Received: 7 February 2025 | Revised: 17 July 2025 | Accepted: 25 July 2025

DOI: 10.1111/jfb.70172

REGULAR ARTICLE

JOURNAL OF FISH BIOLOGY fsbi

Quantitative insights into the spatio-temporal variation of Atlantic salmon (*Salmo salar*) biomass in a river catchment using eDNA metabarcoding

William Bernard Perry[1,2] | Nigel Milner[2,3] | Ifan Bryn Jâms[1] | Mark de Bruyn[4] | Steve J. Ormerod[1] | Kristy Deiner[5] | Holly M. Bik[6] | Gary Carvalho[2] | John Colbourne[7] | Bernard Jack Cosby[8] | Isabelle Durance[1] | Simon Creer[2]

[1]Water Research Institute, Cardiff University, Cardiff, UK

[2]Molecular Ecology and Evolution at Bangor, School of Environmental and Natural Sciences, Bangor University, Bangor, UK

[3]APEM Ltd, Stockport, UK

[4]School of Environment and Science, Griffith University, Brisbane, Queensland, Australia

[5]Department of Environmental Systems Science, ETH Zurich, Zürich, Switzerland

[6]Department of Marine Sciences and Institute of Bioinformatics, University of Georgia, Athens, Georgia, USA

[7]Centre for Environmental Research and Justice, School of Biosciences, University of Birmingham, Birmingham, UK

[8]Centre for Ecology & Hydrology, Environment Centre Wales, Bangor, UK

Correspondence
William Bernard Perry, Water Research Institute, Cardiff University, Cardiff, CF10 3AX, UK.
Email: perryw1@cardiff.ac.uk

Nigel Milner, Molecular Ecology and Evolution at Bangor, School of Environmental and Natural Sciences, Bangor University, Bangor, UK.
Email: n.milner@apemltd.co.uk

Simon Creer, Molecular Ecology and Evolution at Bangor, School of Environmental and Natural Sciences, Bangor University, Bangor, LL57 2UW, UK.
Email: s.creer@bangor.ac.uk

Funding information
Natural Environment Research Council, Grant/Award Number: NE/N006216/1

Abstract

Effective species conservation and management requires comprehensive biomonitoring, enhanced by combining traditional and newer methodologies, such as environmental DNA (eDNA) analyses. A seasonal pulse of spawning adult Atlantic salmon (*Salmo salar*) was detected by normalised eDNA 12S reads from metabarcoding, which facilitated estimation of spatial patterns in salmon biomass. A strong relationship was found between normalised reads in the lower section of the River Conwy (Wales, UK) and whole-river adult biomass (estimated from rod catch data and a fish counter), explaining 61% of the variation in a linear regression. Moreover, the positive linear relationship between adult biomass and partial effect on normalised reads occurred after the biomass estimate exceeded 1500 kg, indicating a threshold where normalised reads become representative of biomass. The relationship observed between normalised reads and biomass, as well as the unique profiles of normalised reads at each of the sites, supports the hypothesis of limited eDNA transport among sampling sites that were 2–4 km apart. River pH showed a significant non-linear relationship with normalised reads, with a peak in partial effect on normalised reads at pH 6.5. Partial effect on normalised reads also showed a positive linear relationship with flow (discharge), while also peaking at the highest average monthly air temperatures (14°C). These trends are contrary to what would be expected from eDNA decay, dilution or transport, demonstrating that metabarcoding is robust to such

influences, reinforcing the interpretation of trends driven by Atlantic salmon ecology and physiology. For example, pH effects reflect beneficial conditions for eggs and perhaps habitat preference for spawners, flow effects reflect the annual return of salmon during higher flows which aid upstream migration, tributary entry and spawning, and, finally, temperature effects reflect higher metabolic rates and greater shedding of eDNA.

KEYWORDS
flow, freshwater, lotic, pH, river Conwy, temperature, waterfall

1 | INTRODUCTION

Analysing environmental DNA (eDNA) to monitor communities of fish and other organisms is now well established (Keck et al., 2022). The ability to effectively detect eDNA and subsequently monitor fish presence in rivers while being non-destructive, easy to sample and suitable for automation is a key improvement for global riverine bioassessment (Altermatt et al., 2020; David et al., 2021). This is particularly important given the urgent need for more data on riverine biodiversity to counter the many anthropogenic pressures eroding freshwater, and especially fish, diversity (Sayer et al., 2025; Su et al., 2021). The quantification of fish diversity and how populations vary spatio-temporally is essential for river ecosystem and fisheries management (Best, 2018).

An approach using eDNA that is often implemented to investigate single species is quantitative PCR (qPCR), which has demonstrated strong quantitative relationships with non-molecular fish-surveying techniques in freshwater environments, especially for salmonids (Berger et al., 2024; Levi et al., 2019; Su et al., 2024; Tillotson et al., 2018), making qPCR a valuable tool for predicting salmon abundance. However, due to the targeted, low-throughput nature of qPCR assays, surveying multi-species communities can be challenging. When surveying broader communities, metabarcoding is often preferred, which utilises universal primers that amplify fragments of eDNA from an array of taxa for DNA sequencing. For fish, targeting the mitochondrial 12S gene has proven particularly effective and is able to detect a diverse range of fish species (Collins et al., 2021; Gibson et al., 2023; Hallam et al., 2021). However, analyses often focus on community-level metrics rather than single species. Moreover, the relative number of sequencing reads assigned to species in these datasets is often criticised as being only semi-quantitative due to PCR bias in the library preparation process (Lamb et al., 2019). Therefore, if representative information on the real-world abundance of individual species can be extracted from metabarcoding datasets, in addition to broad species detection (Goutte et al., 2020), then such data mining could facilitate informed conservation strategies for key species of interest, further endorsing the use of eDNA data in fish-based ecological assessments (Pont et al., 2021).

This study explores the application of eDNA metabarcoding to monitor Atlantic salmon (*Salmo salar*), a species of major cultural and economic importance (Andersen & Dervo, 2019) that has seen considerable decline in recent decades around most of the North Atlantic due to a combination of factors at sea and in freshwater (Olmos et al., 2019). Recently, in line with global trends (Dadswell et al., 2022), the Atlantic salmon population size in Wales (UK) has reached record low levels (Milner & de Leaniz, 2023) and its IUCN classification has been downgraded from 'Least Concern' to 'Near Threatened' at a global scale, and 'Endangered' in Great Britain (IUCN, 2023).

The River Conwy in North Wales is an iconic salmon river in the UK, one of 64 designated as Principal Salmon Rivers (Cefas/EA/NRW, 2023a). The Conwy has a catchment area of 678 km^2, a main channel length of 55 km and drains upland peat moorland, flowing through a rural landscape with a mixture of sheep and cattle farming and mixed woodland (https://catalogue.ceh.ac.uk/). Despite the installation of a fishpass, which opened an additional 40% of nursery area upstream of Conwy Falls in 1993 (Milner, 1985) to give a total of 63Ha of wetted area (Cefas/EA/NRW, 2023a), annual salmon egg deposition has declined (Natural Resources Wales, 2021) from approximately 4.91 million eggs in 1990 to 0.38 million in 2021 (Cefas/EA/NRW, 2023a), therefore, conservation and restoration are urgently required, along with information on spatial and temporal distribution within this catchment. Data can be obtained from juvenile electrofishing surveys (Figure S1), but the necessary spatial granularity is resource intensive and constrained to streams of a certain size, consequently making it challenging to quantify spatial patterns. In most rivers, adult salmon runs are sampled by some combination of angling rod catches and fixed counters (Cefas/EA/NRW, 2023b). Catch data are subject to bias due to reporting errors and assumptions about exploitation rate and fishing effort (Milner et al., 2001), although such errors can be partially accounted for by calibrating against rivers with independent run estimates from traps and counters (Gregory et al., 2023). Generally, catches are reported as a seasonally stratified whole-river estimate of the annual run for the whole catchment. Total run assessment, while adequate for river-scale management, is often insufficient for understanding perturbations in freshwater environments, which occur at finer spatial scales.

Atlantic salmon are anadromous, resulting in two main riverine components of salmon biomass: juveniles, present all year in overlapping cohorts, and adults returning between spring and autumn. The annual return migration of adults is thus anticipated to cause a seasonal eDNA signal on top of juvenile biomass. This signal, which

metabarcoding seeks to detect, has a predictable spatio-temporal pattern. The dominant recurring annual pattern of adult presence is primarily driven by the inherited seasonal timing of sea return leading to a predictable monthly pattern of rod catch and an accumulation of adults throughout the accessible main stem and larger tributaries prior to final maturation and spawning. Over this inherent pattern lies annual variation that may be river- or site-dependent according to river structure and stochastic variation in flow and temperature, among other factors. Adult salmon migration in spate rivers like the Conwy typically follow a three-phase pattern of (i) river entry, beginning in spring, (ii) gradual upstream passage in the main stem and major tributaries interspersed with long holding periods for most of the summer, and (iii) a final spawning migration in autumn to spawning sites (Milner et al., 2012; Thorstad et al., 2008). During phase (ii), small summer spates induce further upstream movement, but many fish may not move for long periods in deep holding pools or downstream of partial barriers, particularly if passage is restricted by low summer river discharge. The final spawning migration in October and November is typically initiated by high flows, such that abundance in small tributaries and the uppermost river sections peaks shortly before spawning. This overall process can be envisaged as an annual pulse of adult biomass entering the river, dispersing and diffusing upstream, with the timing of peak abundance crucially varying according to location within the catchment, and the influence of natural holding areas and partial barriers.

The use of eDNA to monitor salmonid ecology spatiotemporally has been well studied in Pacific salmon (Levi et al., 2019; Shelton et al., 2019; Su et al., 2024; Tillotson et al., 2018), but less so for their Atlantic counterpart (Berger et al., 2024). Using a state-of-the-art, exemplar eDNA metabarcoding dataset constructed from an intensive yearlong sampling regime in the River Conwy, Wales, UK (Perry et al., 2024), we examined (i) the quantitative associations between seasonal eDNA signals and estimates of biomass of adult and juvenile Atlantic salmon, (ii) the ability of eDNA to describe spatial patterns in biomass, inferred from migration ecology and locations of principal holding and rearing areas, and (iii) the significance of environmental conditions on the eDNA signal. Finally, we comment on the complementarity of metabarcoding with conventional biomonitoring techniques.

2 | METHODS

2.1 | Ethics statement

An ethical statement relating to animal welfare is not applicable in this instance because there were no animals sampled directly in this study.

