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# Characterizing Rocky Intertidal Biodiversity Using Environmental DNA Metabarcoding From Local to National Scales

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## ABSTRACT

Efficient and scalable methods for monitoring marine biodiversity are critical for understanding ecological change in coastal environments, given the limited resources available. Environmental DNA (eDNA) metabarcoding shows promise for monitoring coastal taxa, but its ability to differentiate communities from different locations remains insufficiently understood, particularly in dynamic marine environments. Here, we evaluate the effectiveness and resolution capacity of eDNA metabarcoding in detecting rocky intertidal taxa across three spatial scales—national, regional, and local—in the United Kingdom. Onshore surface-water samples were collected from 32 sites across five UK Regional Seas from rockpools in high and low shore zones, as well as directly from the sea. We detected 1026 target taxa within 442 families and 19 phyla using two established markers targeting invertebrates (COI) and macroalgae (18S). Distinct eDNA signals were found at all spatial scales, indicating local discreteness even between vertical shore heights within the same sites. Communities were more discrete at larger scales (i.e., between regions) than at smaller scales (i.e., between shore heights). eDNA signals were more strongly structured by geographical location than by vertical shore height as a probable consequence of greater DNA homogenization over the tidal cycle at smaller spatial scales. Established ecological zonation patterns were reflected in eDNA signals, with higher richness at lower shore heights, reflecting abiotic stress gradients. Detections of cold-affinity boreal species increased with latitude, while warm-affinity lusitanian species declined with latitude. Our work supports the utility of eDNA metabarcoding for multiscale biodiversity monitoring in dynamic marine environments and for detections beyond this study's target taxa. We recommend the adoption of scale-appropriate sampling protocols to optimize the benefits of eDNA, such as prioritizing open water sampling at high tide for broad-scale assessments and rockpool sampling at low tide for capturing local-scale patterns. Future work should validate detections through direct visual comparisons.

## 1 | Introduction

Monitoring marine biodiversity at multiple spatial scales (e.g., local, regional, or national scales) offers a more comprehensive understanding of biological community responses to environmental change compared to single-scale approaches (Perry and Ommer 2003; Anderson 2018). For example, large-scale monitoring can reveal changes in the biogeographic distributions of marine species, such as shifting range edges, while small-scale monitoring can uncover local extinctions of native species or species introductions. Specifically, coastal marine ecosystems are more vulnerable to anthropogenic impacts than pelagic or deep-sea ecosystems due to their close proximity to major sources of human activity (Halpern et al. 2008; Mieszkowska 2021). Thus, efficient and scalable methods for monitoring coastal biodiversity are essential for detecting and managing ecological change, including shifts in species dominance and community composition (Perry and Ommer 2003; Anderson 2018).

Environmental DNA (eDNA) is a powerful molecular tool for detecting biodiversity in marine environments, with advances in using eDNA to monitor macro-organisms in recent years (Takahashi et al. 2023). Specifically, eDNA is defined as a complex mixture of organismal (i.e., whole organisms) and extra-organismal (i.e., shed by organisms) genetic material found in environmental media such as seawater (Rodriguez-Ezpeleta et al. 2021). An established technique for multi-species detection is eDNA metabarcoding which can capture marine biodiversity over large spatial scales (Agersnap et al. 2022; Cole et al. 2021; Deiner et al. 2017). Monitoring methods that use eDNA can overcome challenges faced by visual methods, such as reducing dependency on in situ species identification and standardizing ecological data collection (Takahashi et al. 2023). Although eDNA methods have been applied across the tree of life, ecologically and climatically important indicator groups like invertebrates and macroalgae remain understudied compared to vertebrates (Takahashi et al. 2023).

