

# High frequency environmental DNA metabarcoding provides rapid and effective monitoring of fish community dynamics

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## Abstract

Long-term monitoring is critical to measure the response of biodiversity patterns and processes to human-mediated environmental pressures. This is particularly pertinent in freshwaters, where recent estimates indicated a third of all fish species are threatened with extinction, making ongoing biomonitoring essential for conservation management. High frequency annual monitoring is critical for identifying temporal changes in fish community composition; however, traditional survey methods are typically less practical over such timeframes. While environmental (e)DNA measurement represents a potentially powerful tool for monitoring temporal community dynamics, studies are lacking. To address this deficit, we generated a high frequency time-series dataset of entire fish communities using eDNA metabarcoding, to directly assess the repeatability and sensitivity of this method for detecting annual population trends. We targeted two differing environments (freshwater vs. intertidal) within the Thames catchment, UK, where detailed historical records from traditional monitoring were available for comparison. To test how robust eDNA data is for inferring the known community, we applied a hierarchical, nested design encompassing short and longer-term variation in eDNA data. Our analyses showed that irrespective of environment, eDNA metabarcoding represented known seasonal shifts in fish communities, where increased relative read abundance of eDNA coincided with known migratory and spawning events, including those of the critically endangered native species *Anguilla anguilla* (European eel). eDNA species detections across a single year included over 75% of species recorded in a ca. 30-year historical dataset, highlighting the power of eDNA for species detection. Our findings provide greater insight into the utility of eDNA metabarcoding for recovering temporal trends in fish communities from dynamic freshwater systems and insight into the potential best sampling strategy for future eDNA surveys.

## KEYWORDS

biodiversity, eDNA metabarcoding, fish communities, freshwater, temporal biomonitoring

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## 1 | INTRODUCTION

A detailed understanding of biodiversity processes across both space and time is fundamental for explaining present-day biodiversity patterns and making assessments of ecosystem health and function (Blowes et al., 2019; Hillebrand et al., 2018). This is particularly crucial in light of human-mediated environmental pressures (He et al., 2019; Reid et al., 2018) that have increased the pace and variance of environmental change (Eriksson & Hillebrand, 2019). While this understanding is critical across all habitats, global freshwater biodiversity is being affected disproportionately, threatening key ecosystem services such as food production supporting human livelihoods, health, and welfare at local to global scales (Albert et al., 2021; Darwall et al., 2018). Recent estimates indicate that a third of all freshwater fish species (freshwater fishes comprise a quarter of all vertebrate species on Earth) are threatened with extinction (Hughes, 2021), making detailed, reliable, and cost-effective biomonitoring essential for their conservation and management (Evans et al., 2017; Reid et al., 2018). Time series datasets are particularly valuable to identify causal relationships driving ecological dynamics and environmental change (Bálint et al., 2018; Runge et al., 2019), where they can be used to investigate biological events such as migrations and spawning, and the response to anthropogenic events, such as pollution spills. However, studies collecting data on community composition from at least two points in time are not common (Chevillot et al., 2016; Henderson, 2017; Magurran et al., 2015). In all cases of biomonitoring, insufficient surveillance is commonly presented as an obstacle where emerging technologies may represent the best solution to threat-mitigation efforts (Dudgeon et al., 2006; Littlefair et al., 2023; Reid et al., 2018).

Environmental DNA (eDNA) metabarcoding has proven to be a valuable tool in the biomonitoring of entire communities (Thomsen & Willerslev, 2015), where it has been shown to provide measurements congruent with traditional methods (Keck et al., 2022) and in many cases outperforms them (reviewed in Fediajevaite et al., 2021). Studies have largely focused on its performance for spatial biomonitoring, and it has been successfully applied to various aquatic environments, predominantly monitoring fishes, including lacustrine (e.g., Doble et al., 2020; Sard et al., 2019), lotic (e.g., Hallam et al., 2021; Piggott et al., 2021; Pont et al., 2018) and marine habitats (e.g., Afzali et al., 2020; Port et al., 2015). Due to the ease of collection and relatively low cost compared with traditional sampling techniques (e.g., Smart et al., 2016), eDNA also offers a promising method for high frequency sampling and/or long-term biomonitoring. However, despite the potential power of harnessing time-series datasets using eDNA metabarcoding, temporal community dynamics based on such data are a largely neglected area of research (but see Mathieu et al., 2020 and references therein), probably due to the novelty of eDNA as a biomonitoring tool. To date, only a handful of temporal studies have been carried out and, in most, sampling frequency is sparse (generally monthly or seasonally) and/or duration limited. While lower sampling frequency (per season, i.e., four times annually) may suit studies focussing on invertebrate communities

(Salonen et al., 2018; Seymour et al., 2021), it is unlikely to detect rapid fluxes, such as migrations and spawning events in fishes.

Existing time-series studies of eDNA have applied a range of short-term, high frequency sampling e.g., hourly across days (Ely et al., 2021; Jensen et al., 2022), to longer-term sampling, with less frequency e.g., weekly or fortnightly across 5 to 18 months (Djurhuus et al., 2020; Sigsgaard et al., 2017; Stoeckle et al., 2017; Ushio et al., 2018) and up to 3 years (Di Capua et al., 2021), to twice monthly for 2 years (Ushio et al., 2023). Although, Searcy et al. (2022) employed higher frequency daily sampling across a year. High frequency sampling strategies have, for example, detected clear diel changes in marine fish community composition (sampling hourly across 32 h: Jensen et al., 2022), as well as significant variation in eDNA concentrations in the context of a dynamic biological event, such as salmon spawning (sampling daily for 1 month using qPCR: Tillotson et al., 2018). For longer-term studies, metabarcoding has demonstrated detailed recoveries of seasonality in various coastal fish communities (Sigsgaard et al., 2017; Stoeckle et al., 2017; Ushio et al., 2018, 2023), and oceanic communities across all domains of life (Djurhuus et al., 2020).

Despite the strong potential for eDNA to be used for biomonitoring, heterogeneity of eDNA in the environment, environmental factors, and sampling design, remain a challenge for eDNA metabarcoding, as evidenced by relatively large differences that have been reported between sampling replicates within the same study system (e.g., Beentjes et al., 2019). Seasonal changes in species activity patterns, population densities, and environmental conditions have all been shown to influence eDNA concentrations and detectability (Yao et al., 2022). To draw robust conclusions from eDNA detections, a deeper understanding of the factors influencing temporal variation in eDNA is needed, using a robust sampling design applied to natural systems. In natural systems, many factors may influence measurements using eDNA, for example, temperature and turbidity may affect eDNA detections by reducing eDNA retention time in the environment, or sampling effort respectively (Collins et al., 2018; Sanches & Schreier, 2020). This is particularly important for animals from dynamic aquatic environments such as large catchments, which, to our knowledge, have not been investigated from a temporal perspective to date. These environments present challenges for eDNA monitoring due to marked differences in physical and chemical properties of the water over space and time (see Hallam et al., 2021). Seasonal patterns of flow in lotic habitats have been shown to alter detectability; for example, in a targeted qPCR study, high flow decreased eDNA concentrations and produced false negatives (Curtis et al., 2021), whereas a metabarcoding study found higher flows inflated species richness (Milhau et al., 2019). While eDNA detections from environments affected by tidal cycles have shown strong site fidelity to known community compositions (Jeunen et al., 2019; Kelly et al., 2018), a more detailed approach is needed to test the prediction of site fidelity across weekly, daily, and hourly time frames.

To address the question of temporal turnover of eDNA in a dynamic environment, we apply a novel, nested design of high

frequency sampling across the year to a large-scale temperate lotic system (The Thames catchment, UK) focusing on its fish communities. This system encompasses both fresh and intertidal waters and is regularly monitored by traditional surveys allowing an indirect comparison between traditional and eDNA detections. Seasonal patterns of change in fish communities have been recorded using traditional methods (Araújo et al., 1998, 2000; Tillotson et al., 2018), as fish move to exploit habitat and resource availability and, for some species such as *Anguilla anguilla* (European eel), longer distance migrations (Naismith & Knights, 1988). We selected two sections of river with differing environmental characteristics, to determine if congruent signals are identified from eDNA irrespective of environment. These attributes allowed us to address the following questions: (1) Does eDNA represent temporal changes in the fish communities over longer and shorter timeframes? and (2) Is eDNA sensitive enough to detect dynamic biological events such as migratory shifts in species?

