Received: 17 February 2020 | Revised: 6 July 2020 | Accepted: 25 August 2020

DOI: 10.1002/edn3.136

ORIGINAL ARTICLE

Environmental DNA
Open Access
Dedicated to the study and use of environmental DNA for basic and applied sciences

WILEY

Application of eDNA metabarcoding in a fragmented lowland river: Spatial and methodological comparison of fish species composition

Caterina M. Antognazza[1] | Robert J. Britton[1] | Daniel S. Read[2] | Tim Goodall[2] | Anna Mantzouratou[1] | Vanessa De Santis[1,3] | Peter Davies[1] | Miran Aprahamian[1] | Elizabeth Franklin[1,4] | Emilie A. Hardouin[1] | Demetra Andreou[1]

[1]Department of Life and Environmental Sciences, Faculty of Science and Technology, Bournemouth University, Poole, UK

[2]Centre for Ecology & Hydrology (UKCEH), Wallingford, UK

[3]Department of Theoretical and Applied Sciences, University of Insubria, Varese, Italy

[4]Centre for Applied Zoology, Cornwall College Newquay, Newquay, UK

Correspondence
Caterina M. Antognazza, Department of Life and Environmental Sciences, Faculty of Science and Technology, Bournemouth University, Poole, BH12 5BB, UK.
Email:cantognazza@bournemouth.ac.uk; cm.antognazza@gmail.com

Funding information
Severn Rivers Trust; Bournemouth University; "Unlocking the Severn for LIFE," LIFE Nature Programme, Grant/Award Number: LIFE15/NAT/UK/000219; HEIF 5+1+1; Natural Environment Research Council, Grant/Award Number: NE/R016429/1

Abstract

Assessments of fish communities tend to rely on capture-based methods that, due to sampling biases, can underestimate actual species richness. Alternatively, environmental DNA (eDNA) based metabarcoding is a noncapture approach that infers species richness and distribution by collecting and sequencing DNA present in the ecosystem. Here, eDNA metabarcoding was applied to the lower River Severn, a highly modified and impounded river, to identify the species present in the fish assemblage. Using a universal primer for fish (12S mtDNA region), comparisons were made between the species identified as present by eDNA metabarcoding versus long-term data available from fisheries monitoring data based on capture methods. Depending on the stringency of detection thresholds applied, the two methods detected between 15 and 25 fish species present in the river, with the eDNA metabarcoding detecting most species previously reported in the capture surveys, although with differences in the relative abundance of species between the methods. Notably, eDNA metabarcoding detected species of high conservation importance that were never sampled by capture techniques, including native European shads (*Alosa* spp.). Differences in the similarity indices of species detection were greater between the sampling methods than between sampling sites on each river. These results highlight the high potential of eDNA metabarcoding to provide an effective monitoring tool for biodiversity and conservation in rivers, but also indicate the need for complementary multi-method sampling for robust estimates of fish species richness.

KEYWORDS

eDNA, fish monitoring, habit fragmentation, lotic system, metabarcoding, river

1 | INTRODUCTION

The monitoring of biodiversity is becoming increasingly important in response to rapid environmental change (Hänfling et al., 2016; Lawson Handley et al., 2019; McDevitt et al., 2019). For fish, capture methods (e.g., trapping, netting and electric fishing) are necessary when biometric and demographic data are required (Radinger et al., 2019). However, for estimating the species richness and relative abundance of fish assemblages, environmental DNA (eDNA) metabarcoding has recently emerged as an innovative and effective monitoring tool (e.g. Shaw et al., 2016; Taberlet, Coissac, Hajibabaei, & Rieseberg, 2012; Thomsen & Willerslev, 2015; Valentini et al., 2016). Applied to both lotic (Balasingham, Walter, Mandrak, & Heath, 2018; Nakagawa et al., 2018), semi-lotic (canals; McDevitt et al., 2019), and lentic (Hänfling et al., 2016; Harper et al., 2018; Lawson Handley et al., 2019) systems, evidence suggests it provides an efficient, noninvasive monitoring tool (Yamanaka & Minamoto, 2016). Metabarcoding of eDNA is proving especially useful for detecting the presence of rare, endangered or invasive species that are often present in low abundance (Taberlet et al., 2012), with the advantage of it requiring minimal taxonomic information prior to analysis (Bayley & Peterson, 2001; Gu & Swihart, 2004; Mackenzie & Royle, 2005), a contrast to capture techniques where high proficiency in species identification techniques can be required (Radinger et al., 2019).

There are, however, a number of questions remaining regarding the spatial and temporal distribution of eDNA in lotic systems, given how they differ from other habitat types due to their continuous and directional water flow. In lotic systems, organisms release genetic material which is expected to disperse downstream until it is chemically and/or biologically decomposed (Deiner & Altermatt, 2014; Jane et al., 2015; Wilcox et al., 2016). Therefore, at a sampling site, eDNA collected from water is likely to represent the composition of both local fish communities and those located upstream (Civade et al., 2016; Nakagawa et al., 2018). This is in contrast to more traditional fish monitoring techniques based on capture methods that provide highly localized data at the time of sampling (Radinger et al., 2019). Temporal changes in fish behavior (e.g. spawning migrations, summer vs. winter habitat use, diel migrations) and varying rates of DNA degradation due to biotic and abiotic factors might strongly influence the detectable levels of eDNA (Shogren et al., 2017).

In river fish assemblages, a major anthropogenic pressure is the presence of impoundments (e.g. dams, weirs) that were originally constructed for navigation and hydrological regulation, impact longitudinal connectivity, and fragment habitats (Oliveira, Baumgartner, Gomes, Dias, & Agostinho, 2018; van Puijenbroek, Buijse, Kraak, & Verdonschot, 2019). Even single barriers can interrupt the longitudinal connectivity of a river (Jager, Chandler, Lepla, & Van Winkle, 2001), leading to species isolation (Falke & Gido, 2006) and restricting the natural movements of fish for reproduction, feeding, and habitat colonization and can potentially lead to genetic impoverishment. In Western Britain, the lower River Severn basin was subjected to considerable river engineering in the 19th Century through the construction of a series of weirs that enabled navigation further upstream for industrial purposes (Figure 1; Aprahamian, 1988). These inhibited the spawning migration routes of a number of diadromous fishes, including allis shad (*Alosa alosa*), twaite shad (*Alosa fallax*), and sea lamprey (*Petromyzon marinus*) (Maitland & Lyle, 2005; Aprahamian, Aprahamian, & Knights, 2010). There is now a plan of river reconnection in place ("*Unlocking the Severn*") that either removes these weirs or provides fish passes that facilitate the upstream passage of migratory fish (Antognazza et al., 2019; Environment Agency, 2019a). Such reconnection schemes in river systems can lead to changes in the fish community (Catalano, Bozek, & Pellett, 2011; Magilligan, Nislow, Kynard, & Hackman, 2016). Therefore, knowledge on the community composition and distribution of fishes prior to reconnection are required to enable postconnection to be assessed and the management evaluated. There are also considerable spatial differences evident in the river habitats across the lower River Severn basin, ranging from the River Teme tributary providing a relatively narrow channel of pool and riffle characteristics, though to the main River Severn providing relatively deep, impounded sections (Figure 1; Gutmann Roberts, Hindes, & Britton, 2019). These spatial differences in habitat typologies are then also likely to be reflected in considerable differences in the fish assemblages (Noble, Cowx, & Starkie, 2007).

Consequently, the aim of this study was to apply eDNA-based metabarcoding to characterize the distribution of fish species of the lower River Severn and its River Teme tributary, above and below major impoundments and prior to their river reconnection. The objectives were to identify the fish species present across the two rivers using eDNA and compare the fish species composition and relative abundance with long-term data obtained from approximately 26 years of fish surveys completed using capture methods. It was predicted that while both methods would demonstrate considerable spatial differences in the fish assemblage across the study area, eDNA would be more powerful at detecting fish of high conservation importance given the likelihood of these species being in low abundance (Jerde et al., 2013; Wilcox et al., 2013; Sigsgaard, Carl, Møller, & Thomsen, 2015; Deiner et al., 2017).

2 | MATERIAL AND METHODS

2.1 | Sampling sites

Water sample replicates were collected every 2 weeks across the River Severn and its tributary, River Teme during May and June 2018 (Figure 1; Table 1). An additional 15 water samples were collected in May and June 2017 for one site ("Powick") (Table 1). Samples collected during May and June 2018 are the same samples used for monitoring shads through a real-time assay specific to *Alosa* spp. (Antognazza et al., under review), while samples collected in 2017 were used during the development of a real-time assay specific to *Alosa* spp. (Antognazza et al., 2019).