Despite its advantages, eDNA metabarcoding has inherent limitations that can restrict its application to answer ecological questions effectively. In high-energy marine environments strongly influenced by tides, geology, and environmental factors, eDNA signals can be difficult to interpret over space and time (Scriver et al. 2023). For example, eDNA can be transported away from its source organism, increasing the risk of false-positive detections (Goldberg et al. 2016). Conversely, the concentration and quality of eDNA can be reduced by biotic and abiotic factors, increasing the risk of false negatives (Harrison et al. 2019; Scriver et al. 2023). There is growing evidence that eDNA signals are spatially discrete (i.e., discriminate dissimilar communities from nearby locations) even in highly connected marine systems (Larson et al. 2022; O'Donnell et al. 2017; Shea and Boehm 2024; Stat et al. 2019; West et al. 2020), with detections potentially reflecting the movement of organisms (Scriver et al. 2024). Specifically, spatial discreteness has been found at fine spatial scales (< 5 km and < 40 m, Jeunen et al. 2019; Shea and Boehm 2024, respectively). While broad-scale eDNA studies exist, those specifically testing spatial resolution have been limited to single geographical areas, typically within one site or region. Therefore, to implement eDNA as a biodiversity monitoring tool, it is crucial to understand its utility at multiple spatial scales and the environmental factors that may affect detection.

An appropriate marine system to explore the spatial discreteness of eDNA is rocky intertidal reef due to its unique physical characteristics, such as distinct vertical zonation, ubiquity across coastlines at temperate latitudes, and continuing value in global change research (Hawkins et al. 2020). Rocky intertidal communities are among the most threatened coastal habitats due to their sensitivity to ocean warming, caused by organisms living close to their thermal tolerance limits and regularly being exposed to a wide range of environmental conditions (Falkenberg et al. 2021; Halpern et al. 2007; Mieszkowska 2021). Unique physical features that are important in determining the structure of communities, such as the presence of rockpools and vertical zonation, can be utilized to test the precision of eDNA at small spatial scales (Hawkins et al. 2019; Martins et al. 2007). For example, high shore zones typically support lower species diversity than low shore zones due to greater exposure to environmental stressors such as temperature extremes and desiccation (Underwood and Kennelly 1990; Martins et al. 2007; Hawkins et al. 2019). Thus, if eDNA signals accurately reflect spatial patterns, an increase in species richness from high to low shore zones should be detectable. Conversely, due to the widespread distribution of some species and the potential for DNA homogenization, some overlap in community composition may be expected across spatial scales. A better understanding of the spatial dynamics of eDNA on rocky shores will support its integration into routine ecological monitoring for high-energy coastal ecosystems.

We conducted nationwide sampling across the UK to assess the ability of eDNA metabarcoding to characterize intertidal rocky shore diversity across three spatial scales (local, regional, and national). Through a multi-gene approach, we focused on taxonomic groups known to be sensitive to ocean warming, broadly targeting marine invertebrates (COI) and macroalgae (18S). Onshore surface-water samples were collected across 32 rocky shore sites in five UK Regional Seas. Samples were collected from spatially discrete shore heights, including rockpools in high and low shore zones, as well as directly from the sea to determine how eDNA signals change over small spatial scales. We aimed to expand the application of eDNA-based methods for intertidal monitoring by (i) assessing the ability of eDNA methods to accurately identify intertidal taxa and (ii) investigating the spatial discreteness of eDNA to describe rocky intertidal diversity at multiple spatial scales while accounting for environmental factors (temperature and pH) that could influence eDNA detection.

## 2 | Methods

Detailed protocols are available that provide comprehensive guidance on how to reproduce the methods described here, including sample collection (Simons et al. 2024), laboratory processing (NEOF-NERC Environmental Omics Facility et al. 2025), and computational processing (see NEOF 2023 for bioinformatics;

Simons 2025 for formatting, filtering, and quality control). A publicly accessible GitHub repository (Simons et al. 2025) contains all data and code (in an R Markdown file) necessary to fully reproduce the analyses.