## 2 | MATERIALS AND METHODS

### 2.1 | Study system

The Thames, is a large UK catchment (13,000 km<sup>2</sup>) encompassing fresh and tidal waters that contains a diverse fish fauna (125 species) including rare, migratory, and non-native species (Kirk et al., 2002), for which excellent historical survey records (ca. 30 years of catch data) provide a robust baseline for eDNA comparisons (see Hallam et al., 2021). Two sites were selected (1) Lea Rowing Club (GPS 51.57348, -0.05903) a stretch of the lower freshwater River Lee—a tributary of the Thames which joins the main stem mid-estuary, and (2) Richmond Lock (GPS 51.46186, -0.31665) an upper tidal stretch of the main stem of the River Thames (Figure S1). These sites differed in their habitat, water chemistry, and the diversity of fish species recorded. The distinct fish communities that inhabit each site are known to exhibit seasonal changes in species abundance, including the migration of the endangered species *A. anguilla*. Historical survey records and site accessibility were factored into the selection of sites, with existing survey data from the Lea catchment spanning from 1978 to 2019, and for Richmond from 1992 to 2018.

### 2.2 | Sampling design and eDNA sampling

eDNA samples were collected over 12 months following a nested design, to give greater power regarding quantification of temporal trends of eDNA. For the Lea Rowing Club (Lee) site, the following sampling strategies were undertaken: (1) weekly sampling on the same day each week; (2) in 1 week every 3 months (to account for the seasons), samples were collected each day; (3) nested within the week of daily sampling, 1 day of high-intensity sampling, where samples were collected every 3 h (06:00–18:00). For strategies

1 and 2, sampling was carried out in the morning (06:57–10:14). For the Richmond Lock (Richmond) site we followed a similar protocol, with some differences to accommodate the tidal nature of the site: (1) weekly sampling on the same day each week at high tide; (2) for 1 day every 6 months, high-intensity sampling, every 3 h (06:00–18:00). Differences in the sampling strategy used at the two sites were dictated by the tidal nature of the Richmond site: at the Richmond site, samples taken for strategy 1 were collected during the slack tide period, where there was no tidal movement in either direction. As water temperature was not taken at the time of collection, we used air temperature as a proxy for water temperature.

On each occasion that eDNA was assessed, three 1-liter biological replicates were collected in cleaned and sterilized Nalgene containers. A blank field control (1 liter of deionized water filtered in the same manner as the field samples) was included with every nine field samples. For each biological replicate (and blank field control), 1 liter of water was filtered through a 0.45 μm Sterivex filter (Millipore Corp, USA) using a peristaltic pump with sterilized tubing (Masterflex, Cole-Parmer, USA). Time between collection and filtering was ca. 60 min for Lee samples (where samples were stored on ice) and ca. 10 min for Richmond samples, with variation due to travel distance. At times when water turbidity was high, such that suspended sediment preventing timely filtering, the maximum amount of water that could be filtered in 20 min was processed and recorded. Water was then expelled from the filter units, which were sealed in individual sterile bags and immediately frozen at -20°C. On average 734.8 mL (SD = 264.9) of water was filtered for the Lee and 477.4 mL (SD = 179.4) for Richmond (Figure S2). All sampling equipment was sterilized between sampling events by washing in a 30% commercial thin bleach solution (containing <3% sodium hypochlorite). In total, 314 samples (95 sampling events) were collected from the Lee from 06/02/2019 to 26/02/2020 including 26 blank field controls, and 203 samples (57 sampling events) were collected from Richmond from 01/03/2019 to 27/02/2020 including 20 blank field controls.

### 2.3 | DNA extraction, PCR amplification, and mock community

DNA was extracted from the Sterivex filters with the protocol used by Hallam et al. (2021), based on Cruaud et al. (2017) and Doble et al. (2020). Extractions were performed in a pre-PCR room, in a laminar flow cabinet that was wiped down with 25% bleach solution between each sample extraction. A DNA extraction negative control was included in every batch of samples and all extractions and controls were quantified using a Qubit v2 with the Qubit dsDNA HS Assay Kit (Invitrogen). MiFish-U primers (Miya et al., 2015) were selected, having previously performed well in the same catchment area (Hallam et al., 2021). Each PCR consisted of 7.5 μL of Taq reaction buffer, 0.6 μL of each forward and reverse primer, 1.8 μL of template eDNA diluted 1:5 with nuclease-free water, and 4.5 μL of nuclease-free water, for a total reaction volume of 15 μL. PCR

conditions involved an initial denaturing step at 95°C for 15 min, then 40 cycles of 94°C for 30 s, 90 s annealing temperature at 63°C, 60 s at 72°C for extension, final extension at 72°C for 5 min and a final hold at 4°C. Two negative PCR controls of nuclease-free water were included in each 96-well plate. All PCR products were visualized using gel electrophoresis on 2% agarose gel stained with ethidium bromide. Three replicate PCRs were run per biological sample and pooled in equal volumes for sequencing. Field blanks had one replicate per sample. Although no amplification was observed in the field blanks and negative controls, these samples were still included in the sequencing run.

A mock community (MC) was constructed to assess contamination, investigate potential amplification and sequencing bias, and gauge our assumptions about sample variation. The MC was created in a designated post-PCR laboratory once PCR amplification of the eDNA samples had been completed. Tissue-derived DNA from 10 fish species with a diverse phylogenetic history, yet highly unlikely to occur at the sampling sites, were combined in equal quantities (400 ng per species, measured with Qubit), processed following the same PCR methodology, and sequenced alongside the eDNA samples (see Table S1).

## 2.4 | Sequencing

In total 576 samples were sequenced. These comprised 314 samples collected from the Lee site (including 26 field controls), and 203 samples collected from the Richmond site (including 20 field controls). Five replicates of the mock community were included, as were 25 sequencing negative controls, 14 DNA extraction negative controls, and 15 PCR negative controls (see Table S2). The library was checked for quality and size using Qubit and TapeStation and sequenced using an Illumina MiSeq with a 2 × 250 bp v2 chemistry, and 10% PhiX spike-in. The raw reads were demultiplexed at the sequencing facility, filtered to remove low-quality reads, and converted to FASTAQ files using Illumina software. In total 12.9 million reads (12939910) were generated.

## 2.5 | Bioinformatic methods

All bioinformatic and data filtering steps were carried out in R v4.0.5 (R Core Team, 2021) using RStudio v1.4.1106 (RStudio Team, 2021). DADA2 (Callahan et al., 2016) was used to process the sequencing data into amplicon sequence variants (ASVs). The quality profiles of the unmerged forward and reverse files were visualized to gauge a reasonable length for truncation. Forward reads were truncated at 240 bp, and reverse reads at 200 bp, which kept the median quality scores above 30. Any sequences containing ambiguous nucleotides were discarded using the default `maxN=0` argument. The `maxEE=2.2` argument was used to discard any reads with higher than two expected errors after truncation. PhiX genome was also removed at this stage.

The error rates of the forward and reverse data sets were modeled, and the estimated error rates were checked with plots. Identical reads were dereplicated, and the error models created were used to infer the true sample composition of ASVs. The matching forward and reverse reads were then merged with a minimum overlap of 100 bp (`trimOverhang="True"`), and the ASV matrix was checked for chimeric sequences. As the quality of the reads was high and there was no significant difference between the default setting of 12 bp overlap vs. 100 bp (paired *t*-test,  $t = 1.06$ ,  $df = 575$ ,  $p = 0.28$ ), we preferred to use the more stringent merging parameter. We used the default function `pool="False"`, and when compared to `pool="True"` found no difference in overall fish species lists and richness for the samples. A reference database of 125 sequences for UK fish species was created using the Meta-Fish-Lib pipeline (Collins et al., 2021), and the `assignTaxonomy` argument was used to assign the taxonomy of the most similar reference sequences to the ASVs.