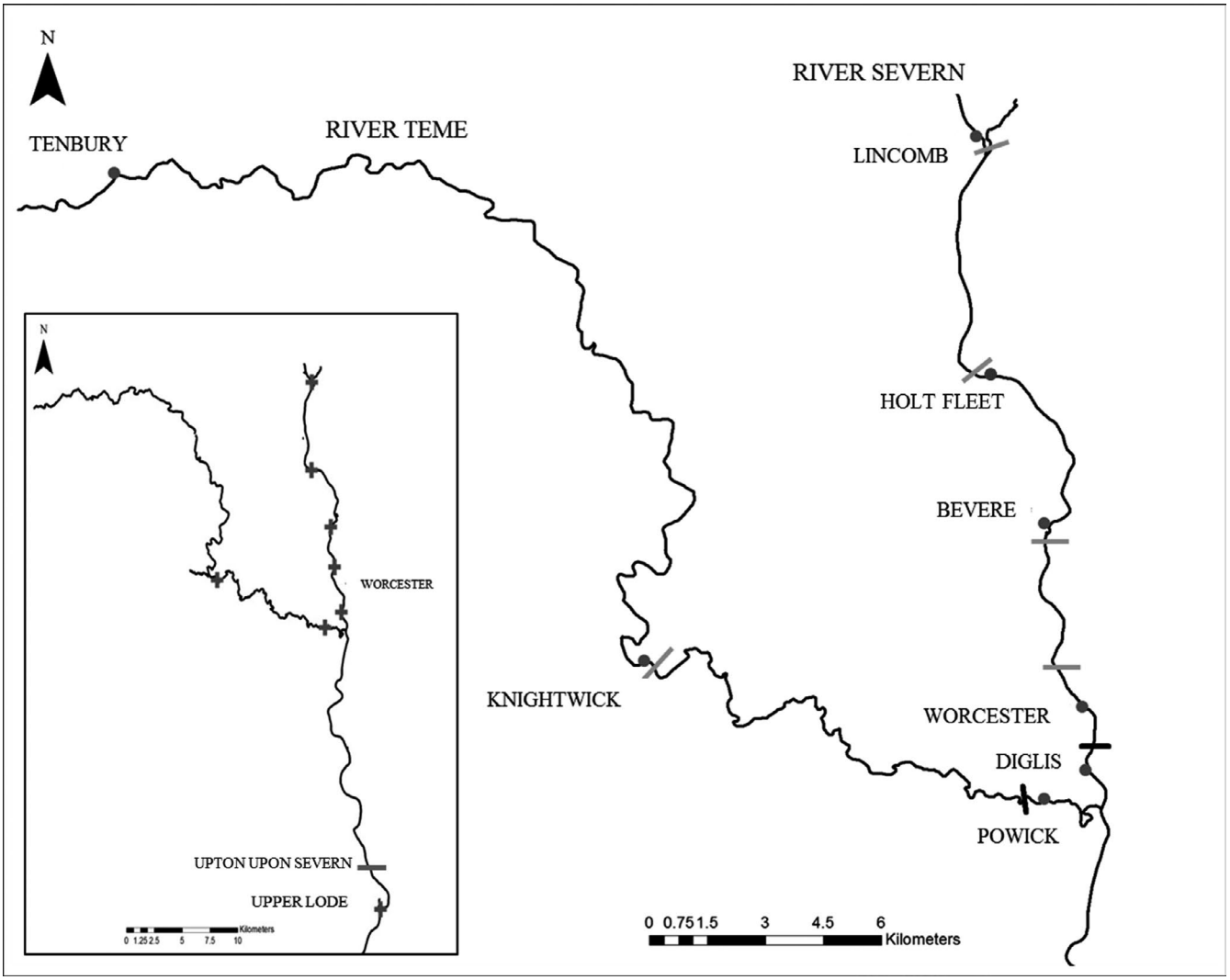

FIGURE 1 Locations of sampling sites on the River Teme and River Severn (gray circles) where the water samples were collected for the eDNA metabarcoding and the fish surveys were performed. Thick black lines refer to the two main impoundments on the Teme and Severn, being Powick and Diglis Weirs, respectively. Light gray lines refer to minor impoundments present on both rivers

The River Teme is approximately 130 km in length and is impounded in its lower reaches by a weir ("Powick Weir") that has a head of approximately 1.5 m. This weir, located 3 km from its confluence with the River Severn, is considered as largely impassable for most fish in the river (Figure 1). The Powick sampling site was located just below this weir. The other sampling site on the River Teme was "Tenbury," located approximately 48 km upstream of Powick Weir, with a further weir ("Knightwick Weir") located between them (Figure 1). Note Knightwick Weir is considered as less of a barrier to fish movements due to a lower head (Figure 1). The River Severn, approximately 354 km in length, has a series of six weirs in its lower reaches that disrupt its longitudinal connectivity. The most downstream sampling site on this river was the second most downstream weir on the nontidal section of the river ("Diglis Weir"), situated around 2 km upstream of the River Teme confluence. This weir was used as the most downstream site on the river, rather than the most downstream weir (Upper Lode Weir), as the latter represents the tidal limit of the river under most flows and is considered passable to most fish in the river, including all of the anadromous fishes in the river, especially during large spring tides when the weir is flooded (Bolland et al., 2019) (Figure 1). Correspondingly, sampling sites were located up- and downstream of Diglis Weir, and then upstream of the weirs at Bevere (10 km upstream from Diglis), Holt Fleet, and Lincomb (15 and 30 km upstream from Diglis, respectively) (Figure 1). In subsequent analyses and evaluations, Diglis (Severn) and Powick (Teme) were therefore considered as the weirs that potentially represented the principal blockages to the movements of fishes between the different sections of each river. The fish species known to be present for at least some of the year in both rivers are provided in the Table S2.

TABLE 1 Sampling sites description

Location	Sampling method	GPS Coordinates	Position according to the main weir	Date	Samples	Negative
Tenbury Wells	Bridge	52.313900, −2.594711	Above	03-May 2018	5	2
				14-May 2018	5	2
				29-May 2018	5	2
				11-June 2018	5	2
				25-June 2018	5	2
Powick	Bridge	52.170497, −2.242295	Below	03-May 2018	5	2
				14-May 2018	5	2
				29-May 2018	5	2
				11-June 2018	5	2
				25-June 2018	5	2
				12-June 2017	3	1
				19-June 2017	6	1
				02-July 2017	4	1
				18-July 2017	1	1
Lincomb	Riparian zone	52.323832, −2.267581	Above	21-May 2018	5	2
				04-June 2018	5	2
Bevere	Riparian zone	52.233704, −2.240804	Above	21-May 2018	5	2
				04-June 2018	5	2
Worcester	Bridge	52.190953, −2.226180	Above	03-May 2018	5	2
				21-May 2018	5	2
				04-June 2018	5	2
				25-June 2018	5	2
Diglis	Bridge	52.176248, −2.224893	Below	03-May 2018	5	2
				21-May 2018	5	2
				04-June 2018	5	2
				25-June 2018	5	2

Note: Locations, method of water collection, GPS coordinates, sampling position regarding the main impoundment, date of sampling, number of samples, and field negative controls (negative) are indicated.

2.2 | Contamination control

In order to minimize the probability of contamination, clean and consistent field collection protocols were established. Negative controls were included in the field, water filtration, DNA extraction, and DNA amplification steps. These were then sequenced, resulting in 36% of the total sequenced samples being negative controls. In addition to intermittent negative controls during the filtration process, negative controls were included at the start and end of field sampling for each site, water filtration, and DNA extraction.

Field equipment were stored and prepared for field sampling in a laboratory that is located in a separate wing to any DNA and tissue handling laboratory. Re-usable plastic bottles, ropes, and weights were used to collect water in the field after undergoing a stringent decontamination protocol that involved cleaning all equipment with 10% commercial bleach solution (immersion for a minimum of 30 min), followed by thoroughly rinsing them with sterilized water and then autoclaving them. Prior to field sampling, each bottle was prepared and stored in a sterile plastic bag, which was wiped on the outside with 10% Microsol detergent (Anachem). All sampling equipment per site was stored separately in large sterile bags that were wiped on the outside with 10% Microsol detergent and sealed until their use at the specific site. Each site also had its dedicated equipment which were sterilized and were held in site specific sterile bag, including single-use disposable gloves, spray bottle with 10% Microsol detergent, tissue paper, plastic bucket (cleaned with 10% Microsol solution) for storing weights after sampling, scissors, duct tape, and cable ties for finishing the set-up of sample bottles (see the next section for details), a sterile plastic bucket for storing all used equipment during sampling (this ensured the equipment did not come in direct contact with the field environment), a rubbish bag, and a sterile ice cooler for storing the collected water samples. After water collection, each bottle was wiped with 10% Microsol solution, placed in an individual sterile bag and placed a cool box filled with ice. Once in the laboratory, water samples were placed in a sterile fridge (5°C; cleaned with 10% Microsol solution). Each bottle was

again cleaned with 10% Microsol solution before opening it for the filtration step.

