Comparison of different eDNA signal sources and electrofishing data in the detection of riverine fish fauna

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BACKGROUND

- Monitoring biodiversity relies on effective tools to identify species
- Environmental DNA (eDNA) may be an efficient way to detect species (Yao et al. 2022), especially for cryptic taxa and/or in environments that are difficult to sample
- As eDNA degrades in the environment, this method may provide 'snapshot' of the site's biodiversity unless large volumes of the sample (e.g. air, water) are collected

SPONGES MAY SAMPLE eDNA?

- Freshwater sponges (Spongillida: Demospongiae) (Fig. 1) comprise some 240 known species that inhabit natural and artificial substrates in continental waters (except Antarctica).
- Sponges are efficient filter feeders that collect particles from their aquatic environment.
- Teleost eDNA has been recovered from marine sponges (Mariani et al. 2019)

GENERAL AIM

- To determine whether freshwater sponges by filtering potentially large volumes of water can act as 'natural eDNA samplers'

STUDY DESIGN

- To compare results from eDNA surveys, we selected 10 streams where the teleost community is monitored by electrofishing by LUKE: 5 locations in Uusimaa, 4 in Central Finland, and 1 in Pirkanmaa
- At each stream, we (1) surveyed for sponges (and collected a sample from sponges if they were present) and collected a (2) water sample (Fig. 2, 3) and (3) sediment sample from 3 different locations.
- The teleost communities that are estimated at each site using eDNA (sponge, water, sediment) will be compared with those estimated by electrofishing.

RESEARCH QUESTIONS

- Will eDNA methods detect a similar community composition as electrofishing? If not, what taxa are overlooked?
- Which type of sample of eDNA sample is most efficient at detecting most species within the teleost community?

HYPOTHESIS

- A broader range of teleost taxa can better be detected by eDNA from sponges due to the greater amount of water filtered by sponges compared with filter and/or a sediment sample.

Fig. 1. A colony of the sponge *Spongilla lacustris.*

Fig. 2 & 3 credits: Sinikka Tikkanen

Fig. 2. Water samples pass through a filter to collect particles / eDNA.

Fig. 3. Filter filled with DNA/RNA Shield to preserve eDNA.

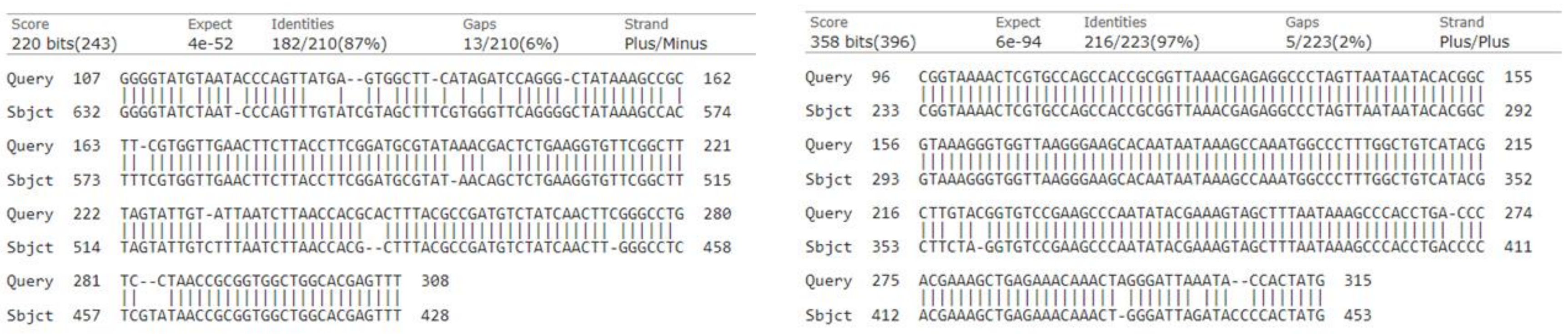
Fig. 4. MinION nanopore sequencing device.

METHODS

- eDNA extracted from sponge samples (QIAGEN DNeasy PowerSoil Pro Kit)
- Amplicon sequencing of Tele02 and MiFish primer pairs that amplify an *ca*. 170 bp region of the 12S locus (Duarte et al. 2018; Miya et al. 2015)
- Sequencing on MinION nanopore sequencing device (Fig. 4).
- Identity of amplicons examined against a custom database of teleost mitochondrial DNA sequences (from NCBI) (Fig. 5).

