

eDNA-based monitoring metrics for migratory and resident fish following full and partial removal of river barriers

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ABSTRACT

The fragmentation of river systems by artificial barriers is contributing to the global decline of migratory fish species. In response, many barriers are now being considered for removal or mitigation to restore connectivity, but the effectiveness of such interventions is unclear due to a lack of monitoring before and after mitigation. We used two complementary monitoring methods based on environmental DNA (eDNA) analysis, detection (determined by number of positive qPCRs) and abundance (based on the analysis of copy numbers), to assess the response of two migratory fish (the European eel, *Anguilla anguilla*, and the Atlantic salmon *Salmo salar*) as well as the (predominantly) potadromous brown trout (*Salmo trutta*) to the partial or full removal of twelve barriers in five Welsh rivers. We observed species-specific responses, and differences based on the assessment method (detection or abundance) and mitigation type (easement or removal). Trout detection and abundance increased following barrier mitigation, while salmon and eel detection declined, with salmon abundance remaining the same. Analysis using the number of copies offered higher resolution than relying on qPCR positives alone, indicating that eDNA monitoring which relies solely on detection may obscure subtle changes in populations following barrier mitigation. Our abundance estimates comparing the removal and easement of small weirs indicate that full barrier removal leads to an increase in trout abundance one year after mitigation when compared to easement, but even small mitigation interventions of in-stream barriers influence both migratory and resident fish populations, which may benefit from increasing habitat availability and connectivity.

1. Introduction

Artificial in-stream barriers are a common occurrence globally (Birnie-Gauvin et al., 2017b), with over 1.2 million barriers estimated to be fragmenting Europe's rivers alone (Belletti et al., 2020). Many of these were constructed without any ecological consideration (Hogg et al., 2015) and despite their socioeconomic importance (O'Hanley, 2011), their presence is now recognised as a major issue contributing to biodiversity loss in freshwater systems (Reid et al., 2019).

Artificial barriers are one of the most important anthropogenic stressors of river ecosystems (Radinger et al., 2018). Their cumulative impacts pose a significant threat to freshwater fish (Miranda et al., 2022), altering the hydromorphological characteristics of rivers, including natural sediment transport and flow regimes (Lim and Do, 2024; Mueller et al., 2011), which can degrade fish spawning habitats and reduce food availability (Garcia de Leaniz, 2008; O'Mara et al.,

2021). River barriers also reduce longitudinal connectivity and cause the fragmentation of freshwater habitats, preventing the movement of fish within their catchments (Sun et al., 2023). Limiting dispersal in this way impedes access to critical feeding and spawning areas (Barbarossa et al., 2020) and restricts gene flow between previously connected populations (Horreo et al., 2011; Meldgaard et al., 2003). Moreover, even if fish are capable of passing some barriers, the process is time consuming and energy intensive, potentially reducing the energy available for successful spawning (McLaughlin et al., 2013).

River barriers represent a significant challenge for potadromous fish which require free movement within the river system to undertake spawning migrations (Kowal et al., 2024; Kowal et al., 2025) and are also known to contribute to the decline of catadromous and anadromous migratory species, like the European eel (*Anguilla anguilla*) and the Atlantic salmon (*Salmo salar*) (Jubb et al., 2023; Koed et al., 2019; Smialek et al., 2021). Habitat fragmentation is considered one of the

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main factors responsible for the global decline of eels and salmon (Forseth et al., 2017; Jacoby et al., 2015; Nunn et al., 2023; Thorstad et al., 2021), which were classified as critically endangered (European eel) and near threatened (Atlantic salmon) on the 2020 IUCN red list (Pike et al., 2020).

To recognise the impacts of reduced connectivity for freshwater species, the restoration of river habitat connectivity has become a key objective of the European Water Framework Directive (WFD; 2000/60/EC) and the EU Nature Restoration Law. The removal of obsolete barriers, while often challenging, is becoming an important restoration tool (Duda and Bellmore, 2021), which can also have financial benefits as the upkeep costs of maintaining ageing dams can exceed the cost of removal (Bellmore et al., 2017).

Removing barriers is often the only way to fully restore the four dimensions of river connectivity (Dodd et al., 2017; Thieme et al., 2024), but social and political constraints including licensing requirements, insurance, landowner permissions and access, make full removal unfeasible in many cases (Magilligan et al., 2017; McKay et al., 2020), requiring some form of barrier prioritization (García de Leaniz and O'Hanley, 2022). Many barriers are still in use because they provide water supply, transportation, hydropower, irrigation and/or flood control services (Bednarek, 2001; O'Hanley, 2011). Although these barriers may not be suitable for removal, some of their impacts can be reduced with the appropriate interventions. The installation of fish passes, the retrofitting of more passable barriers, and partial removal have all been used as alternatives to full removal (King et al., 2021; Thieme et al., 2024). These strategies are also deployed in projects that seek to mitigate historical barriers where, due to technical or financial limitations, full removal is not possible. Yet, the connectivity benefits achieved by barrier easement are not equal to full removal and frequently only benefit a few target species (Kemp and O'Hanley, 2010; Silva et al., 2018).

The wider context of the river network must also be considered when planning barrier mitigation work, as even the complete removal of a barrier may have little effect for fish connectivity if there are downstream barriers which are impassable for the target species (Kemp and O'Hanley, 2010). Similarly, the mitigation of multiple smaller barriers may also be more cost effective, and improve connectivity to a greater extent than targeting fewer, large barriers (García de Leaniz and O'Hanley, 2022). However, these smaller structures are often the most underrepresented in barrier inventories, as they are challenging to detect remotely (Parks et al., 2024).

It is critically important to understand not only the impact of barriers, but also the response of fish to their removal or mitigation, in order to better prioritise those mitigation efforts that provide the greatest restorative gains (Branco et al., 2014; Kemp and O'Hanley, 2010; Lutter et al., 2024). Accurate monitoring of barrier remediation requires the timely application of appropriate biological monitoring techniques, both before and after mitigation (Muha et al., 2021). To assess the impact on fish species, several methods are available, including electrofishing, netting, tagging and hydroacoustic surveys (England et al., 2021). However, monitoring is rare (Bernhardt et al., 2005; Gardner et al., 2013), likely because of restrictions imposed by local policy such as the requirement for licences (Rees et al., 2014), insufficient technical or financial resources (Kraft et al., 2019), or a lack of knowledge or awareness of local species requirements and life-history traits (Feio et al., 2021). Environmental DNA (eDNA)-based monitoring is a sensitive, non-destructive method for the detection of aquatic species, which has proven to be a powerful tool for assessing the outcomes of barrier removal (Duda et al., 2021; Muha et al., 2021). Although still requiring specialised knowledge, eDNA can offer a more affordable, non-invasive, alternative to conventional methods which requires no licensing permits (Harper et al., 2019; Penaluna et al., 2021).

Here, we use two eDNA quantitative-PCR (qPCR) approaches, one based on species detections (measured by the number of positive qPCRs), and the other estimating abundance (based on read copy

numbers), to assess the response of two migratory fish species (Atlantic salmon and the European eel) and a freshwater resident (potadromous) fish (brown trout), to the mitigation of twelve barriers in five Welsh rivers. We compare the effectiveness of barrier mitigation and full barrier removal, providing evidence to inform future mitigation and monitoring programmes.