## 2.1 | Sampling Locations

Thirty-two intertidal rocky sites in five UK Regional Seas (Figure 1a) were sampled in the summer (June–September) of 2022 and 2023. Sampling sites were selected based on four criteria: being extensive intertidal rocky reefs (sometimes with the inclusion of hard coastal structures); distanced from human development (e.g., docks, ports, buildings) and estuarine outputs to reduce acute anthropogenic activity; accessible for researchers to safely traverse the shore with equipment by foot; and located across a wide geographical range to enable detection of regional differences. Sites had variable shore exposures and weather conditions during collection (Table S1). Samples were collected across two summer field seasons and during different weeks, which could have introduced temporal variation (Jensen et al. 2022). However, visual surveys often detect minimal short-term annual variation in the rocky shore taxa studied here (Mieszkowska et al. 2021), and coastal eDNA studies have demonstrated annual consistency in community composition (Sevellec et al. 2025). Although the data are summer-biased, we followed protocols developed in the intertidal visual monitoring programme MarClim (Mieszkowska 2020) which recommends safer site access during this season.

Up to nine samples per site were haphazardly collected within a 100 m section of each shore to capture site heterogeneity. Three replicate samples were collected at three shore heights: high and low shore rockpools, and directly from the sea (referred to as open water samples hereafter; Figure 1b,c). We selected rockpools that were fully isolated from each other at low tide, more than 10 m apart, and contained sufficient water volume. Shore heights were classified through visual identification of species typically found at specific heights (e.g., *Fucus spiralis* at high shore, *Fucus serratus* at low shore) and opinion from expert rocky shore field ecologists. To ensure maximum spatial discreteness, high shore rockpool samples were collected near the high-water mark while open water samples were taken as far below the low-water mark as possible. Open water samples were collected predominantly on foot, except one site where samples were collected in the subtidal zone from a boat ($n = 3$).

## 2.2 | Sample Collection and Filtration

Before sample collection at each site, all equipment was sterilized using a 20% bleach solution (Fisher Scientific UK Ltd., Leicestershire, UK), rinsed with ultrapure water (Milli-Q UltraPure System, Merck KGaA, Darmstadt, Germany), dried, and transported in clean plastic Bryson Packaging bags (Fisher Scientific UK Ltd., Leicestershire, UK).

We collected 265 × 1 L surface water samples using a custom on-site protocol. The chosen sampling volume of 1 L is common for aquatic studies as it sufficiently captures diversity in nearshore