2.3 | Sampling methods

Water samples were collected using 1-L sterile plastic bottles by sampling the river across its width using road bridges that traversed its entire width, with some samples also collected from the riverbank. Both methods were described and compared in Antognazza et al. (2019). Briefly, sampling the river across bridges involved lowering sampling bottles from bridges using sterile ropes. The bottom of each bottle was weighted (using a sterile weight of approximately 700 g) and then attached to a rope for lowering into the river (20 m length at Powick, 40 m at other sites). Each sterile plastic bottle was prepared with sterile ropes and weights in the laboratory and placed individually in a sterile bag. At each sampling site, two negative controls were also prepared consisting of 1-L sterile plastic bottles filled with sterile water and were treated in the field in the exact manner as sample collection bottle (i.e. they were opened for 5 s, closed, and then dipped in the river). The first negative control was collected at the beginning of sample collection and the second one at the end. Five samples were collected from each bridge at equal distances to cover the width of middle section of the river. All water samples were immediately wiped with 10% Microsol solution (by wiping the bottle's exterior), stored in individual sterile bags and in sterile ice cooler box and then in a sterilized fridge overnight (5°C).

At two sites on the River Severn, Lincomb and Bevere, no bridges were available from which samples could be safely collected and so water samples were collected by samplers standing on the river bank, with water collected in a sterile plastic bottle attached to an extendible pole (from 1.8 to 3.7 m), with the bottle submerged sufficiently to allow collection through the water column. Sampling equipment was cleaned after each sample using 10% Microsol detergent. A total of five water samples were collected from each site. Samples were alternately collected with the pole at its shortest and at its longest length. Two negative controls were also collected, one before starting water sample collection (with the pole at its shortest length) and one at the end (with the pole at its longest length). These negative controls consisted of 1-L sterile plastic bottles filled with sterile water which were treated in the same way as sample collection bottles; the lid was removed and put back on the bottle, and the closed bottle was then dipped in the water. The sampling equipment was changed between each sampling point with the pole sterilized using 10% Microsol solution. All samples were immediately stored on ice in a sterilized cooler and then in the sterile fridge overnight (5°C).

2.4 | eDNA filtering and extraction

All samples were filtered within 24 hr of their collection by filtering the water through a 0.45 μm cellulose nitrate filter membrane (WhatmanTM). Filtration negatives (1 L distilled water) were run before the first filtration and then after every sixth sample, plus at the end in order to test for contamination during the filtration step. Filtration and DNA extraction were performed under a biological flow cabinet (Nuaire Labgard Class II biological safety cabinet) in a laboratory not dedicated to any DNA processing. Prior to filtration, all equipment was sterilized by submersion in 10% commercial bleach solution for 15 min and then washed with sterile water, followed by being placed under the flow cabinet with UV light for 20 min. Following each sample filtration, the filter paper was removed using sterile tweezers and placed in an individual power bead tube for DNA extraction and stored in a refrigerator. Tweezers were sterilized after each use in 10% Microsol solution, for at least 10 min and then washed with distilled water. Filtration equipment was sterilized after each sample in a 10% commercial bleach solution for 15 min, followed by flushing with tap water and then followed by two washes with distilled water.

The day after filtration, DNA was extracted using the DNeasy PowerWater Kit (Qiagen), according to the manufacturer guidelines, and was eluted in 100 μl elution buffer. Samples were quantified using the Nanodrop and stored at −20°C for a maximum of 3 months prior to their amplification.

2.5 | Amplification steps

All DNA amplification and sequencing steps were performed at the UK Centre for Ecology & Hydrology, Wallingford, in a laboratory dedicated to environmental DNA analysis. The universal primer MiFish-U-F/R (Miya et al., 2015), which amplified a 199 bp long fragment of the 12S mtDNA region, was used in a two-step PCR approach. The primer pair was first tested on 10 water samples collected in 2018, randomly chosen, to assure positive amplification. Then, all samples were amplified with modified primers (amplicon primers with Illumina MiSeq sequencing primer and preadaptor), allowing the second stage of PCR to add on the barcodes (Illumina MiSeq index) and flow-cell adaptor. A total of 124 samples and 27 field negative controls were amplified, as well as two extraction negative controls and 39 negative controls at the filtering step (Table 1). First step PCR amplifications were performed with Q5 High-Fidelity DNA polymerase (New England BioLabs); amplifications were performed in 25 μl reaction volume containing 4 μl undiluted sample, PCR buffer and high GC buffer, 0.05 μM dNTPs (Bioline), and 0.1 μM of each modified amplicon primer. Thermal cycler conditions included an initial denaturation at 95°C for 3 min followed by 35 cycles of denaturation at 94°C for 20 s, annealing at 61.5°C for 15 s and extension 72°C for 15 s, followed by a final extension at 72°C for 5 min. PCR results were visualized on a 2% agarose gel. Amplicons were cleaned using the Zymo ZR-96 clean-up kit (ZYMO-Research). The second PCR amplification was performed using the Sigma Taq polymerase (Sigma), with an optimized 10x reaction buffer including $MgCl_2$ (15 mM). Amplifications were performed in 50 μl reaction volume containing 1 μl of DNA template from first step amplification,

0.1 μM dNTPs, and 0.125 μM of each barcoding primer. Thermal cycler conditions consisted of an initial denaturation at 95°C for 3 min followed by 8 cycles of denaturation at 5°C for 30 s, annealing at 55°C for 30 s, and extension 72°C for 30 s followed by a final extension at 72°C for 5 min. PCR results were visualized on a 2% agarose gel. Amplicons were normalized using SequalPrep normalization plates (Invitrogen, Life Technologies). Samples were subsequently pooled into single tubes, concentrated, gel extracted (Qiagen), quantified using the Qubit HS Quantification Kit (Thermo Fisher), and sequenced on an Illumina MiSeq using V3 2 X 300 bp chemistry using a 12pM load with 20% Illumina PhiX control library.

2.6 | Bioinformatics and data analysis

Raw reads were processed through the DADA2 pipeline ver. 1.8 (Callahan et al., 2016) in R (R Core Team, 2018). Briefly, amplicon reads were trimmed to maintain Q score > 30, at 220 and 160 bases, forward and reverse, respectively, and filtered with DADA2 default settings, with the exception of the maximum number of Ns (maxN) = 0 and maximum number of expected errors (maxEE) = c(1,1). Primer sequences were removed using trimLeft = c(21,27). Sequences were dereplicated and the DADA2 core sequence variant inference algorithm applied. Processed forward and reverse sequences were merged using the mergePairs function, and a sequence table was constructed from the resultant, merged amplicon sequence variants (ASVs). Chimeric sequences were removed from the ASV table using remove BimeraDenovo with default settings. After processing, 6,595,003 merged sequences were recorded in 6,490 ASVs.

To assign taxonomy, a megablast against the NCBI database was performed initially using the Galaxy platform online (usegalaxy.org; Afgan et al., 2018), aiming to find a match for each of the 6,490 sequences. The set expectation cutoff value (e-value) was set at $1e^{-06}$. A match for all 6,490 sequences was not achieved, but was for 99.3% of the sequences (6,445 sequences) at 76% identity cutoff (e-value) and the minimum query per hsp, allowing gaps. The remaining unmatched sequences (45 sequences) were re-analyzed separately to confirm the absence of any match with fishes before they were discarded. Blast using a custom database (Table S2), created using previous published European freshwater fish (Hänfling et al., 2016), was carried out with the 6,445 sequences; all nonfish assignments sequences, mainly bacteria, were removed. The final dataset yielded 71 ASV.

2.7 | Semi-quantitative eDNA analysis

Following the approach adopted in Balasingham et al. (2018), eDNA detections for target species were interpreted in a semi-quantitative way. The number of the eDNA sequences for a target species at a specific sampling location was divided by the total number of eDNA sequences returned at the same sampling location. This approach can be used to estimate the abundance of the signal of a target species as it gives a proportion of a specific species in relation to the total number of reads at one location (Balasingham et al., 2018). Therefore, if in a particular location, and eventually further upstream, a species occurs in high abundance, water samples collected should reflect a higher proportion of eDNA sequence returns for the species as a measure of the relative signal (Balasingham et al., 2018).

2.8 | Fish survey

In the last 20 years, electric fishing surveys have been completed on the River Teme in the areas close to where the eDNA surveys were completed. With the River Severn being a much larger and deeper river than the Teme, it is more challenging to sample by electric fishing. Consequently, fyke net surveys (fished overnight) and micromesh seine netting of 0+ fish at the end of the summer (Table S3) complemented electric fishing data for the River Severn.

Electric fishing surveys on the River Teme were completed consistently at Tenbury (n = 6; 2003–2015) and Powick (n = 5; 1993–2008) (Figure 1) (Environment Agency, 2019b). In the River Severn, a mixture of electric fishing and micromesh seine net surveys have been completed downstream of Holt Fleet Weir (4 electric fishing and 8 micromesh seine net surveys; 1992–2018) and at Upton upon Severn (downstream of Diglis Weir) (1 fyke net, 4 electric fishing and 5 micromesh seine net surveys; 2002–2018) (Table S3). While these locations do not completely match the eDNA sampling locations, they were considered as representing each of the reaches concerned where water samples were collected and also in relation to the upstream (u/s) or downstream (d/s) split in the rivers caused by Powick and Diglis Weir. Although combining fish data across different capture techniques is not ideal, it had to be done for these River Severn locations due to the paucity of data that would have resulted from the use of only one capture method. This is coupled with the use of different methods providing a greater likelihood that a broader range of fish species were captured in such a large river that is inherently challenging to sample using capture methods (Radinger et al., 2019). The capture data were collated from across a 26 year period to provide more of a long-term perspective on the assemblage composition (Table S2), especially as some of the species present (e.g. *Barbus barbus*, *Squalius cephalus*) are long-lived (>20 years). The data used from each fish survey were the species captured and their numerical abundance, with each survey completed by the Environment Agency, the inland fisheries regulator of England (Environment Agency, 2019b).