2. Methods

2.1. Study sites, water collection and DNA extraction

Twelve redundant barriers (three culverts, seven weirs, and two bridge footings) were identified as suitable targets for removal or easement across five catchments in South Wales (Fig. 1). These sites were selected as there were no barriers present downstream which were impassable for the three target species under normal flow conditions. These barriers were mitigated over two years. Pre-mitigation water samples were collected during April–May 2022 for eight barriers and during April–May 2023 for the remaining four. Post-mitigation water samples were taken around one year after mitigation, i.e. in May 2023 or 2024. Care was taken to ensure that samples were collected at approximately the same time each year to minimise seasonal variation, which has been shown to greatly influence eDNA composition and yield (Muha et al., 2021). Sampling dates and further site information are included in Table 1 and site images are included in fig. S1.

At each site, three field replicates of 1 L water samples were collected upstream, and downstream of each barrier. Samples were taken as close to the barrier (or where the barrier used to be) as was accessible without entering the river. Water was filtered on site through 0.45 µm cellulose nitrate filters (Sartorius) inserted into a filter funnel (Nalgene) using a peristaltic pump modified for use with a battery powered drill. To minimise potential contamination by eDNA carry-over between sites, all disposable equipment was discarded after use and all shared kit was disinfected with Virkon (Antec International Limited) and commercial bleach. A field blank consisting of 1 L ultra-pure water was taken at each site. After each sample was taken, the filters were carefully removed from the filter funnel using sterile forceps and placed in a tube containing Qiagen lysis buffer. Ice packs were used to keep the sample tubes cold during transport, upon arrival, samples were stored frozen and processed as soon as possible. A Hanna 9829 multiparameter probe (Hanna instruments) was used to collect water chemistry parameters including temperature (°C), pH and dissolved oxygen (DO, mg/l) at each site.

For the 2022 sampling, amplifications of salmon and trout eDNA were carried out within two weeks of the extraction, but the eel analysis could not be carried out until a year later and these samples were excluded from all subsequent eel analysis; this includes sites USK16, USK16d, USK12a, TF8, EC13, EC13a, EC9, WC7. Only sites A4, A5, A6 and Uskextra2 were kept for the eel analyses as their pre, and post mitigation samples were extracted and analysed immediately after collection. eDNA quality remained consistent for all salmon and trout amplifications, for which both pre and post mitigation were extracted and processed within 2 weeks. Therefore, samples were analysed from twelve sites for brown trout and Atlantic salmon, and four sites for European eel.

2.2. DNA extraction and amplification

eDNA was extracted from the collected filters using a DNeasy Powerlyzer Powersoil kit (Qiagen) following the manufacturer's instructions with a 30 µL elution step. An extraction blank was included in each batch of samples to detect potential contamination. Extractions were carried out within a designated laminar flow hood which was sterilised with bleach and UV disinfected for at least 20 min before use. A targeted qPCR approach was used to test for the presence of three target species, brown trout, Atlantic salmon, and European eel. Brown trout is

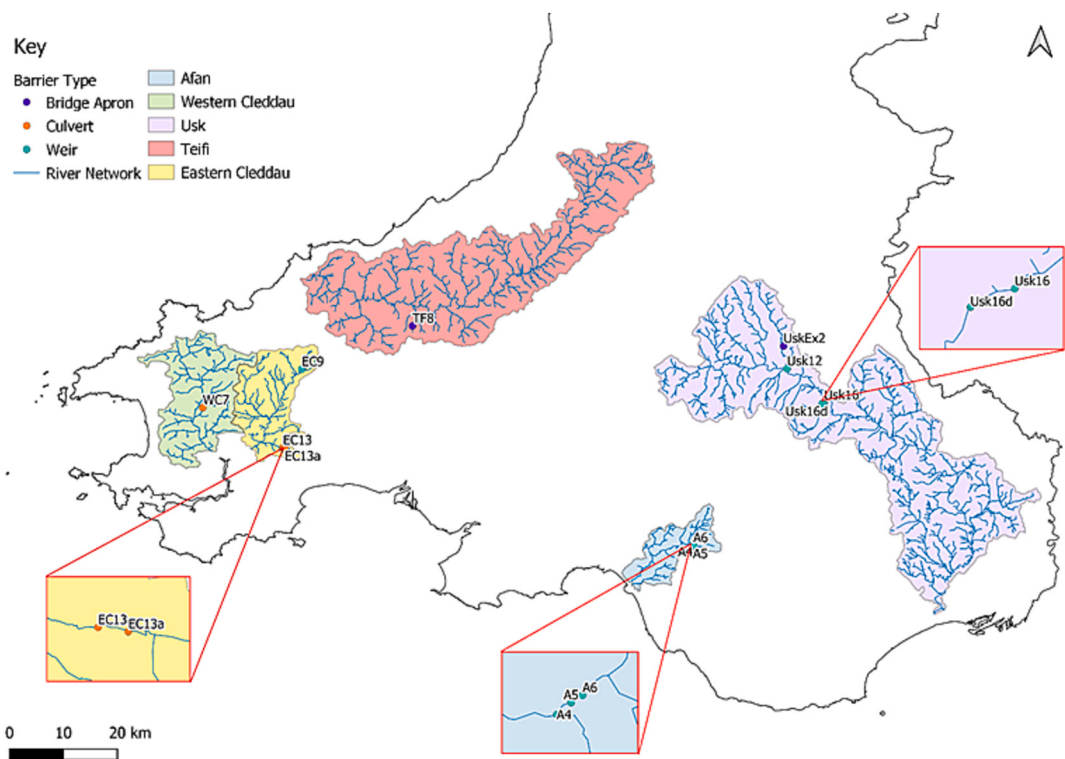


Fig. 1. Map of targeted barriers within Welsh river catchments, including barrier types and river networks.

Table 1
Characteristics of barriers targeted for mitigation and monitored in this study.

Site	Grid reference	Date sampled pre-mitigation	Action	Date sampled post-mitigation	Barrier type	Approximate distance to river mouth (km)
EC13	SN1087614087	20/04/2022	Pre-barrage	04/05/2023	Culvert	12.5
EC13A	SN1120614041	20/04/2022	Pre-barrage	04/05/2023	Culvert	12.9
EC9	SN1436428638	13/04/2022	Removal	09/05/2023	Weir	28.2
TF8	SN3489236649	27/04/2022	Pre-barrage	11/05/2023	Bridge footing	36.4
USK16	SO1145622578	04/05/2022	Pre-barrage	03/05/2023	Weir	85.3
USK16d	SO1089222300	05/05/2022	Baulk attached	03/05/2023	Weir	86
WC7	SM9602421520	11/04/2022	Removal	09/05/2023	Culvert	18.1
USK12a	SO0436728754	29/04/2022	Notch	09/05/2023	Bridge footing	97.5
A4	SS8686395898	02/05/2023	Removal	08/05/2024	Weir	18
A5	SS8692895952	02/05/2023	Removal	08/05/2024	Weir	18.1
A6	SS8698095985	17/04/2023	Removal	08/05/2024	Weir	18.2
UskEx2	SO0372132944	17/04/2023	Removal	09/05/2024	Weir	103.6

ubiquitous in Welsh rivers and as most populations are freshwater resident, it served as an additional positive control to assess the quality of the eDNA after storage, filtration, and extraction.