2.2 | eDNA metabarcoding

The sequencing data used here was generated in a previous study; the data are publicly available from the European Nucleotide Archive (accession PRJEB48362). Detailed methodology on sample collection, metabarcoding library preparation and bioinformatics can be found in Perry et al. (2024). Briefly, water samples were taken longitudinally along the River Conwy, Wales, UK, at 14 sites (Figure 1). Each site was sampled 19 times over a 356-day period starting on 27 April 2017 and ending on 18 April 2018. In an Atlantic salmon context, the river can be split into three zones: the upper river (sites E07-E09), Conwy Falls (site E10) and the lower river (sites E11-E14). Three water samples were taken at each sample site for each of the timepoints, and eDNA was captured using 0.22 μm Sterivex filters. The DNA was then extracted from the filters and purified using a modified Qiagen DNA blood and tissue extraction protocol (Spens et al., 2017). Metabarcoding libraries were based on 12S MiFish-U forward (5′-GTCGGTAAAACTCGTGCCAGC-3) and reverse (5′-CATAGTGGGGT ATCTAATCCCAGTTTG-3) primers (Miya et al., 2015) and sequenced on an Illumina HiSeq. To assign taxonomy to amplicon sequence variants (ASVs), the DNA sequences were scored based on their alignment to the MitoFish database entries using BLAST (Sato et al., 2018), requiring a >90% percentage identity, >90% query cover and an *e* value of <0.001. ASVs were then clustered based on taxonomic assignment. If a species had less than 0.05% of the sample reads, it was removed from the analysis. Additionally, if an ASV had fewer than 20 reads, it was also removed, as were samples containing fewer than 1000 reads. The three replicate water samples for each sample site and time point were then aggregated. Atlantic salmon reads were divided by sum sample reads (post filtering) to generate normalised salmon read depth, referred to hereon in as normalised reads. Therefore, normalised reads refer to the number of reads for Atlantic salmon relative to the total number of fish reads in a sample.

A total of 66 negative controls were taken in the field using deionised water. These negative controls were processed in the same way as samples. Also processed and sequenced were 68 negative controls introduced during the laboratory processing stage. For all samples, pre-PCR steps were performed in a PCR-free, eDNA clean room, in a separate building to where PCRs were undertaken. Access to the clean room was restricted to trained users who wore PCR-free overcoats, hair nets, shoes, gloves and masks, and the room was regularly cleaned with bleach.

2.3 | Salmon biomass

The biomass estimates comprised two main components, (1) juvenile and (2) adult (products derived from the annual run), which were also summed to give (3) a combined biomass value. These three variables were used to test for relationships between biomass and normalised reads.

2.3.1 | Juvenile biomass estimation

Terminology follows Allan and Ritter (1977). Juvenile biomass comprised newly recruited 0+ parr (young of the year of the current year

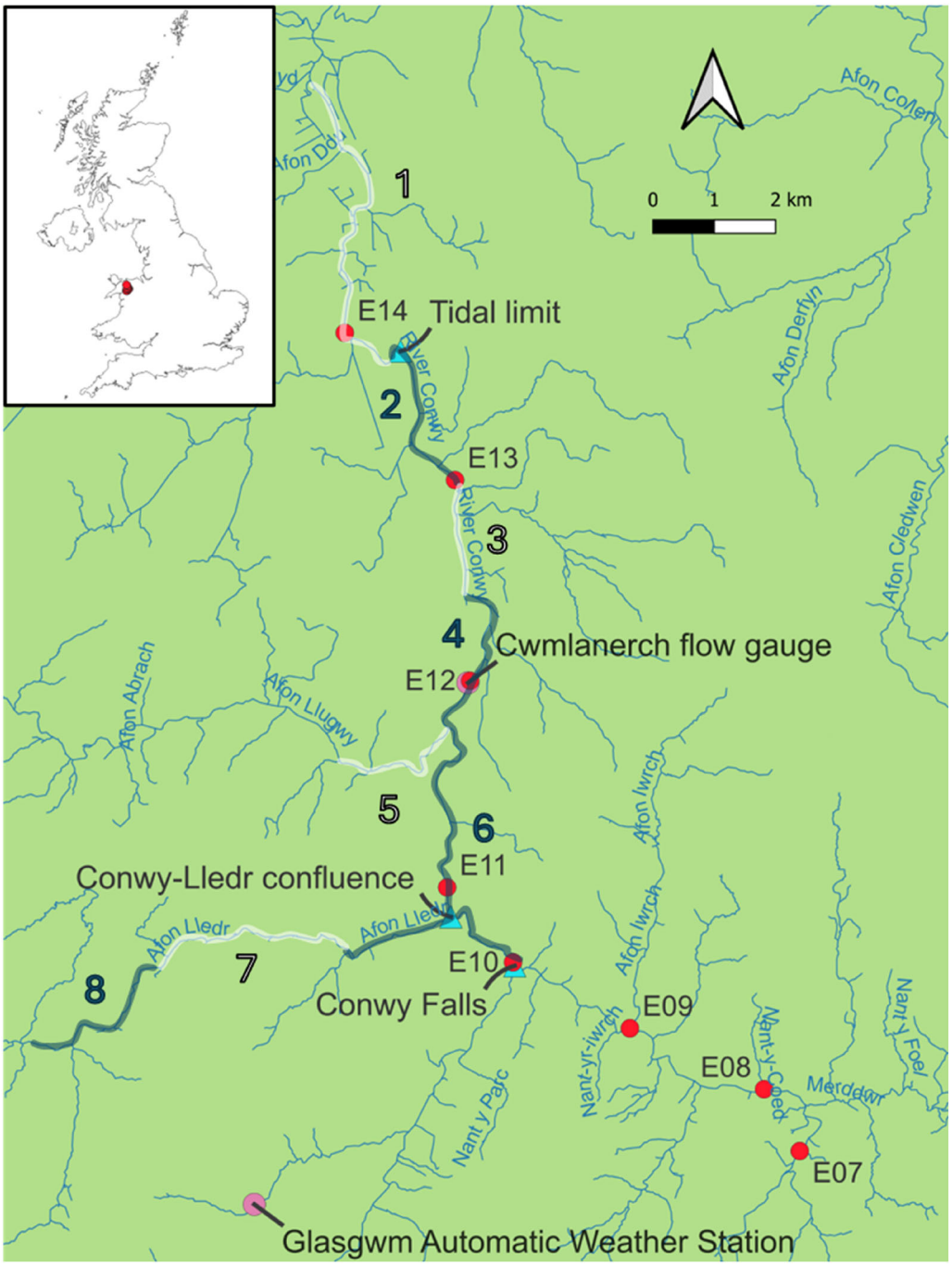

FIGURE 1 Location of eDNA sampling sites E07 to E14 (red circles), eight sectors (blue and white) from which angling catches were reported, hydrological features such as falls and confluences (blue triangles) and sites relevant to other measured environmental variables (pink circles). No salmon angling is permitted upstream of Conwy Falls (site E10). Inset map is of the United Kingdom with red circles indicating the location of the eDNA sampling sites. Contains OS data © Crown copyright and database right 2023 as well as Natural Resources Wales information © Natural Resources Wales and Database Right. All Rights Reserved. Contains Ordnance Survey Data. Ordnance Survey Licence number AC0000849444. Crown Copyright and Database Right.

class, 2017 in this study) and older fish (1+ parr of the 2016 year class). No salmon older than 1+ were recorded by electrofishing surveys by Natural Resources Wales (NRW) in 2017. Juveniles are present in the Conwy all year round, each cohort commencing around April with emerging fry. Their biomass is the outcome of losses from mortality and gains from growth. Smolts leave the River Conwy as 1+ and 2+ fish in April and May.

The methods and timing of electrofishing surveys in the Conwy have been too inconsistent to obtain reliable models of survival and growth, although surveys were considered suitable to derive average abundance in 2017 to calibrate the modelled abundance. Survival and growth models were adapted from data on the River Wye, mid-Wales (Gee et al., 1978a).

Starting reference points to calculate juvenile standing stock (total abundance, N) and density (relative abundance, N 100 m^2) for

2017 0+ and >0+ were total Conwy salmon egg depositions reported by NRW (Cefas/EA/NRW, 2023a), being 1,561,770 (0+ in 2016) and 1,165,500 (0+ in 2015), respectively, in a wetted area of 630,000 m^2. Emergence was assumed to be completed by 1 May and mortality between then and 1 June was assumed to be 50% (Fleming & Einum, 2011).

After 1 June, daily instantaneous loss rate (z in $N_t = N_{t-1}.e^{-zt}$, where t = time in days) was taken as the average (0.0062) for upper River Wye tributaries and main stem sites from tab. 3 in Gee et al. (1978a) and assumed to be constant until age 1+, as reported by Gee et al. (1978b).

The 2016 cohort abundance was adjusted for 1+ smolt loss in May 2017 using an assumed cohort smolting rate of 0.6. Change in relative abundance (N 100m^2) estimated with the above assumptions was validated against observed mean abundances reported from electrofishing surveys by NRW in 2017 (Figure S2), having a mean date of 26 July (range 26 June to 29 August) averaged across 36 surveyed sites.

Seasonal growth in length of 0+ and 1+ parr was assumed to be the same as reported by Gee et al. (1978b) from which growth in individual wet weight (W, g) was derived from $\log10(W) = \log10(L) \times 3.193 - 2.052$ (L = fork length [cm]; Figure S3). Biomass (B) at the end of each month for each cohort (0+ and 1+ in 2017) was calculated from total standing stock (N) as $B = N \times W$). Biomass estimates for 0+ and 1+ parr were summed to give total juvenile biomass expressed in kilograms.

2.3.2 | Adult biomass estimation

The adult component included live fish, decaying fish that died before spawning, post-spawning carcasses, eggs and kelts (post-spawning survivors), together forming the annual adult run contribution, referred to as adult biomass. Returning adults are predicted to show a pulse in biomass beginning with first arrivals in early spring, peaking in late summer and decreasing to near zero as fish die after spawning or migrate back to sea as kelts, leaving their eggs incubating in gravels.

