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Temporal and Spatial eDNA Analysis of Fish Assemblages in Postindustrial, Urban Coastal Habitats

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ABSTRACT

Urban coastal habitats experience substantial disturbances due to their proximity to human settlements and activities. Yet, despite the negative impact of urbanization on coastal environments, industrial structures can also provide artificial habitats. These are often easily accessible to regular surveys, including water sampling for environmental DNA (eDNA) analysis, an emerging and powerful tool for monitoring biodiversity in aquatic ecosystems. In this study, we utilized eDNA metabarcoding to investigate temporal and spatial trends in fish assemblages within urban coastal habitats between the Dee and Mersey estuaries (United Kingdom), historically one of the most anthropogenically impacted postindustrial coasts in the world. Over a 12-month period, we conducted nine water sampling trips at two locations: the Albert Docks in central Liverpool, and the Marine Lake in West Kirby. Illumina sequencing was used to analyze PCR amplicons generated using the fish-targeted Tele02-12S metabarcoding region. We found significant changes in fish community composition across the different months. Fish communities also significantly differed between the two sites, with the patterns of temporal changes varying substantially between them. Seasonal appearances/disappearances of specific taxa (e.g., European eel, sand smelt, flounder, and herring) shed light on important ecological and behavioral processes that may have management implications. Results also corroborate previous findings on the importance of "molecular bycatch" (nontarget sequences) in expanding our understanding of the anthropogenic influences on the natural environment. Overall, our findings emphasize the value of eDNA monitoring as a noninvasive, affordable, and sensitive approach for routine monitoring of temporal trends in fish assemblages, facilitating the stewardship of resilient urban coastal zones, and recognizing interventions that could increase biodiversity.

1 | Introduction

The world's oceans are under unprecedented pressure from numerous climatic and anthropogenic stressors (Bijma et al. 2013), with coastal habitats especially affected (Harley et al. 2006). These highly productive environments often include elements such as (but not limited to) seagrass beds, kelp forests, and tidal flats, providing vital ecosystems for fish species at all stages in their life cycles, from spawning to nurseries and adulthood (Henseler et al. 2019). The deterioration of these habitats has led to environments that can no longer support crucial nursery, feeding, or reproductive functions which are vital for the fishery yield of species of transnational importance, as assessed by the International Council for the Exploration of the Sea (Seitz et al. 2014).

In many regions globally, human-made structures (e.g., shipping docks, seawalls, groynes, and jetties) have significantly altered natural dynamics in coastal areas (Crain et al. 2009; Bulleri and Chapman 2010; Pardal-Souza et al. 2017; Todd

et al. 2019), and the introduction of pollutants has led to imbalances in nutrient cycles, resulting in the degradation of coastal fish habitats (Brown et al. 2018). In order to monitor and mitigate these negative outcomes, the development of tools to assess coastal fish communities accurately and effectively is crucial (Hoffmann 2022). Industrial, human-made structures are also particularly suited to support these monitoring efforts as they often offer easier access to water for surveys than more remote sampling sites.

The rising popularity of environmental DNA (eDNA) as a research tool has provided invaluable ecological insights into the world's ecosystems (Thomsen et al. 2012; Valentini et al. 2016; Deiner et al. 2017). eDNA metabarcoding can reveal the trace DNA left behind by all organisms present in a given environment (Pawlowski, Apothéloz-Perret-Gentil, and Altermatt 2020), offering a nondestructive alternative to traditional survey methods that rely on the capture of organisms (Collins et al. 2022), and opening unprecedented opportunities for both routine and targeted biomonitoring (Taberlet et al. 2012). Additionally, eDNA analysis holds an advantage over less intrusive underwater visual censuses since it does not rely directly on expertise in morphological identification for precise species identification (Thomsen and Willerslev 2015; Miya 2022) and is less biased toward certain ecological traits (Aglieri et al. 2021).

Despite the rapid expansion of eDNA research and its promising advancements, there are limitations that must be considered. Although eDNA persistence in water is generally fairly well understood, more research may be needed across a broader range of environments, especially urban human-made structures, where the interplay of factors such as temperature, pH, salinity, and microbes affects DNA stability in peculiar ways (Collins et al. 2022). Additionally, eDNA cannot distinguish between live and dead organisms or different life stages, which can impact the scope of detection (Kamoroff and Goldberg 2018). Also, biases can arise during PCR amplification, potentially inflating sequencing reads for certain taxa and skewing the results (Fonseca 2018; Bessey et al. 2020). This has led to concerns about the use of sequencing reads from eDNA metabarcoding to accurately quantify species abundance. Some researchers address this by using presence/absence data to reduce bias (Compson et al. 2020), although this may overlook key ecological insights, limiting the method's full potential (Guri et al. 2024). Techniques such as data transformation and/or modeling (Shelton et al. 2023) can help mitigate biases, allowing read count data to offer valuable insights into species' functional traits, community composition, and diversity (Sard et al. 2019). Still, using read counts as abundance proxies requires caution, with appropriate acknowledgment of the biases involved (Skelton et al. 2023).

eDNA studies have consistently demonstrated the effectiveness of eDNA metabarcoding in characterizing fish diversity in marine ecosystems (Stat et al. 2017; Bessey et al. 2020), with some research showing that eDNA surveys can detect greater fish diversity than traditional methods (Thomsen et al. 2016; Port et al. 2016; Zou et al. 2020; Liu et al. 2022), thereby improving fish diversity estimates across various ecological, spatial, and temporal scales (Collins et al. 2022; Port et al. 2016; Sigsgaard et al. 2017). Furthermore, eDNA metabarcoding analysis has been shown to successfully detect a broader spectrum of functional traits within coastal fish communities (Aglieri et al. 2021), and has proven effective in detecting rare fish species that are often challenging to find using conventional sampling methods (Boussarie et al. 2018; Rees et al. 2014; Oka et al. 2021). Such versatility, universality, and effectiveness of the eDNA approach may soon turn it into a staple tool used routinely by environmental managers.

Our study investigates fish communities in two urban coastal habitats constructed in the nineteenth century: The commercial docks in the city of Liverpool and the recreational marine lake in West Kirby, Northwest England. Samples were collected multiple times per season, over a 12-month period, during which eDNA data revealed diverse assemblages and different phenological patterns of resident and migratory species. Our main hypotheses were that: (i) seasonal eDNA metabarcoding analysis would be sensitive to temporal changes in urban coastal fish assemblages; and (ii) the eDNA approach would be able to reveal spatial differences in ichthyofauna that reflect the historical and environmental features of urban infrastructures, thereby enhancing local-scale ecological heterogeneity. The findings showcase how these new, low-impact molecular technologies can inform the public and local authorities on the diversity of coastal marine organisms that thrive alongside us in urban settings, and how accessible multipurpose human-made structures may become convenient platforms for biodiversity monitoring.