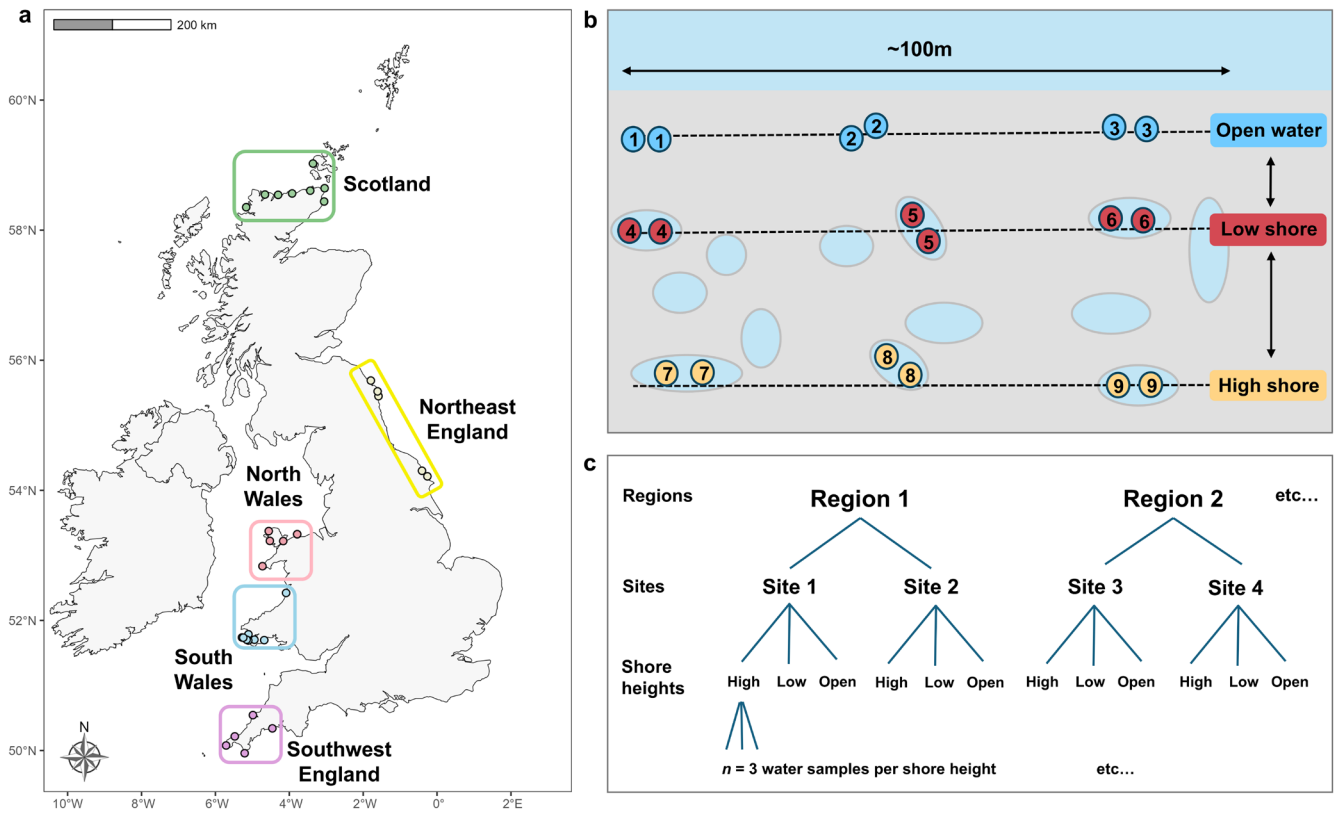


**FIGURE 1** | (a) Location of sampling sites within five major UK regions, (b) local-scale sampling design showing nine 1 L replicates (consisting of two 500 mL subsamples per replicate) per site, with three from each shore height, and (c) schematic diagram of the nested sampling design.

marine environments (Govindarajan et al. 2022; Patin and Goodwin 2023; Takahashi et al. 2023). Due to high turbidity, a maximum volume of less than 1 L (between 200 and 999 mL) was filtered before blockage for 24.5% of the field samples. Since diversity can still be resolved from low-volume water samples (Dan et al. 2024), these samples were retained.

For each sample, 1 L of surface water was collected in a sterile HDPE collection bottle (Wide Neck, Fisher Scientific UK Ltd., Leicestershire, UK). To increase sampling heterogeneity, two 500 mL subsamples were collected in the same bottle at two different locales (i.e., at opposite sides of the same rockpool; Figure 1b). The samples were filtered on-site within 1 h after collection, where possible. When on-site filtering was not possible, samples were kept cool and protected from sunlight to prevent DNA degradation, then filtered within 5 h of collection (4% of samples). The water temperature and pH were recorded onshore using a pre-calibrated ExStik II sensor (Extech EC500, FLIR Systems UK, Kent, UK) directly after sample collection which prevented contamination from equipment entering the water.

The water samples were filtered across a 0.22 μm Sterivex-GX unit (Merck KGaA, Darmstadt, Germany) using a modified sealant gun (a custom plastic adaptor was fitted to narrow the gun's opening for a better syringe fit), dried by pushing air through the capsule, preserved in high-grade 100% ethanol (as recommended by Spens et al. 2017), and capped using sterile combi-stoppers (UKMEDI, Norwich, UK). A negative field control was collected at each site to check for contamination. For each control, we rinsed a sterile collection bottle using sealed bottled drinking water, then filtered 200 mL of the bottled water using the same procedures as the samples. All samples were kept refrigerated until they could be stored at −20°C, with a maximum of 10 days between collection and freezing. The samples remained in the freezer for a maximum of 7 months before DNA was extracted from the filter membranes.