The total adult run biomass (living, dead and decaying adults and eggs) arriving in 2017 was reconstructed from estimates of total annual run, rod catch and spawning escapement used by NRW to report egg deposition (Cefas/EA/NRW, 2023a, 2023b). Escapement is the spawning run after losses from retained angling catch, post-release mortality of angled fish and natural mortality. Retained catch (estimated at 31 fish in 2017) was removed from the biomass estimates. Cumulative annual escapement (874 salmon in 2017) plus losses due to post angling capture-release (annual loss = mortality rate = 0.20) and natural mortality (annual rate = 0.091) were allocated to months based on proportions of monthly rod catch data from angling catch returns to give the seasonal biomass. Because rod catches are seasonally constrained by the angling season (closure in late October) an adjustment was made using run data from a resistivity fish counter at Conwy Falls (53.064992, −3.778941) for those years with full monthly counts (2005, 2007, 2014, 2016, 2020) which showed 4% of the run occurred after the annual closure of the rod fishery. These adults comprised a mixture of salmon and sea trout (anadromous *Salmo trutta* L. [brown trout]), but no adjustment for species composition was possible and the late season proportion was assumed to apply equally to both. Loss of female body weight following spawning was estimated from the gonado-somatic index reported by Jonsson and Jonsson (2003). The decay of salmon carcasses post-spawning was estimated from decay rates reported by Chaloner et al. (2002). No correction for downstream drift of carcasses (Williams et al., 2010) was made because the biomass estimates were for the whole river and although some may have been displaced downstream of site E14 into the upper estuary, the edge effect of this was considered too small to require correction. All egg deposition and post-spawning mortality was assumed to occur in December and carcass decay to begin on 1 January. Atlantic salmon are iteroparous: a small proportion survive spawning to become kelts that migrate back to sea, some of which return to spawn the following year (Persson et al., 2023). The incidence of kelts was not known but a post-spawning in-river survival rate of 0.1 was assumed, which at 0.5 marine survival would give a percentage of previous spawners the following year of about 4%, which lies close to the mean (3.8%) and within the range (0%–26%) reported for Norwegian rivers by Persson et al. (2023). Data on the percentage of previous spawners in the Conwy are not recorded, but on the nearby Welsh Dee, a salmon Index River, the proportion of previous spawners in the annual runs has been <5% over the last 32 years (Davidson et al., 2024). Kelts were assumed to be lost from the Conwy system, beginning in December, at a monthly rate of 0.55, an arbitrary rate iterated to ensure they all left the river by the end of March, which anecdotally matches their occurrence in rod catches. Egg hatching was assumed to be completed by the end of April 2018. Initially, biomass estimates were calculated to the end of each month and then interpolated to the day of eDNA sampling, assuming linear change between each successive pair of monthly estimates. The seasonal pattern of all the sub-biomass compartments is given in Figure S3.

2.3.3 | Spatial distribution of salmon adults and juveniles in the river Conwy

The temporal pattern above captures the mean monthly presence averaged across the areas where adults are sampled by angling (Figure 1) and where juveniles are sampled by electrofishing, but does not convey how salmon were spatially organised within the river. Both adult and juvenile biomass estimates are measures for the whole catchment, thus parsimoniously are assumed to be distributed uniformly within it, when in reality there is spatial pattern. There is a well-understood spatial pattern of angling catch and spawning activity within the Cowy catchment, which has been conserved over decades with some minor variation. For example, most fish are caught in sectors 3, 4 and 7 (Figure 1) (Davidson & Milner, 1985). This consistency arises because channel structure and topography govern how the fish pass through the river and their vulnerability to angling, and because

spawning is tethered to gravel substrates having suitable hydraulic features (Armstrong et al., 2003). The consistency in spatial distribution in spawning is reflected in the spatial variation of fry recorded in electrofishing surveys (Davidson & Milner, 1985; Figure S1). No angling is permitted above Conwy Falls.

Juvenile salmon abundance is determined by factors including channel habitat features that govern spawning locations (through location and suitability of gravels) and the carrying capacity for free-swimming juveniles (through in-channel and riparian features that govern survival and growth) (Armstrong et al., 2003). In the River Conwy, historic spawning and juvenile habitat have been surveyed and reported (Davidson & Milner, 1985; Davidson & Milner, 1989). Qualitatively, therefore, the expected distribution of juveniles is known and supported by electrofishing surveys (Figure S1). Historical data from redd counts and juvenile surveys (Davidson & Milner, 1985, Davidson & Milner, 1989) show that before the fishpass was opened in 1993, spawning was concentrated in the main River Conwy stem between E011 and E013, and in the Lledr (the largest tributary), which enters the Conwy at a large, deep confluence, which is a salmon holding pool, immediately above E11 (Figure 1). Several minor tributaries also support locally intense spawning. Since 1993, spawning and rearing have occurred in the Merddwr and upper Conwy, upstream of their confluence (APEM, 2011). Fishpass counter records (NRW unpublished) show that 18% (range 6%–37%) of the Conwy run spawns upstream of Conwy Falls, but these include an unspecified proportion of sea trout (*Salmo trutta*). Crucially, the main Conwy upstream of the Lledr confluence has extensive bedrock and torrential flow sections, most prevalent upstream of E10, E09 and E08. This stretch of the river is not appropriate for electrofishing which, except for a few short sections, has few characteristics of spawning or rearing habitat, but such habitat is abundant at and upstream of E07 (APEM, 2011).

2.4 | Environmental conditions

Average daily flow (m^3/s) (discharge) on the day of eDNA sample collection was measured at the flow gauge at site E12 (Cwmlanerch, 53.106375, −3.7920625). Flow estimates at sites upstream of E12 were extrapolated from E12 based on the catchment area of each of the upstream sites. For sites downstream of E12 (E13 and E14), the same flow as E12 was used because discharges into this section of the river are small and were assumed to contribute very little to the main river flow. Average monthly air temperature estimates, referred to subsequently as temperature, were based on measurements taken at the Glasgwm Automatic Weather Station (53.029018, −3.8408066). Air temperature could not be estimated for site E14. pH measurements were taken in situ at each site as the eDNA samples were collected.

2.5 | Linear models

Before more complex modelling, linear correlations were explored among estimated biomass and normalised reads at each site, for adults and juveniles (separately). Pearson correlation coefficients (r) and bivariate plots (see Figure S5) were derived using the chart.Correlation function of the PerformanceAnalytics package in R.

2.6 | Generalised additive models

Modelling was carried out in R v. 4.2.2 (R Core Team, 2022) and the code is available on GitHub (https://github.com/WillPerryMEFGL/salmon_eDNA.git). Three generalised additive models (GAMs) were constructed using the mgcv package (Wood, 2010). The first GAM, which included all sites, had normalised salmon reads as the response variable and the following smooth functions: two separate biomass estimates, (1) juveniles and (2) adults (including eggs), daily flow, pH, the distance a sample was taken from the river source (a lake) as well as the number of days after first sample, all of which were assigned six knots. Interaction terms between each of the biomass estimates and site code were included, as well as between days after first sample and site code, and between flow and site code. The second GAM was the same as the first, other than the biomass estimate, which was (3) juveniles and adults summed. The summed biomass estimate was included in its own model to avoid multicollinearity between the partitioned biomass estimates. The second GAM containing the summed biomass had a higher Akaike information criterion (−333.19) than the first GAM containing the partitioned biomass (−373.35). Therefore, the second GAM was dropped and not used for further analysis. The third GAM was also the same as the first but had an additional smooth function for average monthly air temperature estimates and did not include site E14 due to temperature data being unavailable for this site. The only results presented from this third GAM are for the temperature effect, all other results are from the first GAM. The fourth and final GAM was the same as the first, but sites were aggregated into river regions relevant to Atlantic salmon: upper (site E07, E08, E09), Conwy Falls (E10) and lower (E11, E12, E13 and E14). The aggregation makes the normalised reads more comparable to the whole-river biomass estimates, which is based on rod catch data from the lower river (Figure 1). To aggregate site data, a mean of normalised reads and environmental variables was used. Due to the consolidation of data points caused by the aggregation, the knots assigned in the GAM were reduced to 3. After the fourth GAM, to get an understanding of effect size, a linear regression was performed between adult biomass and normalised reads for the lower river section.

Results from the GAMs are discussed in the context of partial effect, which is the isolated effect of a single predictor variable on the response variable (normalised reads) after accounting for all other variables in the model.

3 | RESULTS

3.1 | Spatial variation in normalised reads

Utilising linear correlations, the distributions of normalised reads varied significantly among sites ($F_{1,7} = 4.152$, $p < 0.01$) (Figure 2). E11

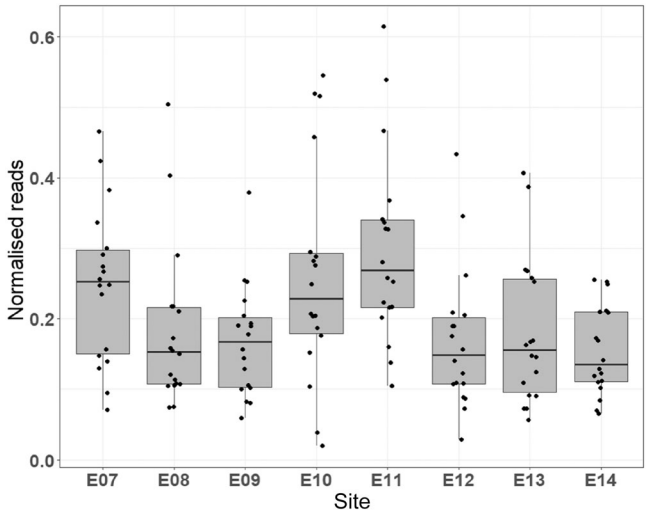

FIGURE 2 Boxplots of normalised Atlantic salmon eDNA reads from eight sites of the River Conwy.

was significantly higher than all others, except E10 and E07 (Tukey's Honestly Significant Difference test). Although site variances were homogeneous, the residuals were not normally distributed and so the results should be viewed in this context. The overall significance was maintained in a non-parametric Kruskal–Wallis test ($\chi^2 = 28.39$, $p < 0.01$). Spatial variation was also highlighted in the GAMs. The first GAM, which performed well (adjusted $R^2 = 0.78$, deviance explained = 88%), showed that distance the site was away from the source had a significant effect on normalised reads ($F = 17.72$, $p < 0.01$) (Figure 3a).

3.2 | Temporal variation in salmon biomass and normalised reads

Two components of salmon biomass, juvenile and adult, peaked at 1812 kg in July and at 3471 kg in late November, respectively (Figure 4a). Combined biomass peaked at 4655 kg in early November. As the return from sea migration progressed the proportion of adult-origin biomass increased from 0.06 in May to peak at 0.80 in December (Figure S4). Normalised reads displayed monthly fluctuations (Figure 4b,d) with a seasonal pattern that was, visually, broadly consistent among sites except for E10 (Figure 4c), which peaked earlier than others at the start of September. There was also a statistically significant effect of the number of days since the first sample on normalised reads in the first GAM ($F = 3.00$, $p = 0.01$) (Figure 3b). At all sites, except for E10, the highest normalised reads were seen in November and December (Figure 4b–d), corresponding with peak estimated adult run biomass (Figure 5).