2 | Methods and Materials

2.1 | Field Collection

Sampling was conducted at least twice per season (spring, summer, autumn, and winter) in two distinct locations in Northwest England, United Kingdom. The first location, the Royal Albert Dock, which was opened in 1846, has long been the center of Liverpool's maritime industries, and it remains a major tourist destination, experiencing high levels of human activity. The water in this area is enclosed within high dock walls (approximate depth: 5 m) but is connected to the estuary of the river Mersey through a sluice gate, allowing water to mix during boat entry or exit.

The second location, West Kirby Marine Lake, is a Victorian-period structure built in 1899. It has remained popular for its recreational aquatic activities such as sailing, kayaking, windsurfing, and coastal walks. Here, there is also a sluice gate, which is regulated to allow tidal water to enter the lake twice a day at high tide and be retained inside the lake when water retreats at low tide (approximate depth: 1.5 m) which determines frequent hydrological changes, resulting in a more open coastal environment.

In each location, three sampling sites were selected. For the Albert Docks, the coordinates for each site were as follows: 53.400964–2.992001, 53.400185–2.993644, and 53.401701–2.990286. For West Kirby, the coordinates for each site were as follows: 53.370971–3.189751, 53.367476–3.189022, and 53.368756–3.186189. At each sampling location, two Sterivex filters (0.45 μm, PES membrane) were collected from each of the three sites (Figure 1). A Sterile syringe was used

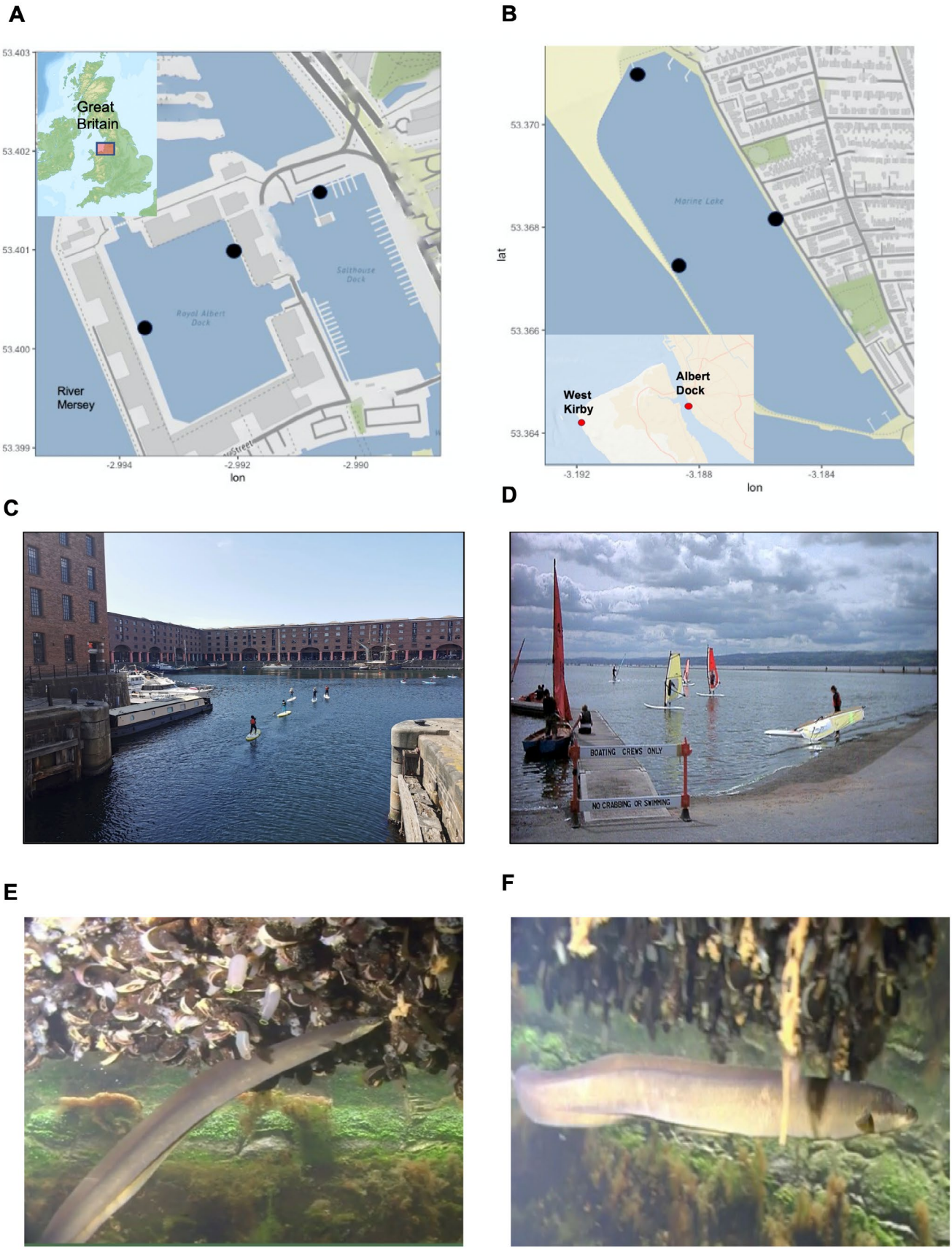

FIGURE 1 | Map showing the two sampling locations: (A) Albert Docks, Liverpool, UK, and (B) Marine Lake West Kirby, UK. The two insets illustrate the broader geographical background of the area, with the black circles representing the three different sampling sites within each location. Images (C) (Albert Docks) and (D) (West Kirby) offer a view of the two study locations. The two pictures of European eel at the bottom, E and F, are taken from BBC Springwatch (2016), which was filmed at the Albert Docks.

to pass 3 L of water through each Sterivex filter in the field, amounting to a total of 18 L of water per location, per sampling event.

Prior to sampling, all field equipment was sterilized with 10% bleach followed by 70% ethanol. Laboratory-grade gloves were worn and regularly changed after filtering each sample. To monitor contamination at each site, a field blank was taken, in which purified water was pushed through a Sterivex filter, with blanks treated identically to other samples throughout the collection and extraction process. Sterivex filters were placed inside two sterile bags and immediately stored on ice. All samples were stored at −20°C in the lab until further processing.