The R package `tidyverse` (Wickham, 2019) was used to merge the ASV table with the assigned taxonomy table and the sample metadata. The reads of any ASVs detected in the negative controls were considered as contamination, and these read counts were removed from the corresponding ASVs across all samples. The proportion of any reads from mock community DNA found in the samples was then used to set a second threshold for contamination. Mock community DNA was found in 14 of the samples, 10 of which were samples from the Lee, physically located next to the mock communities on the same 96-well plate. Since this contamination was localized and of obvious origin, the 10 samples that were contaminated were discarded. This resulted in a 1.2% threshold (based on *Lates niloticus* reads in sample R140) that was used to remove any ASV present at a proportion of <1.2% of the read count of a sample. Finally, the remaining ASVs were consolidated to the species level. To investigate the effect of sequencing depth on diversity detections, rarefaction curves of species richness with read count were plotted for each site for a random 10% of subsets of eDNA samples, with all samples plateauing before reaching the sequencing depth of the lowest sample (Figure S3).

## 2.6 | Statistical analysis

Species tables were constructed for the Lee and Richmond sites, which were thereafter analyzed separately. These tables included the date, season, and time at which samples were collected, the air temperature recorded at the time of sample collection, and the volume of water filtered per sample. Variation partitioning (Borcard et al., 1992) was used to investigate the contribution of the environmental variables to variation in the data sets and was implemented in the R package `vegan` v2.5-7 (Oksanen et al., 2019). The function `capscale` was used to perform a partial redundancy analysis on each of the one-year datasets and the `ordistep` function was subsequently used to identify significant variables. Variation partitioning was initially performed using the function `varpart` with "month", and "volume filtered" having been selected as categorical factors in

the models. Then, the individual components were tested for significance using the RDA function. The effects of time, temperature, and water volume filtered, were further investigated for subsets of the data: (1) across a day of high-intensity sampling, (2) across a week of sampling, (3) across a month of sampling, and (4) across seasons.

For diversity analyses, to minimize the possibility of false positives, only species present in two out of three biological replicates were retained (Ficetola et al., 2014). Therefore, the species tables were consolidated, so that data from the three biological replicates (taken per sampling event) were merged, resulting in the sum of the read counts for each species, and a new column of mean water volume filtered for the three biological replicates.

Historic fish survey data collected by the UK Environment Agency (EA) was downloaded from <https://data.gov.uk/> against which eDNA detections were compared. Fish survey data from sites within 2.25 km upstream and downstream of the Lee eDNA site was used as a reference for fish diversity in the area, comprising 38 fish surveys taken at 12 sites between 1992 and 2019. Richmond and its neighboring site 4.5 km downstream at Kew were used as references for fish diversity at Richmond Lock, comprising 93 surveys conducted between 1989 and 2018.

The eDNA read count data was Hellinger transformed using the *decostand* function, which has been shown to be the most appropriate data transformation to use for metabarcoding read count data (Laporte et al., 2021). The site-by-species matrix was transformed into relative values per site by dividing each read count by the site sum and then taking the square root of the resulting value (Legendre & Legendre, 2012). This reduces the importance of species with high read counts and corrects for biases produced by zeros, both of which are characteristic of eDNA metabarcoding data (Afzali et al., 2020; Laporte et al., 2021). Species richness and Shannon diversity were calculated for each eDNA sampling event. Generalized linear models (GLM) with Gaussian distribution were used to test the significance of change in richness across the year, with the volume of water filtered included as an explanatory variable, and Kruskal-Wallis tests were used to test for significant differences between Shannon diversity and season. Fish community composition at different temporal scales was plotted with nonmetric multidimensional scaling (nMDS) using Bray-Curtis distance, to visualize patterns across time. Analysis of similarities (ANOSIM) was then performed to test if there were statistically significant differences between communities. Similarity percentage (SIMPER) analyses were conducted to identify taxa contributing to dissimilarity, which were then individually tested for significance with Kruskal-Wallis tests.

The package iNEXT v2.0.20 (Hsieh et al., 2016) was used to assess sampling completeness, with the function *estimateD* used to estimate sampling effort with respect to sample coverage. Sample-sized based rarefaction and extrapolation (R/E) curves (Chao et al., 2014) were calculated to compare species richness and sampling coverage for the two sites. Beta diversity in the form of Sørensen dissimilarity, was calculated using *betapart* v1.5.4 (Baselga & Orme, 2012), to investigate the difference between the samples when taken using the different sampling strategies. A value of

0 indicates an identical community composition, while a value of 1 indicates total dissimilarity. Two further measures that partition dissimilarity were calculated: Simpson dissimilarity which accounts for dissimilarity due to species turnover, and the nestedness-resultant fraction of Sørensen dissimilarity, a measure of the fraction of total dissimilarity that is caused by species nestedness rather than replacement (Baselga, 2012). Differences in average Sørensen dissimilarity for the different sampling strategies were tested for significance with Kruskal-Wallis tests.

## 3 | RESULTS

### 3.1 | Mock community

The five replicates of the mock community contained an average of 21,573 reads following standard initial bioinformatic processing. All 10 species in the mock community were detected, however, *Synodontis multipunctatus* was detected in only one of the five mock communities and very low abundance (three reads). Among the other nine species, we observed only slight differences between the observed and expected proportions of species, which were not significantly different (paired t-test:  $p=0.99$ ; Figure S4 and Table S3). As a consequence, we used read counts as a proxy for abundance rather than transforming the data into presence/absence. Reads from the four most abundant fish ASVs from the eDNA samples (*Anguilla anguilla*, *Gasterosteus aculeatus*, *Perca fluviatilis*, and *Rutilus rutilus*) were detected in four of the five mock communities (6 to 26 reads,  $M=6.2$  reads). Across the five replicates, contamination accounted for only 0.26% of the reads and ranged from 38 to 90 reads ( $M=59$ ). This contamination exceeded the detection of *S. multipunctatus* and, therefore, read counts should be treated cautiously.

### 3.2 | Variation partitioning

After filtering, trimming, merging, and chimera removal, ~8.9 million reads were retained for analysis spanning 6700 ASVs. Model fitting permutation tests on the data identified time, particularly over longer periods, and (air) temperature as generally significant variables influencing variation in eDNA detections across the two sites, with less effect from (filtered water) volume, across the different temporal sampling schemes (Table 1). Over short time periods, air temperature at the time of sampling explained a large proportion of the variance, but its explanatory power was reduced over longer time periods where temporal effects (e.g., season) tended to become more important. However, aside from the year, most of the variation was not accounted for by any of the recorded variables. For the entire year, temporal variation ("month") explained a similar fraction for both sites (Lee: 28.3% and Richmond: 25.4%) of overall variation ( $p \leq 0.05$ ), whereas temperature ( $p \geq 0.05$ ) and volume ( $p \leq 0.05$ ) contributed a very small fraction, such that 46.1% and 48.3% (Lee and Richmond) of the variation in eDNA across

TABLE 1 Results of variation partitioning of the fish communities detected by eDNA in the River Lee, and Richmond Lock, River Thames sites.

Site	Sampling strategy	Timeframe	Unit	Temporal variable	Temperature	Volume	Residuals
River Lee	Day (HI)	May	Hour	22.2*	9.9*	0	57.8
		Aug	Hour	5.7	9.5*	4.7	81.5
		Nov	Hour	5.2	22.3*	0	74.1
		Feb	Hour	6.8	14.3*	0	78.1
	Week	May	Day	30.2*	18.6*	7.5	63.4
		Aug	Day	3.9	29.2*	9.5*	63.7
		Nov	Day	5.8	12.4*	13.1*	67.0
		Feb	Day	17.9*	2.4	5.7	74.4
	Season	Spring	Month	12.5*	2.3*	2.7*	77.9
		Summer	Month	23.5*	1.9*	1.5*	74.0
		Autumn	Month	12.9*	0	10.9*	63.0
		Winter	Month	5.2*	0	7.7*	83.4
	Year	All	Month	28.3*	0.3*	2.4*	46.1
River Thames, Richmond Lock	Day (HI)	May	Hour	14.3	27.7*	1.0	72.7
		Nov	Hour	9.0*	1.9	6.4	87.0
	Season	Spring	Month	14.6*	1.1	0.9	58.0
		Summer	Month	12.8*	2.7*	0.3	80.4
		Autumn	Month	11.8*	2.8*	0.2	58.7
		Winter	Month	11.2*	1.6	2.5*	85.9
	Year	All	Month	25.4*	0.2	0.5*	48.3

Note: HI, high intensity (sampling every 3h for 12h).

