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

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21

22 Abstract

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

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

31

32 Keywords: canals, eDNA, electrofishing, fish survey

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

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

52 Environmental DNA (eDNA) metabarcoding has emerged as an innovative and
53 effective biodiversity monitoring tool that enables the rapid classification of multiple
54 taxa without the assistance of a taxonomist or local fishing knowledge (Taberlet *et al.*,
55 2012). A cost-effective, fast and non-invasive eDNA protocol could prove extremely
56 useful to provide a constantly updated and broad monitoring of the aquatic biodiversity
57 of canals and its changes through time. The present study focuses on the detection

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

62

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

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

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

92

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

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

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

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

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

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

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

150

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157

158 **Author Contributions:**

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

164

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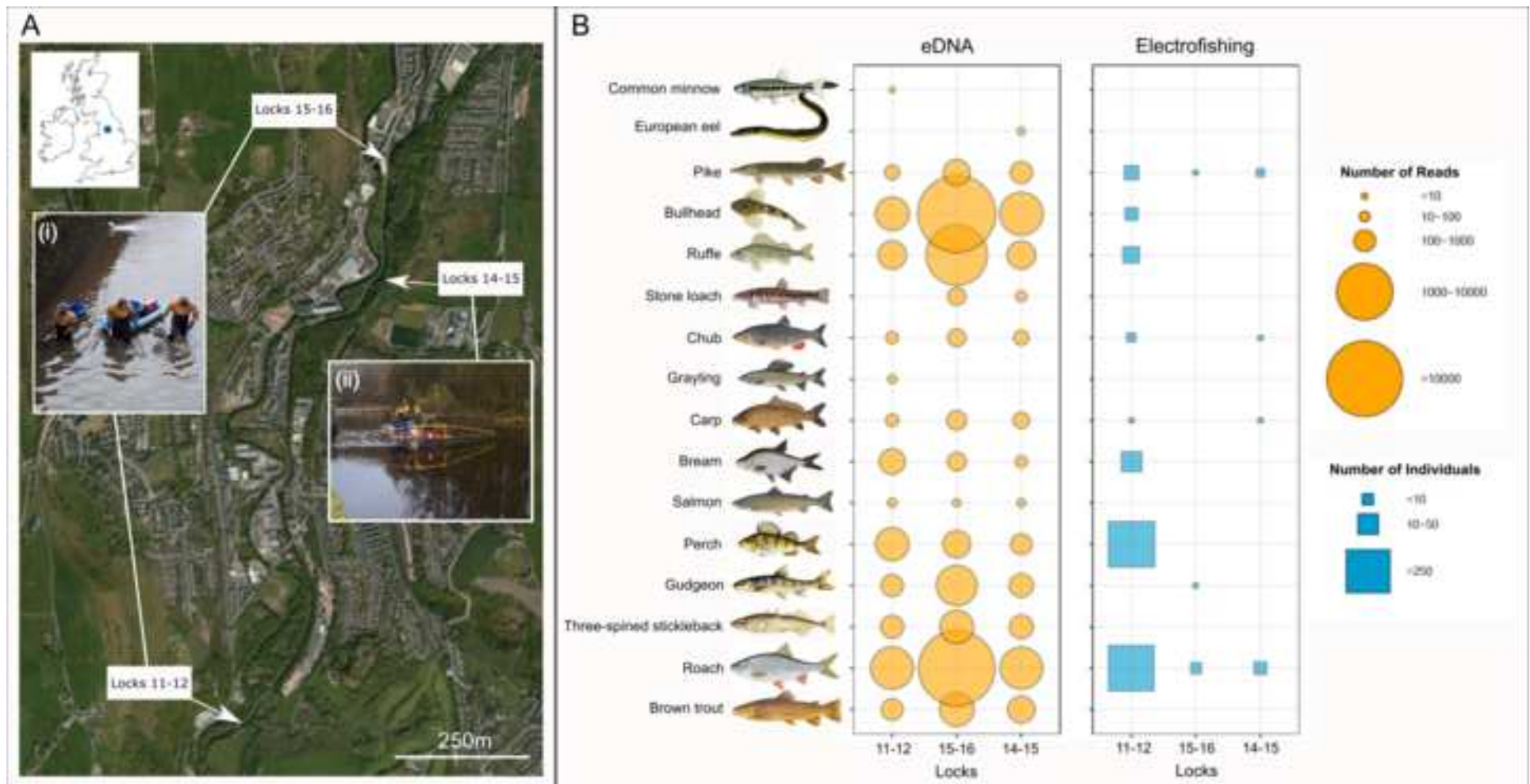
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252 Figure legend

253

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



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21 **Supplementary Material**

22

23 ***Sampling sites***

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

28

29 ***Electrofishing***

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

36

37 ***eDNA Laboratory Methods***

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

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

58

59 ***Bioinformatics***

60 Bioinformatics analysis were based on the OBITools metabarcoding package (Boyer
61 *et al.*, 2016). Alignment of paired-end reads and removal of primer sequences were
62 performed using *illuminapairedend*. Short fragments originated from library
63 preparation artefacts (primer-dimer, non-specific amplifications) and reads containing
64 ambiguous bases were removed applying a length filter selecting fragments of 140-
65 190bp using *obigrep*. Clustering of strictly identical sequences was performed using
66 *obiuniq* and a chimera removal step was applied in *vsearch* (Rognes *et al.*, 2016)
67 through the *uchime-denovo* algorithm (Edgar *et al.*, 2011). Molecular Operational
68 Taxonomic Unit (MOTU) delimitation was performed using the SWARM algorithm with
69 a distance value of $d=3$ (Sales *et al.*, 2018, Siegenthaler *et al.*, 2019) and *ecotag* was
70 used for the subsequent taxonomic assignment. A total of 2,998,146 reads were

71 obtained for the library including the canal samples. For the canal samples, 1,113,066
72 were recovered (read depth averaged ~38.8k reads/sample). A conservative approach
73 was applied to our analyses to avoid false positives and exclude MOTUs/reads
74 putatively belonging to sequencing errors or contamination. MOTUs containing less
75 than 10 reads and with a similarity to a sequence in the reference database (GenBank)
76 lower than 98% (minidentity 0.98) were discarded, the maximum number of reads
77 detected in the controls was removed for each MOTU from all samples, and obvious
78 non-target species (e.g. mammals) and those from likely originating from carry-over
79 contaminations (e.g. oceanic fishes, South American species) were excluded from
80 further analyses (Li *et al.*, 2018; Ushio *et al.*, 2018). After these stringent filtering steps,
81 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.

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

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