2.2 | DNA Extraction

All extraction procedures were carried out in a sterile, eDNA-only laboratory with stringent decontamination protocols. Only pre-PCR materials were handled in this area. Laboratory equipment was sterilized and UV-treated prior to DNA extraction. DNA extraction from Sterivex filters was based on the mu-DNA protocol by Sellers et al. (2018). Firstly, the eDNA filters were removed using pincers and the filter paper was removed using sterile dissecting scissors and forceps. Filters were cut up into small, ~20 mm^2 pieces. Half of the filter was used in DNA extraction with the other half archived at −20°C to allow for future testing if needed (Nguyen et al. 2020). 750 μL lysis solution, 250 μL lysis additive, and 20 uL proteinase K (concentration 100 μg/mL) per sample were combined in a 1.5-mL Eppendorf tube to make the lysis master mix, which was added to each sample. These were placed on a thermomixer for 12 h at 55°C at 650 rpm and then centrifuged at 10,000 x g for 1 min at room temperature. The supernatant was transferred to new tubes, 0.3x volume flocculant solution per sample was added, and then placed on ice for 10–30 min. Samples were then centrifuged at 10,000 x g for 1 min and the supernatant was transferred to 2-mL Eppendorf tubes. A 2x volume of the tissue binding buffer was added and vortexed. The sample was then transferred to a spin column and centrifuged at 10,000 x g for 1 min. This step was repeated until the entire volume had passed through the filter. 500 μL of wash solution was added to each sample and centrifuged. This was repeated twice. 100 μL of elution buffer was added directly to the spin column which was then incubated at room temperature for 5 min. The sample was then centrifuged at 10,000 x g for 1 min and the supernatant was retained.

2.3 | Target Amplification and Library Preparation

PCR amplification was conducted in triplicate in a designated post-PCR molecular lab, using the Tele02 fish targeted primers (F: 5′-AAACTCGTGCCAGCCACC-3′, R: 5′-GGGTATCTAATCCCAGTTTG-3′), which amplify a ~167 bp fragment of the 12S rRNA mitochondrial region (Taberlet et al. 2018). Positive controls were put in place for each PCR batch. We used extracts of iridescent shark catfish (*Pangasianodon hypophthalmus*) DNA at 0.05 ng/μl, choosing this organism as it is a tropical freshwater fish, and has no close relatives in UK coastal waters. Primer pairs were uniquely indexed with 8 bp tags, which were shared by both reverse and forward primers to reduce tag switching and cross-contamination during sequencing, facilitating downstream bioinformatic analysis. Each tag differed by at least three base pairs from each other and featured degenerate base Ns at the beginning of the sequence to improve clustering efficiency. The 20 μL reaction mix included: 10 μL Myfi mix (2x), molecular-grade water 5.84 μL, BSA 0.16 μL, 1 μL of each primer (10 μM), and 2 μL of eDNA template. The thermocycling profile featured: 95°C for 10 min; 40 cycles of 95°C for 30 s, 60°C for 45 s, and 72°C for 30 s; and a final extension of 72°C for 5 mins. The positive control was amplified with the same PCR conditions and was used to confirm that the PCR, sequencing, and bioinformatic steps were functioning and without contamination as expected. PCR products were run on 2% agarose gels stained with SYBR safe. PCR replicates were then pooled. Samples were purified at a 1:1 ratio with 30 μL Mag-Bind TotalPure NGS magnetic beads and 30 μL of the three pooled PCR products from each sample. The concentration of each purified PCR was quantified using a Qubit 4 Fluorometer dsDNA HS assay kit and pooled in equimolar amounts. The pooled PCR product, containing all samples at equimolar concentration, was quantified using a Tapestation 4200 using the high-sensitivity D1000 assay. Then, a further 1x bead clean-up was performed on the pooled PCR product. Illumina libraries were made using the NEXTFLEX Rapid DNA-Seq Kit 2.0 (PerkinElmer), using 1 μg as starting concentration of the pooled PCR product following the manufacturers' guidelines with library amplification. The library and 20% PhiX control were quantified by qPCR using the NEBNext Library Quant Kit for Illumina (NEB). The final library and PhiX control were diluted to 85 pM and loaded onto an Illumina iSeq 100 Reagent v2 (300-cycle).

2.4 | Bioinformatics and Downstream Analysis

The bioinformatic process was based on the OBITOOLS pipeline (Boyer et al. 2016). FASTQC was used to assess the quality scores of the fastq files. ILLUMINAPAIREDEND was then used to merge the paired-end reads and remove alignments with low (< 40)-quality scores. NGSFILTER was used to demultiplex samples. To remove sequences that were not in the target base pair range, we filtered the sequence lengths to 120–200 bp by using OBIGREP. We dereplicated the samples using OBIUNIQ. Chimeras were then removed using the uchime-denovo chimera search function in VSEARCH (Rognes et al. 2016). Molecular operational taxonomic unit (MOTU) clustering was implemented using SWARM with "-d 3" (Cai et al. 2022; Mahé et al. 2014; Maiello et al. 2024). Taxonomic assignment per sample was carried out using ECOTAG with a 12S reference database which was constructed using "ecoPCR" in silico against the EMBL database (Release version r143 October 2023). The taxonomic assignments included seven levels of biological classification along with a percentage indicating the likelihood of accurate classification. After taxonomic assignment, nonnative and unexpected taxa were cross-checked by manual BLAST against the NCBI nucleotide database (https://www.ncbi.nlm.nih.gov/nucleotide). MOTUs that could not be assigned to genus or species level were also cross-checked manually against the NCBI nucleotide database.

All downstream analyses were conducted using R version 4.2.2. The package DECONTAM in R was used on the raw MOTU