\* $p < 0.05$ .

the year was not explained by any of the recorded variables. Although seasonal temporal variation ("month") contributed considerably more variation in community composition than the other variables ( $p \leq 0.05$ ), its contribution was highly variable across the freshwater site compared to the intertidal site (Lee 5.2% – 23.5% vs. Richmond 11.2% – 14.6%). Despite this, residual unexplained variation was similar at both sites, ranging from 63.0% to 83.4% (Lee) and 58% to 85.9% (Richmond). For the seasonal sampling, temperature explained very little of the variation (Lee: 0–2.3%; Richmond: 1.1% – 2.8%) and was not always significant (Table 1), and although the same was true for volume at Richmond (0.2% – 2.5% of the variation, with only winter as significant), the proportion explained was more variable at the Lee (1.5% – 10.9,  $p \geq 0.05$ ). This was likely to be due to the more constant volume filtered at the Richmond site (Table S1) due to the higher and more consistent suspended sediment load in the tidal water than in the freshwater Lee. Across all sampling schemes, temperature explained much less variation in community composition in the cooler months, especially winter, but this result was not always significant (Table 1). In contrast with the longer temporal sampling schemes (i.e., annual, and seasonal), temperature explained a greater fraction of the variation in community composition over weekly ("day") and daily ("HI day," diurnal 3 hourly sampling for 12h) temporal sampling, and together with time, explained a similar fraction of the

overall variation in community composition. Similar to the longer time-series data, shorter frequency data identified volume as typically explaining a small fraction of overall variation for both sites and while non-significant for the HI days, volume was significant for the Lee for two of the daily weekly sampling events (Table 1).

### 3.3 | Site diversity detected by eDNA metabarcoding

In total 45 and 41 fish species were detected in at least one biological replicate from the Lee and Richmond (based on 283 and 179 samples) respectively. However, as only species present in two out of three biological replicates were retained (reducing the likelihood of false positives, see Methods), a total of 19 and 20 species remained (see Tables S4 and S5). These numbers based on eDNA are congruent with historical survey data from the EA, which recorded 23 species (and three hybrids) and 27 (and one hybrid) species from the Lee, and Richmond and Kew Sites based on surveys from 1992 to 2019 (41 surveys) and 1989 to 2018 (93 surveys), respectively. Species rarefaction/estimation curves, and sample completeness curves based on sampling coverage, estimate that 99.2% sampling coverage was achieved for the Lee, and 100% coverage for Richmond Lock (Figure S5). Over the sampling period, species

richness at the Lee River detected by eDNA varied from three to eight species on each sampling event ( $M=5.7$ ,  $SD=1.14$ ), whereas at Richmond it ranged from three to 11 species ( $M=6.56$ ,  $SD=1.95$ ). No significant change in species richness across the year ( $p=0.71$ ) was identified at the Lee, and Shannon diversity ranged from 0.85 to 2.03 ( $M=1.6$ ,  $SD=0.2$ ) and did not change significantly across the seasons ( $p=0.7$ ). This contrasted with the Richmond site where species richness increased significantly across the year ( $p<0.003$ , Figure S6), and changed significantly across seasons ( $p<0.0001$ ). At Richmond, Shannon diversity ranged from 0.98 to 2.36 ( $M=1.76$ ,  $SD=0.31$ ;  $p<0.0002$ ) and changed significantly across season also ( $p<0.0001$ ).

Although diversity differed between the sites due to the differing habitats (freshwater vs. intertidal) (see Hallam et al., 2021; Kirk et al., 2002), *A. anguilla* was the most abundant species in the Lee and the second most abundant species at Richmond based on read counts, with a similar pattern in occurrence, where it was detected at 99% and 72% of the sampling events respectively. In the Lee, *A. anguilla*, along with *G. aculeatus* (three-spined-stickleback), *Perca fluviatilis* (perch), *Rutilus rutilus* (roach), *Gobio gobio* (gudgeon) and *Abramis brama* (common bream), made up 92.8% of the fish read counts. In contrast, at Richmond the six most abundant species based on read counts were *A. brama*, *A. anguilla*, *R. rutilus*, *Platichthys flesus* (flounder), *Cottus gobio* (bullhead), and *P. fluviatilis* which made up 78.75% of all fish read counts. The remaining 13 species from the Lee and 14 species from Richmond were detected less frequently, and each made up <1% and <5% of the read counts respectively. These were predominantly species recorded by the EA as occurring in the area, with the following exceptions: *Salmo salar* (Atlantic salmon), *Scomber scombrus* (mackerel), *Sprattus sprattus* (sprat), and reads identified to Gadidae (the cod family) were detected at the Lee site, and *Oncorhynchus mykiss* (rainbow trout) was detected at Richmond.

The majority of fish species lost from the Lee due to only retaining species present in two out of the three biological replicates had not been previously been recorded in the area by the EA, and are likely false positives. These included common menu species, the marine species: *Chelon auratus* (golden gray mullet), *Merlangus merlangus* (whiting), *Microstomus kitt* (lemon sole), *Mullus surmuletus* (red mullet), *Pagellus erythrinus* (sea bream), *Pleuronectes platessa* (plaice), *Sardina pilchardus* (pilchard), *Solea solea* (Dover sole), *Sparus aurata* (gilt-head sea bream), and *Trisopterus luscus* (pouting), as well as the freshwater species: *Oncorhynchus mykiss* (rainbow trout), and Asian freshwater catfish *Pangasius bocourti* (commonly sold in the UK as basa). Similarly, at Richmond, the following menu species that had not been recorded by the EA were also lost due to our more conservative approach regarding the biological replicates: *Hippoglossus hippoglossus* (halibut), *Oncorhynchus gorbuscha* (pink salmon), *Thunnus albacares* (yellowfin tuna), gilt-head sea bream, lemon sole, mackerel and sprat.

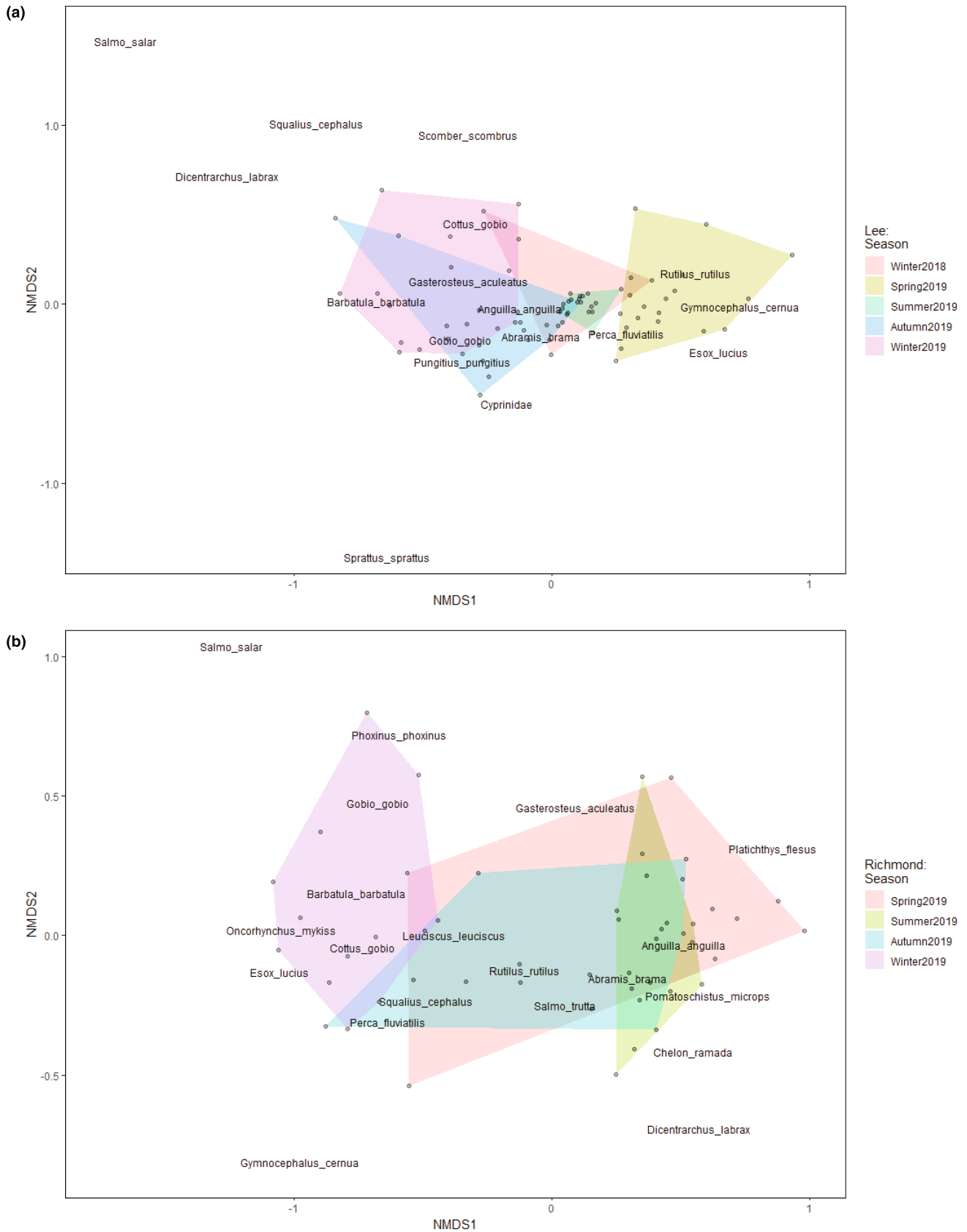
Species known to be present at the sites, but lost by including species from two out of the three biological replicates (and therefore could be considered false negatives) included from the Lee:

