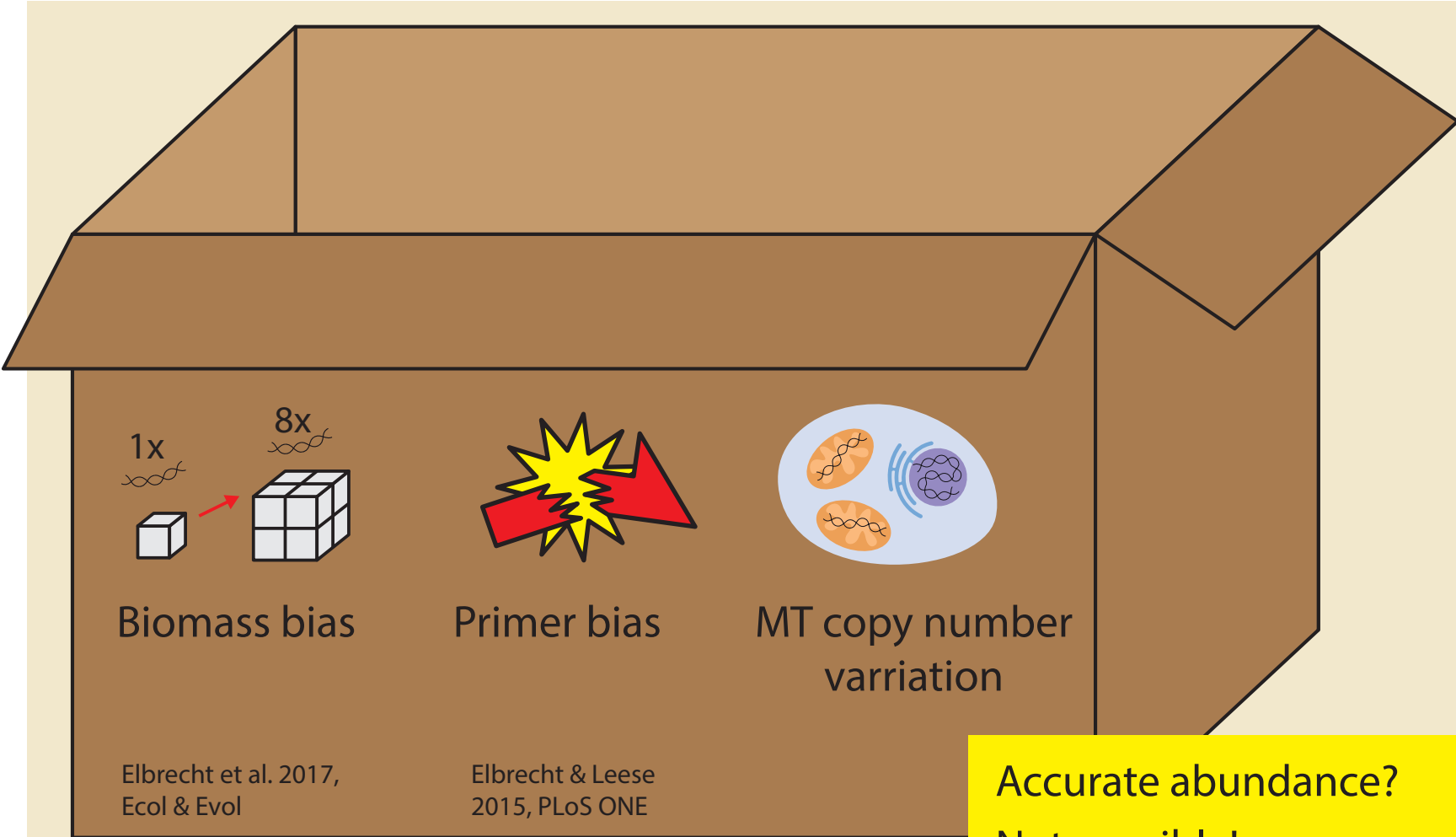


Vasco's backgroud: Method validation ~~eDNA~~ Metabarcoding



Elbrecht et al. 2017, MEE

Tissue based metabarcoding: Well understood biases



Accurate abundance?
Not possible!

eDNA metabarcoding

Adding (unknown) biases on top!