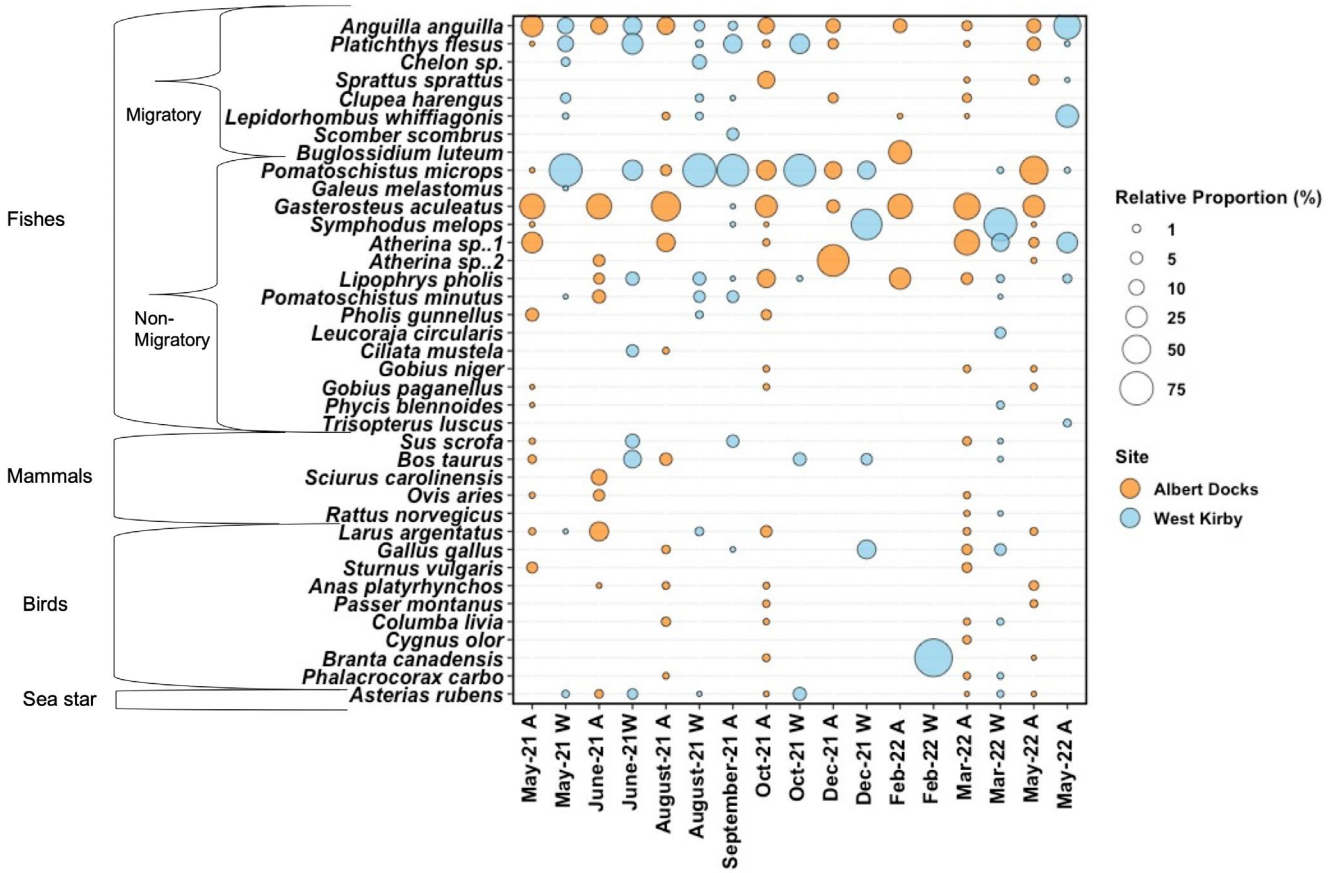

FIGURE 2 | Proportional read counts of species detected at the Albert Docks and West Kirby throughout the different sampling seasons starting from Spring 2021 until Spring 2022. The size of the bubbles indicates the proportional read counts that species represented in a sample. Fish species are grouped by migratory or nonmigratory.

output (Davis et al. 2018). MOTUs were filtered by retaining assignments with > 97% identity match for biological classification. Alpha-diversity indices (richness and Shannon) were calculated using the PHYLOSEQ and VEGAN package (McMurdie and Holmes 2013; Oksanen 2013). ANOVA was used to test for significant differences among seasons and locations. Prior to downstream analysis, the data were transformed using the Hellinger approach, which converts species abundances from absolute to relative values followed by square rooting for standardization.

To visualize spatial (Albert Docks vs. West Kirby) and temporal (seasons) differences among eDNA samples, we used nonmetric multidimensional scaling (NMDS) based on both Bray–Curtis and Jaccard distances. We tested differences between locations and seasons using permutational multivariate analysis of variance (PERMANOVA, 999 permutations) on pairwise distance matrices using the function *adonis* in VEGAN. Finally, to identify whether there were any species significantly associated with certain months, we used an indicator species analysis in R using the *multipatt* function in the INDISPECIES package (De Caceres et al. 2016).

3 | Results

Samples were sequenced across one Illumina iSeq100 run alongside an unrelated eDNA project. The samples from this study made up 89% of the overall sequencing run. There were 134 samples altogether: 108 eDNA filters, 18 field blank controls, three extraction blanks, three PCR blanks, and two positive controls which yielded 1,579,456 raw sequencing reads, with a mean of 14,625 reads per sample. To minimize false positives and contamination, low abundance OTUs (< 5 reads) were removed from downstream analysis.

All human reads were also removed (which made up 66% of the total raw reads). Across the negative controls, there were just < 100 human reads. Six samples from the Albert Docks (all from the sampling trip in September 2021) contained no sequencing reads and were removed. After the removal of the above reads and quality filtering, 533,342 reads were retained for downstream analysis across 102 samples.

From the Albert Docks, we recovered 36 taxa altogether: 20 fishes, 9 birds, 5 mammals, and 1 sea star. From West Kirby, we recovered 24 taxa altogether: 17 fishes, 4 birds, 2 mammals, and 1 sea star (Figure 2). Around 1% of all filtered reads belonged to four taxa that are typical of more offshore environments: *Galeus melastomus* (102 reads), *Leucoraja circularis* (1580 reads), *Lepidorhombus whiffiagonis* (3402 reads), and *Phycis blennoides* (401 reads), and these were omitted from further analyses (see Section 4).

In the Albert Docks, the three-spined stickleback *Gasterosteus aculeatus* and European eel *Anguilla anguilla* were detected throughout