3.3 | Abiotic influences on normalised reads

In the first GAM, pH ($F = 2.55$, $p = 0.04$) and flow ($F = 10.52$, $p < 0.01$) both had a significant effect on normalised reads. There were also significant interaction terms between flow and site at E07 ($F = 24.78$, $p < 0.01$), E08 ($F = 16.77$, $p < 0.01$), E10 ($F = 6.32$, $p < 0.01$), E12 ($F = 8.06$, $p < 0.01$) and E14 ($F = 4.43$, $p = 0.05$). In the third GAM, which performed well (adjusted $R^2 = 0.79$, deviance explained = 88%), which excluded E14 but included a temperature term, the relationship between average monthly air temperature and normalised reads was significant ($F = 2.54$, $p = 0.04$).

3.4 | Biomass–eDNA comparison

Utilising linear correlations, normalised reads were significantly positively correlated with adult biomass at sites E14, E13, E11 and E08 ($p < 0.05$). The associations were the strongest at the sites downstream of the Lledr, particularly at E11 (Pearson's $r = 0.80$). In contrast, juvenile biomass was correlated with normalised reads at only one site (E10), and combined juvenile and adult biomass was driven by the adult component.

Exploring these relationships further, the first GAM (which accounted for non-linear relationships, pH, flow, days, distance from the river source as well as juvenile and adult biomass estimates) showed that normalised reads had a significant relationship with adult and juvenile biomass (Figure S6), but none of these relationships were positive or linear. However, in the fourth GAM, once the site data was aggregated by river section to more closely fit the biomass data, normalised reads had a significant non-linear relationship with adult biomass in the lower river ($F = 10.78$, $p < 0.01$) characterised by a positive relationship with partial effect after a biomass of 1500 kg (Figure 6c). There were no significant relationships with adult biomass in other river sections (Figure 6a,b) or with juvenile biomass in any river sections (Figure 6d–f). Overall, the fourth GAM also performed well (adjusted $R^2 = 0.82$, deviance explained = 90%).

4 | DISCUSSION

Metabarcoding datasets provide a wealth of information on biological communities, and with over a decade of advances in methods refinement, database construction and primer design, the effective assessment of fish biodiversity is an exemplar of the approach (Collins et al., 2021; Deiner et al., 2016; Gibson et al., 2023; Hallam et al., 2021; Miya et al., 2015; Sato et al., 2018). However, it remains unclear how representative metabarcoding datasets are for describing the distribution and biomass of single species. Here, we provide evidence that sequenced mitochondrial 12S genes, amplified by PCR from DNA obtained from the environment, can identify trends representative of Atlantic salmon biomass estimates, especially adult biomass, at the catchment scale. The 12S gene reads produced by sequencing, after being normalised to adjust for differing read depths among samples, showed a significant relationship with biomass estimates. To our knowledge, we present the first application of eDNA metabarcoding to estimating salmonid biomass distribution in a river system, with previous studies having utilised qPCR.

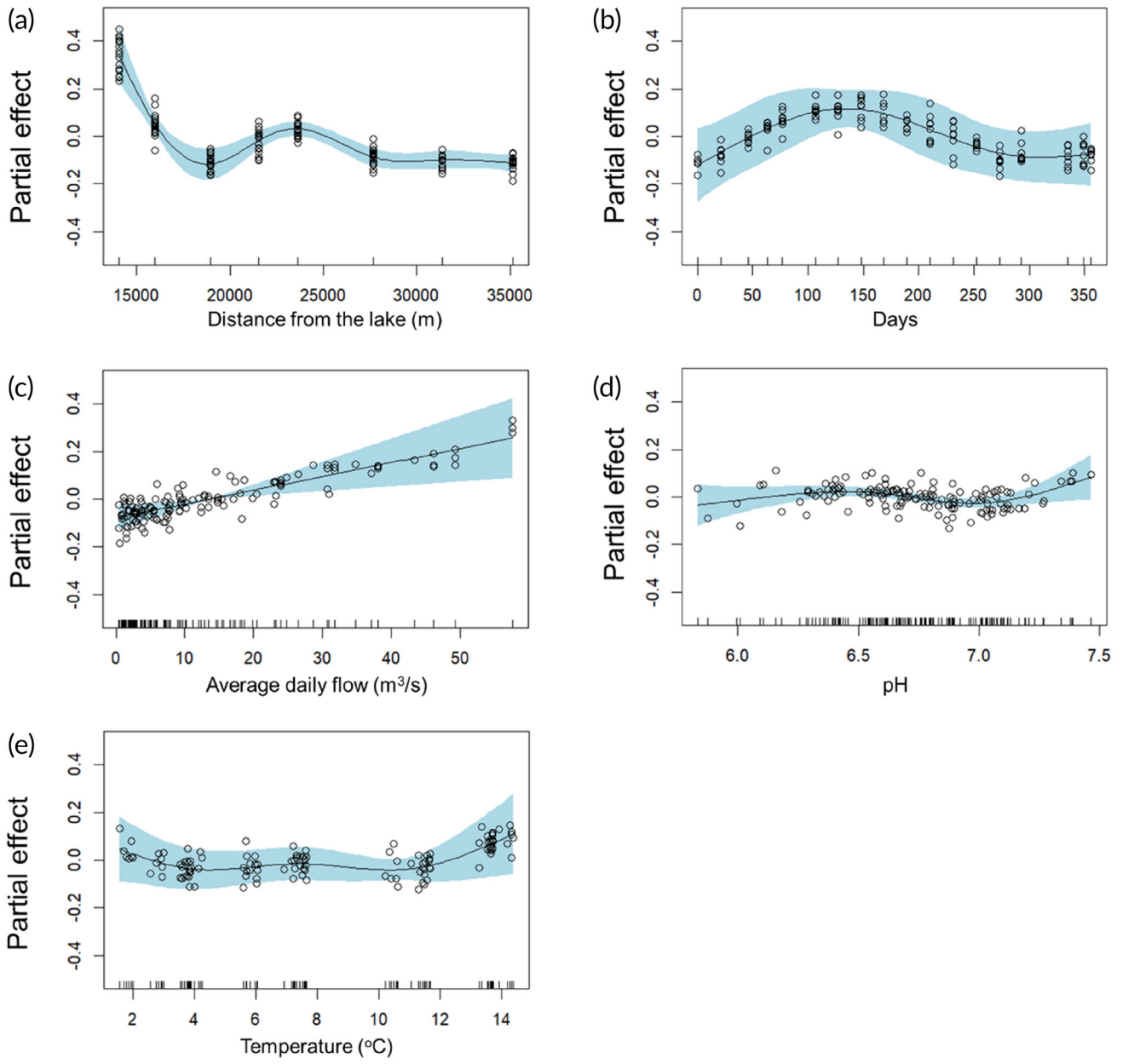

FIGURE 3 Outputs from the first generalised additive model (GAM) showing the partial effect of (a) distance from the river source, (b) days since the first sample and the statistically significant abiotic conditions, (c) flow, (d) pH and (e) temperature on normalised Atlantic salmon reads. The output for (e) temperature was taken from the third GAM, which did not include site E14 due to the lack of temperature data at this site.

Briefly, qPCR measures the quantity of a specific DNA fragment in a sample by using primers and a fluorescent dye or probe, which allows the qPCR machine to measure the fluorescence level caused by target fragment amplification. Using a known standard, the fluorescence can quantify the concentration of that fragment. Metabarcoding, however, is sequencing-based, meaning that specific fragments are not targeted. Instead, universal primers are used to amplify, in a PCR reaction, a broad array of similar fragments. Here, the sequencing-by-synthesis approach (Illumina HiSeq platform) was used, whereby the sequencing machine reads the nucleotide bases making up the fragments, producing 'reads'. These reads do not allow for the concentration of the fragments to be calculated. However, more reads of a specific fragment are expected if there is more of that fragment in the sample. One would also expect a proportional representation of a specific fragment (i.e. salmon DNA fragment) in a water sample to the biomass of organisms in the environment. However, a correction must first be applied among samples based on differing levels of sequencing effort. This correction is achieved by normalising the reads from each fragment by the total number of the reads within that sample to make them comparable, resulting in a relative measurement of normalised reads.