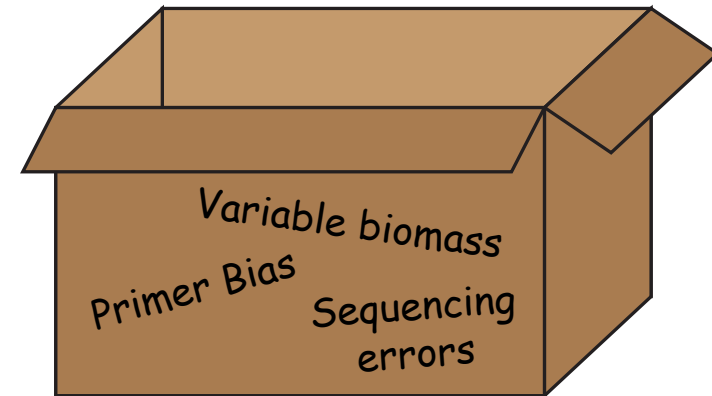
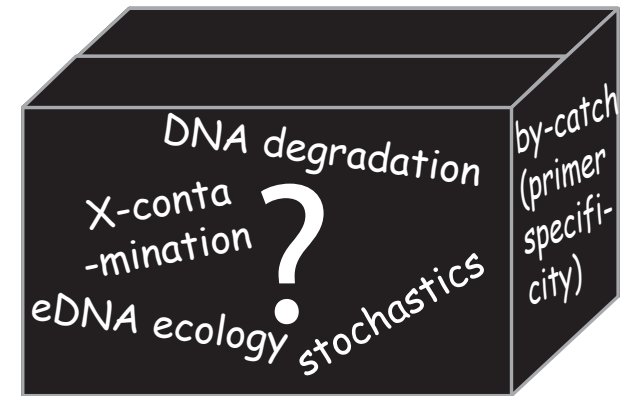


- eDNA metabarcoding:
 - Metabarcoding biases (previous slides)
 - Many eDNA biases and unknowns!
- Reviews:
 - **Ecology of eDNA:** Barnes & Turner 2015, Conserv Genet
 - **eDNA abundance / good introduction:** Deiner et al. 2017, Mol Ecol
 - **eDNA uncertainty:** Cristescu & Hebert, 2018 Annual Reviews
- Less a problem for targeted qPCR / ddPCR

eDNA Metabarcoding = amazing tool!

- But let's use it cautiously
- Maybe a bit over-hyped ;)

eDNA biases (black box)



Metabarcoding biases

Presence / absence vs relative abundance

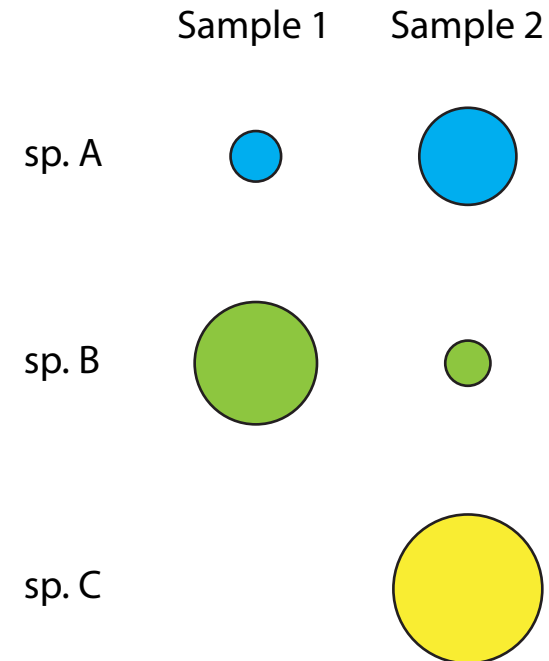


IMO: What can we (reliably) say with eDNA metabarcoding?

- Exact number of fish? **NO**
 - Biomass of each fish? **NO**
 - With species specific markers: **Maybe**
- Levi et al. 2018, bioRxiv
- Presence / absence of fish species? **YES!**
 - Relative abundance? **YES!**

eDNA has it's biases!
Will **not** capture everything

How to use relative abundance?



- 1.) Sample 1 contains more sp. A!
- 2.) But due to primer bias, species abundance is unknown!