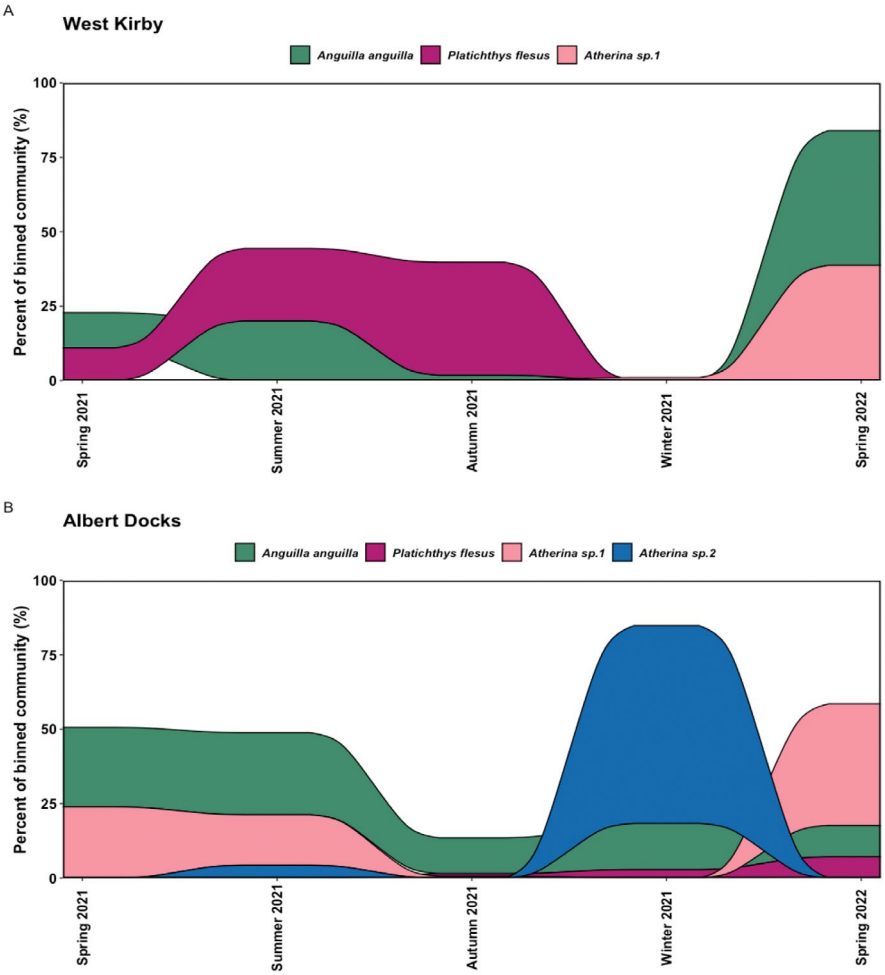

FIGURE 3 | Percentage of the binned community, representing the proportion of taxa within the total community based on proportional Hellinger transformed read counts, for three dominant species: *Anguilla anguilla*, *Platichthys flesus,* and *Atherina* sp. 1. (A): West Kirby and (B): Albert Docks. For the Albert Docks only, *Atherina* sp. 2 is also displayed. The figure shows the change from Spring 2021 to Spring 2022.

all seasons. In West Kirby, the assemblage was dominated by the common goby *Pomatoschistus microps*, which amounted to 72.1%, 68%, and 66.1% of the proportional read counts in Spring 2021, Summer 2021, and Autumn 2021, respectively (Figure 2). We identified high abundances of the common goby in both locations. In the Albert Docks, its dominance is diluted by a greater diversity of other species throughout the year, whereas in West Kirby, it has highest abundance throughout the warmer months (Figure 2). Two species of sand smelt (*Atherina* sp.) were detected across locations and in most seasons and are likely to represent *A. presbyter* and *A. boyeri*, but due to uncertainties around their taxonomy and the reference sequence data, they were precautionarily recorded as "sp. 1" and "sp. 2" (see Section 4). As *Chelon labrosus* and *Chelon ramada* share an identical 12S reference, making it impossible to differentiate between the two, we have documented this as "*Chelon* sp." The critically endangered European eel was identified in both locations and across all seasons in the Albert Docks while absent in winter in West Kirby (Figure 3).

In both locations, diversity drops pronouncedly during the coldest months (Figure 4) (Shannon index, ANOVA $F = 14.22$; $p < 0.001$). The diversity is consistently higher in the Albert Docks throughout all seasons (Figure 5) (Shannon index, ANOVA $F = 5.352$; $p < 0.001$).

There was a significant difference in community composition detected between the two sites (PERMANOVA $F = 6.3667$ $p = 0.002$) (Figure 6). Indicator species analysis showed that two fish species were statistically more abundant in Albert Docks: three-spined stickleback ($p = 0.0123$) and the *Atherina* sp. 1 ($p = 0.0314$).

When fish community structure is compared across the various seasons, we found significant temporal (seasonal) differences (PERMANOVA $F = 1.7082$; $p = 0.037$). The interaction between season and location is also significant (PERMANOVA $F = 4.5276$; $p = 0.002$) (Figure 6).

4 | Discussion

The importance of sampling in coastal urban habitats is increasingly apparent, particularly due to the escalating pressures of pollutants, climate shifts, and infrastructure construction. Coastal marine fish populations are in decline (Strain et al. 2018; Cowley, Tweedley, and Whitfield 2022), a phenomenon aggravated by human-made developments and commercial activities, which drive ecological changes across a variety of habitats (Bulleri and Chapman 2010). Artificial ocean sprawl,

