Environmental DNA metabarcoding as an effective and rapid tool for fish monitoring in canals

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Abstract

Canal systems are among the least-studied environments in terms of biodiversity in Britain. With environmental DNA (eDNA) metabarcoding emerging as a viable method for monitoring aquatic habitats, we focus on a case study along an English canal

comparing eDNA metabarcoding with two types of electrofishing techniques (wade-and-reach and boom-boat). In addition to corroborating data obtained by electrofishing, eDNA provided a wider snapshot of fish assemblages. Given the semi-lotic nature of canals, we encourage the use of eDNA as a fast and cost-effective tool to detect and monitor whole fish communities.

Keywords: canals, eDNA, electrofishing, fish survey

England's biodiversity depends on diverse habitats that are currently protected as SSSI (Sites of Special Scientific Interest). Among these designated areas, there are several canal systems, which are monitored for habitat quality and the occurrence of certain indicator species (Mainstone *et al.*, 2018). Long-term and routine monitoring of canal systems is critically important, as these can be key in the assessment of invasive, migratory and/or endangered species, as well as safeguarding against the spread of diseases through the early detection of pathogens. However, despite there being over 3,000km of canals in the United Kingdom, little has been done to assess their entire biodiversity (Natural England, 2011).

Traditionally, teleost populations have been monitored through live capture and subsequent morphological identification of specimens (Hill *et al.*, 2005). However, these practices are intrusive, can compromise the health of targeted species and induce stress (Goldberg *et al*., 2016). The selectivity of equipment used during traditional surveying practices can also lead to inaccuracies when monitoring freshwater ecosystems because specialized equipment can exclude the sampling of specific species (due to size, microhabitat use, and low abundances), thus leading to an insufficient representation of the community (Evans & Lamberti, 2017). Furthermore, the limited access to specialized equipment (such as electrofishing gear) and funding can make traditional surveys expensive and restrictive (Shaw *et al*., 2016).

Environmental DNA (eDNA) metabarcoding has emerged as an innovative and effective biodiversity monitoring tool that enables the rapid classification of multiple taxa without the assistance of a taxonomist or local fishing knowledge (Taberlet *et al.*, 2012). A cost-effective, fast and non-invasive eDNA protocol could prove extremely useful to provide a constantly updated and broad monitoring of the aquatic biodiversity of canals and its changes through time. The present study focuses on the detection

capability of eDNA metabarcoding compared to two different types of electrofishing for the detection of fish species along a stretch of the Huddersfield Narrow Canal in the UK (Fig. 1A). This canal is a designated SSSI and has very limited data available in terms of fish assemblage and biodiversity.

Three stretches of the canal were chosen for a Canal and River Trust-commissioned fish survey between December 2017 and January 2018. Electrofishing surveys were conducted using two methods: 'backpack' electrofishing with water levels lowered and surveyors wading through the canal bed between Locks 11-12 and 15-16 (Fig. 1A(i)) and a boom boat with water levels maintained between Locks 14-15 (Fig. 1A(ii)). Three sweeps were undertaken at each stretch and fish were identified to species level (see Supplementary Material for further details). One to 16 hours prior to these surveys being conducted (and before water levels were lowered), water temperature and pH were measured, and water (5 × 2L) and sediment (3 × ~10g) samples were taken from each of the three stretches of the canal. We chose to test both water and sediment for eDNA detection as taxonomic composition can vary depending on the substrate analysed due to the habitat preferences and life histories of different species (Koziol *et al.,* 2019). Water samples were filtered (250-400ml) within three hours of sampling in a decontaminated laboratory using Sterivex 0.45µM filters that were then kept at -20°C; sediment samples were stored in 100% ethanol at room temperature. To avoid cross contamination between samples, appropriate decontamination measures/precautions were taken: gloves were worn at all times, equipment and surfaces were treated with bleach (10%) and three field blanks were also analysed.