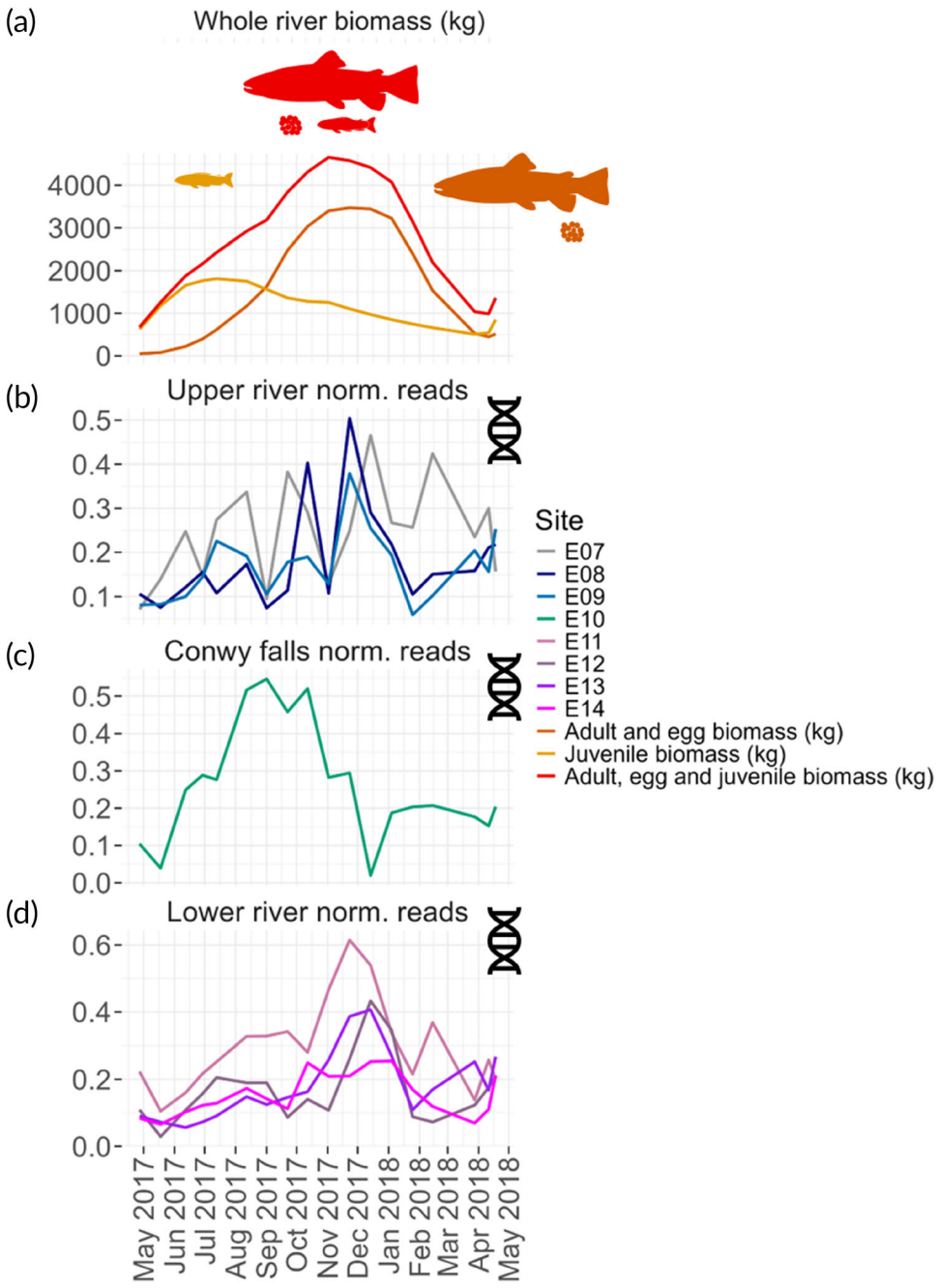

FIGURE 4 (a) A time series of biomass estimates, coloured by the life stage they represent. Also included is a time series of normalised reads grouped into different sections of the River Conwy, with (b) upstream of Conwy Falls, (c) immediately downstream of Conwy falls and (d) lower river, downstream of the Lledr confluence.

4.1 | Spatio-temporal variation in normalised eDNA reads

Normalised reads were detected at all eight eDNA sampling sites, implying that salmon were present at distances of up to approximately 40 km upstream of the mouth of the Conwy Estuary at Deganwy. The overall variance of normalised reads includes seasonal fluctuations in salmon presence, although E11 stood out with higher mean normalised reads (mean 0.299, standard deviation [SD] = 0.135, $n = 18$) compared to other sites (Figure 2). This site is downstream of a major pool for adult salmon at the junction of the Lledr and the main stem of the River Conwy. It is also the first significant large holding site for salmon that may migrate back downstream, having been temporarily delayed at Conwy Falls. The Falls site (E10) returned the second highest mean normalised reads (0.262, SD = 0.157, $n = 18$) and this, in addition to its unique seasonal pattern, may point to this location as a partial barrier for early summer running fish, despite the fishpass at the Falls, as is seen with artificial barriers with fishpasses (Twardek

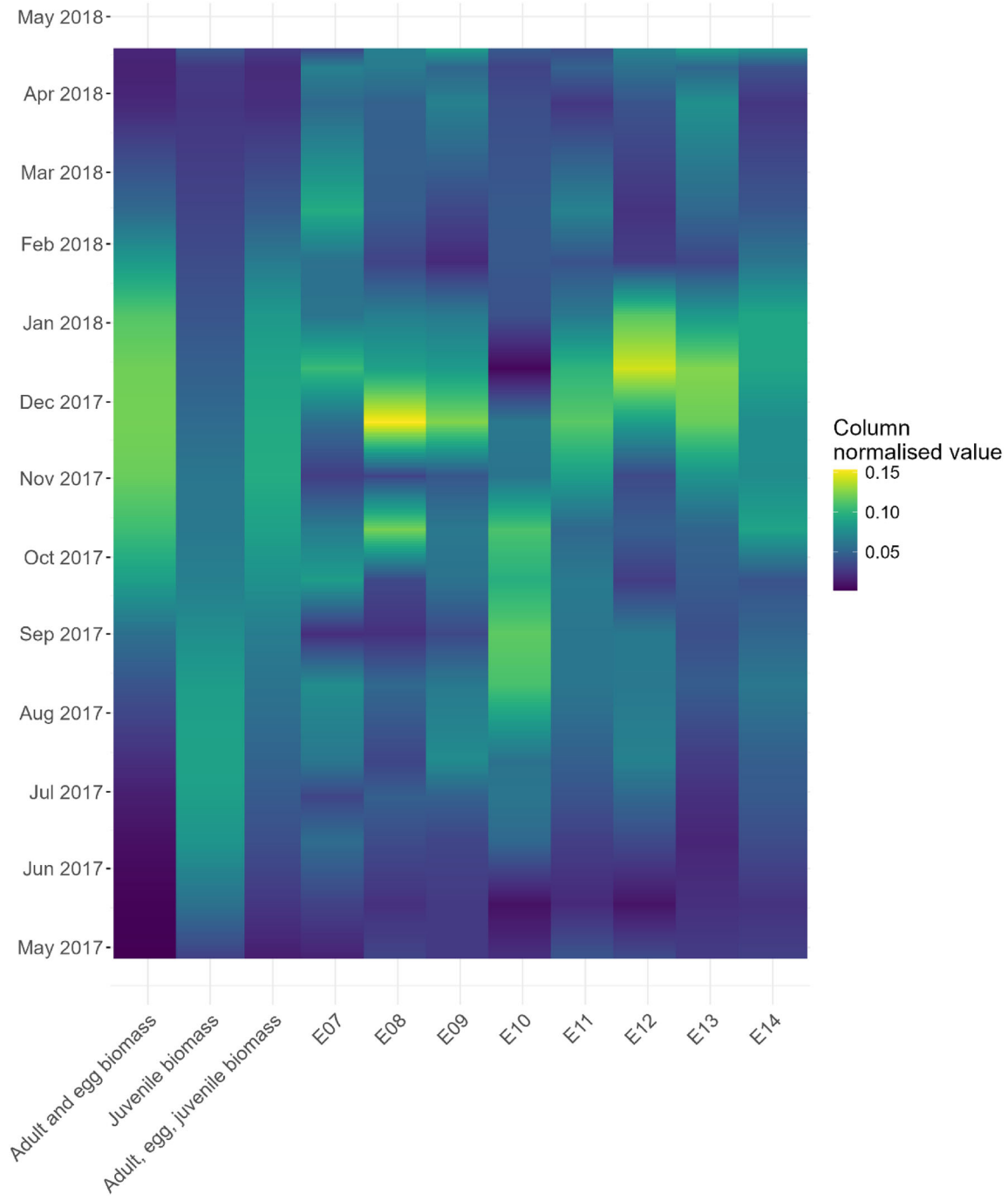

FIGURE 5 Heatmap showing within-column scaled values of salmon biomass and eDNA normalised reads at each eDNA sampling site (E07–E14). Each of the values is divided by the sum of all values belonging to the associated variable being displayed on the *x* axis. To create a continuum, linear interpolation was used to generate values for dates among samples.

et al., 2021). Furthermore, the rapid decline in normalised reads at E10 (which has no spawning or rearing habitat) from September to January corresponded with an increase in normalised reads at all other sites, suggesting a departure of presumptive spawners either upstream through the fishpass or downstream to the lower Conwy or Lledr spawning sites.

High normalised reads at E07 after February 2018 distinguished it from the other sites upstream of the falls (Figure 4b). Indeed, site E07 showed the highest partial effect on normalised reads, demonstrating it has the highest normalised reads after accounting for the effects of other variables (Figure 3a). This site is at the end of the most productive spawning and rearing 2 km of the upper Conwy (APEM, 2011), which is located downstream of impassable falls at Ysbyty Ifan (NGR SH 8300 4840). Thus, E07 would be expected to have a high abundance of adult spawners and juveniles. However, high normalised reads at E07 were maintained well into March 2018,

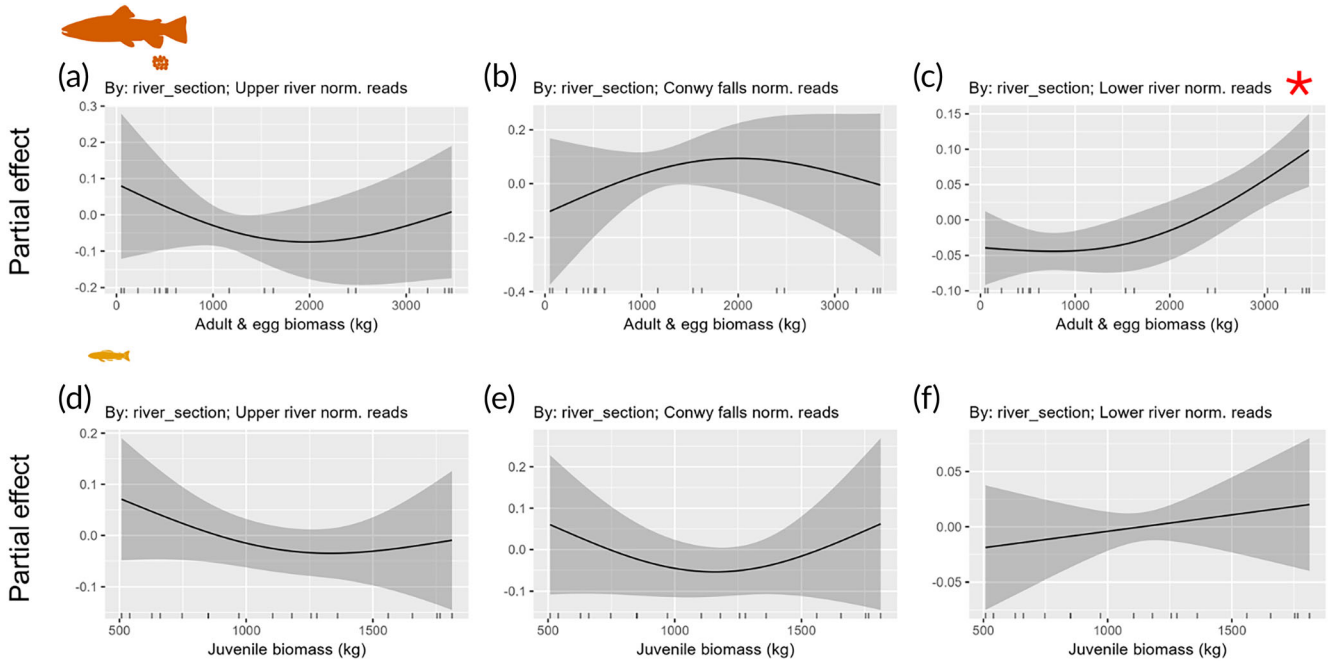

FIGURE 6 Partial effect plots from the fourth generalised additive model (GAM) where sites were aggregated into river sections: upper (a, d), Conwy Falls (b, e) and lower (c, f). Normalised salmon reads is the response variable and plots show the relationship with the independent variables, (a–c) adult and (d–f) juvenile biomass in the form of partial effects, which account for the other variables included in the model. The red asterisk indicates a significant relationship ($p < 0.05$).

which is considerably later than the known spawning time and may be indicative of the high abundance of salmon fry and parr in that section. Previously, qPCR approaches have shown a strong correlation between Atlantic salmon juvenile biomass and eDNA concentration in tributaries ($R^2 = 0.82$) (Berger et al., 2024). In the Conwy upstream of the Merddwr, at four sites adjacent to E07 in 2010, late-summer salmon fry (density = 17.2100 m^{-2}, SD = 2.58) contributed 0.94 of the salmon and trout abundance, and salmon parr (density = 1.1100 m^{-2}, SD = 0.28) contributed 0.48 of total salmonid abundance (APEM, 2011). Salmon dominance appears to be a consistent feature of this reach, stemming from a particularly favourable salmon spawning habitat. In 2014, a survey showed salmon contributed 0.99 and 0.54 of the total salmonid fry and parr, respectively (CCRT, 2014).