2.9 | Comparing eDNA metabarcoding detection and fish capture survey techniques

The species recorded during the fish surveys and detected in the eDNA-based metabarcoding were compared based on presence/absence of each species detected and then the proportion of species according to their numerical abundance within each survey.

To enable this, the eDNA data were managed in two ways: (a) sequences which gave at least one read count (singletons), prior to the application of a minimum number of reads count threshold (threshold set at 320 reads, see section "Library quality and raw data control" for details), and (b) only sequences which passed the minimum number of reads count threshold (determined by the maximum reads of contaminations 320 reads, see section "Library quality and raw data control" for details). Data from the fish surveys were summarized in three ways using detection thresholds in as similar manner as possible to the metabarcoding, although it is recognized that the physical capture of a fish species is not equivalent to an erroneous recording in eDNA. However, this is countered by the appearance of a single fish species in only one sample that is never captured in subsequent samples being either rare or highly transient and thus would not be considered as a long-term member of the natural fish assemblage. Correspondingly, the thresholds applied were (a) the species was considered present in the site if captured in at least one survey at that location; (b) the species was considered present in the site if captured in at least 50% of all surveys; and (c) the species was considered present in the site if retrieved in at least 80% of all surveys. This categorization allowed comparing the data on three levels, increasing the level of stringency: (a) eDNA data without applying reads count threshold were compared with species found at least once in the fish surveys (less-stringency scenario); (b) eDNA data after applying reads count threshold with species found at least in 50% of the fish surveys (moderate-stringency scenario), and (c) eDNA data after applying reads count threshold with species found at least in 80% of the fish surveys (high-stringency scenario). In the literature, there remains no standard method to select the minimum reads counts for considering a species to be present; methods vary from excluding only singleton to a certain percentage aiming to identifying contaminants in the controls (e.g. Balasingham et al., 2018; Civade et al., 2016; Lawson Handley et al., 2019). Therefore, the data without any threshold applied were associated with fish survey data which considered a species present if retrieved in at least one survey, leading to the less-stringency scenario. The purpose of this comparison was purely explorative.

To enable comparisons between the two sampling methods, the two rivers were divided according to their main impoundment (Powick and Diglis Weirs). Thus, comparisons on the Teme were between Tenbury and Powick (and only samples collected during 2018) and on the Severn were between u/s Diglis Weir (and combining data from the Lincomb, Bevere and Worcester sites combining) and d/s Diglis Weir (Diglis). Two sets of comparisons were then made. Firstly, a qualitative approach was used that compared the presence/ absence records of the species between the eDNA and fish survey methods, with calculation of Sørensen's similarity coefficient (S_S) (Sørensen, 1948):

$$SS = \frac{2a}{2a+b+c}, \text{ where}$$

a = number of species common to both detected communities,

b = number of species unique detected with eDNA, and.

c = number of species unique detected with electrofishing.

Values of S_S vary between 0 (no similarity on species composition between the methods) and 1 (perfect similarity between the methods), and was applied to comparisons between the two survey methods and, for the eDNA metabarcoding data, to each river to compare composition of the fish assemblage between the sampling areas (eDNA with the threshold of minimum reads applied and fish surveys with the threshold of species presence in at least 50% of surveys). Data collected in Powick in 2017 were only compared to data collected there in 2018. Secondly, a semi-quantitative approach was used where the relative abundance of the fishes detected as present in the river were compared between the methods using a bubble plot. For eDNA, this was based on proportion of the number of reads (after bioinformatics filtering and minimum threshold reads applied as described above). For fish surveys, it was the proportion by species (according to numerical abundances) captured by the sampling method.

3 | RESULTS

3.1 | Library quality and raw data control

After quality filtering and merging, a total of 6,490 amplicon sequence variants were retrieved, with only 71 ASVs belonging to fishes. These 71 ASVs assigned to 20 fish species and were compiled in a table of "amplicon sequence variants" (Table S1). Out of all 68 negatives analyzed, only five displayed contamination (7.4%), which for two of them (negatives PBN9 and WBN5) the level of contamination was negligible. Specifically, field negative PBN9 displayed 71 reads in total (divided between two species: 32 reads assigned to *Phoxinus phoxinus*, 39 reads assigned to *Alburnus alburnus*) and field negative WBN5 displayed 465 reads in total (divided in two species: 312 assigned to *Alosa* spp. and 153 reads assigned to *Pseudorasbora parva*) (Table S1). Therefore, the reads threshold was set at 320 (i.e. all samples with less than 320 reads were discarded from further analyses). The additional three negative samples that were contaminated were: (a) an extraction negative (NE2), which displayed high level of contamination with >20,000 reads assigned to *Alosa* spp.; (b) a filter negative (N28, reads >8,000 assigned to *Alosa* spp.); and (c) a filter negative N7 with 6,000 reads each assigning to *Alosa* spp. and *P. parva* (Table S1).

In order to detect the source of contamination, the filtering, extraction, and amplification workflow were reviewed, and samples suspected to be contaminated (i.e. samples associated with a contaminated negative control) were removed from further analyses, resulting in six samples being removed (after sequence quality check and merging): two samples from Diglis, two samples from Bevere and two samples from Lincomb (Table S1). The selection of the 320 reads threshold resulted in five species having read numbers below this threshold and they had to be considered as undetected by eDNA metabarcoding at the applied level of detection threshold. The five species were *Gasterosteus aculeatus* (L., 1758), *Perca fluviatils* (L.,

1758), *Salmo trutta* (L., 1758), *Salmo salar* (L., 1758), and *Thymallus thymallus* (L., 1758).

3.2 | Species detection with eDNA

Species assignment was not possible for eight of the 71 final ASV due to a nonconcordant match within the Blast analyses (Table 2). Specifically, five sequences initially identified as *A. alburnus* (L., 1758), two sequences identified as *Blicca bjoerkna* (L., 1758) and one sequence identified as *Cottus aleuticus* (Gilbert, 1896) were discarded from further analysis, as no clear identification was possible (Table 2). Following these steps, a total of 15 fish species were considered as being detected by the eDNA-based metabarcoding across the entire study area: *Abramis brama* (L., 1758), *A. alburnus*, *Alosa* spp., *Anguilla anguilla* (L., 1758), *Barbatula barbatula* (L., 1758), *B. barbus* (L., 1758), *Cottus* spp., *Esox lucius* (L., 1758), *Gobio gobio*, *Gymnocephalus cernuus* (L., 1758), *Leuciscus* spp., *Rutilus rutilus* (L., 1758), *P. marinus* (L., 1758), *Phonixus phonixus* (L., 1758), and *P. parva* (Temminck and Schlegels, 1825). Specifically, *G. cernuus*, *E. lucius*, *A. anguilla*, and *P. parva* were detected only in the River Severn, while *Barbatula* spp., *Cottus* spp., and *P. marinus* were only detected in the River Teme; the other eight species were detected in both rivers (Figure 2; Table S4). The species with the highest number of reads was *P. phoxinus* in the Teme and *R. rutilus* in the Severn (Figure 2; Table S4).

The Sørensen's similarity coefficient between eDNA data collected at Powick in 2017 and 2018 was high (S_S = 0.94), with only one species difference (*B. barbatula* identified during sampling in 2018 but not in 2017; Table S4). In both years, the main species present at Powick was *P. phoxinus*, while *Alosa* spp. had higher detection in 2018, and *A. alburnus*, *B. barbus*, and *Leuciscus* spp. had higher detection in 2017 (Table S4).

TABLE 2 Sequences with unambiguous matches between databases

ID	Galaxy Blast	Custom Database Blast
2	*A. alburnus*	*Leuciscus leuciscus*
324	*A. alburnus*	*L. leuciscus*
468	*A. alburnus*	*L. leuciscus*
1715	*A. alburnus*	*L. leuciscus*
5778	*A. alburnus*	*L. leuciscus*
1373	*Cottus aleuticus*	*Gobio gobio*
51	*Blicca bjoerkna*	*L. leuciscus*
103	*B. bjoerkna*	*L. leuciscus*

Note: Sequence ID (this study), best species match against Galaxy database Blast, custom database blast and GenBank Blast are detailed. Pairwise identity (Identity) obtained from blast against custom database and GenBank are indicated. Galaxy species assignment is according to the details in Section 2.6.

3.3 | Comparing eDNA metabarcoding detection and fish capture survey techniques

Comparisons of the eDNA metabarcoding versus the fish survey data under the less-stringency scenario resulted in the highest values of the Sørenson coefficient, ranging from 0.60% to 0.81% (Table 3). Under the moderate-stringency scenario, this reduced to 0.13 and 0.71%, while under the high-stringency scenario it ranged from 0% to 0.71% (Table 3). Generally, similarity decreased as the stringency level increased, except at Powick where it remained relatively high. Across both methods and the lowest level of stringency, the total number of fish species identified in the river was 25 (Table 3; Table S5a), but again this number declined as the level of stringency increased (17 at the high-stringency scenario), especially for the fish surveys (Table 3; Table S5a–S5c).