### 2.3 | DNA Extraction and Metabarcoding

We conducted DNA extractions, PCR amplification, library preparation, and sequencing at the UK NERC Environmental Omics Visitors Facility (NEOF, Sheffield, UK). Before molecular work, all surfaces and equipment were sterilized with a 20% bleach solution and daily UV exposure. All DNA extractions and PCR plate preparations were performed in a PCR-free clean laboratory room under a laminar flow hood. Rigorous precautions were implemented to prevent cross-contamination, including the incorporation of field, extraction, and PCR negative controls.

DNA was extracted directly from filter membranes using a modified protocol with the DNeasy Blood and Tissue Kit in combination with a QIAshredder and eluted in 50 μL AE buffer (all QIAGEN GMBH, Hilden, Germany). The filters were separated from their casing following the guidance of Cruaud et al. (2017), air-dried to remove trace ethanol, and incubated overnight (> 16 h) with proteinase K and ATL buffer (QIAGEN GMBH, Hilden, Germany). A negative extraction control consisting of molecular-grade water was included in each of the 10 extraction batches. DNA concentrations were measured by fluorometry on a multimode microplate reader (Thermo Fisher Scientific Inc., Waltham, MA, USA).

Two pairs of metabarcoding primers were used in a two-step amplification protocol to allow a broad characterization of marine invertebrates and macroalgae diversity. For invertebrates, we amplified a ~313-bp fragment of the mitochondrial COI gene using the highly degenerate Leray-XT primer set (Forward: GGWACWRGWTGRACWITITAYCCYCC; Reverse: TAIACYTCIGGRTGICCRAARAAYCA), which provides high taxonomic resolution in diverse marine invertebrate communities (Wangensteen et al. 2018). For macroalgae, we amplified a ~133-bp fragment from the V7 hypervariable region of the 18S rRNA gene using the Euka02 primer set (Forward: TTTGTCTGSTTAATTSCG; Reverse: CACAGACCTGTTATTGC), which provides good coverage for macroalgae taxa and, to our knowledge, the only macrophyte-specific primer tested in eDNA metabarcoding (Ortega et al. 2020). Both markers were amplified in an initial PCR reaction (PCR1) using the primers mentioned above tailed with an overhang adapter. A second amplification (PCR2) used indexed primers that incorporate the overhang adapter from PCR1 and Illumina sequencing primers to allow sample multiplexing for sequencing. To reduce PCR inhibitors and improve amplification performance, serial dilutions were performed on extracts prior to PCR amplification, with 18S extracts diluted by 1:8 and COI extracted by 1:16.

For PCR1 reactions, we used a total volume of 20 μL, consisting of 10 μL of Multiplex PCR Master Mix (QIAGEN GMBH, Hilden, Germany), 2 μL of 5 μM forward primer (Merck KGaA, Darmstadt, Germany), 2 μL of 5 μM reverse primer (Merck KGaA, Darmstadt, Germany), 5 μL of ddH2O, and 1 μL of diluted DNA. Due to budgetary limitations, single PCR1 replicates were performed on a representative subset comprising 5% of the total samples. Optimized PCR thermocycling conditions were used for each primer (Table 1). PCR1 amplification was carried out in 10 batches, with each batch including a negative PCR1 control consisting of molecular-grade water. DNA extracts from four taxa outside the target communities were obtained as positive controls for each primer, including two terrestrial invertebrates (stalk-eyed fly [*Teleopsis dalmanni*] and tropical ant [*Philidris nagasau*]), an Arctic microalga (Snow algae [*Chlorella spp*]), and a terrestrial grass [*Vulpia myuros*]. All sample replicates and controls were amplified in separate wells alongside the main samples within the same PCR runs.

Post-PCR1, successful amplification, correct product sizes, and absence of contamination were confirmed by agarose gel electrophoresis. PCR1 products were cleaned using a 1.5× volume of Pronex magnetic beads (Promega Corporation, Madison, WI, USA). PCR2 was performed using tailed primers (dual-plexed Fi5 and Ri7) to add unique barcodes and Illumina adapter sequences to each sample, using 10 μL reactions with MyTaq HS Mix (Meridian Bioscience, Cincinnati, OH, USA), 0.5 μL each of 10 μM primers, 1 μL sterile $ddH_2O$, and 8 μL of PCR1 product as template. The samples were then again cleaned with Pronex magnetic beads and pooled to approximately equal molarity, using fluorometry and qPCR (KK4873 kit, KAPA Biosystems on a QuantStudio 12 K Flex instrument, Thermo Fisher Scientific Inc., Waltham, MA, USA)