The presence of brown trout was tested using a qPCR based TaqMan assay outlined in [Matejusova et al. \(2007\)](#). A 95 bp segment of the cytochrome oxidase B (cytb) region was targeted using a universal salmonid forward primer and a brown trout specific reverse primer and probe. Each site was then tested for the presence of Atlantic salmon. For this, a 96 bp segment of the same region was targeted using the salmonid forward primer with an Atlantic salmon specific reverse primer and probe. The reaction mixes for both assays consisted of 12.5 µL TaqMan fast advance master mix (Applied Biosystems), 5.5 µL water, 2.25 µL of each primer, 1 µL probe and 1.5 µL of DNA in a total well volume of 25 µL. qPCR was performed using a Bio-Rad CFX96 thermal cycler and began with a 3-min denaturation step at 95 °C, followed by 50 cycles of 95 °C for 10 s, 58 °C for 60 s, and 72 °C for 60 s. Extracted eDNA was also analysed for the presence of European eel using the TaqMan assay designed by [Halvorsen et al. \(2020\)](#) targeting a 101 bp region of the cytb gene. Reactions consisted of 2.25 µL primer, 1.5 µL probe, 4.5 µL water, 12.5 µL TaqMan fast advance master mix and 2 µL template DNA in a 25

µL reaction volume. qPCR began with a 95 °C denaturation for 3 min, followed by 50 cycles of 95 °C for 15 s, 57 °C for 30 s and 72 °C for 60 s. Primer and probe sequences are included in [Table 2](#).

Samples were tested in triplicate and each qPCR-plate contained field blanks, extraction blanks, negative controls, and positive controls. Positive control oligonucleotides consisting of a known concentration of the target region were synthesised for each species (G blocks, IDT) ([Han](#)

Table 2
Primer and probe sequences used for the detection of *Salmo trutta* and *Salmo salar*.

Name	Sequence
Salmonid Forward	5'-CGG AGC ATC TTT CTT CTT TAT CTG T-3'
<i>Salmo trutta</i> Reverse	5'-CTC CGA TAT TTC AGG TTT CTT TAT ATA GG-3'
<i>Salmo salar</i> Reverse	5'-ACT CCG ATA TTT CAG GTT TCT TTA TAT AGA-3'
<i>Salmo trutta</i> Probe	5'-VIC-CGA GGA CTC TAC TAT GGT TC-MGB-3'
<i>Salmo Salar</i> Probe	5'-FAM-CGA GGA CTT TAT TAT GGT TC-MGB-3'
<i>Anguilla anguilla</i> Forward	5'-CAC CCA TAC TTC TCC TAC AAA GAC CTA-3'
<i>Anguilla anguilla</i> Reverse	5'-TCT GGG TCT CCA AGC AGG TT-3'
<i>Anguilla anguilla</i> Probe	5'-FAM-TTC ATT ATC ATG CTC ACC-MGBEQ-3'

et al., 2023). Limit of detection (LOD) and limit of quantification (LOQ) were calculated for each assay individually, following the recommendations and the R script provided by Klymus et al. (2020). Copy number was calculated by multiplying the number of moles of each oligo (provided in the specification sheet) by Avogadro's number (Boulter et al., 2016). This number was then divided by the resuspension volume to obtain the copy number/ul. Quantification was achieved by converting the ct value to copy number using the equation $ct = \text{slope} \times \log \text{copy number} + \text{Intercept}$, where the slope and intercept were obtained from a linear regression of the standard curve (Smith et al., 2006). We used a $10 \times$ serial dilution series of positive control oligo to form standard curves which ranged from 9.3×10^7 to 9 copies in Trout, 1.2×10^9 to 12 copies in Salmon and 1.45×10^9 to 14 copies in eel. A standard curve was included in each analysis plate to ensure consistency in amplification for the assays over time.

2.3. Statistical analysis

Two strategies were used to statistically assess the response of fish to barrier mitigation via eDNA monitoring: using 1) the total number of positive qPCRs (detection) and 2) the number of copies (abundance). All statistical analysis was performed in R (R version 4.3.0).

Collinearity between the environmental variables pH, temperature and dissolved oxygen (DO) was then determined using the `check_collinearity` function of the *performance* package. This determined low correlation for pH (VIF = 1.66) and temperature (VIF = 2.92), however DO was excluded from subsequent analysis due to its higher VIF of 3.9 and larger number of missing values.

1) Analysis using the total number of positive qPCRs.

For each species, we first tested if variation between technical replicates (qPCRs) needed to be accounted for by comparing the fit of a Generalised Linear Mixed Effect (GLMM) model that included replicate ID nested within site, with a model that only included site as a random effect using the `glmer` function of the *lme4* package (Bates et al., 2015) and the `anova` function. This identified if replicates could be pooled for subsequent analysis.

For each species, we then modelled the total number of replicates that were positive for that species (of a possible nine when technical replicates were not pooled, or three when replicates were pooled) as a binomial response with the fixed factors sampling position (upstream vs downstream), sampling time (before vs after mitigation), and the interaction between sampling position and sampling time as predictors. These models were then compared to mixed models (GLMM) with the addition of site as a random factor. We decided to include site as random effect for all species, even if there were 12 tested sites for salmon and trout, but only four sites for eel, as we wanted to account for potential site variation influence which could still be biologically meaningful (Gomes, 2022). Additionally, the Akaike information Criterion (AIC) was used to compare models with and without random effects, and the most appropriate was selected. Collinearity between variables was checked using the `cor` function and the `check_collinearity` function in the *performance* package (Lüdtke et al., 2021).

2) Analysis using copy number.

These analyses were only carried out for salmon and trout as the copy numbers for eel, based on the synthetic oligo, were inconsistent between plates.

As with previous analysis, we first tested if individual technical replicates (qPCRs) needed to be included in the model as a random factor. For this, a generalised linear mixed effect model (glmm) with the tweedie distribution was compared to one without qPCR replicate with the `anova` command using the `glmmTMB` function from the *glmmTMB* package. Glmm with tweedie distribution were used for all subsequent

models for each species and included sampling position (upstream/downstream), sampling time (pre/post mitigation), and the interaction between sampling position and sampling time, as above. Models with and without site as a random factor were compared and the model providing the best fit was determined based on AIC comparisons.

Both types of analysis (total qPCR and copy number) for all species were repeated including the additional environmental variables measured in ten of the sites (excluding A6 and USK16 where these values could not be collected pre-mitigation). The `glmulti` function of the *glmulti* package (Calcagno and de Mazancourt, 2010) was used for automatic model selection. For each species, the most plausible model from *glmulti* was then compared to an identical model containing site as a random factor using AIC using *anova*.

3) Effect of the type of mitigation.

Weirs were the only barrier type in the study that offered the potential to observe a direct comparison between removal and easement, as the other barrier types (culverts and bridge footings) had too few replicates to accurately compare intervention strategies. Seven weirs were mitigated in the study, five of which were removed, and two mitigated through the addition of a pre-barrage and a baulk respectively. In order to test the effects of the intervention type (removal or easement) on both the detection (number of positive qPCRs) and abundance (copy number) of trout and salmon, data was first subset to include only post removal values for weirs, each species was then tested individually. To determine the influence of mitigation type on the qPCR detection data, the total number of replicates that were positive for each species (of a possible nine) were modelled as a binomial response using the `glmmTMB` function (binomial family) of the *glmmTMB* package. For abundance data, *glmmTMB*s were also used with the same factors but with a tweedie distribution family. All models contained only intervention as fixed factor, models were then repeated with the addition of catchment as a random effect, the final, most appropriate model was then selected based on AIC.

Figures were created using *ggplot2* (Villanueva and Chen, 2019) and the package *emmeans* (Piaskowski, 2025) was used to generate posthoc significance between sites in the figures using the Holm correction for multiple testing (Holm, 1979).