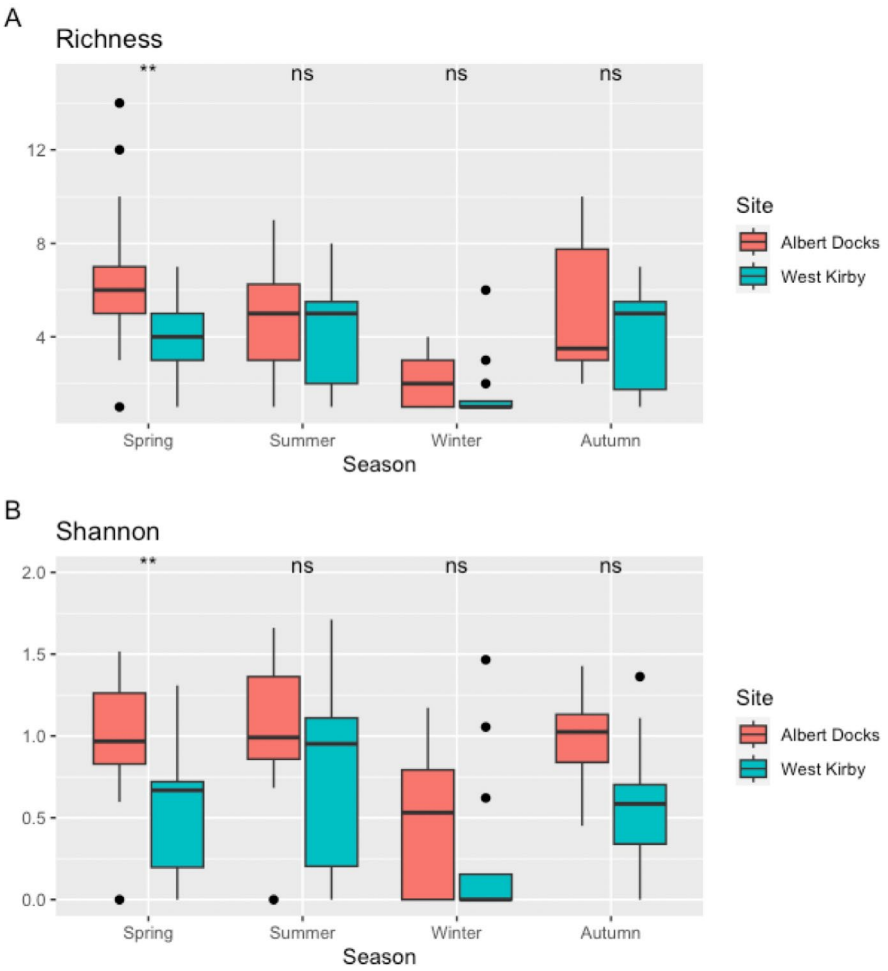

FIGURE 4 | (A) Species richness and (B) Shannon index alpha diversity between Albert Docks and West Kirby locations for each season. The plots include error bars indicating the variability or dispersion of the data points for each season measured. Statistical results were calculated with ANOVA ($p > 0.5 = \text{ns}$; $p < 0.05$**).

such as in West Kirby and Liverpool, is commonplace in estuaries and coastal waters worldwide (Kennish 2002, Kennish 2021; Kelly and Bliven 2003) and is essential for various industrial and recreational activities; with shipping docks often associated with adverse environmental impacts, (i.e., pollution and invasive species; Todd et al. 2019). Nevertheless, these areas can also play a role in providing habitats and supporting aquatic communities through alterations in environmental conditions (Logan et al. 2022). Given the complex dynamics of urban coastal ecosystems, it is particularly important to advance the development of efficient, scalable, and reproducible methods for detecting and monitoring fish communities.

By using eDNA analysis, 37 distinct taxa were identified across the two locations. The observed fish species were consistent with the anticipated composition for these environments (Baldock and Dipper 2023), although there is evidence suggesting that fish assemblages associated with artificial structures differ from those that inhabit more natural coastal environments (Clynick, Chapman, and Underwood 2008). This divergence can lead to variations in the populations that establish themselves in these areas. Indeed, the two coastal urban infrastructures investigated exhibited significant spatial disparities in their fish communities. Despite the similarity in location, the nature of the infrastructures themselves may account for the difference in the communities that inhabit these sites.

The Albert Docks exhibited a consistently higher species richness and diversity overall, a difference that was maintained across all seasons. The consistently greater species diversity suggests that this habitat is more complex and ecologically stable compared to West Kirby. In the winter season (December 2021 to February 2022), the Albert Docks contained five migratory fish species (*Anguilla anguilla*, *Platichthys flesus*, *Clupea harengus*, *Lepidorhombus whiffiagonis*, and *Buglossidium luteum*) and four nonmigratory fishes (*Pomatoschistus microps*, *Gasterosteus aculeatus*, *Atherina* sp. 2, and *Lipophrys pholis*). In contrast, West Kirby during the same winter period, only had two nonmigratory fish species detected: *Pomatoschistus microps* and *Symphodus melops*. Although species richness declined at both locations during winter, the docks still maintained greater diversity, with increases in spring, summer, and autumn following typical seasonal patterns for cold-temperate coastal habitats, where diversity peaks in late summer (Jovanovic et al. 2007). These shifts are largely driven by migration and species' reproductive cycles (Connor et al. 2019). However, the presence of unexpected migratory species during winter in the docks may be

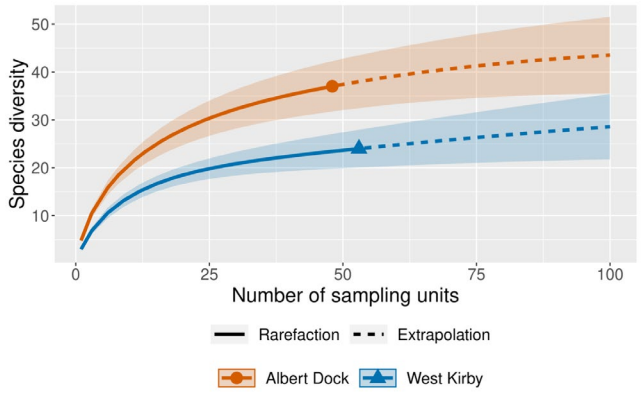

FIGURE 5 | Rarefaction curve between Albert Dock and West Kirby. The dashed line represents the extrapolated estimate calculated with R package iNEXT (Hsieh et al. 2016) and the shaded section shows the 95% confidence interval for the estimation.

linked to the infrastructure, which could be trapping fish and altering their ecological functions.

We found significant distinctions in fish community composition across the seasons, observing seasonal appearances and disappearances of specific taxa. The European flounder *Platichthys flesus* exemplifies this, migrating to warmer waters in winter, before spawning in spring. During summer, shallow coastal waters and estuaries become feeding grounds for its larvae, juveniles, and adults (Orio et al. 2017). In West Kirby, we observed increased abundances of *P. flesus* in summer and autumn of 2021, aligning with its expected behavior. We also detected *P. flesus* in all months apart from winter which is again, consistent with its expected migratory behavior. However, in the Albert Docks, *P. flesus* was detected even in winter, indicating that the depth of the docks allows these fish to maintain a local population all year round. A similar pattern is also observed