Comparison of the proportion of the fish species identified by the two methods (with the threshold applied for eDNA reads) and upstream and downstream of the weirs revealed some contrasting results. *P. parva* were not detected in any fish survey and *P. marinus* was detected only once (albeit it was recorded as a "lamprey" due to taxonomic ambiguity in identification); both species were detected using eDNA. While *Alosa* spp. were detected at all sites by eDNA detection, they were only recorded twice in fish surveys and only at Powick. Also, while eDNA-based metabarcoding detected the presence of *B. barbus* in all sites, this was not the case for fish surveys (Figure 2; Table S5a–S5c). In fish surveys, *E. lucius*, *A. anguilla*, *A. alburnus*, and *G. cernuus* were more prevalent than suggested by our eDNA-based results (Figure 2). Indeed, there appeared more differences by sampling method within each river than were apparent between the two rivers (Figure 2). Spatial comparisons of the fish species detected between the two sampling areas of each river, and both methods revealed that similarity in species composition was higher in the River Severn (S_S = 0.89 for eDNA, S_S = 0.86 for fish survey) than the River Teme (S_S = 0.71 for eDNA, S_S = 0.53 for fish survey).

4 | DISCUSSION

The eDNA-based metabarcoding approach used here was able to detect most of the species that have been detected using fish capture methods in the two studied rivers over the last 26 years. Applying a range of stringency levels to both the metabarcoding data and the fish survey data revealed that at relatively low stringency levels, a larger number of species was detected with both methods (n = 25 by fish surveys, 20 by metabarcoding); at the highest stringency level, this decreased to 15 species by metabarcoding and nine species by fish surveys. In the low stringency scenario, a no-minimum reads threshold for the metabarcoding data was applied and was considered as the equivalent of using all the fish capture data to represent the species richness of the fish assemblage (including occasions of when the capture of a single individual fish species occurred in a single survey). Application of the high-stringency scenario represents

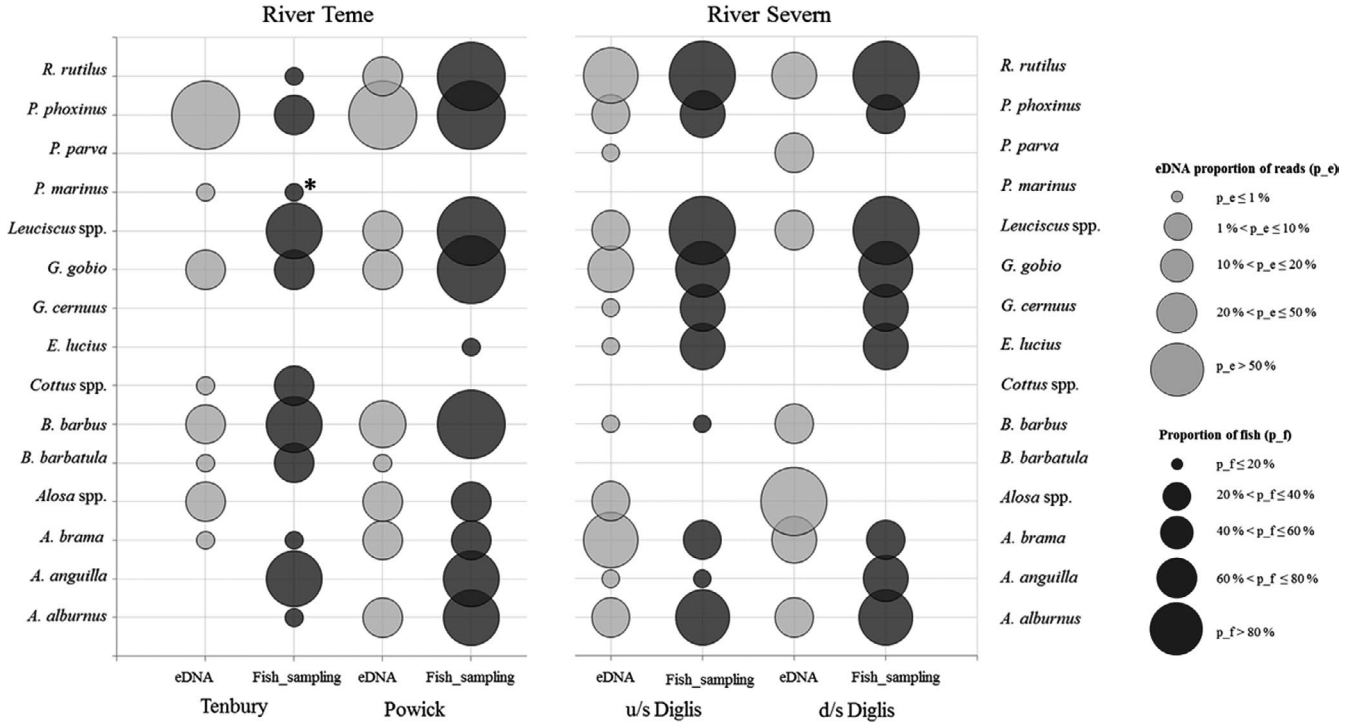

FIGURE 2 Bubble graph showing the relative abundance of 15 fish species detected by eDNA metabarcoding and fish survey above and below the main impoundment on both rivers. For eDNA, this was based on proportion of the number of reads (after bioinformatics filtering and minimum threshold reads applied); for fish surveys, it was the proportion of individuals caught through fish surveys (total number of species detectable through eDNA metabarcoding). *Lamprey detected at Tenbury without a clear species assignment

results where greater rigour has been applied in analyses, ensuring that only species which were regularly present in fish catches and at relatively high proportions in the metabarcoding data were used to describe the composition of the fish assemblage.

A criticism of eDNA-based detection is the possibility of false positives due to contamination; therefore, decontamination procedures, designed to significantly limit contamination (Goldberg et al., 2016), were followed in the field and laboratory processes for this study. There were 68 negative samples sequenced (a combination of field, filtration, DNA extraction and PCR negatives), representing 36% of the total samples analyzed (a higher proportion than what is commonly reported in the literature (e.g. Balasingham et al., 2018; Hänfling et al., 2016; Nakagawa et al., 2018). Often, negative samples are only inspected on agarose gel after the first round PCR and are not sequenced or include a small proportion of only field negatives (i.e. Balasingham et al., 2018; Nakagawa et al., 2018). Although sequencing all of the negative samples increases sample sizes and the costs of studies, the inclusion of a high number of negative controls in the sequencing analysis of metabarcoding studies should be standard practice and will allow a better assessment of contamination rates.

Here, significant contamination (i.e. high number of reads) was only present in three negatives, representing 4.4% of all negatives, with these assigned to *Alosa* spp. (found in 4.4% of negatives) and *P. parva* (detected in 1.4% of negatives). No other target species was detected in the negatives, providing confidence that the overall results were not compromised by the level of contamination detected. Moreover, *Alosa* spp. were detected in 29.5% of the samples, with this detection considerably higher than the 4.4% contamination of the negative samples. Moreover, *Alosa* spp. detection was also confirmed by single species qPCR detection in both rivers (Antognazza et al., 2019; Supp. Table S6).

In contrast, *P. parva* was detected in 2.6% of the samples (at Diglis and Worcester), but also in 1.4% of the negative samples. Therefore, its potential presence in the River Severn has to be considered carefully, especially as it is a highly invasive species across Europe (Gozlan et al., 2010) whose presence in England has been minimized though a programme of eradicating lentic populations to prevent their dispersal into lotic systems (Britton, Davies, & Brazier, 2010). Notwithstanding, *P. parva* populations have been known to be present in the lower River Severn basin, with a population eradicated from a pond connected to the River Teme in 2005 (Britton, Brazier, Davies, & Chare, 2008), with a more recent population present in a pond connected to the River Severn that was eradicated in early spring 2017, just prior to our water sampling (Canal River Trust, 2018). As these ponds have connection to the Severn, there was some likelihood of their dispersal from the pond into the river, as this dispersal mechanism is common in this species (Davies & Britton, 2016). For example, in the Meuse River in the Netherlands, floodplain lakes are used by *P. parva* as spawning, nursery, and adult habitats, with river channels mainly used as dispersal corridors (Pollux & Kurosi, 2006). Given the high invasiveness of *P. parva*, allied to it hosting a novel,

TABLE 3 Comparison of the number of species detected (yes) and not detected (no) between eDNA and fish survey for the four stretches of the River Teme and Severn across the three stringency levels, and relative Sørensen's similarity coefficient (S_S), showing the similarity of the detected fish species assemblage between the two methods