**TABLE 1** | Thermocycler conditions for primers and PCR steps used in metabarcoding.

| PCR cycle | Dilution | Denaturation | Cyclic denaturation | Cycles | Final extension and cooling |
|---|---|---|---|---|---|
| PCR1 (Leray-XT) | 1/16 | 95°C/15 min | 95°C/20 s | 35 | 72°C/10 min |
| | | | 50°C/90 s | | 10°C/indefinite |
| | | | 72°C/90 s | | |
| PCR1 (Euka02) | 1/8 | 95°C/15 min | 95°C/20 s | 35 | 72°C/10 min |
| | | | 53°C/90 s | | 10°C/indefinite |
| | | | 72°C/90 s | | |
| PCR2 | NA | 95°C/2 min | 98°C/10 s | 12 | 72°C/5 min |
| | | | 65°C/30 s | | 10°C/indefinite |
| | | | 72°C/30 s | | |

for quantification. Positive and negative controls were added at a volume of 0.5 μL to prevent overwhelming the pool. A single library per primer pair was prepared, resulting in two final libraries. The size of the DNA fragment was checked on an Agilent 4200 TapeStation (Agilent Technologies, Santa Clara, CA, USA), which found primer dimer in the COI library. Consequently, the COI fragment was size-selected using a BluePippin (Pippin Prep, Sage Science Inc., Beverly, MA, USA). We acknowledge that size selection is not optimal and could have resulted in a non-normalized library pool prior to sequencing.

COI and 18S libraries were sequenced on an MiSeq platform (2 × 300 bp paired-end reads, Illumina UK, Cambridgeshire, UK) and a MiniSeq platform (2 × 150 bp paired-end reads, Illumina UK, Cambridgeshire, UK), respectively. A 10% PhiX spike-in was included in both runs to increase the sequence diversity. Laboratory work was carried out separately for each field season. Hence, two sequencing runs per primer pair were conducted. Samples from South Wales and Scotland were sequenced first, and then samples from Northeast England, North Wales, and Southwest England were sequenced second. All conditions were consistent across sequencing runs, and sample replicates were included between runs to check for consistency.

Across the two libraries, a total of 675 PCR samples were sequenced (Table S2). The COI library contained a total of 353 PCR samples, with 231 field samples, 53 negative controls, 17 positive controls, 44 replicates, and 8 failed repeats (Table S2). The 18S library contained a total of 322 PCR samples, with 231 field samples, 50 negative controls, 15 positive controls, 25 replicates, and 1 failed repeat (Table S2).

## 2.4 | Bioinformatics, Filtering and Quality Control

Bioinformatic analyses were conducted on the University of Sheffield's High Performance Computing Cluster (Bessemer). We used the DADA2 bioinformatics pipeline to filter, trim, dereplicate, and denoise sequences (Callahan et al. 2016; NEOF 2023). BLAST searches were performed against the MIDORI2 vGB259 database (Leray et al. 2022) for COI amplicons and the SILVA v132 database (Quast et al. 2013) for 18S amplicons. Both curated databases are quality-controlled, regularly updated, and technically validated. We used a strict sequence identity threshold of 98% to reduce false positive detections. Taxonomic assignments were assessed using the software MEGAN6 (Huson et al. 2016), where we used the LCA algorithm default parameters, except for adjusting the top percentage identity to 1% to improve species-level taxonomic matches. Analysis pipelines with different parameters, including using the NCBI database (Sayers et al. 2022), retaining a sequence identity of 80%, and adjusting the top percentage identity to 10%, did not show differences except for improved taxonomic assignments with curated databases and stricter settings.