3. Results

The average efficiencies and R^2 values were within acceptable ranges (Taylor et al., 2010), 101.8% and 0.995 for the trout assay, 95.9% and 0.990 for the salmon assay and 95.75% and 0.994 for eel. The LOD for the trout assay was determined to be below the minimum standard of 9 copies as every replicate at this standard amplified, the LOQ for the assay was 37 copies. For salmon, the LOD and LOQ were determined to be 151 copies. For eel, the limit of detection was 8.4 copies, and the limit of quantification was 21 copies. Any amplifications with copy numbers below the LOD were regarded as absences for the presence analysis, this removed 25 pre-mitigation and 14 post-mitigation technical replicates (qPCRs) for trout, and 3 pre-mitigation and 2 post-mitigation replicates for salmon.

At some sites there were amplifications below the LOQ but above the LOD, this occurred in five pre-mitigation and five post-mitigation sites for trout (pre-mitigation: TF8, USK16, USK16D, WC7, USKEX2; post-mitigation: A4, TF8, USK16, USK16D, WC7) and in two pre-mitigation sites and one post-mitigation site for salmon (pre-mitigation: USK12, WC7; post-mitigation: EC9).

Of the twelve tested barriers, only six were fully removed, the remaining barriers were mitigated through the installation of a pre-barrage, baulk, or a notch. We considered a species to be present at a site if at least two positive qPCR replicates were observed within the triplicate qPCRs of a single field replicate. This threshold was intended to minimise false positives. Following this criterium, brown trout were

present upstream and downstream of every barrier except WC7 and continued to be present at the same eleven sites after barrier mitigation. Atlantic salmon was present in seven of the twelve sites pre-mitigation, both upstream and downstream at four sites, only downstream at two sites, and only upstream at one site. Post mitigation, salmon were detected at only two sites, upstream and downstream of one barrier, and only upstream of another barrier (Fig. S2). Eel were present at all four sites pre-mitigation, both upstream and downstream of three barriers and only downstream of one other. Post mitigation, eel were present in two of the four sites, both upstream and downstream of one barrier and only downstream of the other (Fig. 2, Fig. 3).

1) Analysis using the total number of positive qPCRs.

Model comparisons showed that nesting the technical replicates within the site variable did not improve the model fit: Trout ($X^2_1 = 0$, $p = 1$), salmon ($X^2_1 = 0.792$, $p = 0.374$), eel ($X^2_1 = 0$, $p = 1$) AIC showed minor differences between models (within 2 AIC). Therefore, the sum of positive qPCRs was used for each field replicate, providing three upstream and three downstream values for each species. The proportion of positive qPCR detections, relative to the total number of qPCRs was then modelled as a binomial response.

After comparison using AIC, including site as a random factor improved the model fit for both trout and salmon but not for eel. In all three models the sampling time and position interaction term was not significant and was therefore excluded. For trout, sampling time and therefore barrier mitigation, was associated with a significant increase ($Z_{44} = 5.420$, $p < 0.001$). In contrast, sampling position ($Z_{44} = 1.111$, $p = 0.267$) did not have a significant impact. For salmon, the number of qPCR positives declined after mitigation ($Z_{44} = -4.850$, $p < 0.001$), sampling position was also not a significant predictor ($Z_{43} = -0.143$, $p = 0.886$). For eel, the number of positive qPCRs also decreased after mitigation ($Z_{13} = -3.081$, $p = 0.002$) and sampling position was significant ($Z_{13} = -2.415$, $p = 0.015$) with more qPCR detections downstream of the barrier.

Analysis was repeated using the subset of samples for which both temperature and pH were collected alongside the variables used previously. The glmulti function was then used to simplify the model. For trout, the best model included sampling time and pH. In salmon the best model included sampling time, temperature and pH and for trout, the model containing the terms sampling time and sampling position. As above, including site as a random factor improved the model fit for trout and salmon, but not for eel.

For trout, the number of qPCR positives increased after mitigation

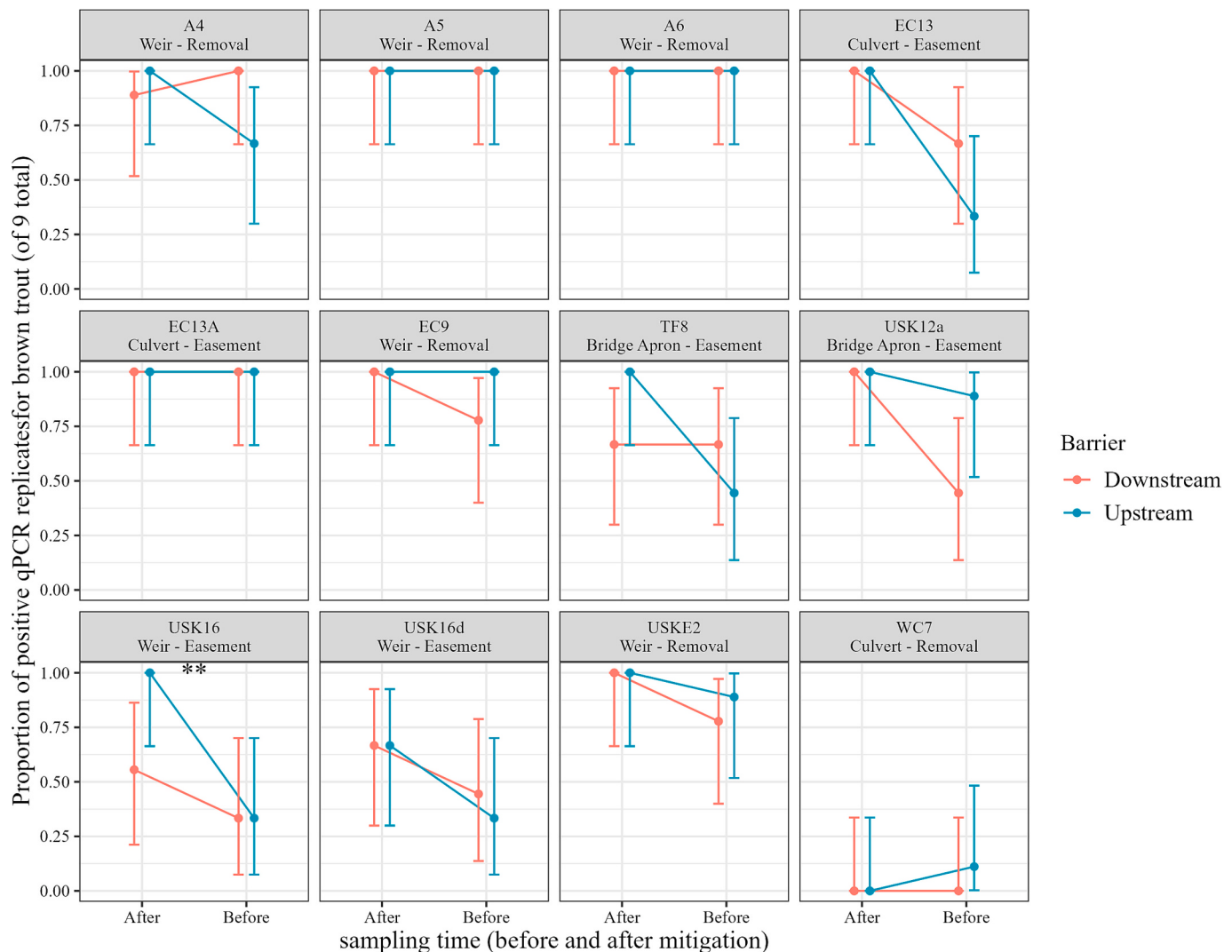


Fig. 2. Mean proportion of positive qPCR replicates (of a total 9) for trout (a), salmon (b), and eel (c) across each site, before and after mitigation, including 95% binomial confidence intervals, Fig. 2c includes only the four sites in which eel could be tested. Barrier type and mitigation strategy are included in the titles of each sub-plot. Significant differences between pre and post mitigation at each site are also included (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$).