Example: Primer bias

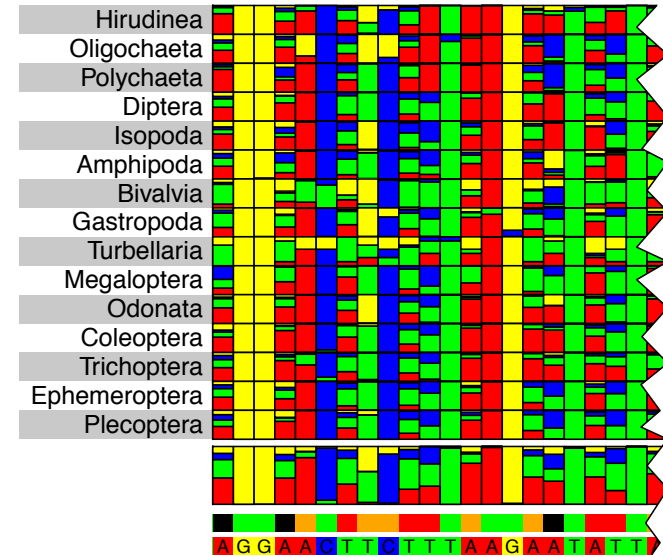
- “Solving” primer bias;
 - We need multiple markers!
Alberdi et al. 2017, MEE, Zhang et al. 2018, Ecol Appl
 - Ribosomal markers are great!
Deagle et al. 2014, Biol Letters
 - My COI primer (without degeneracy) is great
Jusino et al. 2018, Mol Ecol Resour

Maybe true in *some* cases!

But how about extensive primer validation!?

- Tissue bulk sample containing 374 taxa (BINs)
- Metabarcoding with 21 different primer sets!

