The true picture of environmental DNA, a case study in harvested fishponds

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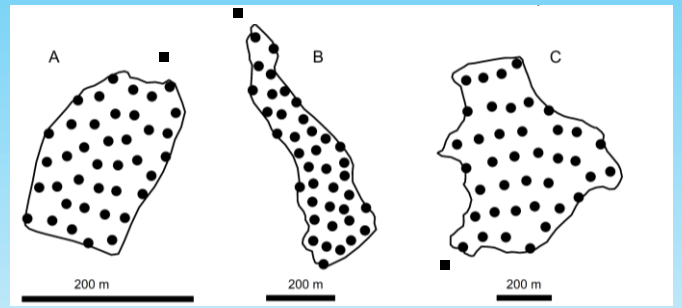
Introduction

eDNA metabarcoding is a high sensitivity method for species detection. While this has been compared in controlled conditions, and validated via comparison with traditional sampling, absolute comparisons in natural settings are rare. In this study, we compare the absolute fish community with eDNA metabarcoding outputs.

Material and methods

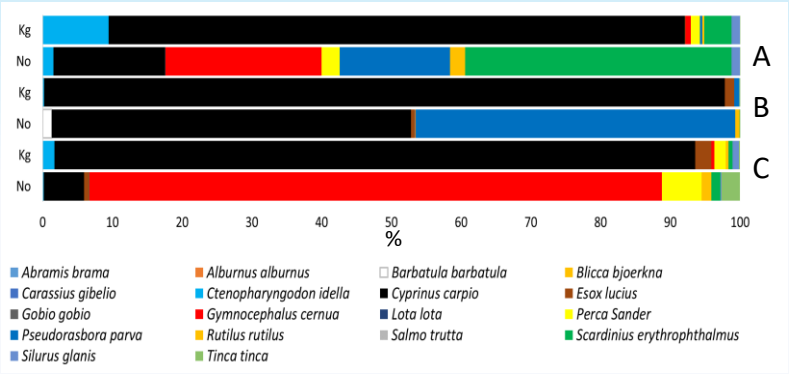
In summer and autumn 2019 water samples in 39 sites and an inflow across ponds A, B, C in the Czech Republic were collected. 500 ml from each sample was filtered through 0.45 µl membrane filters. Uniquely indexed vertebrate primers aligning mitochondrial 12S ribosomal RNA gene were utilised for eDNA amplification. Sequence reads were analysed using metaBEAT pipeline. The sequences were assigned to species level with exception *Perca fluviatilis* and *Sander lucioperca* which could not be differentiated.



Location of sampling points in each pond (circles) and inflows (square).

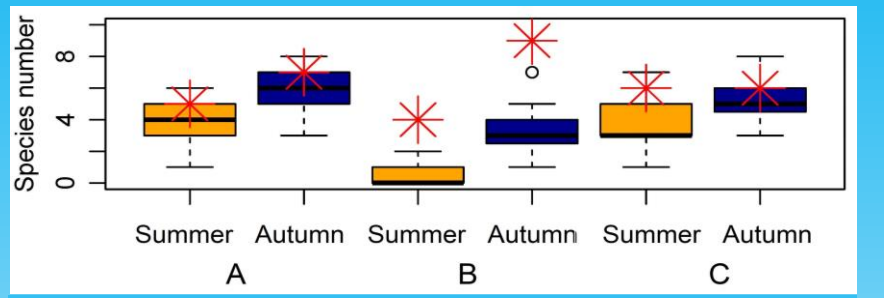
Results

319,833 fish of 27,053.8 kg were harvested in the ponds.

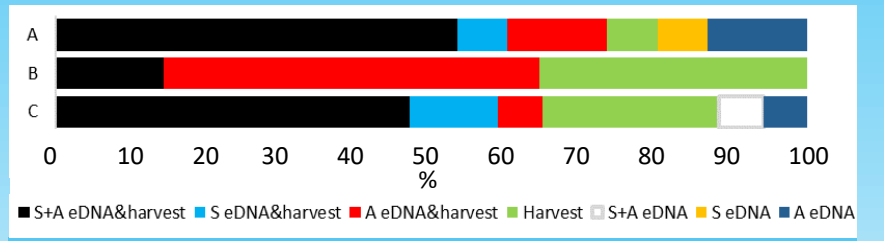


Barplot of relative species abundance and biomass harvested in ponds.