DNA was extracted from the water samples using the DNeasy PowerWater Kit and from the sediment samples using the DNeasy PowerMax Soil Kit (both Qiagen) in

the lab. All field blanks were extracted first, and extractions were completed following the manufacturer's protocol. Due to the nature of the sediment it was not always possible to collect 10g free of macroremains. Amplification of a fragment of the mitochondrial 12S rRNA gene was conducted using the MiFish 12S primer set (Miya *et al.*, 2015) and library preparation were conducted according to the protocol described in Sales *et al*. (2018). A total of 29 samples (including collection blanks and laboratory negative controls) were sequenced in a single multiplexed Illumina MiSeq run along with samples from a non-related project. See Supplementary Material for details on laboratory methods and bioinformatic analyses.

Water temperature ranged from 4.6-5.2 °C and pH from 6.13-6.68. A total of nine species were identified with the two electrofishing methods. With the boom boat, pike (*Esox lucius*), roach (*Rutilus rutilus),* chub (*Squalius cephalus*) and carp (*Cyprinus carpio*) were captured between Locks 14-15. Using the other electrofishing method (wade-and-reach) between Locks 11-12 and 15-16, perch (*Perca fluviatilis*), gudgeon (*Gobio gobio*), bream (*Abramis brama*), ruffe (*Gymnocephalus cernuus*) and bullhead (*Cottus gobio*) were captured in addition to the previous four species. Only roach and pike were captured across all three electrofishing sessions (Fig. 1B).

A total of 104,055 sequence reads (after all filtering steps; see Supplementary Material) were retrieved, allowing for the detection of 16 species in the eDNA survey. All nine species from the electrofishing survey were identified, with the addition of brown trout (*Salmo trutta*), common minnow (*Phoxinus phoxinus*), European eel (*Anguilla anguilla*), grayling (*Thymallus thymallus*), salmon (*Salmo salar*), stone loach (*Barbatula barbatula*) and the three-spined stickleback (*Gasterosteus aculeatus*). The results provided by eDNA were more consistent, with 12 out of the 16 species being

detected in all three sampling sessions (Fig. 1B). Electrofishing failed to detect seven species, and a low number of species and individuals within each species were recorded in two of three stretches of canal (Fig. 1B; Table S1). In addition, the selectivity of the method may hamper the detection of species difficult to capture due to their morphological or behavioural characteristics (small body size fish species such as *P. phoxinus*, *G. aculeatus*, or solitary and nocturnal fish such as *B. barbatula*).

Due to the expected relatively fast degradation of DNA molecules (Seymour *et al.* 2018), the detection of species through this method suggests their recent presence and provides an overview of the contemporary fish community. However, eDNA molecules might persist in the water column for more than a few days and thus, allow the detection of transient species not necessarily present in the system at the collection time (Dejean *et al*., 2011). DNA molecules can be transported long distances so fish may be detected far away from their occurrence (Jane *et al*., 2015) or even originating from different sources. Therefore, the detection of certain species (e.g. brown trout and salmon) in this study could be due to an external source, such as human consumption. Putative false positives should be taken into account and carefully analysed before drawing a conclusion about the occurrence of these species in the Huddersfield Canal, and to understand their origin (e.g. endogenous or exogenous, regional or local).

As demonstrated in previous studies, eDNA obtained from the water column yielded better results when compared to sediment samples (Shaw *et al.,* 2016; Koziol *et al.,* 2019), with 14 out of 16 species recovered, but sediment samples outperformed water samples only by detecting eel and minnow. Environmental DNA recovered from sediment samples allowed the detection of only five species (eel, brown trout, salmon, minnow and stone loach; Table S1). These could originate from historical depositions

rather than contemporary records (Turner *et al.,* 2015). Given the associated effort and costs of obtaining sediment samples from aquatic environments, we would not recommend incorporating them in future biomonitoring using eDNA in canals.