All downstream formatting, quality control check, and data analyses were completed using R v4.4.2 (R Core Team 2024) in the RStudio v2024.12.1 + 563 integrated development environment (Posit Team 2024). Taxonomic names were cleaned and validated against the World Register of Marine Species (WoRMS; WoRMS Editorial Board 2024) using the packages *worrms* v0.4.3 (Chamberlain and Vanhoorne 2024) and *taxize* v0.9.1 (Chamberlain et al. 2012). Broad ecological taxonomic groups associated with taxa (known hereafter as taxonomic groups) were obtained through a custom function using the package *worrms* (Chamberlain and Vanhoorne 2024; Webb 2024). Subsequently, the reads, sample metadata, and cleaned taxonomy were wrangled into a phyloseq object using the package *phyloseq* v1.48.0 (McMurdie and Holmes 2013).

To further reduce the risk of false positives, a decontamination pipeline was applied (as suggested by Gold et al. 2022). The samples were decontaminated by batch (field, extraction, and PCR) using a custom function which removed all taxa that contained a higher read count in negative controls than in the corresponding sample. To prevent non-target taxa from influencing biodiversity estimates, taxa were filtered to only include target macroinvertebrate and macroalgae phyla, as well as those organisms larger than 1 mm (i.e., macrobenthos only).

A series of quality control checks was performed on the filtered data using the packages *phyloseq*, *metabaR* v1.0.0 (Zinger et al. 2021) and *vegan* v2.6.6 (Oksanen et al. 2022). We confirmed the success of positive controls to at least a family

level, though some sequences required manual BLAST searching when absent from the curated databases. We visualized read depths across primers (Figure S1), regions (Figure S2), and samples (Figures S3 and S4) to evaluate the overall sequencing quality and to identify a suitable threshold for further filtering. Thus, samples with a read depth below 3000 reads (20 samples) were removed to prevent bias in diversity estimates. Rarefaction curves were constructed across each region and primer set to verify sufficient sampling effort using *vegan* (Figure S5). The similarity of technical replicates (between runs and within runs) was explored by calculating the pairwise Jaccard and Bray dissimilarity in *vegan* (Figure S6), as well as by inspection of compositional taxa plots in *phyloseq* (Figures S7 and S8). Data were not rarefied to prevent reduction in statistical power and reduce the risk of false positive rates (McMurdie and Holmes 2014). Conclusions were not affected by any of these steps, as checked by replicating analyses with rarefied data, unfiltered data, and alternative decontamination steps. All replicates were retained in the statistical analyses.

## 2.5 | Statistical Analyses

To assess the ability of our employed eDNA methods to identify a wide range of intertidal macroinvertebrates and macroalgae, we visualized taxa detected across key phyla and taxonomic groups (macroalgae, macrobenthos, and others) using bar plots in the *ggplot2* v3.5.1 package (Wickham 2016). We also visualized taxa detected across COI and 18S markers using Euler diagrams in the package *eulerr* v7.0.2 (Larsson et al. 2024).

To evaluate the spatial discreteness of eDNA signals, we explored how alpha and beta diversity varied across different scales while accounting for environmental variables. When reporting results, we use the graded evidence language outlined by Muff et al. (2022), which defines the following approximate categories: no evidence ($p > 0.10$), weak evidence ($0.05 < p \leq 0.10$), moderate evidence ($0.01 < p \leq 0.05$), strong evidence ($0.001 < p \leq 0.01$), and very strong evidence ($p \leq 0.001$).

We tested for differences in alpha diversity across shore heights using a mixed modeling approach that could handle both nested random effects and unbalanced data. Two diversity indices were calculated using the package *phyloseq* and modeled as outcome variables using the package *lme4* v1.1.35.3 (Bates et al. 2024): richness (number of species) and Shannon diversity index (which takes account of both species richness and evenness). For both models, explanatory variables were shore height (a categorical fixed effect with three levels), region (a categorical fixed effect with five levels), temperature (a continuous explanatory variable), and pH (a continuous explanatory variable). Variation across sampling sites was accounted for by incorporating a nested random effect.

