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Conference Abstract

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

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Abstract

The applications of environmental DNA (eDNA) metabarcoding are constantly increasing. Many validation studies have been performed in laboratories, however, field experiments are necessary to verify the robustness of eDNA based monitoring. In summer and autumn 2019 water samples in 39 sites and an inflow across three ponds of sizes of approximately 3 (A), 10 (B) and 29 (C) hectares in the Czech Republic were collected. 500 ml from each sample was filtered through 0.45 µl membrane filters and extracted using the Mu-DNA water protocol. Uniquely indexed vertebrate primers aligning mitochondrial 12S ribosomal RNA gene were utilised for eDNA amplification. PCR negative and positive controls were included to each sublibrary. 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, further referred as *Perca+Sander* and used as a single species.

At the end of autumn, ponds were harvested and 319,833 fish of 27,054 kg of 22 species were captured. The most abundant species was *Gymnocephalus cernua* (on average 34.8%) followed by *Cyprinus carpio* and *Pseudorasbora parva*. *Cyprinus carpio* was also the dominant species in biomass (on average 90.7%), followed by the *Ctenopharyngodon idella* and *Esox Lucius*.

The total number of sequences across 240 eDNA samples was 35,627,310. 21,540,396 sequences remained after bioinformatic filtering with an average read count per sample of 68,111. All samples detected 1–6 species, except for summer pond B where 64% were negative. In total, more species were detected in autumn than summer in pond A 12/10 and pond B 9/2 and the same in pond C 11/11. The only species detected in all campaigns was *Cyprinus carpio* with the highest average reads count of 28,961. *Ctenopharyngodon idella*, *Rutilus rutilus*, and *Scardinius erythrophthalmus* were detected in all campaigns except the summer pond B samples. The site occupancy values were variable between ponds and seasons with exception of ubiquitous *Cyprinus carpio*.

The numbers of detected species in inflows were higher in autumn compared to summer in pond A (7/5) and B (9/4) and identical in pond C (6/6). The detected species in inflows were mostly the same as species detected in ponds. *Gobio gobio* was the only detected species in all campaigns. *Lota lota* was detected in eDNA in summer and autumn only in inflow to pond A. In pond B *Perca+Sander* and in pond C *Pseudorasbora parva* were detected in eDNA inflow in both periods and in harvest, but not in eDNA in ponds. The percentage of shared species detected by eDNA and at pond harvests was >50% in all ponds. During the pond harvest 1, 5 and 4 species were captured not detected in ponds´ eDNA compared to 3, 0, and 2 species detected only in ponds´ eDNA in ponds A, B and C, respectively. Significant positive relationships were observed between fish read counts/species site occupancy and fish abundance/biomass with exception of data from pond B in autumn.

This study highlights the importance of field experiments and methods validation. Dominant species detection is relatively straightforward; however, detection of rare species is more challenging depending on species behaviour, habitat complexity. It further supports that eDNA should be applied in optimal conditions to achieve highest overall detection, which has important implications for applying this method to aid management and policy initiatives.

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Keywords

community ecology; environmental DNA; water; species detection

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