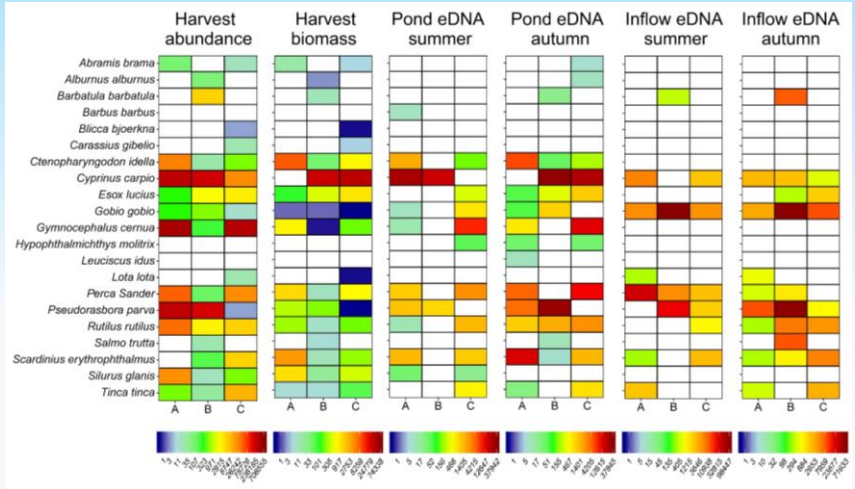
35,627,310 raw sequences, 21,540,396 after bioinformatic filtering, Ø 68,11 per sample



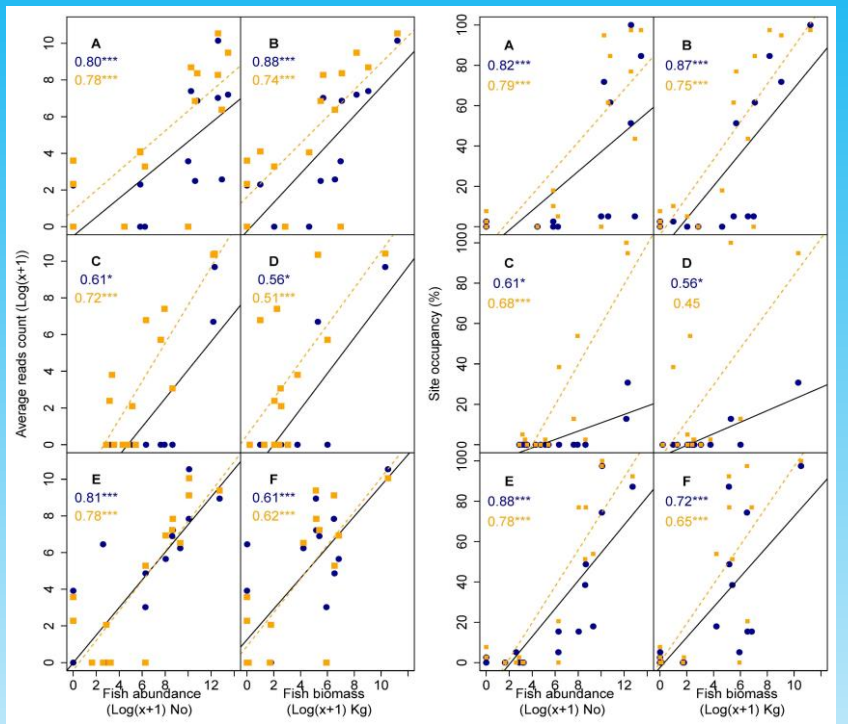
Boxplots with number of species detected in pond eDNA samples in different seasons. Red stars indicate number of species detected in inflows.



Barplot with proportions of detected species by pond eDNA in summer (S), autumn (A) and during the harvest.



Heatmap with detected species in studied ponds based on harvest and eDNA matabarcoding in summer and autumn.



Relationships between average reads count / site occupancy and fish abundance (A, C, E) / biomass (B, D, F) in ponds A (A, B), B (C, D) and C (E, F) in summer (orange dots, solid line) and autumn (blue squares, dashed line). Spearman's correlations are added to each relationship with significance $p < 0.001$***, <0.05*.

Conclusion

This study provides evidence of the factors which influence the efficiency of eDNA metabarcoding campaigns. eDNA metabarcoding data detected common species in the communities. The data correlates with real fish abundance and biomass, but the detections depend on environmental variables. More species were detected in conditions of lower temperature, more technical replicates, and in running compare to standing water. This research highlights timing and sample coverage as essential steps to achieve highest overall detection.

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