*Alburnus alburnus* (bleak), *Blicca bjoerkna* (silver bream), *Carassius auratus* (goldfish), *C. carassius* (crucian carp), *Leuciscus leuciscus* (dace), and *Phoxinus phoxinus* (minnow). A detection of Siluriformes DNA (444 reads) was also lost, and may have represented one of four introduced catfish species to this area. Likewise, various species recorded by the EA for the Richmond site were also lost through retaining species from two of the three biological replicates, including bleak, *Barbus barbus* (barbel), silver bream, *Osmerus eperlanus* (smelt), *Pungitius pungitius* (10-spined stickleback), *Sander lucioperca* (zander), *Scardinius erythrophthalmus* (rudd) and *Tinca tinca* (tench). DNA from these species together made up <0.2% of each of the total read counts for fish at Richmond. Two invasive species of high interest, *Ameiurus melas* (black bullhead catfish), and *Silurus glanis* (wels catfish) were each detected at two separate sampling events but were each only present in one of the three biological replicates and so discarded.

### 3.4 | Statistical differences in fish community between samples

Significant differences in communities across seasons were observed in nMDS plots and detected by ANOSIM at both the Lee and Richmond sites (Figure 1, Figure S6). When using all data from the whole year of sampling, despite overlap observed in nMDS ordination between the seasons, the communities remained significantly different at both the Lee (nMDS 2D stress=0.14, ANOSIM  $R=0.47$ ,  $p<0.001$ , and Richmond (nMDS 2D stress=0.11, ANOSIM  $R=0.45$ ,  $p<0.001$ , Figure 1). There were statistically significant differences in the communities detected on the four Lee HI days (nMDS 2D stress=0.07; Figure S7a, ANOSIM  $R=0.71$ ,  $p<0.001$ ), and on the two Richmond HI days (nMDS 2D stress <0.0001, ANOSIM  $R=1$ ,  $p<0.01$ ). For the Lee, these data were extended to include the samples collected daily for a week (sampling strategies 2 and 3). Significant differences in the communities were still detected by ANOSIM ( $R=0.62$ ,  $p<0.001$ ) but a greater overlap was observed in the nMDS plot (2D stress=0.14; Figure S7b).

The Sørensen dissimilarity between the detected communities was first calculated for the HI days, where dissimilarity varied with season, with the two sites exhibiting quite differing variations in the communities. At the Lee site, average dissimilarity for the four HI days ranged from 0.13 for the November (Autumn) HI day where the community detected across the day was very similar, to 0.50 for samples collected across the February (Winter) HI day, and was predominantly attributed to nestedness (beta.SNE, Table 2). In contrast, the dissimilarity for the five Richmond samples collected across the May (Spring) HI day was 0.11 dominated by nestedness (beta.SNE=0.11) indicating the high similarity of the samples, despite being collected at different stages of the tidal cycle. The five November (Autumn) HI day samples had a much higher dissimilarity of 0.49 between the samples, with turnover contributing to the majority of dissimilarity (beta.SIM=0.43) (see Table 2).



**FIGURE 1** Nonmetric multidimensional scaling (nMDS) ordinations representing fish community dissimilarities across the year at (a) the River Lee site (freshwater), and (b) Richmond Lock, River Thames (tidal) sites. Points represent a single eDNA survey, and colored polygons indicate season. Lee nMDS stress (2d)=0.14. Richmond nMDS stress (2d)=0.11.



**TABLE 2** Sørensen dissimilarity for the nested samples taken at four periods during the year, at the River Lee site, and at two periods during the year at the Richmond Lock, River Thames site.

Sampling strategy	Month	Sørensen dissimilarity		
		Beta.SOR	Beta.SIM	Beta.SNE
River Lee				
3: High intensity (HI), sampling every 3 h (Day)	May	0.24	0.07	0.16
	Aug	0.33	0.18	0.15
	Nov	0.13	0	0.13
	Feb	0.5	0.21	0.29
2: Sampling once a day for 6 days (Week)	May	0.42	0.28	0.13
	Aug	0.13	0	0.13
	Nov	0.46	0.35	0.11
	Feb	0.57	0.55	0.02
1: Sampling once a week for a month (Month)	May	0.58	0.41	0.18
	Aug	0.42	0.1	0.33
	Nov	0.61	0.5	0.11
	Feb	0.76	0.64	0.12
All months		0.92	0.87	0.05
River Thames, Richmond Lock				
2: High intensity (HI), sampling every 3 h (Day)	May	0.11	0	0.11
	Nov	0.49	0.43	0.07
1: Sampling once a week for a month (Month)	May	0.49	0.35	0.14
	Nov	0.58	0.53	0.05
All months		0.92	0.88	0.04

Abbreviations: Beta.SOR, total Sørensen dissimilarity; beta.SIM, Simpson dissimilarity measuring species turnover; beta.SNE, dissimilarity accounting for species nestedness.

For the 4 weeks of the year where eDNA sampling was conducted daily for a week at the Lee site, the average Sørensen dissimilarity between the samples was 0.40. The week of sampling events in August were the most similar (0.13, all attributed to nestedness) while the other 3 weeks ranged from 0.42 to 0.57 with turnover contributing to most of the dissimilarity.

Across the whole year of data, at both sites, Sørensen dissimilarity between the sampling events was 0.92 and dominated by turnover. There were no statistically significant differences detected between the average dissimilarity and sampling strategy for either the Lee (Chi-square=6.82,  $p=0.08$ ,  $df=3$ ) or Richmond samples (Chi-square=3.05,  $p=0.22$ ,  $df=2$ ) (Figure S8).