In contrast, E08 and E09 lie in sections characterised mainly by bedrock channels and torrential flow that would not be expected to support significant juvenile production or offer major holding areas. At these two sites, normalised reads decreased rapidly after the November peak (Figure 4b), suggesting it was more indicative of adult migrant passage on the way to spawning in the Merddwr and upper Conwy. After accounting for the effects of other variables, overall site E08 did show the second highest partial effect on normalised reads (Figure 3a) in the system, which is likely due to its proximity to the Merddwr. The rapid post-November decline in E11, E12, E13 and E14, which are wide main stem channels, probably reflects their primary role as conduits for arriving and departing adults. However, at all these sites, some continuous presence of juvenile salmon is likely to arise from local spawning.

4.2 | Biomass-eDNA comparison

Due to the difference in spatial resolution between normalised reads (eight sites along the Conwy) and biomass (whole river), insights from directly comparing the two measures are limited. Despite this, when normalised reads were aggregated into river sections that more closely resembled the whole river biomass estimate, a strong relationship between the two metrics emerged. Moreover, by partitioning biomass by life stage, strong relationships can inform what life stages are contributing most to normalised reads. Biomass best explained the variation in normalised reads in the lower river (Figure 6c) and specifically adult biomass. The relationship here was overall non-linear, but positive and linear after a biomass of 1500 kg was reached. When fitting a linear regression, 61% of variance was explained. Normalised reads in the lower river being the most representative of the whole river adult biomass estimate is logical, given that the rod catch data underpinning the biomass estimate is collected from the lower river. The lower river is also home to a major holding pool for adult salmon. The positive nature of the relationship only occurring after 1500 kg of biomass also suggests that there is a threshold, after which normalised reads are more representative of biomass. This density dependency has also been seen in qPCR studies looking at returning Pacific salmon spawners, where

lower numbers of individuals (daily counts of less than 100) led to a weaker correlation with eDNA levels (Su et al., 2024).

4.3 | eDNA transport

Across applications of eDNA in aquatic systems it is important to assess the extent to which signals may represent patterns transported from non-target sites due to water transport. Significant relationships between normalised salmon reads and biomass, as well as the unique profiles of normalised salmon reads at each of the sites, supports the hypothesis that the transport of eDNA from upstream is not impairing localised trends downstream. The lack of eDNA transport detected at downstream sites, or accumulation over time, supports prior qPCR studies assessing Pacific salmonid species (Levi et al., 2019; Tillotson et al., 2018) and caged Atlantic salmon (Wood et al., 2021). In addition, previous work in the River Conwy assessing eDNA transport using exogenous eDNA and the 12S marker showed that there was greatly reduced abundance and detection of eDNA after just 1 km, and no detection beyond 5 km (Perry et al., 2024). Given the spatial structuring and significant relationship with biomass seen in this study, and the eDNA sample sites being distributed between 2 and 4 km away from each other, the results of this study support previous findings indicating limited eDNA transport. These results also provide further confirmation that eDNA quickly becomes undetectable in lotic environments and is therefore representative of biodiversity in the immediate vicinity of the site sampled.

4.4 | Environmental conditions

Previous qPCR studies have shown that the eDNA signal of salmonids is heavily influenced by the river flow rate (Levi et al., 2019; Su et al., 2024; Tillotson et al., 2018), with increased flows associated with higher dilution, therefore reducing eDNA concentration (Wood et al., 2021). Here, flow had a significant effect on normalised reads, but higher normalised reads were associated with higher flows. This contradictory result is likely due to the use of metabarcoding and normalised reads rather than absolute concentrations provided by qPCR in the previous studies. The relative nature of normalised reads, unlike qPCR, which is an absolute measure, means that so long as eDNA from all other species is being diluted by flow, normalised salmon reads will not be significantly affected. Reads from other species are therefore acting as flow-adjusted environmental standards. Such an approach has multiple benefits, which includes less reliance on flow data (which can be hard to acquire) to normalise the eDNA signal, contrary to using qPCR, where high-resolution stream flow data is required to accurately calculate salmonid abundance from eDNA (Levi et al., 2019).

As the significant effect of flow was not primarily driven by the ecology of the eDNA molecule, we expect, therefore, that it is driven by the ecology of the salmon. In surface-fed rivers like the Conwy salmon annual migration is broadly timed to coincide with the annual hydrograph (Figure S7) such that in-river numbers increase as flows increase from summer lows to autumn/winter high flows that aid upstream migration, tributary entry and spawning (Thorstad et al., 2008). Therefore, some correspondence between flow at the season scale and eDNA reads would be anticipated. The sites furthest upstream (E07 and E08) showed significant positive linear relationships between partial effect on normalised reads and flow in the first GAM. This relationship could be driven by spawning migration to access spawning sites, as some are in smaller channels where higher flows facilitate movement, mate selection and egg deposition (Milner et al., 2012; Thorstad et al., 2008; Webb & McLay, 1996), as in the Merddwr (adjacent to E07 and E08) and upper Conwy. Flow also significantly affected normalised reads at E10, but this relationship was non-linear and was characterised by a drop-off in normalised reads at high flows. As E10 is downstream of Conwy Falls and its associated fishpass, particularly high flows could deter fish from using the fishpass and thus entering this stretch of the river. Flow also had a significant effect on normalised reads at site E12 and E14, where there was a linear negative correlation. Lower normalised reads at higher flows in this most downstream section of the river could reflect that, at higher flows, much of the salmon biomass is dispersing further upstream and leaving the lower reaches. Likewise, while dilution of eDNA does not appear to be the primary driver of the flow trends, it is possible that it could have some effect in these lower reaches where the river is larger.

It is important to note that, as flow estimates were based on a single site and scaled to other sites in the catchment, local spatial variation in flow that can occur between sub-catchments will not have been captured, especially during storm events, therefore interpreting these results and their meaning for salmon ecology does come with caveats.

Finally, pH and temperature also had a significant effect on normalised salmon reads. While acidity is known to degrade eDNA (Seymour et al., 2018), the relationship seen here suggests that normalised salmon reads were lowest at pH neutral (pH 7.0), after accounting for the effects of other variables, and highest at pH 6.5, the opposite of what one would expect from the ecology of the eDNA molecule. Again, like flow, this may be due to the relative nature of the normalised salmon reads, with the trend being driven by salmon ecology. The optimal pH for Atlantic salmon eggs and alevins has been documented as being between pH 6.2–6.8 (Benchmark Genetics, 2019). Therefore, sites with a pH in this range could be more attractive to spawners, thus increasing the amount of eDNA. Similarly, while higher temperatures are associated with higher eDNA degradation rates (Strickler et al., 2015), the relationship seen here suggests that normalised salmon reads were highest at the highest temperatures (14°C), M after accounting for the effects of other variables. Rather than the ecology of the salmon driving this trend it is likely their physiology, with higher metabolic rates at higher temperatures, increasing the rate of eDNA shedding (Rourke et al., 2022), a trend which has already been observed in Atlantic salmon with qPCR approaches (Berger et al., 2024).

4.5 | Limitations of the biomass estimates and metabarcoding

In discussing the results, two important caveats apply. First, the observed biomass estimates are for the whole river where salmon occur, and interpretation of spatial patterns of biomass within the River Conwy is constrained by a combination of observed normalised reads and the actual salmon spatial distribution and ecology within the river. Inferences drawn regarding the processes that may lead to normalised read variation and its anomalies within the river are subject to assumptions made regarding salmon ecology. This presents more of a problem for juveniles than for adult salmon because the former are present at background levels all year and are known to be spatially highly variable (Figure S1) as influenced by local habitat features. Modelling seasonal biomass change, at sufficient granularity to be relevant to the eDNA sampling sites, is therefore extraordinarily difficult due to the paucity of local robust demographic data. In contrast, the annual migrants represent a readily identifiable and large annual pulse of biomass that can be estimated and is known (through juvenile surveys) to have wide dispersal throughout the spawning locations of the catchment, although also having some spatial organisation as the run develops seasonally and fish aggregate in spawning locations.

Second, the normalised reads for salmon are relative to the reads of other fish species in the system. Therefore, if the sum reads of all other non-target species show a net change, the relative abundance of the species of interest would be impacted. Such an effect may be exacerbated in systems with fewer species or those that are dominated by migratory species. Indeed, marginally lower species found in the River Conwy's upper reaches could contribute to the noisy eDNA signal at sites E07 to E09 (more on the fish species richness of these sites can be found in Figure S8).

There were approaches throughout the eDNA methodological pipeline used to produce the data in this study which we expect will have reduced the impact of stochasticity. Among the most important is replication (Perry et al., 2023). Three water samples were taken at each sample point, each being sequenced and later aggregated to provide a more robust representation of the sample. In addition to this, during library preparation, each sample replicate had an additional three replicate PCR reactions which were combined before adding the Illumina indexes. We expect that replication during the PCR stage will also help minimise stochasticity and provide a more representative sample (Ficetola et al., 2015). Stochasticity introduced by PCR amplification makes read counts less quantitative than other methodologies such as qPCR. However, the results here demonstrate that normalised reads have the potential to provide data that can represent real-world abundance, even at fish densities where qPCR techniques have struggled for Pacific salmon (Su et al., 2024).