Braukmann et al. 2018, bioRxiv



Elbrecht & Leese 2016, MEE

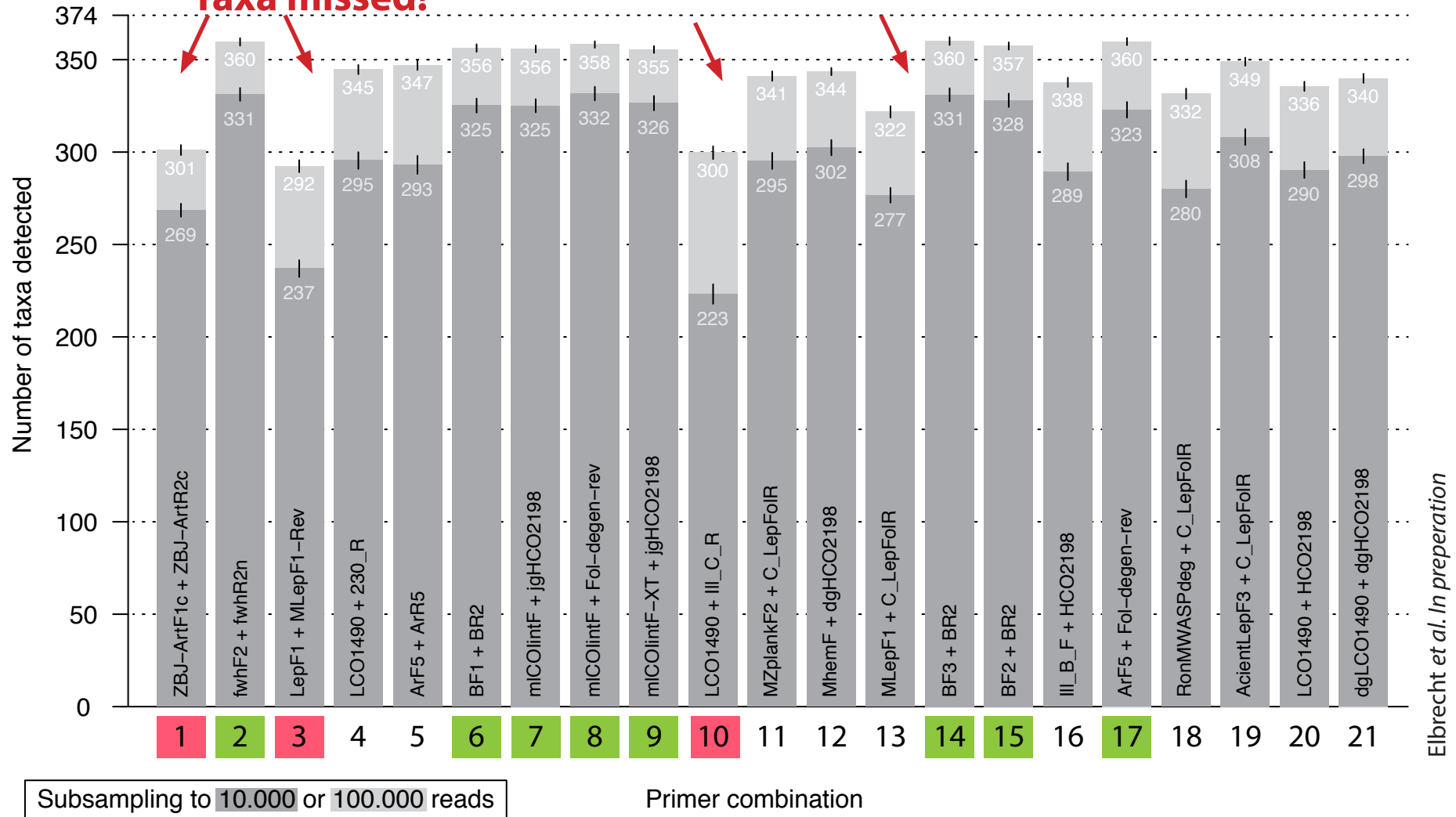


Primer bias!

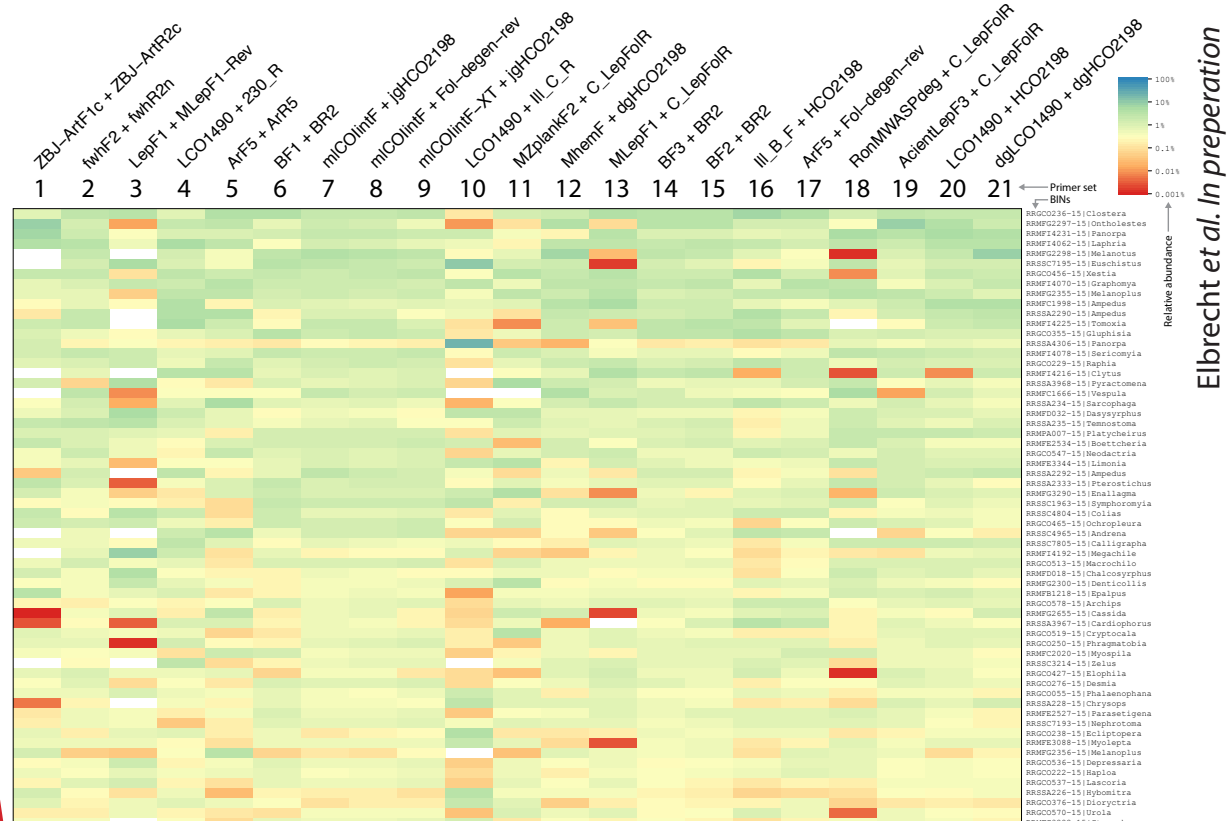
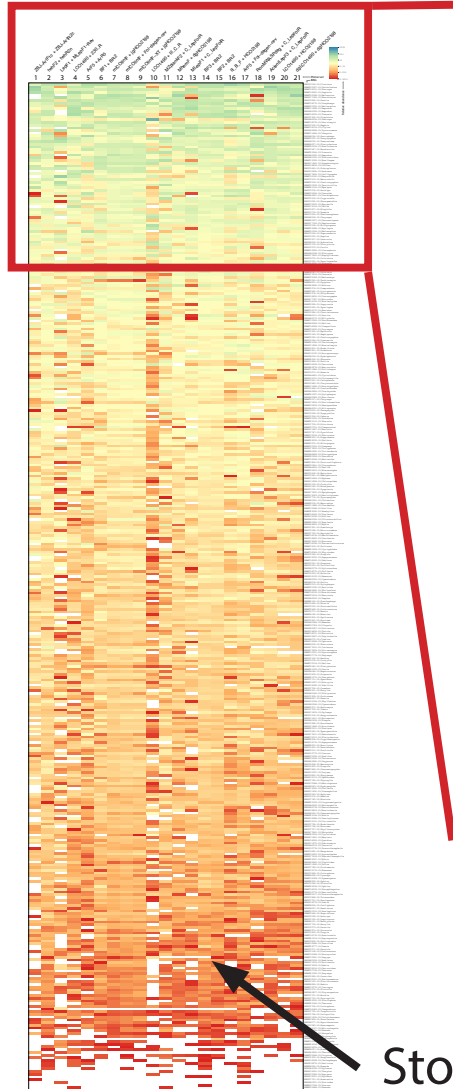
21 Primer sets evaluated on 374 bulk insect taxa