	Less-stringency					**Moderate-stringency**					**High-stringency**				
d/s Diglis		eDNA			S_S		eDNA			S_S		eDNA			S_S
	Fish survey	Yes	No	Total		Fish survey	Yes	No	Total		Fish survey	Yes	No	Total	
	Yes	9	8	17		Yes	4	3	7		Yes	4	3	7	
	No	4	—	4		No	4	—			No	4	—		
	Total	13	8	**21**	0.60	Total	8	3	**11**	0.53	Total	8	3	**11**	0.36
u/s Diglis		eDNA			S_S		eDNA			S_S		eDNA			S_S
	Fish survey	Yes	No	Total		Fish survey	Yes	No	Total		Fish survey	Yes	No	Total	
	Yes	11	1	12		Yes	6	1	7		Yes	2	0	2	
	No	4	—			No	6	—			No	10	—		
	Total	15	1	**16**	0.81	Total	12	1	**13**	0.63	Total	12	0	**12**	0.29
Powick		eDNA			S_S		eDNA			S_S		eDNA			S_S
	Fish survey	Yes	No	Total		Fish survey	Yes	No	Total		Fish survey	Yes	No	Total	
	Yes	12	0	12		Yes	6	2	8		Yes	6	2	8	
	No	8	—			No	3	—			No	3	—		
	Total	20	0	**20**	0.75	Total	9	2	**11**	0.71	Total	9	2	**11**	0.71
Tenbury		eDNA			S_S		eDNA			S_S		eDNA			S_S
	Fish survey	Yes	No	Total		Fish survey	Yes	No	Total		Fish survey	Yes	No	Total	
	Yes	12	5	17		Yes	1	6	7		Yes	0	2	2	
	No	3	—			No	7	—			No	8	—		
	Total	15	5	**20**	0.75	Total	8	6	**14**	0.13	Total	8	2	**10**	0.00

Note: The total number of species highlighted in bold character is the sum of detected species using both methods.

generalist pathogen (Sana et al., 2018), then more work is recommended to determine more definitively whether *P. parva* and/ or its pathogens are now present in the river.

In order to track contamination of water samples, negative samples were included at each step (i.e. water sampling, filtering, DNA extractions, first and second PCR, sequencing) (Goldberg et al., 2016). However, a consistent pattern of contamination could not be detected, preventing it being tracked back to a specific stage in analytical process (e.g. filtration, DNA extraction or PCR stage). As not all of the analyzed negative samples amplified *Alosa* spp. DNA (Table S6), then contamination during filtration and DNA extraction can be excluded. As such, the source of contamination has to remain speculative, with it potentially occurring at the library preparation stage and/ or during multiplexing prior to sequencing (due to 96-well plates being used), or it could be due to tag-jumping (i.e. Schnell, Bohmann, & Gilbert, 2015). As the contamination was dealt with by removing all of the samples that were associated with contaminated negative samples, then the subsequent discussion points are based on data analyses that can be considered as robust.

In general, the eDNA-based metabarcoding detected most of the species that had been recorded in fish surveys completed over a 26 years period and also had increased detection of *Alosa* spp. and *P. marinus* when compared with fish capture methods. This emphasizes the utility of using eDNA methods for detecting the presence of invasive, endangered, and rare species (i.e. Jerde et al., 2013; Wilcox et al., 2013; Sigsgaard et al., 2015), with both *Alosa* spp. and *P. marinus* being native species with high conservation designations in the River Severn basin (Antognazza et al., 2019; Guo, Andreou, & Britton, 2017). It should be noted, however, that the period of river occupancy of *Alosa* spp. is highly seasonal (spring) and so they would be considered as unlikely to be captured by fish surveys if these were completed at other times of the year. Moreover, the collection of the water samples for eDNA was during the peak period of occupancy of spawning adults in the basin. In the present study, the sampling sites for eDNA at Powick and Diglis were located approximately 500 m downstream of the weirs and so while the positive detection of fish species could have been reflective of their DNA from water upstream of the weir, it would also have been strongly influenced by the fish present downstream. At the moderate-stringency scenario, in the River Teme, more species were detected downstream of Powick weir than upstream (9 vs. 8 species), with *A. alburnus*, *Leuciscus* spp., and *R. rutilus* only detected at Powick, but with *P. marinus* and *Cottus* spp. only detected upstream. It is, however, difficult to attribute these species differences only to weir presence, given the sampling sites were approximately 50 km apart in distance. Moreover, *Leuciscus* spp. are known to be present upstream from the fish surveys and from angler catches (e.g. Gutmann Roberts & Britton, 2018), with *P. marinus* regularly observed to spawn in areas downstream of Powick Weir. At the moderate-stringency scenario, in the River Severn, more species were detected upstream than downstream of Diglis Weir (12 vs. 8 species), although all those fish species that were not detected downstream are known to be present in the river from either the fish surveys or from angler catches (e.g. Nolan, & Britton, 2018). As such, while the eDNA data have shown some high promise in detecting species that were not apparent in fish surveys, their data need to be complemented from species detections using other sampling methods if reliable spatial comparisons of total fish species richness are to be made. In addition, the completion of fish surveys and angler catches in recent years on both rivers have provided biometric data on fish populations that have high utility for fisheries management (e.g. Amat Trigo, Roberts, & Britton, 2017; Nolan et al., 2019), aspects which cannot be provided by eDNA methods.

The effect of multiple environmental factors on the efficiency of eDNA detection (e.g. pH, temperature, UV, PCR inhibitors, organic materials) has been recently investigated (Barnes et al., 2014; Jane et al., 2015; Strickler, 2015; Takahara, Minamoto, Yamanaka, Doi, & Kawabata, 2012), as their impact ultimately determines the concentrations of DNA in the environment. Pivotal to this is the need for increased knowledge on the transport and degradation rate of eDNA within fluvial systems (Deiner et al., 2017; Shaw et al., 2016). Given that many of the factors affecting eDNA presence and detection are environmental and seasonally variable, then the timing of surveys remains an important factor in determining the efficiency of species detection. However, this is also the case for fish surveys based on capture methods, where there can be considerable differences in seasonal habitat use between species and within species, with potential for high habitat partitioning between different life-stages of fishes (eggs, larvae, juvenile, adult) (Radinger et al., 2019). Many adult fishes are also highly vagile, with species such as *B. barbus* having home ranges of over 12 km and showing considerable movements upstream in early spring for spawning (Gutmann Roberts et al., 2019). Thus, the completion of sampling events for eDNA at discrete times of the year, such as spring in this study, are likely to be too simplistic to provide a comprehensive perspective on the fish assemblage and for a more robust spatial and temporal description to be made, is likely to require increased sampling frequency throughout the year, and across a greater number of sampling sites.

In summary, the results of the eDNA metabarcoding revealed that it provided a relatively robust description of the composition of the fish assemblage from a limited number of water samples collected over a discrete 2 months sampling period. Indeed, depending in the level of stringency applied to the data, the results were similar to those retrieved from over 20 years of fish capture surveys. eDNA metabarcoding also detected some fish that rarely, if ever, get sampled by capture techniques, such as *Alosa* spp. and *P. marinus*. The eDNA data also provided a snapshot of the fish assemblage on the two rivers prior to the outlined works on river reconnection, providing baseline data on fish distributions in spring for subsequent comparisons postreconnection. For future investigations, the inclusion of multiple marker loci for a more robust community richness estimate can be considered. Although increasing the number of markers could increase the overall cost and increase the risk of contamination, it would concomitantly increase species detection sensitivity by reducing primer bias (Evans et al., 2016; Miya et al., 2015; Hänfling et al., 2016; Lawson Handley et al., 2019). As the reconnection of

the River Severn has the potential to influence the composition of the fish assemblage through most of the basin, then refined methods should enable improved assessments that will ultimately support management conservation actions.

ACKNOWLEDGMENTS

Financial support for this study was by a studentship provided by the Severn Rivers Trust and Bournemouth University, with funding from "Unlocking the Severn for LIFE," LIFE Nature Programme (LIFE15/NAT/UK/000219), and HEIF 5+1+1 funding. D.S.R. was supported by the Natural Environment Research Council award number NE/R016429/1 as part of the UK-SCAPE programme delivering National Capability.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The raw data will be available at the repository BORDaR.

ORCID

Caterina M. Antognazza https://orcid.org/0000-0001-5344-8245
Robert J. Britton https://orcid.org/0000-0003-1853-3086
Daniel S. Read https://orcid.org/0000-0001-8546-5154
Tim Goodall https://orcid.org/0000-0002-1526-4071
Vanessa De Santis https://orcid.org/0000-0001-5419-9850
Peter Davies https://orcid.org/0000-0003-3739-5352
Miran Aprahamian https://orcid.org/0000-0002-6190-2628
Elizabeth Franklin https://orcid.org/0000-0003-4175-2236
Emilie A. Hardouin https://orcid.org/0000-0002-2031-5160
Demetra Andreou https://orcid.org/0000-0001-9242-4692

REFERENCES

Afgan, E., Baker, D., Batut, B., van den Beek, M., Bouvier, D., Čech, M., … Blankenberg, D. (2018). The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2018 update. *Nucleic Acids Research*, *46*(W1), W537–W544. https://doi.org/10.1093/nar/gky379

Amat Trigo, F., Roberts, C. G., & Britton, J. R. (2017). Spatial variability in the growth of invasive European barbel *Barbus barbus* in the River Severn basin, revealed using anglers as citizen scientists. *Knowledge & Management of Aquatic Ecosystems*, *418*, 17.