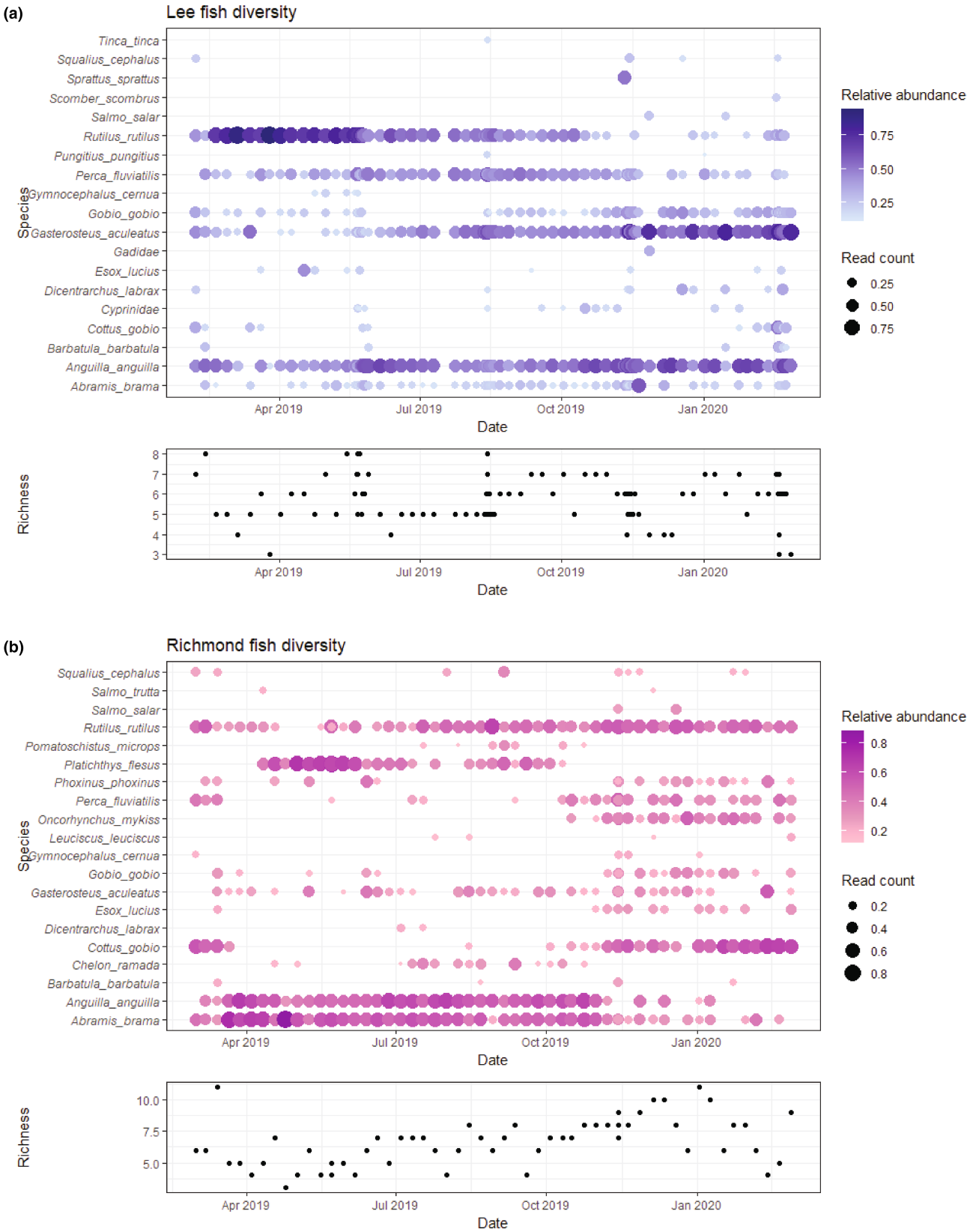
Differences between the fish communities among seasons were investigated further with SIMPER analysis, with the results driven by the most abundant species at both sites that showed common trends across the sampling period. The majority of dissimilarity in the Lee communities was attributed to the seven most abundant species and ranged from 25% to 51% between seasons ( $M=36.2\%$ , see Table S6), with six of these influential species showing significant changes in their abundance across the sampling period (Table S7). At Richmond, the 11 most abundant species contributed most of the dissimilarity, which ranged from 37% to 69% ( $M=52\%$ , see Table S8), and of these, 10 had significant changes in their relative abundance across the sampling period (Table S9).

At the Lee, variation in the relative abundance of the common river species *R.utilus* was consistently most influential in the dissimilarity between groups, with the exception of an increase in *G. aculeatus* across spring and summer 2019 that accounted for 25% dissimilarity, an increase in *P. fluviatilis* between winter 2018 and summer 2019 (16% dissimilarity), and a subsequent decrease in perch between autumn and winter 2019 (17% dissimilarity: Figure 2, Figure S6a).

Reflecting the difference in the fish community at the tidal Richmond site, marked variations in the relative abundance of the migratory estuarine species *P. flesus* and *A. anguilla* had the most frequent influence on dissimilarity between groups, with the exception between spring and winter where 13% dissimilarity was attributed to a decrease in the relative abundance of *A. brama* (Figure S6b).

## 4 | DISCUSSION

In the light of anthropogenic impacts on freshwater systems globally (Darwall et al., 2018) monitoring and understanding changes to species composition is urgently needed and will help to define conservation priorities (Dornelas et al., 2014). Time-series data for these ecosystems have previously been limited by the practicalities of traditional methods. In this study, we demonstrated that the



**FIGURE 2** Changes in eDNA sequencing read count (Hellinger transformed and used as a proxy for abundance) across a year for fish species comprising communities in (a) River Lee, and (b) Richmond Lock, River Thames sites. Color intensity and larger points both indicate greater abundance of sequencing reads. Richness is based on species present in 2 of the 3 biological replicates.

application of high frequency temporal eDNA sampling using a robust sampling design can be used to infer annual community dynamics in large catchments. We showed that eDNA represented shifts in the fish communities within the Thames catchment that correspond to known seasonal patterns of change, irrespective of water properties (i.e., fresh vs. tidal), including strong eDNA signatures that coincided with the times of the year that migratory and spawning events have been recorded (Araújo et al., 1998, 2000; Naismith & Knights, 1988; Thomas, 1998). Dissimilarity between samples and seasons varied and provided insight into the potential best sampling strategy for future eDNA surveys.

#### 4.1 | eDNA metabarcoding as an effective monitoring tool for community dynamics

We demonstrated that eDNA species detections across a single year included over 75% of the species recorded in a ca. 30-year historical dataset, highlighting the power of eDNA to detect species, although we acknowledge that this is not a direct comparison and therefore does not measure timescale or effort. Our findings support previous temporal studies using aquatic eDNA metabarcoding (Di Capua et al., 2021; Seymour et al., 2021; Sigsgaard et al., 2017) that have demonstrated congruence between molecular identification and traditional assessments, indicating, as with spatial studies, that eDNA metabarcoding can provide a robust method for assessing changes in community composition, typical in fishes. Through repeated temporal eDNA sampling, the dynamic nature of the fish communities was described at a high resolution that otherwise requires considerable effort when applying traditional methods e.g., electrofishing (Hallam et al., 2021), and/or is restricted to a handful of sites, e.g., station cooling water intakes (Araújo et al., 1998, 2000; Thomas, 1998), eel-traps (Naismith & Knights, 1988).