Taxa missed!



One good primer set, multiple primers only for multiple large groups!



All good (degenerated) primers recover $\leq 95\%$ of the taxa!

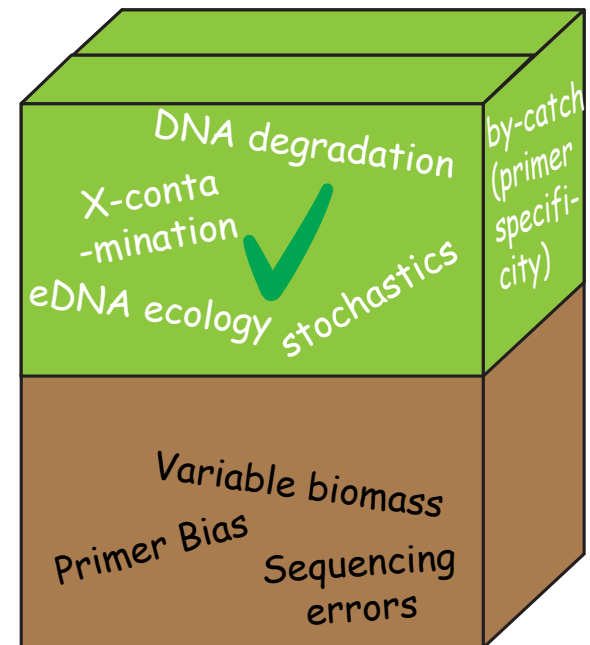
Stochastic effects Leray & Knowlton et al. (PeerJ 2017)

Conclusions



- Metabarcoding or single species detection?
- Primer validations are key!
- Need to understand biases in:
 - eDNA "ecology"
 - Lab-methods & bioinformatics
- eDNA based methods already useful, embrace them!

"As this study clearly shows, there are excellent [#metabarcoding](#) primers out there, we just have to use them."
[@VascoElbrecht](#) via Twitter



Thanks for your attention



Former involved people

- Florian Leese
 - Janis Neumanns
 - Bianca Peinert
 - Arne Beermann
 - Kristian Meissner
 - Edith Vamos
 - Cristina Hartmann-Fatu
 - Kathrin Theissinger
 - Anna Kästel
 - Jenny Makkonen
 - Pierre Taberlet
 - Eric Coissac
- Red brackets on the right side of the list group the names into four categories: TierMix (first two), Schmalenau (next two), Finland (next two), and Chironomids (next two). A final bracket labeled '16S' groups the last two names.

The new stuff:

- Dirk Steinke
- Paul Hebert
- Tom Braukmann
- Sean Prosser
- Evgeny Zakharov
- Nataly Ivanova
- Jayme Sones
- Steph deWaard
- Linda Lait
- Brianne Jacques
- Vanessa Bouwer
- Marsha Kuzmina
- and many more
(sorry running out of space ;)

Funding:

- Food From Thought



Thank you!
=)