PRELIMINARY RESULTS

- Sponge distribution is variable, with sponges found in 5 of the 10 study streams and at 10 sites
- Of the 540 sponge colonies found, 500 (93%) occurred in just 2 streams
- Preliminary amplicon sequencing uncovered teleost eDNA from sponge samples – for perch (*Perca fluviatilis*) and roach (*Rutilus rutilus*) (Fig. 5).
- The primer pair Tele02 yielded teleost sequences (but MiFish amplified other, non-target taxa) – further PCR optimisation is required



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Score            Expect   Identities      Gaps           Strand
220 bits(243)    4e-52    182/210(87%)    13/210(6%)     Plus/Minus

Query  107  GGGGTATGTAATACCCAGTTATGA--GTGGCTT-CATAGATCCAGGG-CTATAAAGCCGC  162
Sbjct  632  GGGGTATCTAAT-CCCAGTTTGTATCGTAGCTTTCGTGGGTTCAGGGGCTATAAAGCCAC  574
Query  163  TT-CGTGGTTGAACTTCTTACCTTCGGATGCGTATAAACGACTCTGAAGGTGTTCGGCTT  221
Sbjct  573  TTTCGTGGTTGAACTTCTTACCTTCGGATGCGTAT-AACAGCTCTGAAGGTGTTCGGCTT  515
Query  222  TAGTATTGT-ATTAATCTTAACCACGCACTTTACGCCGATGTCTATCAACTTCGGGCCTG  280
Sbjct  514  TAGTATTGTCTTTAATCTTAACCACG--CTTTACGCCGATGTCTATCAACTT-GGGCCTC  458
Query  281  TC--CTAACCGCGGTGGCTGGCACGAGTTT  308
Sbjct  457  TCGTATAACCGCGGTGGCTGGCACGAGTTT  428
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Score            Expect   Identities      Gaps           Strand
358 bits(396)    6e-94    216/223(97%)    5/223(2%)      Plus/Plus

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Query  156  GTAAAGGGTGGTTAAGGGAAGCACAATAATAAAGCCAAATGGCCCTTTGGCTGTCATACG  215
Sbjct  293  GTAAAGGGTGGTTAAGGGAAGCACAATAATAAAGCCAAATGGCCCTTTGGCTGTCATACG  352
Query  216  CTTGTACGGTGTCCGAAGCCCAATATACGAAAGTAGCTTTAATAAAGCCCACCTGA-CCC  274
Sbjct  353  CTTCTA-GGTGTCCGAAGCCCAATATACGAAAGTAGCTTTAATAAAGCCCACCTGACCCC  411
Query  275  ACGAAAGCTGAGAAACAAACTAGGGATTAAATA--CCACTATG  315
Sbjct  412  ACGAAAGCTGAGAAACAAACT-GGGATTAGATACCCCACTATG  453
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Fig. 5. BLAST identification of reads from MinION (amplicon sequences of eDNA from freshwater sponge samples) against perch and roach 12S loci

CONCLUSIONS & FUTURE DIRECTIONS

- Freshwater sponges can collect eDNA from teleosts and thus offer a possible way for monitoring diversity of stream ecosystems
- Amplicon sequencing primers need to be optimised for effective biodiversity detection (maximise percent reads from target taxa)
- Analyse other eDNA samples (water, sediment) to quantify possible limitations and biases of different eDNA protocols compared with electrofishing
- Examine the potential of eDNA from sponges to quantify diversity of other aquatic taxa, such as plants, birds, or viruses

LITERATURE CITED

- Duarte, I.A., et al. (2018). Short-term variability of fish condition and growth in estuarine and shallow coastal areas. *Mar Environ Res*, **134**, 130–7.
- Mariani, S., et al. (2019). Sponges as natural environmental DNA samplers. *Curr Biol*, **29**, R401-2.
- Miya, M., et al. (2015). MiFish, a set of universal PCR primers for metabarcoding environmental DNA from fishes: Detection of more than 230 subtropical marine species. *Roy Soc Open Sci* **2**, 150088.
- Yao, M., et al.. (2022). Fishing for fish environmental DNA: Ecological applications, methodological considerations, surveying designs, and ways forward. *Mol Ecol*, **31**, 5132-64.

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BE PREP

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