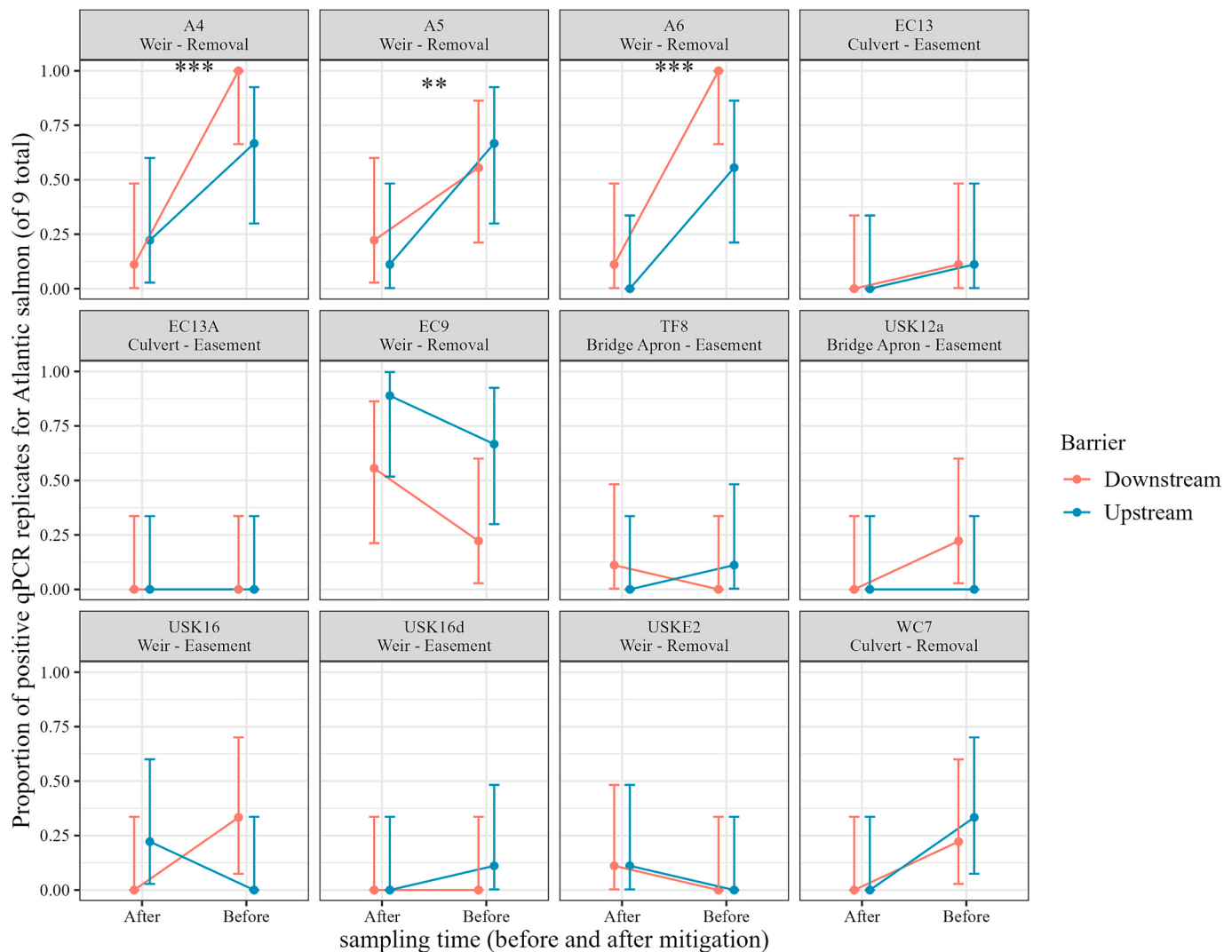


Fig. 2. (continued).

($Z_{40} = 4.332$, $p < 0.001$). pH ($Z_{40} = 0.704$, $p = 0.481$) was not a significant factor. For salmon, detection declined after barrier mitigation ($Z_{39} = -5.452$, $p < 0.001$), and increased alongside temperature ($Z_{39} = 4.147$, $p < 0.001$), pH was not significant ($Z_{39} = -1.517$, $p = 0.129$). Barrier mitigation was also associated with a decline in detection for eel ($Z_{11} = -3.407$, $p < 0.001$), sampling position was also significant with more eel detections downstream of the tested barriers ($Z_{11} = -2.785$, $p = 0.005$).

2) Analysis using copy number.

A comparison between models with and without nested replicates showed a significant difference for both species. Trout ($X^2_1 = 4703.4$, $p < 0.001$), salmon ($X^2_1 = 8961.2$, $p < 0.001$). Replicates were kept separate for all subsequent abundance analysis. As with the above analysis, the sampling time and position interaction terms were not significant in any models and therefore excluded.

Including site as a random factor provided the better fit based on AIC in both trout and salmon data. For trout, there was a significant increase in copy number following barrier mitigation ($Z_{138} = 4.738$, $p < 0.001$), sampling position was also significant ($Z_{138} = 2.333$, $p = 0.02$), with higher copy numbers detected upstream of the tested barriers. For salmon, no factor was a significant predictor of abundance, sampling time ($Z_{138} = -1.186$, $p = 0.236$), sampling position ($Z_{138} = 0.298$, $p =$

0.766).

A model including pH and temperature as additional factors were then performed. After using glmulti, the best model for trout contained the predictors sampling position, and sampling time. AIC determined that including the random factor site improved the model fit. Copy number increased after barrier easement ($Z_{138} = 4.738$, $p < 0.001$) and a higher number of copies were detected upstream of the barriers ($Z_{138} = 2.333$, $p = 0.02$).

A glmulti performed on the same variables for salmon, determined that the best model contained sampling time, temperature and pH. AIC determined that including site improved the model fit. Increased temperature was associated with an increase in salmon copy number ($Z_{125} = 2.190$, $p = 0.03$) but pH ($Z_{125} = -0.218$, $p = 0.827$), and sampling time ($Z_{125} = -1.690$, $p = 0.09$) were not significant.

3) Effect of the type of mitigation.