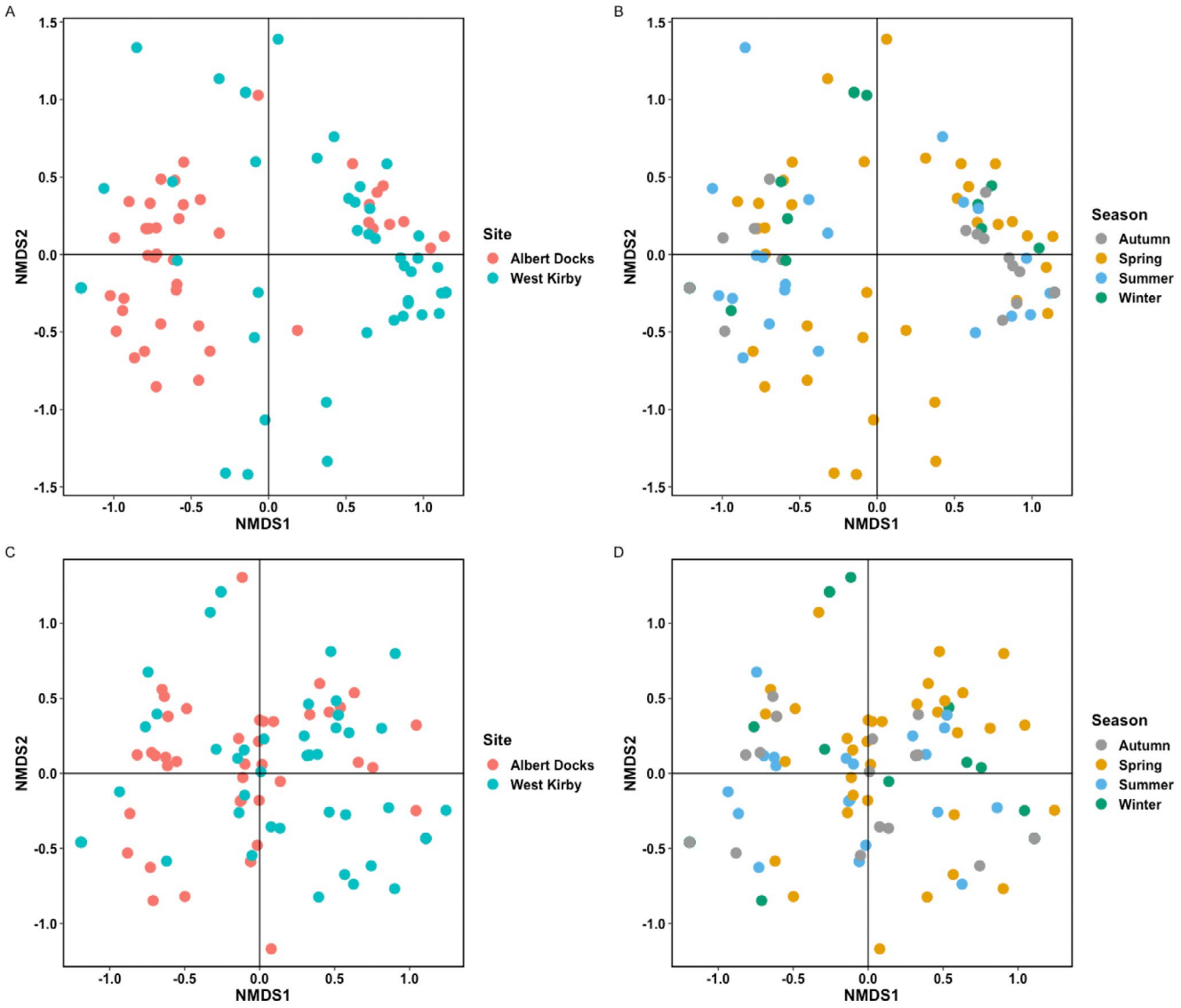

FIGURE 6 | A nonmetric multidimensional scaling (NMDS) analysis was performed using Bray–Curtis dissimilarity (A and B; stress = 0.0993) and Jaccard dissimilarity (C and D; stress = 0.1516). The plots show all eDNA samples collected from Albert Docks and West Kirby and annotated by season. (A) Colors depict the two different sites. (B) Colors depict the four different seasons.

for Atlantic herring *Clupea harengus*, an emblematic pelagic species that has been exploited by European commercial fishers for centuries. Spawning occurs for approximately 4 weeks in autumn within the Irish Sea (Dickey-Collas et al. 2010), but pockets of winter-spawning contingents are also known (Burke et al. 2009). While we observed *C. harengus* in both locations during spring and autumn, winter detections were exclusive to the Albert Docks. These findings shed light on the impact of urban infrastructure on migratory patterns, and their role in detecting variations in the ecological and behavioral patterns of fish communities inhabiting the adjacent shelf seas.

Two distinct species of *Atherina* sand smelt were identified at the two study locations. There are two species of *Atherina* that are known to be present in the United Kingdom: Sand smelt *Atherina presbyter* and big-scale sand smelt *Atherina boyeri*. *Atherina presbyter* is a common UK coastal species, known for its abundance in estuarine habitats. By contrast, *A. boyeri* is believed to be only present in specific UK locations, primarily in more southern regions (Bowers and Naylor 1964). Populations of *A. boyeri* found further north in the United Kingdom are typically situated in urban coastal areas. In this study, we detected two distinct sand smelt species identified through two different DNA reference sequences in the NCBI GenBank database, indicating their existence in urban coastal areas within the Irish Sea. However, due to historical taxonomic misidentification and ambiguities (Ardura 2019) which are also represented in DNA reference databases such as NCBI, we are not able to determine at this point which of the two divergent sequences identified in this study belongs to *A. presbyter* or *A. boyeri*. It is, however, noteworthy that we observe that *Atherina* sp. 2 is only detected in the Albert Docks which suggests potential differences in ecological preferences between *A.* sp. 1 and *A.* sp. 2. Given that *A.* sp. 2 is only observed in the Albert Dock, it raises the possibility that human-made coastal habitats can serve as artificial havens for ecologically significant traits. The occurrence and/or abundance ratio between these species could also plausibly reflect trends in rising water temperatures (given *A. boyeri*'s preference for warmer water). Similar patterns are being documented in commercial fish species, where warm-water species appear in UK waters alongside a decline in cold-associated species (Simpson, Blanchard, and Genner 2013; Townhill et al. 2023). Although further research will be required to comprehensively understand distribution and adaptation of sand smelts, it is possible that these and other species may in the future contribute to devising new tools for environmental monitoring. Notwithstanding the problems with reference sequence data, eDNA analysis could be particularly well suited to monitoring the distribution of sand smelts due to the difficulty identifying them to species level in the field.

Even though urban infrastructures are often associated with having negative impacts on the environment, our results reveal their potential significance as crucial refuges for species of conservation importance. Long-term surveys indicate that mussel biomass and consequent biofiltration rates in the Albert Docks are essential for maintaining water quality, offering habitat for other species, supporting relatively stable ecosystems, and constituting vital refuge for fish (Firth et al. 2024). The European eel was found to be abundant in both locations. Notably, in the Albert Docks, eels were detected in every month sampled, while in the marine lake, there were no detections in Winter 2021. The eels in these habitats have been featured on BBC documentaries (i.e., Springwatch 2016), reinforcing that the species has been finding refuge here for several years. These findings demonstrate the presence of European eels in both areas, which is particularly noteworthy. Although it is not unusual to observe European eels in the United Kingdom, it is, however, valuable that they have been detected, given the population decline which has contributed to its critically endangered status as listed on the IUCN Red List of threatened species (ICUN 2024). There has been a recent push to add eDNA analysis among the methods to assess its occurrence and abundance in UK habitats reaching beyond an academic setting (Hillsdon 2023; Horston 2023; Rodriguez 2023). The substantial presence of this species in both locations is both encouraging and concerning (especially the detection of eels all year round in the Albert Docks), and the insights gained from this assessment may play a pivotal role in shaping the future management of these urban areas. While the existence of an important refuge for such an iconic endangered species in the middle of a bustling city is inspiring, the design and functioning of the Albert Docks raises the possibility that these catadromous animals may become trapped within the structures of the docks during the time of their long reproductive journey (Verhelst et al. 2018). On the other hand, West Kirby's marine lake exhibits a fluctuation in presence and absence, which might be attributed to its location as a more exposed coastal habitat, enabling the ongoing use of migration routes. The presence of the European eel in these urban coastal areas carries significance for their conservation and underscores the need for assessing and monitoring the ecological and behavioral impacts of urban structures within these environments.