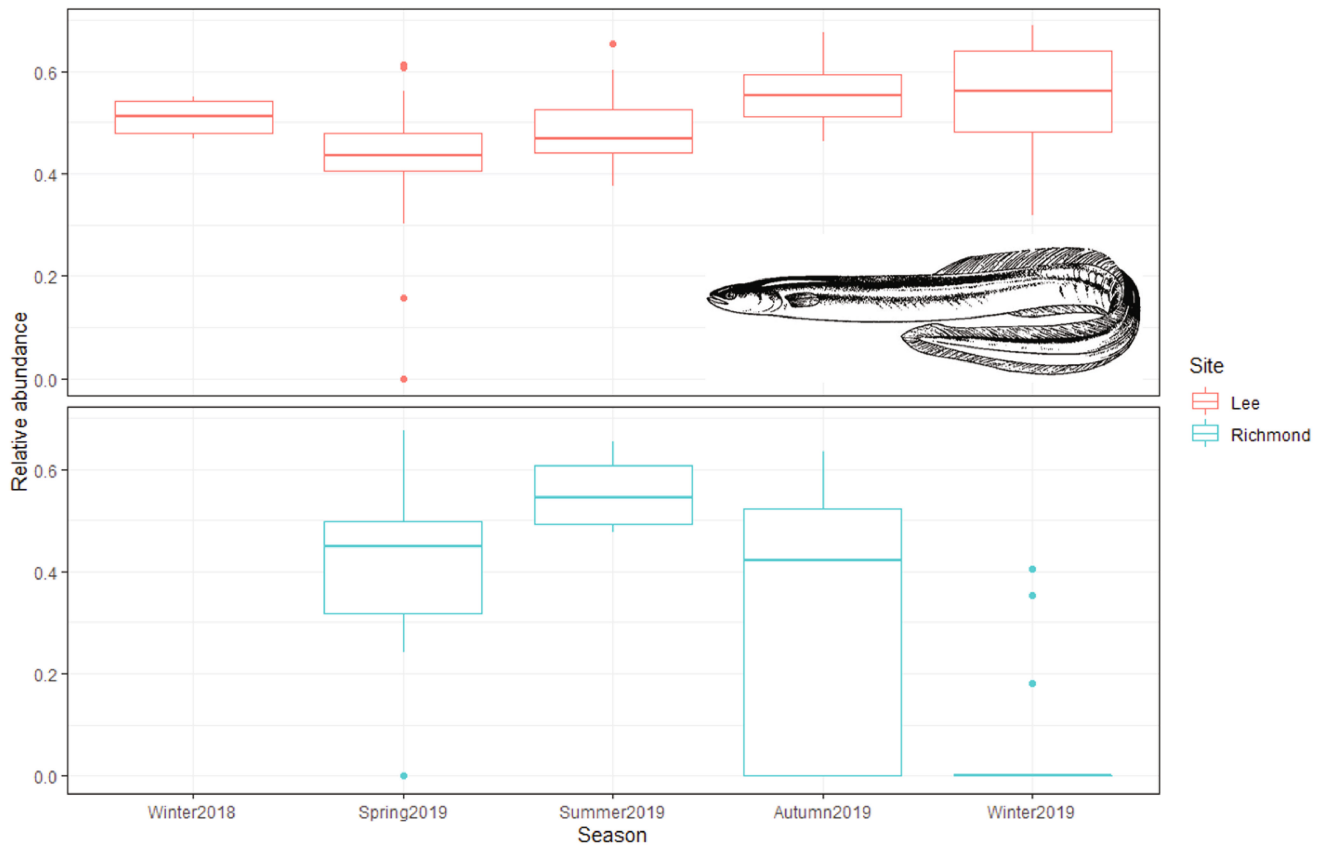
We substantiate the findings of previous eDNA metabarcoding studies that identified significant temporal differences in communities detected by eDNA (e.g., Antognazza et al., 2021; Bista et al., 2017; Lawson Handley et al., 2019; Sales et al., 2021; Seymour et al., 2021). By expanding upon previous temporal studies through our nested sampling design (sampling from 3 hourly time windows up to a year) we were able to both elucidate annual community dynamics and show variation of aquatic eDNA over the short-term. Our comparative approach demonstrated seasonal patterns in eDNA detections, indicating that our results were biologically meaningful. However, environmental conditions (Strickler et al., 2015) and physical characteristics, e.g., seasonal stratification (Lawson Handley et al., 2019), are known to affect eDNA detections, so that an understanding of the impact of these factors is crucial to the interpretation of results in order to identify true changes in diversity and abundance, rather than changes in eDNA detectability. Our study showed that longer-term temporal change ("month") explained the most variation in the detected communities, suggesting seasonal shifts in fish communities.

Our findings support previous temporal studies on Thames fishes based on seine netting, and a power station cooling water intake screen (Araújo et al., 1998, 1999, 2000; Thomas, 1998) that detected core fish communities throughout the year while showing seasonal changes in the abundance of key species. Crucially, our findings indicate changes in the read count abundances of some species that coincided with reproductive periods and known migration behaviors (see Section 4.2). However, despite our study supporting previous trends from these traditional studies, we acknowledge that the influence of downstream movement of DNA has been shown to affect detections through inflation from upstream DNA sources (Milhau et al., 2019), or rapid dispersion of DNA due to high water flow (Curtis et al., 2021), and cannot be disregarded as a potential factor here.

#### 4.2 | Potential detections of fish migration and spawning events using eDNA metabarcoding

Our study identified strong eDNA signatures that appear to correlate with dynamic biological events, such as fish migration based on previous traditional studies (Araújo et al., 1998, 1999; Araújo & Bailey, 2000; Naismith & Knights, 1988; Thomas, 1998). Previously, targeted eDNA-based studies employing qPCR (Thalinger et al., 2019) have successfully detected fish migrations, e.g., of sea lamprey (Bracken et al., 2019), and Danube bleak and vimba (Thalinger et al., 2019), but similar migratory patterns have not been reported with eDNA metabarcoding due to the lack of longer term high frequency sampling. Our findings showed that while the community of freshwater fish species at the Richmond site (main channel of the tidal River Thames) remained stable during the year, notable fluctuations in read counts appeared to correspond with known migration events of several species in the estuary (Araújo et al., 1999; Naismith & Knights, 1988; Thomas, 1998), including *A. anguilla*. Despite its status as a critically endangered native species in the UK. (Pecorelli et al., 2019), *A. anguilla* was the most abundant species by read count in our study (Figure 2), and the relative abundance of read counts was shown to increase through the spring and summer months at both fresh and tidal sites (Figures 3, Figure S6a,b), potentially reflecting the migration of juvenile eels into freshwaters. While *A. anguilla* does migrate through the Lee site, they also remain resident in this habitat (Pecorelli et al., 2019), leading to a regular input of eDNA. In contrast, at Richmond this signal tailed off through autumn and winter, potentially as the fish passed through the tidal waters and took up residency in freshwater habitats further upstream. Traditional data confirming our findings are based on eel traps, which are visited daily and the number and size class of the eels are recorded (Naismith & Knights, 1988).

Additional potential migration events were detected for *Platichthys flesus* (flounder) and *Chelon ramada* (thin lip mullet), again at the Richmond site, with both species showing significant changes in read counts across the year. Similar to *A. anguilla*, the high read counts of *P. flesus* were detected in spring (Figure S6b), which then



**FIGURE 3** Changes in the relative abundance of *Anguilla anguilla* (European eel) read counts across the study period from the River Lee (freshwater tributary of the River Thames) and Richmond Lock, River Thames (tidal) sites.

decreased significantly across the year. This trend in abundance is corroborated from a study that used traditional (seine netting) sampling to determine flounder numbers in the Thames and showed their peak to be around May in the upper Thames estuary (Araújo et al., 1999). In contrast, an increase in read counts was detected across the summer and autumn months for *C. ramada* (Figure S6b), with their complete absence during the winter months, corresponding to when this species is known to overwinter at sea (Colclough et al., 2002; Wheeler, 1979).

As well as being able to detect migrations, spawning activities have been demonstrated to increase target eDNA and detections in PCR (Gingera et al., 2016) and qPCR-based studies (Erickson et al., 2016; Thaling et al., 2019; Tillotson et al., 2018; Wu et al., 2022). A recent metabarcoding study comparing eDNA with ichthyoplankton netting surveys found a high level of agreement between the methods for detecting spawning grounds for offshore fish communities (Ratcliffe et al., 2021), further demonstrating the potential of eDNA metabarcoding as a tool for detecting breeding events of multiple species rapidly and cost-effectively. In a metabarcoding study investigating coastal fish communities (Sigsgaard et al., 2017), detections for lumpfish (*Cyclopterus lumpus*) and garfish (*Belone belone*) mainly coincided with their known spawning periods. Possible spawning activities may also be reflected in some of the detections from the Lee and Richmond sites, where peaks in read count abundance between March and

June coincided with the breeding season of *Rutilus rutilus* (roach), and the increase in *Perca fluviatilis* (perch) read count abundance across the spring and summer also potentially coincided with their period for spawning, hatching and recruitment of juveniles. Investigating how activity patterns and breeding cycles effect eDNA detectability would be a useful next step for the fish species highlighted in our study as showing fluctuations in read count abundance. For example, this has been demonstrated for the endangered UK native white clawed crayfish *Austropotamobius pallipes* (Troth et al., 2021) through the use of mesocosm and controlled field experiments. Mesocosms were also used to show how seasonal variation in the eDNA of great crested newts was linked with breeding period, and abundances of larvae and adults (Buxton et al., 2017). While studies have investigated short-term fluctuations in eDNA under laboratory settings (Klymus et al., 2015; Sassoubre et al., 2016), to our knowledge there have not been any longer-term studies investigating trends in fish eDNA detectability relating to life stages or behaviors.