While many studies have shown the advantages of using eDNA metabarcoding in lotic (flowing streams and rivers; Balasingham *et al*., 2018) and lentic (still lakes and ponds; Harper *et al*., 2018; Hänfling *et al*., 2016) systems, they also raise concerns about the influence of flow in DNA dispersal in fast running water and the need to sample multiple locations in lentic waters. Canals represent man-made environments with a semi-lotic regime and regulated flow, which minimize the risk of detection of species present too far away, while at the same time allowing enough water movement to reduce the need of extra sampling akin to that undertaken in lentic systems. Here we showed that environmental DNA corroborates the data obtained by electrofishing, but also provides a wider snapshot of fish assemblages (Pont *et al*., 2018). While traditional methods cannot be replaced when investigating size, age class distribution, and, for now, abundance, we find that the power, speed and cost-effectiveness of eDNA metabarcoding may often represent a highly efficient tool to assess and monitor whole fish communities in canal systems.

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Author Contributions:

CB and ADM conceived, and ADM, CB, IC, NGS, SSB and SM, designed the study. ADM, SSB and AOS carried out the fieldwork. NGS and ADM performed the laboratory work. NGS and OSW performed the bioinformatics. ADM, NGS, IC and CB analysed the data. ADM, NGS, CB, IC, SSB and AOS wrote the paper, with all authors commenting on the manuscript.

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Figure legend

Figure 1. Map of the study area showing sampling locations for electrofishing (wade-and-reach (i) and boom-boat (ii)) and eDNA between Locks 11-16 of the Huddersfield Narrow Canal (A). A bubble graph (B) is used to represent presence-absence and categorical values of the number of reads retained (after bioinformatic filtering) for eDNA (water and sediment combined) and the number of individuals caught for electrofishing for 16 fish species. Fish illustrations are not shown to scale.

Figure

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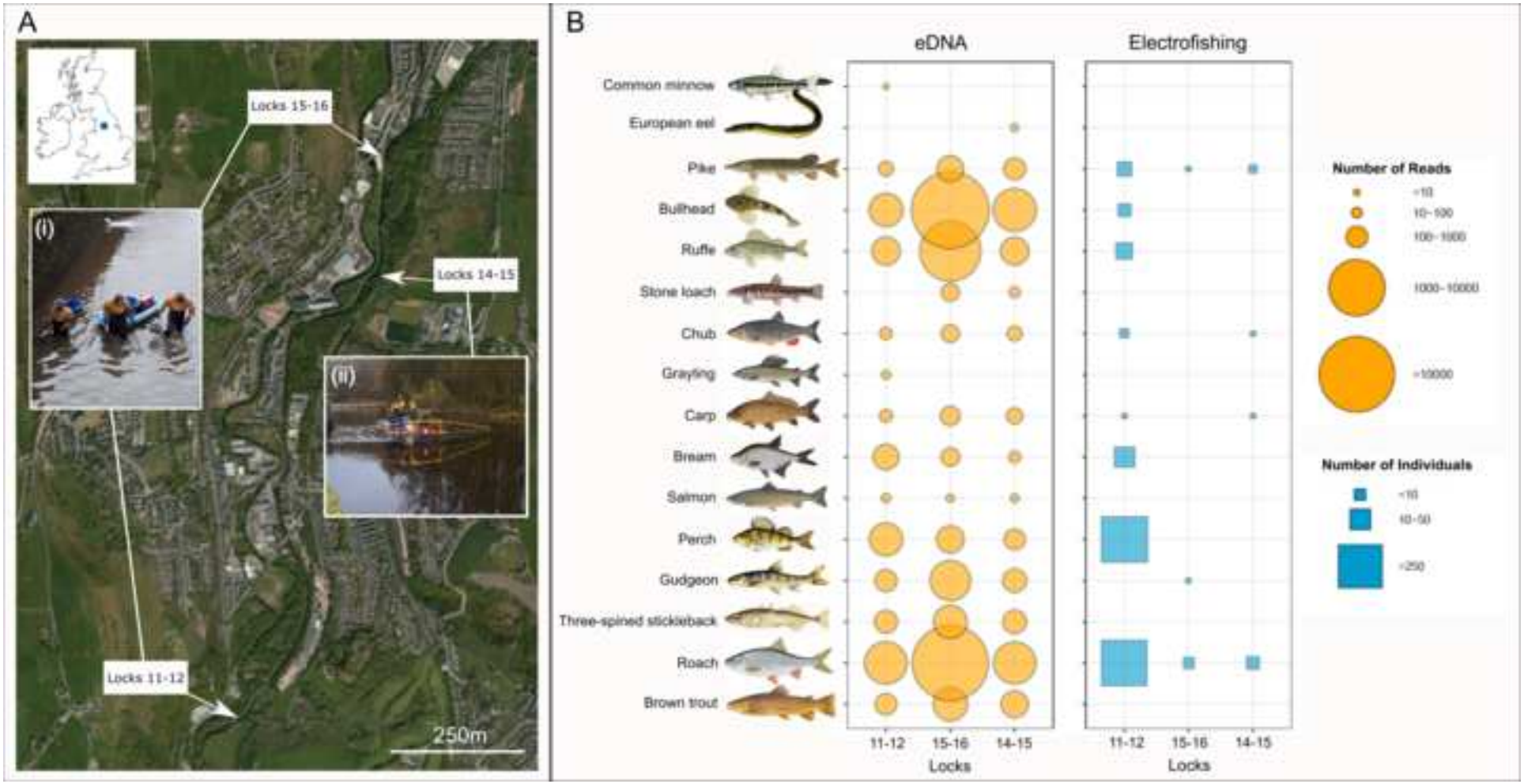