Models assessing the effect of mitigation type (removal or easement) were performed on both the qPCR detection and the copy number data. For qPCR analysis, the sum of qPCRs that tested positive for each site was used for each species separately, for the copy number data, replicates were kept separate. AIC was used to determine the models that provided the best fit, for the trout qPCR and salmon abundance (copy number), this model excluded the random factor catchment. In the

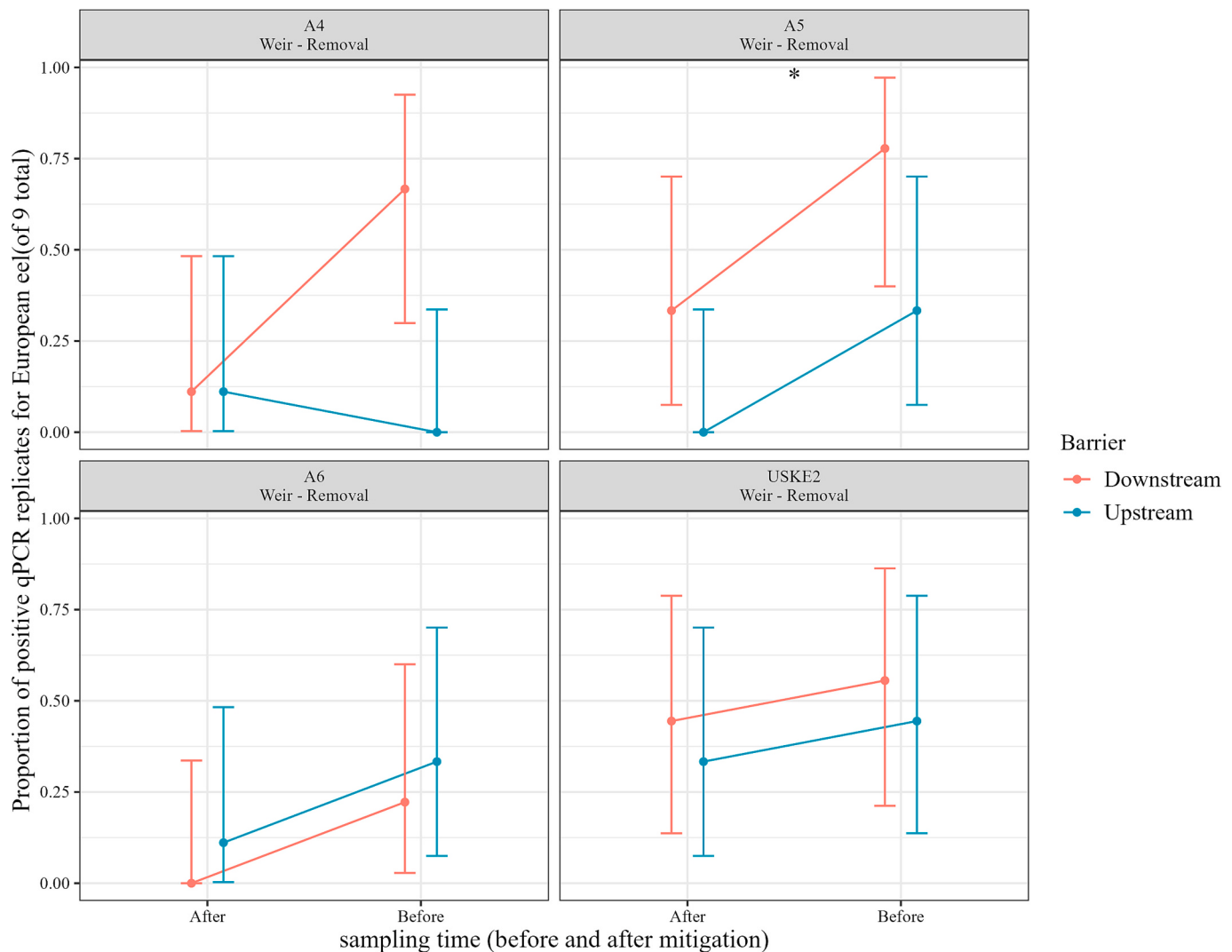


Fig. 2. (continued).

remaining models (Salmon qPCR and trout abundance), catchment was included. These models determined that full removal of weirs caused a significant increase in copy number for trout ($Z_{37} = 2.627$, $p = 0.009$) and salmon ($Z_{38} = 2.421$, $p = 0.015$) both upstream and downstream of the barrier, this was also observed in the number positive qPCRs for trout ($Z_{12} = 3.295$, $p < 0.001$), but not in the number of positive qPCRs for salmon ($Z_{11} = 1.049$, $p = 0.294$). This could not be tested in the eel dataset as it only included fully removed weirs so comparisons between interventions was impossible.

4. Discussion

We assessed how the mitigation of twelve barriers impacted the detection (total number of qPCRs) and abundance (copy number) of trout, salmon, and eel, and found differences between species, site, mitigation type, and method of analysis. When analysis included all barrier types, and all potential mitigation methods, the detection and abundance of trout increased after mitigation, but while salmon and eel detection decreased, salmon abundance was not significantly influenced by barrier mitigation. Furthermore, when barrier intervention methods were directly compared across the seven targeted weirs, full removal was associated with an increase in trout detection and an increase in the abundance of both trout and salmon compared with mitigation. Salmon detection did not change.

Brown trout are widespread across the United Kingdom (Freyhof, 2024) and were used as an internal control to compare responses of salmon and eel, as they were expected to be present at similar levels at each site. Total qPCR analysis determined that trout were present both upstream and downstream of all but one site in this study. An increase in both the detection and abundance of trout was observed after barrier mitigation. Salmon showed a different response as they were present in seven sites before mitigation, but only two after mitigation. However, this significant decline was only observed in the detection analysis, in the areas where salmon were present, the abundance did not change after mitigation. Although eel abundance could not be confirmed, their detection also declined following barrier mitigation. Previous studies have shown that the response of fish to mitigation projects is species-specific, and it may take many years for fish numbers to increase and stabilise following intervention (Bubb et al., 2021; Poulos et al., 2014). Differential responses are based on a number of factors including alternative life histories, habitat requirements and spawning requirements (Flinn et al., 2025). Both salmon and eel are migratory, salmon are known to migrate upstream to fast flowing, headwater streams to find suitable spawning habitat (Lazzaro et al., 2017). A reduction in detection but no change in abundance suggests that individuals might have been able to redistribute within the catchment (Pess et al., 2014) (Fig. 4). A similar effect is expected for eel which also undergo complex migrations (Wright et al., 2022). A decrease in the