By retaining read count data, it was possible from our study to detail seasonal trends in abundance that were corroborated by previous historic survey records (Araújo et al., 1999). Determining if eDNA metabarcoding data can reliably inform on species abundance, including the context-specific factors that influence the eDNA abundance/biomass relationship, has generated promising results (Rourke et al., 2022) and positive correlations between

eDNA concentration and abundance data have been demonstrated across a variety of studies, e.g., by gillnetting (Boivin-Delisle et al., 2021; Hänfling et al., 2016), trawling (Afzali et al., 2020; Salter et al., 2019; Stoeckle et al., 2021; Thomsen et al., 2016), piscicides (Skelton et al., 2022) and controlled mesocosm experiments (Di Muri et al., 2020; Takahara et al., 2012). However, some of these studies also revealed mixed results across sites (e.g., Hänfling et al., 2016) and did not perform well for non-dominant species (e.g., Skelton et al., 2022). As such, the performance of read count data is not without caveats and multiple factors from PCR bias, biotic (e.g., target organism, shedding rates, abundance) and environmental (e.g., fate, transport) factors affect the association between measured eDNA and abundance (Yao et al., 2022 and refs therein). For example, environmental variables have been shown to explain a large proportion of eDNA relative abundance variation in some systems, including rivers (Laporte et al., 2021; Rourke et al., 2022).

### 4.3 | Short-term temporal monitoring

Studies investigating short-term time series (hours to days) are rather limited despite potential daily or even hourly changes in community composition at a site. A recent study (sampling every hour, for 32h) reported clear diel changes in community composition for fishes and eukaryotes regarding relative read frequency data from a shallow coastal European site (Jensen et al., 2022) highlighting the importance of biological variation regarding sampling time. Although we only sampled diurnally on our high intensity (HI) days (sampling every 3h, for 12h), these data typically showed low dissimilarity; however, results were equivocal regarding the tidal site, with low and high dissimilarity reported from Spring and Autumn respectively (Table 2). Although the site is subjected to a daily large tidal range, and thus tidal movement may affect community composition, low and high tides for the HI days were similar (May 0.12–4.77m; November: 0.06–4.91m). It is possible that the contrasting signal may be a consequence of higher freshwater input into the estuary on the Autumn HI day and subsequently greater influence of eDNA from upstream and waste outlets. However, Kelly et al. (2018), found eDNA-detected communities were not affected by exogenous DNA arriving on tides, despite tidal movement theoretically transporting and mixing DNA over wide distances from a nearshore intertidal site. A previous study of a nearshore coastal habitat (Ely et al., 2021) also found mixed results regarding variation in diversity observed across replicates, which could not be explained by tide and/or time of collection, and suggested that the volume of water sampled was a source of variation in detections. Due to the nature of our focal sites having high levels of suspended particles, our filters suffered from clogging and consequently, volumes of filtered water were not uniform (Figure S2). However, despite volume significantly contributing to community variance, it only explained a small fraction for both the Lee (freshwater) and, particularly Richmond (tidal) sites. That volume had a minimal effect in our study maybe due to the comparatively low diversity of fish communities present at the sites, whereas it has

been shown to have a large effect on more diverse tropical communities (e.g., Bessey et al., 2020). Clearly, filter design needs further development in order to optimize field sampling techniques.

In contrast to the longer-term time series data from this study, both temperature and time had an effect on the short-term sampling strategies. Notably, temperature is a major abiotic factor affecting eDNA decay rates, where rates are faster in warmer waters (Lamb et al., 2022). That temperature typically had little effect on the cooler months (except for the Lee “HI” February day), where eDNA decay rates would have likely been slower, suggests that the influence of temperature on community composition during higher intensity sampling warmer periods may have been a result of increased fish activity. We point out, however, that we are using air temperature, which may not always be linearly correlated to water temperature (Harvey et al., 2011). We acknowledge that further investigation of more environmental variables (e.g., Laporte et al., 2021) would be desirable to provide a deeper understanding of changes in eDNA detectability versus true biotic change. Ultimately greater efforts into short-term monitoring are required to ensure the refinement of sampling design for spatial or longer-term studies.

### 4.4 | Detection of false positives

The detection of the common menu marine species, and several freshwater species (e.g., rainbow trout), at the freshwater Lee site are likely false positives, as they have not been recorded by the EA and, therefore are possibly detections originating from wastewater. However, at the intertidal Richmond site, rainbow trout, in which read count numbers are relatively high (Figure 2b), have been recorded at the Thames at Hampton Court (ca. 8 Km upstream) and are present at a nearby (downstream, <2km) fishery to Richmond Lock (Albury Estate Fisheries, Syon Park) (National Biodiversity Network (NBN) Atlas, <https://species.nbnatlas.org/species/NHMSYS0000544715>) and therefore their detections (Figure 2b) may represent wastewater from the fisheries and/or escapees. At this site, although common menu marine species have most likely been detected from wastewater, for several species we cannot discount their presence as they have been recorded in nearby marine waters.

### 4.5 | Is there an optimal survey window where community diversity is best represented?

Optimal sampling strategies vary according to the environment in question, and the results of this study show that consideration of temporal community change may help guide further sampling design. Conducting any biomonitoring at a discrete time of year is likely too simplistic to provide a comprehensive description of an assemblage, and in order to provide a more robust account, increased sampling frequency is required (Antognazza et al., 2021; Radinger et al., 2019). For rivers, less precipitation during the summer months may result in lower flows and less dilution by wastewater

and connections with other aquatic environments. Similar results have been documented during a study of the River Rhone (France), where periods of low flow were linked with better homogeneity in fish communities detected by eDNA (Milhau et al., 2019). As the fish communities detected by eDNA at both the Lee and Richmond sites, were significantly different across the seasons, we suggest that future biomonitoring should be carried out seasonally to gain the best representation of the fish community at a site.

## 5 | CONCLUSION

Given the rapid degradation of freshwater habitats and unprecedented rates of biodiversity change, the need for robust monitoring has never been higher and the value of temporal data is well established (Bálint et al., 2018; Magurran et al., 2010). Our study thus provides key data that demonstrate for the first time the viability of this molecular biomonitoring tool for detecting temporal community changes across a large catchment containing environments of differing water properties, since rivers can be challenging for eDNA monitoring due to downstream transport of eDNA (Deiner & Altermatt, 2014). Specifically, our study builds upon earlier temporal studies (e.g., Di Capua et al., 2021; Djurhuus et al., 2020; Sigsgaard et al., 2017; Ushio et al., 2018), by providing a robust methodology through our nested design and inclusion of environmental data, allowing us to explicitly account for some aspects of the ecology of eDNA over various time frames regarding its effect on accurately inferring the known community, although we acknowledge that variables such as water chemistry and physical properties would be needed for a more thorough assessment. Moreover, the inclusion of read count data as a proxy for abundance, allows community dynamics to be investigated beyond simply species richness.

Due to its relatively low cost, ease of collection and non-invasive attributes, temporal eDNA metabarcoding sampling allows a greater description of seasonal changes in fish or other aquatic biota. In particular, it has the power to be used to answer questions regarding rapid biological or environmental change, for example, dynamic biological events, extreme climate events, and direct anthropogenic events, such as pollution discharges. By highlighting the sensitivity of eDNA metabarcoding to probable migration and spawning events, our study further supports the continued development and expansion of this method as a valuable tool for whole ecosystem biomonitoring.

### AUTHOR CONTRIBUTIONS

All authors designed the study; Jane Hallam and Julia J. Day conducted the fieldwork. Jane Hallam undertook the laboratory work, bioinformatic and downstream analyses; All authors contributed to the writing and editing of the manuscript.

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### CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to declare.

### DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available on the UCL Data Research Repository at <https://doi.org/10.5522/04/c.6817098>.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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