eDNA metabarcoding: Sounds too good to be true?

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

Water Sample ➔ Filtration ➔ DNA & PCR ➔ Barcode (12S, COI, ...?) ➔ HTS ➔ Bioinformatics

Traditional surveys
- Lot’s of work
- Count data (Abundance)

<table>
<thead>
<tr>
<th>Fish A</th>
<th>Fish B</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Fish A</th>
<th>Fish B</th>
<th>Fish C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td>0</td>
<td>300</td>
</tr>
</tbody>
</table>
Tissue based metabarcoding: Well understood biases

Elbrecht & Leese 2015, PLoS ONE
Elbrecht et al. 2017, Ecol & Evol

Accurate abundance? Not possible!

Biomass bias

Primer bias

MT copy number variation
eDNA metabarcoding
Adding (unkown) biases on top!

- eDNA metabarcoding:
  - Metabarcoding biases (previous slides)
  - Many eDNA biases and unknowns!

- Reviews:
  - Ecology of eDNA: Barnes & Turner 2015, Conserv Genet
  - 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)**
- DNA degradation
- X-contamination
- eDNA ecology
- Stochastics
- By-catch (primer specificity)
- Variable biomass
- Primer bias
- Sequencing errors

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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

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1.) Sample 1 contains more sp. B!
2.) But due to primer bias, species abundance is unknown!
Example: Primer bias

- “Solving” primer bias;
  - We need multiple markers!  
  - 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
Primer bias!
21 Primer sets evaluated on 374 bulk insect taxa

Subsampling to 10,000 or 100,000 reads
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