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Supplementary Material

Sampling sites

The stretch of canal between locks 15-16 is approximately 200m in length, and had a depth of ~50cm at the time of eDNA sampling. Between locks 14-15 is ~500m in length and was >1m deep during sampling, and between locks 11-12 is ~300m in length and was >1m deep during sampling.

Electrofishing

For the 'backpack' electrofishing method, 2 x Electracatch (Electrofish Ltd., UK) electrofishing control boxes (with variable amp power and hertz) were placed and pulled along in a 4 m Dory boat (Fig. 1A (i)). For the boom boat, a 41-probe boom boat designed and manufactured by MEM (see Fig. 1A (ii)) was used. This was powered by 2 x Honda 3.0 KVA lightweight silent generators. For both methods, there were three operatives, with two acting as nets people.

eDNA Laboratory Methods

A set of primers pairs with seven-base sample-specific oligo-tags and a variable number (2-4) of fully degenerate positions (leading Ns) to increase variability in amplicon sequences were used. PCR amplification was conducted using a single-step protocol and to minimize bias in individual reactions, PCRs were replicated three times for each sample and subsequently pooled. The PCR reaction consisted of a total volume of 20 µl, including 10 µl AmpliTaq Gold™ 360 Master Mix (Applied Biosystems); 0.16 µl of BSA; 1.0 µl of each of the two primers (5 µM); 5.84 µl of ultra-pure water, and 2 µl of DNA template. The PCR profile included an initial denaturing

step of 95°C for 10 min, 40 cycles of 95°C for 30s, 60°C for 45s, and 72°C for 30s and a final extension step of 72°C for 5 min. Amplification were checked through electrophoresis in a 1.5% agarose gel stained with GelRed (Cambridge Bioscience). PCR products were pooled and a left-sided size selection was performed using 1.1x Agencourt AMPure XP (Beckman Coulter). Illumina libraries were built using a NextFlex PCR-free library preparation kit according to the manufacturer's protocols (Bioo Scientific). Libraries were then quantified by qPCR using a NEBNext qPCR quantification kit (New England Biolabs) and pooled in equimolar concentrations along with 1% PhiX (v3, Illumina). This included 29 samples from the present study, 23 samples from a non-related project (targeting South American fish species) and 3 negative controls. This library was run alongside two other libraries in a single Illumina MiSeq run using a flow cell with 2 x 150bp v2 chemistry at a final molarity of 9pM.