The ability to identify temporal changes in fish community compositions can play a crucial role in developing a better understanding of complex community dynamics. This study highlights the effectiveness of eDNA metabarcoding in detecting and shedding light on these temporal trends, as demonstrated by the observed patterns in flounder and eel populations. Statistical analysis using PERMANOVA shows the presence of significant differences in fish community structure across months and seasons in both sampling locations. Various factors influence the abundance of eDNA. For example, marine organisms that undergo spawning events are expected to shed increased quantities of DNA, through the release of gametes (Collins et al. 2022). Studies have demonstrated the composition and structure of fish communities exhibit seasonal differences in estuarine or coastal ecosystems due to the yearly variations in environmental conditions, spawning, migration, and fishing activities (Hallam et al. 2021; Li et al. 2022; Zamani, Zuhdi, and Madduppa 2022; Jiang et al. 2023; Gibson et al. 2024).

Among our detections, there were some unexpected deeper-sea species: Blackmouth catshark (*Galeus melastomus*), sand ray (*Leucoraja circularis*), megrim (*Lepidorhombus whiffiagonis*), and greater forkbeard (*Phycis blennoides*). These species are not expected in our inshore coastal urban study area and typically inhabit more offshore environments (Froese and Pauly 2000). The sensitivity of eDNA methods is one of its main advantages in being able to detect rare species at low abundances, but with this comes the increased risk of making spurious detections

which are difficult to verify. There are several routes that allochthonous DNA can enter into the sampling and analysis steps. Firstly, field contamination can distort results from the transfer of eDNA between sites via sampling gear or clothing. Laboratory contamination from amplicons generated during previous work can frequently be observed in eDNA studies, despite rigorous decontamination controls being employed. Here, this prior knowledge allowed us to flag *Galeus melastomus* reads as likely coming from a previous project. Another consideration is taxonomic assignment artifacts and reference database deficiencies (Ardura 2019; Jackman et al. 2021), which may lead to misidentifications. Despite global initiatives such as the International Barcode of Life (Weigand et al. 2019), reference databases remain incomplete (Schenekar et al. 2020). Biological explanations are also possible. One factor could be tidal transport, where eDNA from adjacent areas is carried to coastal environments. While there is evidence supporting the influence of tides on eDNA transportation (Jeunen et al. 2019), conflicting studies suggest minimal effects (Larson et al. 2022). This may also especially be the case during reproductive activity, where eDNA quantities are elevated due to spawning (Collins et al. 2022). Nonlocal eDNA may also be brought in via ballast water locally discharged from shipping (Feist and Lance 2021). Future eDNA studies must address these issues and develop new strategies to improve end-user confidence in the results.

Non-target, non-fish species were found in both sampling locations. These "bonus" species consist of several mammals, birds, and even a sea star. A range of different bird species were detected including the cormorant *Phalacrocorax carbo*, a piscivorous bird common in the Mersey and Dee estuaries, as well as other well-known urban species, such as Canada goose *Branta canadensis* and common starling *Sturnus vulgaris*. Mariani et al. (2021) highlight the importance of this molecular by-catch of "bonus" species, showing that eDNA metabarcoding using 12S Tele02 marker is not limited to detecting fish species alone. As the primers used primarily target teleosts, the presence of these "bonus" species is likely underestimated, indicating the need for specific primers if future studies aim to focus on these additional taxa. The detection of terrestrial mammals such as the Eastern gray squirrel *Sciurus carolinensis* and brown rat *Rattus norvegicus*, as well as the four domestic species, namely, pig *Sus scrofa*, cow *Bos taurus*, sheep *Ovis aries*, and chicken *Gallus gallus*—all most likely associated with the human food chain—completes the picture of a typical urban water body. As part of this molecular by-catch, 66% of the total reads were human. Through extensive checks across the control samples, which contained very minimal reads of humans, we conclude that this human DNA was derived from the environment itself. Both sampling locations experience high levels of human activity both within and outside of the water, and we surmise that this is the most likely explanation for these results. Amplification of human sequences by eDNA metabarcoding, especially using the 12S region, is consistently observed (Kelly et al. 2014; Miya et al. 2015; Ragot and Villemur 2022; Zhang, Zhao, and Yao 2020). Although this is potentially a limitation of eDNA metabarcoding in urban coastal environments at present, further advancements in this field—for example, redesigning metabarcoding primers, using primers that do not amplify humans, using human DNA blocking primers, or increasing sequencing depth—could counteract this issue (Zhang, Zhao, and Yao 2020). Given global urbanization trends, it is crucial that we continue sampling in urbanized coastal areas, but researchers should take off-target amplification into account and identify preemptive mitigations for future studies.

5 | Conclusion

Our findings underscore the potential of novel, low-impact molecular technologies to provide valuable insights into the diversity of coastal marine organisms that live alongside us in urban settings, bringing to the forefront the importance of urban infrastructures as multipurpose study subjects and monitoring platforms. Most of the docks, piers, and sea walls in our coastal cities tend to be overlooked as areas of ecological importance but are in fact diverse environments that can serve as a refuge for a plethora of fish species, and they provide easy access for noninvasive sampling methods, such as aqueous eDNA.

Through eDNA metabarcoding, we have compiled a comprehensive list of teleost species present in both the Liverpool Albert Docks and West Kirby's marine lake, which include species of conservation importance. We have unveiled significant changes in fish community composition between habitats and across different seasons, which point to the important source of habitat heterogeneity that is maintained by these human-made structures.

Overall, eDNA metabarcoding in urban habitats offers a promising and versatile approach for evaluating biodiversity in postindustrial coastal environments. Additionally, our results provide valuable insights into species dynamics and seasonal fluctuations that can be used to inform the public and guide local authorities in future assessments and monitoring efforts within these diverse and resilient coastal refuges.

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Ethics Statement

Approval was obtained for the relevant ethics from Liverpool John Moores University.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data and metadata are available at: https://zenodo.org/records/14424384.

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