4.6 | Future work

This study took advantage of a pre-existing dataset (Perry et al., 2023) that was not designed to investigate salmon abundance. Further refinement would benefit from targeted experimental sampling, which would allow for more comprehensive exploration of the system. For example, distinction between juvenile and adult eDNA is not yet possible and would benefit from (i) a purpose-driven experimental and sampling design that matches eDNA sampling with sub-catchments with known contrasts in salmon and non-salmon fish abundance (e.g. brown trout), (ii) a greater description of habitat types in the upstream zone relevant to eDNA dispersal into each site, and (iii) better spatial data on population dynamics (abundance, survival and growth) of juvenile salmon and sympatric species, particularly brown trout, to improve biomass estimates.

The application, specifically in relation to the Conwy, requires validation of the inferences regarding adult migration. The proposed blockage and downstream dispersal of adults at E10, which is entirely in line with conventional principles of salmon upstream migration, could be tested by telemetry to track the movements and eventual spawning locations of adult salmon around the catchment. In addition, enhancements could be made to the counter at Conwy Falls, including video validation to distinguish salmon and sea trout migrants, measure their size distributions and better estimate their eDNA contribution.

5 | CONCLUSION

Normalised reads derived from eDNA detected the seasonal pulse of spawning adult salmon and allowed for the estimation of spatial variation in salmon biomass. In sites that deviated from the overall trend, the ecology of salmon migration and habitat–productivity relationships gave plausible explanations for the anomalies. Despite a mismatch in areas covered and differing spatial resolutions between the two measures, there were significant relationships between normalised reads and biomass, demonstrating that metabarcoding reads can provide species-specific quantitative information and data on broader fish communities. In addition, normalised reads had significant relationships with environmental variables such as flow, pH and temperature that could not be explained by degradation or dilution of the eDNA molecule and are, therefore, expected to be caused by salmon ecology and physiology.

To our knowledge, this is the first account of applying normalised reads to the assessment of Atlantic salmon distribution in a catchment. The results are encouraging, potentially revealing aspects of adult migration in the River Conwy that were previously only suspected. Moreover, individual species reads mined from a metabarcoding dataset and biomass estimated from rod catch and a fish counter are sub-optimal, yet, despite the limitations, when combined in a multimethod approach, with background information on the system, they provide valuable insights into salmon ecology. Finally, the approach demonstrates eDNA metabarcoding is a cost-effective, non-destructive tool that can add capacity and flexibility to the monitoring of the spatial distribution and relative abundance of this important, yet seriously threatened, migratory species.

AUTHOR CONTRIBUTIONS

W.B.P. and N.M. prepared the manuscript. W.B.P and N.M. analysed the data. S.C., B.J.C., J.C. and I.D were awarded the funding. All authors were involved in conceptualising the ideas in the manuscript. No data were collected as part of this study, just analysis of existing datasets.

ACKNOWLEDGEMENTS

The project was funded by a NERC Highlight Topic grant (NE/N006216/1). We acknowledge the support of the Supercomputing Wales project, which is part-funded by the European Regional Development Fund via Welsh Government, as well as the expertise provided by Ade Fewings and Aaron Owen. Natural Resources Wales provided unpublished electrofishing data and counts from the fish counter at Conwy Falls. The high throughput sequencing data used in this study, which was previously published, was generated by EnviSion, BioSequencing and BioComputing at the University of Birmingham.

DATA AVAILABILITY STATEMENT

No new data was created during this study.

ORCID

William Bernard Perry https://orcid.org/0000-0001-9596-3333

REFERENCES

Allan, I. R. H., & Ritter, J. A. (1977). Salmonid terminology. *ICES Journal of Marine Science*, *37*, 293–299. https://doi.org/10.1093/ICESJMS/37.3.293

Altermatt, F., Little, C. J., Mächler, E., Wang, S., Zhang, X., & Blackman, R. C. (2020). Uncovering the complete biodiversity structure in spatial networks: The example of riverine systems. *Oikos*, *129*, 607–618. https://doi.org/10.1111/OIK.06806

Andersen, O., & Dervo, B. K. (2019). *Jegernes og fiskernes forbruk av varer og tjenester i Norge i 2018*, *NINA Report 1605*. Norsk Institutt for Naturforskning (NINA).

APEM. (2011). Electro-fishing survey of the upper Conwy. Report to Afonydd Cymru. Ref. 411306.

Armstrong, J. D., Kemp, P. S., Kennedy, G. J. A., Ladle, M., & Milner, N. J. (2003). Habitat requirements of Atlantic salmon and brown trout in rivers and streams. *Fisheries Research*, *62*, 143–170. https://doi.org/10.1016/S0165-7836(02)00160-1

Benchmark Genetics. (2019). *Atlantic salmon hatchery manual: Egg to first feeding*. Benchmark Genetics.

Berger, C. S., Gagnon, S., Bernatchez, L., & Bergeron, N. (2024). Monitoring and predicting the presence and abundance of juvenile Atlantic salmon in tributaries according to habitat characteristics using environmental DNA. *Environmental DNA*, *6*, e553. https://doi.org/10.1002/EDN3.553

Best, J. (2018). Anthropogenic stresses on the world's big rivers. *Nature Geoscience*, *12*, 7–21. https://doi.org/10.1038/s41561-018-0262-x

CCRT. (2014). *Report on a survey of fish populations in the upper river Conwy*. Clwyd and Conwy Rivers Trust.

Cefas/EA/NRW. (2023a). Salmon Stocks and Fisheries in England and Wales in 2022. Preliminary assessment prepared for ICES. Cefas, Lowestoft, Suffolk., p. 96.

Cefas/EA/NRW. (2023b). Salmon Stocks and Fisheries in England and Wales in 2015 - GOV.UK. Cefas, Lowestoft, Suffolk.

Chaloner, D. T., Wipfli, M. S., & Caouette, J. P. (2002). Mass loss and macroinvertebrate colonisation of Pacific salmon carcasses in southeastern Alaskan streams. *Freshwater Biology*, *47*, 263–273. https://doi.org/10.1046/J.1365-2427.2002.00804.X

Collins, R. A., Trauzzi, G., Maltby, K. M., Gibson, T. I., Ratcliffe, F. C., Hallam, J., Rainbird, S., Maclaine, J., Henderson, P. A., Sims, D. W., Mariani, S., & Genner, M. J. (2021). Meta-fish-lib: A generalised, dynamic DNA reference library pipeline for metabarcoding of fishes. *Journal of Fish Biology*, *99*, 1446–1454. https://doi.org/10.1111/JFB.14852

Dadswell, M., Spares, A., Reader, J., McLean, M., McDermott, T., Samways, K., & Lilly, J. (2022). The decline and impending collapse of the Atlantic salmon (*Salmo salar*) population in the North Atlantic Ocean: A review of possible causes. *Reviews in Fisheries Science & Aquaculture*, *30*, 215–258. https://doi.org/10.1080/23308249.2021.1937044

David, B. O., Fake, D. R., Hicks, A. S., Wilkinson, S. P., Bunce, M., Smith, J. S., West, D. W., Collins, K. E., & Gleeson, D. M. (2021). Sucked in by eDNA – A promising tool for complementing riverine assessment of freshwater fish communities in Aotearoa New Zealand. *New Zealand Journal of Zoology*, *48*, 217–244. https://doi.org/10.1080/03014223.2021.1905672

Davidson, I. C., Brown, B., & Sherratt, S. (2024). *Dee stock assessment Programme angler report 2023*. Natural Resources Wales.

Davidson, I. C., & Milner, N. J. (1985). Conwy fisheries monitoring programme: interim report. Technical Report AFTUN/86/5. Welsh water Authority, Brecon.

Davidson, I. C., & Milner, N. J. (1989). A survey of physical barriers to adult salmonid migration up and downstream of the Proposed Conway Falls fishpass. Technical Report EAN/89/4. Welsh water Authority, Brecon.

Deiner, K., Fronhofer, E. A., Mächler, E., Walser, J. C., & Altermatt, F. (2016). Environmental DNA reveals that rivers are conveyer belts of biodiversity information. *Nature Communications*, *7*, 12544. https://doi.org/10.1038/ncomms12544

Ficetola, G. F., Pansu, J., Bonin, A., Coissac, E., Giguet-Covex, C., De Barba, M., Gielly, L., Lopes, C. M., Boyer, F., Pompanon, F., Rayé, G., & Taberlet, P. (2015). Replication levels, false presences and the estimation of the presence/absence from eDNA metabarcoding data. *Molecular Ecology Resources*, *15*, 543–556. https://doi.org/10.1111/1755-0998.12338

Fleming, I. A., & Einum, S. (2011). Reproductive ecology: Of two sexes. In *Atlantic Salmon Ecology*. John Wiley & Sons.

Gee, A. S., Milner, N. J., & Hemsworth, R. J. (1978a). The production of juvenile Atlantic salmon, *Salmo salar* in the upper wye. *Wales. Journal of Fish Biology*, *13*, 439–451. https://doi.org/10.1111/J.1095-8649.1978.TB03453.X

Gee, A. S., Milner, N. J., & Hemsworth, R. J. (1978b). The effect of density on mortality in juvenile Atlantic salmon (*Salmo salar*). *The Journal of Animal Ecology*, *47*, 497. https://doi.org/10.2307/3796

Gibson, T. I., Carvalho, G., Ellison, A., Gargiulo, E., Hatton-Ellis, T., Lawson-Handley, L., Mariani, S., Collins, R. A., Sellers, G., Antonio Distaso, M., Zampieri, C., & Creer, S. (2023). Environmental DNA metabarcoding for fish diversity assessment in a macrotidal estuary: A comparison with established fish survey methods. *Estuarine, Coastal and Shelf Science*, *294*, 108522. https://doi.org/10.1016/J.ECSS.2023.108522

Goutte, A., Molbert, N., Guérin, S., Richoux, R., & Rocher, V. (2020). Monitoring freshwater fish communities in large rivers using environmental DNA metabarcoding and a long-term electrofishing survey. *Journal of Fish Biology*, *97*, 444–452. https://doi.org/10.1111/JFB.14383

Gregory, S. D., Gillson, J. P., Whitlock, K., Barry, J., Gough, P., Hillman, R. J., Mee, D., Peirson, G., Shields, B. A., Talks, L., Toms, S., Walker, A. M., Wilson, B., & Davidson, I. C. (2023). Estimation of returning Atlantic salmon stock from rod exploitation rate for principal salmon rivers in England & Wales. *ICES Journal of Marine Science*, *80*, 2504–2519. https://doi.org/10.1093/ICESJMS/FSAD161

Hallam, J., Clare, E. L., Jones, J. I., & Day, J. J. (2021). Biodiversity assessment across a dynamic riverine system: A comparison of eDNA metabarcoding versus traditional fish surveying methods. *Environmental DNA*, *3*, 1247–1266. https://doi.org/10.1002/EDN3.241

IUCN. (2023). Freshwater fish highlight escalating climate impacts on species. IUCN Red List.