Antognazza, C. M., Britton, R. J., De Santis, V., Kolia, K., Turunen, O. A., Davies, P., … Andreou, D. (under revision) Environmental DNA reveals the temporal and spatial extent of spawning migrations of European shad in a highly fragmented river basin. *Aquatic Conservation: Marine and Freshwater Ecosystems*, AQC-20-0021.R1.

Antognazza, C. M., Britton, J. R., Potter, C., Franklin, E., Hardouin, E. A., Gutmann Roberts, C., … Andreou, D. (2019). Environmental DNA as a non-invasive sampling tool to detect the spawning distribution of European anadromous shads (*Alosa* spp.). *Aquatic Conservation: Marine and Freshwater Ecosystems*, *29*(1), 148–152. https://doi.org/10.1002/aqc.3010

Aprahamian, M. W. (1988). The biology of the twaite shad, *Alosa fallax fallax* (Lacepede), in the Severn Estuary. *Journal of Fish Biology*, *33*(sa), 141–152. https://doi.org/10.1111/j.1095-8649.1988.tb05568.x

Aprahamian, M. W., Aprahamian, C. D., & Knights, M. (2010). Climate change and the green energy paradox: The consequences for twaite shad *Alosa fallax* from the River Severn, UK. *Journal of Fihs Biology*, *77*, 1912–1930. https://doi.org/10.1111/j.1095-8649.2010.02775.x

Balasingham, K. D., Walter, R. P., Mandrak, N. E., & Heath, D. D. (2018). Environmental DNA detection of rare and invasive fish species in two Great Lakes tributaries. *Molecular Ecology*, *27*(1), 112–127. https://doi.org/10.1111/mec.14395

Barnes, M. A., Turner, C. R., Jerde, C. L., Renshaw, M. A., Chadderton, W. L., & Lodge, D. M. (2014). Environmental conditions influence eDNA persistence in aquatic systems. *Environmental Science & Technology*, *48*(3), 1819–1827. https://doi.org/10.1021/es404734p

Bayley, P. B., & Peterson, J. T. (2001). An approach to estimate probability of presence and richness of fish species. *Transactions of the American Fisheries Society*, *130*(4), 620–633. https://doi.org/10.1577/1548-8659(2001)130<0620:AATEPO>2.0.CO;2

Bolland, J. D., Nunn, A. D., Angelopoulos, N. V., Dodd, J. R., Davies, P., Gutmann Roberts, C., … Cowx, I. G. (2019). Refinement of acoustic-tagging protocol for twaite shad *Alosa fallax* (Lacépède), a species sensitive to handling and sedation. *Fisheries Research*, *212*, 183–187. https://doi.org/10.1016/j.fishres.2018.12.006

Britton, J. R., Brazier, M., Davies, G. D., & Chare, S. I. (2008). Case studies on eradicating the Asiatic cyprinid *Pseudorasbora parva* from fishing lakes in England to prevent their riverine dispersal. *Aquatic Conservation Marine and Freshwater Ecosystems*, *18*(6), 867–876.

Britton, J. R., Davies, G. D., & Brazier, M. (2010). Towards the successful control of *Pseudorasbora parva* in the UK. *Biological Invasions*, *12*(1), 125–131.

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*, *13*, 581–583.

Canal River Trust (2018). Retrieved from https://canalrivertrust.org.uk/enjoy-the-waterways/fishing/related-articles/the-fisheries-and-angling-team/tackling-the-topmouth-gudgeon

Catalano, M. J., Bozek, M. A., & Pellett, T. D. (2011). Effects of dam removal on fish assemblages structure and spatial distributions in the Baraboo River, Wisconsin. *North American Journal of Fisheries Management*, *27*(2), 519–530.

Civade, R., Dejean, T., Valentini, A., Roset, N., Raymond, J.-C., Bonin, A., … Pont, D. (2016). Spatial representativeness of environmental DNA metabarcoding signal for fish biodiversity assessment in a natural freshwater system. *PLoS One*, *11*(6), e0157366. https://doi.org/10.1371/journal.pone.0157366

Davies, G. D., & Britton, J. R. (2016). Assessment of non-native fish dispersal from a freshwater aquaculture site. *Fisheries Management and Ecology*, *23*, 428–430. https://doi.org/10.1111/fme.12176

Deiner, K., & Altermatt, F. (2014). Transport distance of invertebrate environmental DNA in a natural river. *PLoS One*, *9*(2), e88786. https://doi.org/10.1371/journal.pone.0088786

Deiner, K., Bik, H. M., Mächler, E., Seymour, M., Lacoursière-Roussel, A., Altermatt, F., … Bernatchez, L. (2017). Environmental DNA metabarcoding: Transforming how we survey animal and plant communities. *Molecular Ecology*, *26*(21), 5872–5895. https://doi.org/10.1111/mec.14350

Environment Agency (2019a). Retrieved from https://www.gov.uk/government/publications/unlocking-the-river-severn-powick-and-knightsford

Environment Agency (2019b). Retrieved from https://data.gov.uk/dataset/3feebf99-ae62-4d5c-ad41-f308e805a3d6/national-fish-population-database-load-statistics

Evans, N. T., Li, Y., Renshaw, M. A., Olds, B. P., Deiner, K., Turner, C. R., … Pfrender, M. E. (2016). Fish community assessment with eDNA metabarcoding: Effects of sampling design and bioinformatic filtering. *Canadian Journal of Fisheries and Aquatic Sciences*, *74*(9), 1362–1374. https://doi.org/10.1139/cjfas-2016-0306

Falke, J. A., & Gido, K. B. (2006). Effects of reservoir connectivity on stream fish assemblages in the Great Plains. *Canadian Journal of Fisheries and Aquatic Sciences*, *63*(3), 480–493. https://doi.org/10.1139/f05-233

Goldberg, C. S., Turner, C. R., Deiner, K., Klymus, K. E., Thomsen, P. F., Murphy, M. A., … Taberlet, P. (2016). Critical considerations for the application of environmental DNA methods to detect aquatic species. *Methods in Ecology and Evolution*, *7*(11), 1299–1307. https://doi.org/10.1111/2041-210X.12595

Gozlan, R. E., Andreou, D., Asaeda, T., Beyer, K., Bouhadad, R., Burnard, D., … Robert Britton, J. (2010). Pan-continental invasion of *Pseudorasbora parva*: Towards a better understanding of freshwater fish invasions. *Fish and Fisheries*, *11*(4), 315–340. https://doi.org/10.1111/j.1467-2979.2010.00361.x

Gu, W., & Swihart, R. K. (2004). Absent or undetected? Effects of non-detection of species occurrence on wildlife–habitat models. *Biological Conservation*, *116*(2), 195–203. https://doi.org/10.1016/S0006-3207(03)00190-3

Guo, Z., Andreou, D., & Britton, J. R. (2017). Sea lamprey *Petromyzon marinus* biology and management across their native and invasive ranges: Promoting conservation by knowledge transfer. *Reviews in Fisheries Science & Aquaculture*, *25*(1), 84–99.

Gutmann Roberts, C., & Britton, J. R. (2018). Quantifying trophic interactions and niche sizes of juvenile fishes in an invaded riverine cyprinid fish community. *Ecology of Freshwater Fish*, *27*(4), 976–987. http://dx.doi.org/10.1111/eff.12408

Gutmann Roberts, C., Hindes, A. M., & Britton, J. R. (2019). Factors influencing individual movements and behaviours of invasive European barbel *Barbus barbus* in a regulated river. *Hydrobiologia*, *830*(1), 213–228. https://doi.org/10.1007/s10750-018-3864-9

Hänfling, B., Lawson Handley, L., Read, D. S., Hahn,C., Li,J., Nichols,P., … Winfield,I. J. (2016). Environmental DNA metabarcoding of lake fish communities reflects long-term data from established survey methods. *Molecular Ecology*, *25*(13), 3101–3119. http://dx.doi.org/10.1111/mec.13660

Harper, L. R., Handley, L. L., Hahn, C., Boonham, N., Rees, H. C., Gough, K. C., … Hänfling, B. (2018). Needle in a haystack? A comparison of eDNA metabarcoding and targeted qPCR for detection of the great crested newt (*Triturus cristatus*). *Ecology and Evolution*, *8*(12), 6330–6341. https://doi.org/10.1002/ece3.4013

Jager, H. I., Chandler, J. A., Lepla, K. B., & Van Winkle, W. (2001). A theoretical study of river fragmentation by dams and its effects on white sturgeon populations. *Environmental Biology of Fishes*, *60*(4), 347–361. https://doi.org/10.1023/A:1011036127663

Jane, S. F., Wilcox, T. M., McKelvey, K. S., Young, M. K., Schwartz, M. K., Lowe, W. H., … Whiteley, A. R. (2015). Distance, flow and PCR inhibition: eDNA dynamics in two headwater streams. *Molecular Ecology Resources*, *15*(1), 216–227. https://doi.org/10.1111/1755-0998.12285