Bioinformatics

Bioinformatics analysis were based on the OBITools metabarcoding package (Boyer *et al.*, 2016). Alignment of paired-end reads and removal of primer sequences were performed using illuminapairedend. Short fragments originated from library preparation artefacts (primer-dimer, non-specific amplifications) and reads containing ambiguous bases were removed applying a length filter selecting fragments of 140-190bp using *obigrep*. Clustering of strictly identical sequences was performed using *obiuniq* and a chimera removal step was applied in *vsearch* (Rognes *et al*., 2016) through the uchime-denovo algorithm (Edgar *et al.*, 2011). Molecular Operational Taxonomic Unit (MOTU) delimitation was performed using the SWARM algorithm with a distance value of d=3 (Sales *et al*., 2018, Siegenthaler *et al*., 2019) and *ecotag* was used for the subsequent taxonomic assignment. A total of 2,998,146 reads were

obtained for the library including the canal samples. For the canal samples, 1,113,066 were recovered (read depth averaged ~38.8k reads/sample). A conservative approach was applied to our analyses to avoid false positives and exclude MOTUs/reads putatively belonging to sequencing errors or contamination. MOTUs containing less than 10 reads and with a similarity to a sequence in the reference database (GenBank) lower than 98% (minidentity 0.98) were discarded, the maximum number of reads detected in the controls was removed for each MOTU from all samples, and obvious non-target species (e.g. mammals) and those from likely originating from carry-over contaminations (e.g. oceanic fishes, South American species) were excluded from further analyses (Li *et al*., 2018; Ushio *et al*., 2018). After these stringent filtering steps, a total of 104,055 reads were retained for downstream analyses.

Table S1. Species identified using eDNA metabarcoding in water and sediment samples (read number from combined replicates) and electrofishing (number of individuals caught) in the three sampling points between Locks.

		Water eDNA (reads)			**Sediment eDNA (reads)**			**Electrofishing (individuals)**		
Species name	Common name	Locks 11-12	Locks 15-16	Locks 14-15	Locks 11-12	Locks 15-16	Locks 14-15	Locks 11-12	Locks 15-16	Locks 14-15
Salmo trutta	Brown trout	798	2752	1701	143	261	0	0	0	0
Rutilus rutilus	Roach	5135	18549	5030	0	0	0	296	7	12
Gasterosteus aculeatus	Three-spined stickleback	1134	2911	1209	0	0	0	0	0	0
Gobio gobio	Gudgeon	1016	4540	1229	0	0	0	0	1	0
Perca fluviatilis	Perch	2873	1801	882	0	0	0	301	0	0
Salmo salar	Salmon	41	25	0	0	0	36	0	0	0
Abramis brama	Bream	1528	627	142	0	0	0	41	0	0
Cyprinus carpio	Carp	197	718	408	0	0	0	1	0	1
Thymallus thymallus	Grayling	40	0	0	0	0	0	0	0	0
Squalius cephalus	Chub	160	560	328	0	0	0	4	0	1
Barbatula barbatula	Stone loach	0	571	0	0	0	104	0	0	0
Gymnocephalus cernua	Ruffe	1947	11449	1938	0	0	0	24	0	0
Cottus gobio	Bullhead	2956	19372	5277	0	0	0	12	0	0
Esox lucius	Pike	311	1596	1012	0	0	0	16	1	3
Anguilla anguilla	European eel	0	0	0	0	0	21	0	0	0
Phoxinus phoxinus	Common minnow	0	0	0	6	0	0	0	0	0

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