Jonsson, N., & Jonsson, B. (2003). Energy allocation among developmental stages, age groups, and types of Atlantic salmon (*Salmo salar*) spawners. *Canadian Journal of Fisheries and Aquatic Sciences*, *60*, 506–516. https://doi.org/10.1139/F03-042

Keck, F., Blackman, R. C., Bossart, R., Brantschen, J., Couton, M., Hürlemann, S., Kirschner, D., Locher, N., Zhang, H., & Altermatt, F. (2022). Meta-analysis shows both congruence and complementarity of DNA and eDNA metabarcoding to traditional methods for biological community assessment. *Molecular Ecology*, *31*, 1820–1835. https://doi.org/10.1111/MEC.16364

Lamb, P. D., Hunter, E., Pinnegar, J. K., Creer, S., Davies, R. G., & Taylor, M. I. (2019). How quantitative is metabarcoding: A meta-analytical approach. *Molecular Ecology*, *28*, 420–430. https://doi.org/10.1111/MEC.14920

Levi, T., Allen, J. M., Bell, D., Joyce, J., Russell, J. R., Tallmon, D. A., Vulstek, S. C., Yang, C., & Yu, D. W. (2019). Environmental DNA for the enumeration and management of Pacific salmon. *Molecular Ecology Resources*, *19*, 597–608. https://doi.org/10.1111/1755-0998.12987

Milner, N. (1985). Predicted fisheries consequences of Cowy falls fishpass. In *Technical report FTUN/85/4* (p. 21). Welsh Water Authority.

Milner, N., & de Leaniz, C. G. (2023). The identification and characterisation of small salmon populations to support their conservation and management. Nat. Resour. Wales Report No.

Milner, N. J., Davidson, I. C., Evan, R., Locke, V., & Wyatt, R. J. (2001). The use of rod catches to estimate salmon runs in England and Wales, in: The interpretation of rod and net catch data. In *Proceedings of a workshop held at the Centre for Environment, fisheries and aquatic science, Lowestoft, 6th – 7th November 2001*. (pp. 45–65). Atlantic Salmon Trust.

Milner, N. J., Solomon, D. J., & Smith, G. W. (2012). The role of river flow in the migration of adult Atlantic salmon, *Salmo salar*, through estuaries and rivers. *Fisheries Management and Ecology*, *19*, 537–547. https://doi.org/10.1111/FME.12011

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

Natural Resources Wales. (2021). Know Your River – Conwy Salmon & Sea Trout Catchment Summary.

Olmos, M., Massiot-Granier, F., Prévost, E., Chaput, G., Bradbury, I. R., Nevoux, M., & Rivot, E. (2019). Evidence for spatial coherence in time trends of marine life history traits of Atlantic salmon in the North Atlantic. *Fish and Fisheries*, *20*, 322–342. https://doi.org/10.1111/FAF.12345

Perry, W. B., Pillay, K., George, P., Brennan, G., Lowe, A., Jones, L., Holman, L., Gibson, T., de Vere, N., & Creer, S. (2023). Design considerations for eDNA metabarcoding surveys. In O. F. Berry, C. E. Holleley, & S. N. Jarman (Eds.), *Applied environmental genomics* (pp. 2–13). CSIRO Publishing.

Perry, W. B., Seymour, M., Orsini, L., Jâms, I. B., Milner, N., Edwards, F., Harvey, R., de Bruyn, M., Bista, I., Walsh, K., Emmett, B., Blackman, R., Altermatt, F., Lawson Handley, L., Mächler, E., Deiner, K., Bik, H. M., Carvalho, G., Colbourne, J., … Creer, S. (2024). An integrated spatio-temporal view of riverine biodiversity using environmental DNA metabarcoding. *Nature Communications*, *15*, 1–13. https://doi.org/10.1038/s41467-024-48640-3

Persson, L., Raunsgard, A., Thorstad, E. B., Østborg, G., Urdal, K., Sægrov, H., Ugedal, O., Hindar, K., Karlsson, S., Fiske, P., & Bolstad, G. H. (2023). Iteroparity and its contribution to life-history variation in Atlantic salmon. *Canadian Journal of Fisheries and Aquatic Sciences*, *80*, 577–592. https://doi.org/10.1139/CJFAS-2022-0126

Pont, D., Valentini, A., Rocle, M., Maire, A., Delaigue, O., Jean, P., & Dejean, T. (2021). The future of fish-based ecological assessment of European rivers: From traditional EU water framework directive compliant methods to eDNA metabarcoding-based approaches. *Journal of Fish Biology*, *98*, 354–366. https://doi.org/10.1111/JFB.14176

R Core Team. (2022). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing.

Rourke, M. L., Fowler, A. M., Hughes, J. M., Broadhurst, M. K., DiBattista, J. D., Fielder, S., Wilkes Walburn, J., & Furlan, E. M. (2022). Environmental DNA (eDNA) as a tool for assessing fish biomass: A review of approaches and future considerations for resource surveys. *Environmental DNA*, *4*, 9–33. https://doi.org/10.1002/EDN3.185

Sato, Y., Miya, M., Fukunaga, T., Sado, T., & Iwasaki, W. (2018). MitoFish and MiFish pipeline: A mitochondrial genome database of fish with an analysis pipeline for environmental DNA Metabarcoding. *Molecular Biology and Evolution*, *35*, 1553–1555. https://doi.org/10.1093/molbev/msy074

Sayer, C. A., Fernando, E., Jimenez, R. R., Macfarlane, N. B. W., Rapacciuolo, G., Böhm, M., Brooks, T. M., Contreras-MacBeath, T., Cox, N. A., Harrison, I., Hoffmann, M., Jenkins, R., Smith, K. G., Vié, J.-C., Abbott, J. C., Allen, D. J., Allen, G. R., Barrios, V., Boudot, J.-P., … Darwall, W. R. T. (2025). One-quarter of freshwater fauna threatened with extinction. *Nature*, *638*, 138–145. https://doi.org/10.1038/s41586-024-08375-z

Seymour, M., Durance, I., Cosby, B. J., Ransom-Jones, E., Deiner, K., Ormerod, S. J., Colbourne, J. K., Wilgar, G., Carvalho, G. R., de Bruyn, M., Edwards, F., Emmett, B. A., Bik, H. M., & Creer, S. (2018). Acidity promotes degradation of multi-species environmental DNA in lotic mesocosms. *Communications Biology*, *1*1(1), 1–8. https://doi.org/10.1038/s42003-017-0005-3

Shelton, A. O., Kelly, R. P., O'Donnell, J. L., Park, L., Schwenke, P., Greene, C., Henderson, R. A., & Beamer, E. M. (2019). Environmental DNA provides quantitative estimates of a threatened salmon species. *Biological Conservation*, *237*, 383–391. https://doi.org/10.1016/J.BIOCON.2019.07.003

Spens, J., Evans, A. R., Halfmaerten, D., Knudsen, S. W., Sengupta, M. E., Mak, S. S. T., Sigsgaard, E. E., & Hellström, M. (2017). Comparison of capture and storage methods for aqueous macrobial eDNA using an optimized extraction protocol: Advantage of enclosed filter. *Methods in Ecology and Evolution*, *8*, 635–645. https://doi.org/10.1111/2041-210X.12683

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

Su, G., Logez, M., Xu, J., Tao, S., Villéger, S., & Brosse, S. (2021). Human impacts on global freshwater fish biodiversity. *Science*, *371*(80), 835–838. https://doi.org/10.1126/science.abd3369

Su, G. Y., Allison, M. J., Beblow, J., Koch, K., Anderson, J., M'Gonigle, L. K., Cleveland, M., Helbing, C. C., & Marlatt, V. L. (2024). Enumeration potential of environmental DNA for Pacific salmon stock assessments. *Environmental DNA*, *6*, e508. https://doi.org/10.1002/EDN3.508

Thorstad, E. B., Økland, F., Aarestrup, K., & Heggberget, T. G. (2008). Factors affecting the within-river spawning migration of Atlantic salmon, with emphasis on human impacts. *Reviews in Fish Biology and Fisheries*, *18*, 345–371. https://doi.org/10.1007/S11160-007-9076-4

Tillotson, M. D., Kelly, R. P., Duda, J. J., Hoy, M., Kralj, J., & Quinn, T. P. (2018). Concentrations of environmental DNA (eDNA) reflect spawning salmon abundance at fine spatial and temporal scales. *Biological*

Conservation, *220*, 1–11. https://doi.org/10.1016/J.BIOCON.2018.01.030

Twardek, W. M., Lapointe, N. W. R., & Cooke, S. J. (2021). High egg retention in Chinook salmon *Oncorhynchus tshawytscha* carcasses sampled downstream of a migratory barrier. *Journal of Fish Biology*, *100*(3), 715–726. https://doi.org/10.1111/JFB.14985

Webb, J. H., & McLay, H. A. (1996). Variation in the time of spawning of Atlantic salmon (*Salmo salar*) and its relationship to temperature in the Aberdeenshire Dee, Scotland. *Canadian Journal of Fisheries and Aquatic Sciences*, *53*, 2739–2744. https://doi.org/10.1139/F96-240

Williams, K. L., Griffiths, S. W., McKelvey, S., & Armstrong, J. D. (2010). Deposition of Atlantic salmon *Salmo salar* carcasses in a Scottish upland catchment. *Journal of Fish Biology*, *77*, 927–934. https://doi.org/10.1111/J.1095-8649.2010.02725.X

Wood, S. N. (2010). Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models. *Journal of the Royal Statistical Society. Series B, Statistical Methodology*, *73*, 3–36.

Wood, Z. T., Lacoursière-Roussel, A., LeBlanc, F., Trudel, M., Kinnison, M. T., Garry McBrine, C., Pavey, S. A., & Gagné, N. (2021). Spatial heterogeneity of eDNA transport improves stream assessment of threatened salmon presence, abundance, and location. *Frontiers in Ecology and Evolution*, *9*, 650717. https://doi.org/10.3389/fevo.2021.650717

SUPPORTING INFORMATION

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

How to cite this article: Perry, W. B., Milner, N., Jâms, I. B., de Bruyn, M., Ormerod, S. J., Deiner, K., Bik, H. M., Carvalho, G., Colbourne, J., Cosby, B. J., Durance, I., & Creer, S. (2025). Quantitative insights into the spatio-temporal variation of Atlantic salmon (*Salmo salar*) biomass in a river catchment using eDNA metabarcoding. *Journal of Fish Biology*, 1–16. https://doi.org/10.1111/jfb.70172