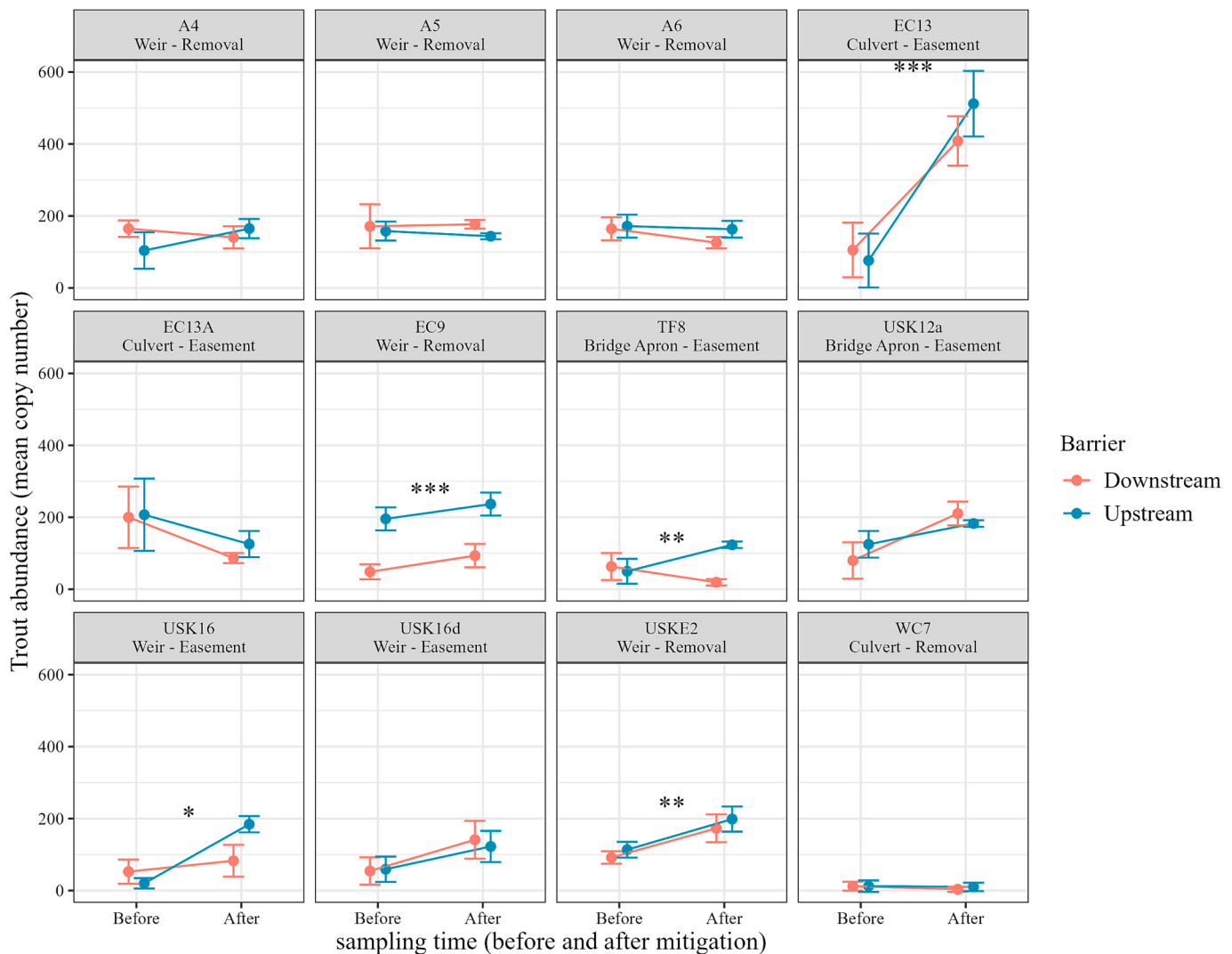


Fig. 3. Change in trout (a) and salmon (b) average copy number at each site, upstream and downstream of each barrier, before and after removal including 95% confidence intervals. Barrier type and mitigation strategy are included in the titles of each sub-plot. Significant differences between pre and post mitigation at each site are also included (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$).

occurrence of positive qPCRs could also be linked to poor recruitment and unfavourable environmental conditions for that particular year, but this can only be determined through a continued sampling effort for several years after barrier mitigation.

An increase in the detection and abundance of salmon was linked to increasing temperature (Fig. S3), however the degrading effects of higher temperatures on eDNA are well understood (McCartin et al., 2022). The observed increases could therefore be associated with species preferences. It is important to note that the collected environmental values are spot measurements taken at the time of eDNA collection which may not reflect the environmental conditions experienced by the target species, or the persistence of eDNA in the rivers. Follow up sampling would be needed to separate the effects of mitigation from background noise generated by annual changes in river conditions and their possible effects on eDNA detection. Critically, the absence of salmon detections post-mitigation is unlikely to have been caused by technical factors (such as eDNA degradation) as the same samples were used for all species and brown trout detections remained high over time and across sites.

Brown trout exhibit a variety of life histories, but are most commonly resident in the study rivers, remaining in the river system for their

lifetime (Ferguson et al., 2019; Smialek et al., 2021). In this case, the increase in both detection and abundance observed after barrier mitigation, suggests that previously isolated populations could now be able to expand their range (Fig. 4), leading to increases in density and a broader spatial distribution. The remediation of a barrier promotes a shift in the river dynamics and can change the habitat immediately upstream from a more lentic to lotic environment, which trout prefer (Birnie-Gauvin et al., 2017a). As the local brown trout are now able to pass the barrier in both directions, the mitigation of the barrier and the creation of more preferable habitat could therefore be encouraging the arrival of trout from further upstream and downstream.

While our results could be consistent with positive responses to barrier mitigation, sampling was limited to before and one year after mitigation, it is therefore not possible to rule out that changes in fish presence and abundance caused by the mitigation of the barrier from natural changes in recruitment. Continued monitoring over multiple years is essential to confirm whether the observed changes reflect long term benefits of barrier mitigation or natural population variability.

Anthropogenic barriers are present in every river catchment in Europe (Belletti et al., 2020), and have contrasting impacts on river fragmentation (Jones et al., 2021). As barrier mitigation projects are

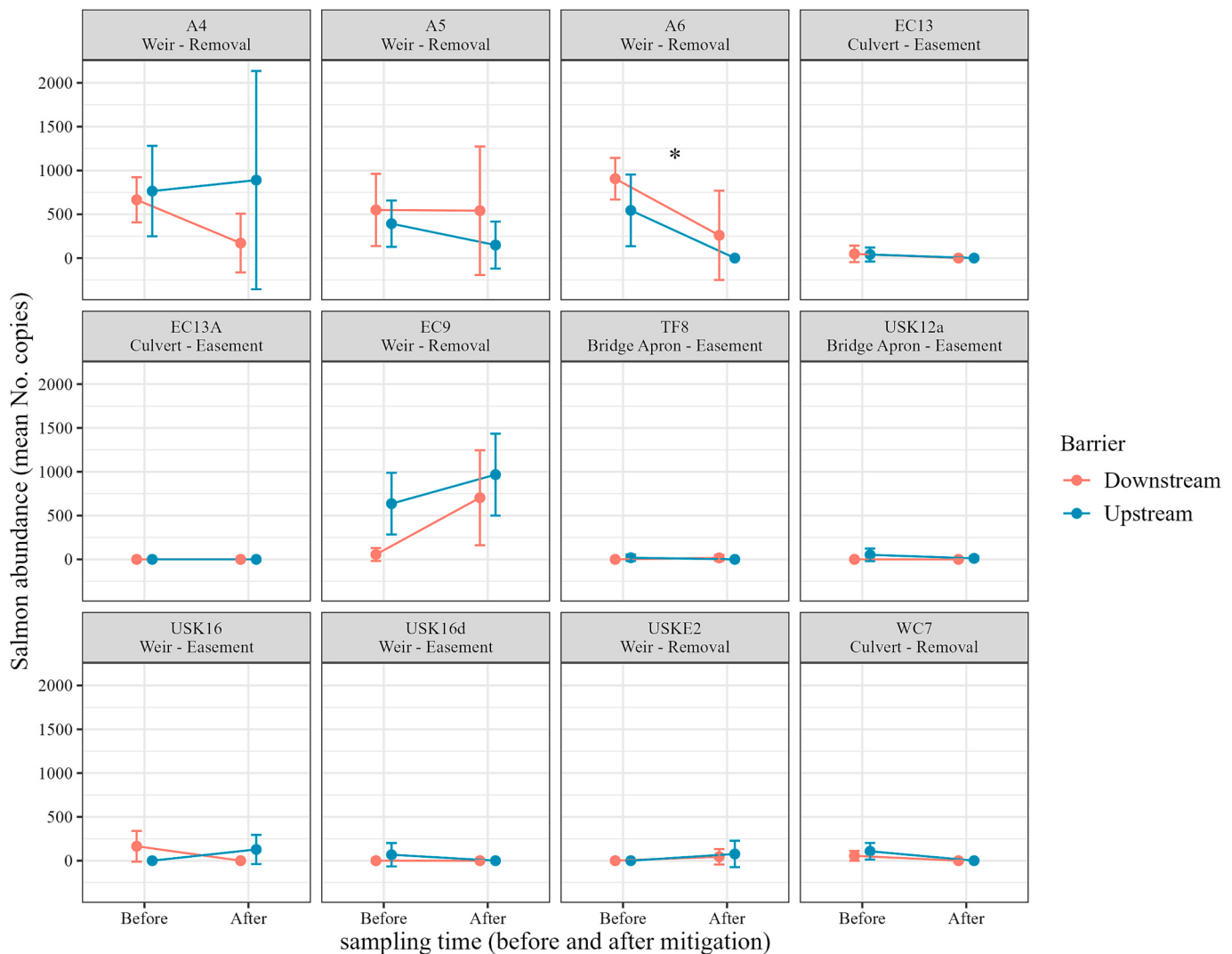


Fig. 3. (continued).