Jerde, C. L., Chadderton, W. L., Mahon, A. R., Renshaw, M. A., Corush, J., Budny, M. L., … Lodge, D. M. (2013). Detection of Asian carp DNA as part of a Great Lakes basin-wide surveillance program. *Canadian Journal of Fisheries and Aquatic Sciences*, *70*(4), 522–526. https://doi.org/10.1139/cjfas-2012-0478

Lawson Handley, L., Read, D. S., Winfield, I. J., Kimbell, H., Johnson, H., Li, J., … Hänfling, B. (2019). Temporal and spatial variation in distribution of fish environmental DNA in England's largest lake. *Environmental DNA*, *1*(1), 26–39. https://doi.org/10.1002/edn3.5

Mackenzie, D. I., & Royle, J. A. (2005). Designing occupancy studies: General advice and allocating survey effort. *Journal of Applied Ecology*, *42*(6), 1105–1114. https://doi.org/10.1111/j.1365-2664.2005.01098.x

Magilligan, F. J., Nislow, K. H., Kynard, B. E., & Hackman, A. M. (2016). Immediate changes in stream channel geomorphology, aquatic habitat, and fish assemblages following dam removal in a small upland catchment. *Geomorphology*, *252*(1), 158–170. https://doi.org/10.1016/j.geomorph.2015.07.027

Maitland, P. S., & Lyle, A. A. (2005). Ecology of Allis Shad Alosa alosa and Twaite Shad Alosa fallax in the Solway Firth, Scotland. *Hydrobiologia*, *534*, 205–221.

McDevitt, A. D., Sales, N. G., Browett, S. S., Sparnenn, A. O., Mariani, S., Wangensteen, O. S., … Benvenuto, C. (2019). Environmental DNA metabarcoding as an effective and rapid tool for fish monitoring in canals. *Journal of Fish Biology*, *95*(2), 679–682. https://doi.org/10.1111/jfb.14053

Miya, M., Sato, Y., Fukunaga, T., Sado, T., Poulsen, J. Y., Sato, K., … Iwasaki, W. (2015). MiFish, a set of universal PCR primers for metabarcoding environmental DNA from fishes: Detection of more than 230 subtropical marine species. *Royal Society Open Science*, *2*(7), 150088. https://doi.org/10.1098/rsos.150088

Nakagawa, H., Yamamoto, S., Sato, Y., Sado, T., Minamoto, T., & Miya, M. (2018). Comparing local- and regional-scale estimations of the diversity of stream fish using eDNA metabarcoding and conventional observation methods. *Freshwater Biology*, *63*(6), 569–580. https://doi.org/10.1111/fwb.13094

Noble, R. A. A., Cowx, I. G., & Starkie, A. (2007). Development of fish-based methods for the assessment of ecological status in English and Welsh rivers. *Fisheries Management and Ecology*, *14*(6), 495–508. https://doi.org/10.1111/j.1365-2400.2007.00574.x

Nolan, E. T., & Britton, J. R. (2018). Spatial variability in the somatic growth of pikeperch *Sander lucioperca*, an invasive piscivorous fish. *Ecology of Freshwater Fish*, *28*(2), 330–340.

Nolan, E. T., Gutmann Roberts, C., & Britton, J. R. (2019). Predicting the contributions of novel marine prey resources from angling and anadromy to the diet of a freshwater apex predator. *Freshwater Biology*, *64*, 1542–1554. https://doi.org/10.1111/fwb.13326

Oliveira, A. G., Baumgartner, M. T., Gomes, L. C., Dias, R. M., & Agostinho, A. A. (2018). Long-term effects of flow regulation by dams simplify fish functional diversity. *Freshwater Biology*, *63*(3), 293–305. https://doi.org/10.1111/fwb.13064

Pollux, B., & Kurosi, A. (2006). On the occurrence of the Asiatic cyprinid *Pseudorasbora parva* in the Netherlands. *Journal of Fish Biology*, *69*(5), 1575–1580.

R Core Team (2018). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from http://www.R-project.org/.Swofford, D., L. 2002.

Radinger, J., Britton, J. R., Carlson, S. M., Magurran, A. E., Alcaraz-Hernández, J. D., Almodóvar, A., … Torralva, M. (2019). Effective monitoring of freshwater fish. *Fish and Fisheries*, *20*, 729–747.

Sana, S., Williams, C., Hardouin, E. A., Blake, A., Davison, P., Pegg, J., … Andreou, D. (2018). Phylogenetic and environmental DNA insights into emerging aquatic parasites: Implications for risk management. *International Journal for Parasitology*, *48*(6), 473–481.

Schnell, I. B., Bohmann, K., & Gilbert, M. T. P. (2015). Tag jumps illuminated - reducing sequence-to-sample misidentifications in metabarcoding studies. *Molecular Ecology Resources*, *15*(6), 1289–1303. https://doi.org/10.1111/1755-0998.12402

Shaw, J. L. A., Clarke, L. J., Wedderburn, S. D., Barnes, T. C., Weyrich, L. S., & Cooper, A. (2016). Comparison of environmental DNA metabarcoding and conventional fish survey methods in a river system. *Biological Conservation*, *197*, 131–138. https://doi.org/10.1016/j.biocon.2016.03.010

Shogren, A. J., Tank, J. L., Andruszkiewicz, E., Olds, B., Mahon, A. R., Jerde, C. L., & Bolster, D. (2017). Controls on eDNA movement in streams: Transport, retention, and resuspension. *Scientific Reports*, *7*(1), 5065. https://doi.org/10.1038/s41598-017-05223-1

Sigsgaard, E. E., Carl, H., Møller, P. R., & Thomsen, P. F. (2015). Monitoring the near-extinct European weather loach in Denmark based on environmental DNA from water samples. *Biological Conservation*, *183*, 46–52. https://doi.org/10.1016/j.biocon.2014.11.023

Sørensen, T. (1948). *A method of establishing groups of equal amplitude in plant sociology based on similarity of species content and its application to analyses of the vegetation on Danish commons*. København, Denmarkl kommision hos E. Munksgaard.

Strickler, K. M. (2015). Quantifying effects of UV-B, temperature, and pH on eDNA degradation in aquatic microcosms (K. M. Strickler, A. K. Fremier, & C. S. Goldberg, Trans.). *Biological Conservation*, *183*, 85–92. https://doi.org/10.1016/j.biocon.2014.11.038

Taberlet, P., Coissac, E., Hajibabaei, M., & Rieseberg, L. H. (2012). Environmental DNA. *Molecular Ecology*, *21*(8), 1789–1793. https://doi.org/10.1111/j.1365-294X.2012.05542.x

Takahara, T., Minamoto, T., Yamanaka, H., Doi, H., & Kawabata, Z. (2012). Estimation of fish biomass using environmental DNA. *PLoS One*, *7*(4), e35868. https://doi.org/10.1371/journal.pone.0035868

Thomsen, P. F., & Willerslev, E. (2015). Environmental DNA – An emerging tool in conservation for monitoring past and present biodiversity. *Biological Conservation*, *183*, 4–18. https://doi.org/10.1016/j.biocon.2014.11.019

Valentini, A., Taberlet, P., Miaud, C., Civade, R., Herder, J., Thomsen, P. F., … Dejean, T. (2016). Next-generation monitoring of aquatic biodiversity using environmental DNA metabarcoding. *Molecular Ecology*, *25*(4), 929–942. https://doi.org/10.1111/mec.13428

van Puijenbroek, P. J. T. M., Buijse, A. D., Kraak, M. H. S., & Verdonschot, P. F. M. (2019). Species and river specific effects of river fragmentation on European anadromous fish species: Effects of river fragmentation on European migratory fish species. *River Research and Applications*, *35*(1), 68–77. https://doi.org/10.1002/rra.3386

Wilcox, T. M., McKelvey, K. S., Young, M. K., Jane, S. F., Lowe, W. H., Whiteley, A. R., & Schwartz, M. K. (2013). Robust Detection of Rare Species Using Environmental DNA: The Importance of Primer Specificity. *Plos One*, *8*(3), e59520. https://doi.org/10.1371/journal.pone.0059520

Wilcox, T. M., McKelvey, K. S., Young, M. K., Sepulveda, A. J., Shepard, B. B., Jane, S. F., … Schwartz, M. K. (2016). Understanding environmental DNA detection probabilities: A case study using a stream-dwelling char *Salvelinus fontinalis*. *Biological Conservation*, *194*, 209–216. https://doi.org/10.1016/j.biocon.2015.12.023

Yamanaka, H., & Minamoto, T. (2016). The use of environmental DNA of fishes as an efficient method of determining habitat connectivity. *Ecological Indicators*, *62*, 147–153. https://doi.org/10.1016/j.ecolind.2015.11.022

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Antognazza CM, Britton RJ, Read DS, et al. Application of eDNA metabarcoding in a fragmented lowland river: Spatial and methodological comparison of fish species composition. *Environmental DNA*. 2021;3:458–471. https://doi.org/10.1002/edn3.136