cost- and energy-intensive, they need to be prioritised to maximise results. When comparing the impact of alternative methods of barrier mitigation, albeit based on a subset of data with a very small sample size (seven barriers), we found that full weir removal led to an increase in both trout and salmon copy numbers compared to alternative mitigation strategies (the addition of a notch or baulk), suggesting that full removal is more effective at improving conditions for salmonid occupancy. This effect, however, was not observed with the salmon detection analysis. With a maximum of nine replicates (three water samples analysed in triplicate qPCRs), it is possible that detection based on qPCR presence/absence at this scale could not provide the resolution needed to compare fine changes between the mitigation methods which was possible through quantification. qPCR detection analysis, therefore, despite being useful for broad-range species assessments, can obscure changes in population abundance, especially near detection thresholds (Bohmann et al., 2014). While detection has proved useful to assess the presence and absence of target species in relation to fragmentation by barriers (Consuegra et al., 2021), it may not be sensitive enough for accurate monitoring of fish responses to barrier removal and should be paired with copy number analysis.

Multiple technologies are currently used for the monitoring of barrier mitigation projects, and their success varies depending on the knowledge of the mitigation project team and their budget. These can include traditional survey methods such as electrofishing, netting, and

red counts (Kemp and O'Hanley, 2010), or molecular methods, such as eDNA metabarcoding (Civade et al., 2016). Each method however, has its limitations; for example, it is possible for rarer animals to be missed during electrofishing (Ficetola et al., 2015) or fyke netting (Eissenhauer et al., 2025). Similarly, highly sensitive eDNA monitoring assays require considerable attention to detail to prevent contamination, particularly in projects involving multiple sites and shared equipment (Goldberg et al., 2016). There is also potential for the abundance of a species to be incorrectly inferred from eDNA assessments. For example, eDNA can wash downstream in lotic areas or pool in lentic areas (Mächler et al., 2019). When using eDNA to detect the presence of a species downstream of a barrier, the potential for that eDNA to originate from an individual upstream of the barrier must be considered. (Deiner & Altermatt, 2014; Pont et al., 2018). However, many factors influence the persistence of eDNA in the water column, including the amount and size of the eDNA itself and environmental parameters such as mineralogy, microbial activity, flow conditions, temperature and pH (Chipurro et al., 2022; Shogren et al., 2017). Standardised eDNA collection, extraction and analysis protocols are required to ensure results are comparable within and between mitigation projects.

Both eDNA detection and abundance analysis concluded that sampling position, upstream or downstream of each barrier, had no impact on salmon, before or after mitigation. This could suggest that the low head-height barriers mitigated as part of this study were not major

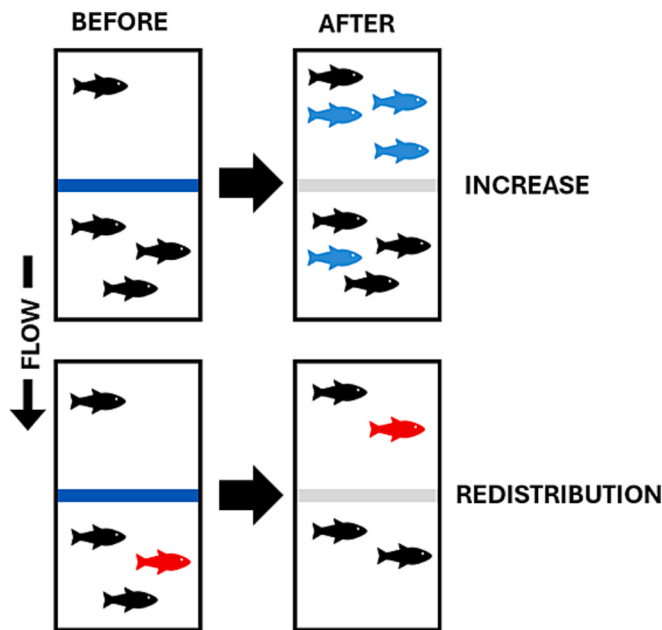


Fig. 4. Two stages in the response of fish to barrier mitigation. In the first stage, fish are merely redistributed and able to exploit novel habitats upstream of the former barrier. In a second stage, there is a genuine increase in abundance both upstream and downstream of the former barrier.

obstacles to movement for this species. Sampling position was however a significant predictor of eel detection and trout abundance, but they were both affected in different ways. Brown trout abundance was higher in the upstream samples before mitigation, which could reflect species specific habitat preferences and a limited distribution downstream. In one case there was only presence of trout upstream after removal of the barrier (WC7, only one positive qPCR downstream). As in this site the detection was also low pre-removal, this result could potentially have been caused by upstream movement of the trout and downstream dilution of the eDNA due to flow increase after removal. However, in this case, poor eDNA quality (which trout is mainly used as a control for) is unlikely, considering the positive detection of Atlantic salmon. Conversely, eel detection was higher downstream of the barriers, which suggests that the subset of barriers which were tested for eel may restrict their movement to a greater extent than they do for salmonids, likely due to their comparatively lower swimming capacity (Jones et al., 2020; Russon and Kemp, 2011). European eel can respond well to barrier removal. Sun et al. (2021) found a significant increase in eel numbers five months after removal of a tidal weir, most likely as a result of their redistribution within the catchment (Bubb et al., 2021). However, this was not the case in the rivers tested as part of this study, which could relate to site-specific physical or behavioural factors preventing eel redistribution.

In summary, our study indicates that relying solely on presence/absence by qPCR detection may not fully capture the response of fish to barrier mitigation, potentially underestimating subtle differences between species and sites. eDNA offers a powerful, non-destructive method of assessing the effect of barrier mitigation, but different analytical approaches can draw different inferences. The effects of barrier mitigation can be highly species-specific (Bubb et al., 2021), but our results indicate that mitigation by full removal is most likely to increase passability and maximise ecological connectivity and should be aimed for whenever possible. Monitoring before and after mitigation, is needed to properly assess the benefits of barrier mitigation and inform the prioritization of future mitigation efforts.

CRediT authorship contribution statement

Benjamin Overland: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Millicent V. Parks:** Writing – review & editing, Investigation. **Carlos Garcia de Leaniz:** Writing – review & editing, Supervision, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Sofia Consuegra:** Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Ethics approval

The experimental plan was approved by the College Ethics Committee/AWERB Group at Swansea University (AWERB IP Reference: 12023 8075 6909).

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ecolind.2026.114694>.

Data availability

All the data is included in the main text and supplementary material

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