Richness was modeled using a generalized linear mixed model with a Poisson response and a log link function in the function glmer(). The model structure is expressed mathematically as:

$$Y_{ijk} \sim \text{Poisson}\left(\lambda_{ijk}\right), u_{ij} \sim N\left(0, \sigma^2\right)$$

$$\log\left(\lambda_{ijk}\right) = \mu + \alpha_i + \beta_1 \cdot \text{ShoreHeight}_{ijk} + \beta_2 \cdot \text{Temperature}_{ijk} + \beta_3 \cdot \text{pH}_{ijk} + u_{ij}$$

where $Y_{ijk}$ is the observed richness for observation $k$ at site $j$ within region $i$, $\lambda_{ijk}$ is the expected richness (the mean of the Poisson distribution), $\mu$ is the overall intercept, $\alpha_i$ is the fixed effect of region $i$, $\beta_1$ is fixed effect of shore height, $\beta_2$ is fixed effect of temperature, $\beta_3$ is fixed effect of pH, and $u_{ij}$ is the random effect of site. Denominator degrees of freedom are not provided when fitting GLMMs with glmer() because they are difficult to define (see Bolker 2025 for discussion). Therefore, for richness analyses, we report only the numerator degrees of freedom.

Shannon diversity index was modeled using a linear mixed model in the function lmer(). The model structure is expressed mathematically as:

$$u_{ij} \sim N\left(0, \sigma^2\right), \varepsilon_{ijk} \sim N\left(0, \Psi^2\right)$$

$$Z_{ijk} = \mu + \alpha_i + \beta_1 \cdot \text{ShoreHeight}_{ijk} + \beta_2 \cdot \text{Temperature}_{ijk} + \beta_3 \cdot \text{pH}_{ijk} + u_{ij} + \varepsilon_{ijk}$$

where $Z_{ijk}$ is Shannon diversity for observation $k$ at site $j$ within region $i$, $\varepsilon_{ijk}$ is the residual error, and $\mu, \alpha_i, \beta_1, \beta_2, \beta_3$, and $u_{ij}$ are defined above. $u_{ij}$ and $\varepsilon_{ijk}$ were assumed to be mutually independent. Denominator degrees of freedom were estimated using the Kenward–Roger approximation for Shannon diversity analyses (Kenward and Roger 1997; Schaalje et al. 2002).

Model assumptions were assessed using diagnostic plots generated with the core functions *plot* or *qqnorm*, including qq-plots of residuals to check normality, residuals versus fitted values to check linearity, and scale-location of residuals to check homoscedasticity. Multicollinearity was checked through the variance inflation factor (VIF) calculated in the package *car* (Fox and Weisberg 2018). In all cases, VIF values were $<2$, which is below the threshold of 10 that indicates collinearity issues in model interpretation (Dormann et al. 2013). Type II Wald chi-square tests were conducted for each model using the Anova() function in the package *car* to evaluate significance.

Two planned orthogonal contrasts were used to determine differences between shore heights in the package *emmeans* (Lenth 2023). The first contrast examined whether diversity in the low shore rockpools differed from that in the high shore rockpools (contrast weights: 0, 1, −1 for open water, high shore, and low shore respectively). The second contrast tested whether diversity in open water differed from that in the high and low shore rockpools (contrast weights: 1, −0.5, −0.5 for open water, high shore, and low shore respectively). We visualized shared species detections across shore heights using Euler diagrams in the package *eulerr* v7.0.2 (Larsson et al. 2024).

While alpha diversity indices provide insight into overall diversity, they do not distinguish the underlying community composition that drives such patterns. Therefore, we explored how beta diversity varied across spatial scales. Due to distinct dissimilarity between COI and 18S derived

communities, we tested differences in beta diversity (specifically Jaccard dissimilarity) separately for each marker. We conducted restricted permutational multivariate analysis of variance (PERMANOVA) tests for multi-factorial analysis using 9999 permutations on a resemblance matrix based on Jaccard dissimilarity in PRIMER 7 with the PERMANOVA+ add-on (Clarke and Gorley 2015). We used Type III sums of squares as recommended by Anderson et al. (2008, section 1.3.4) to appropriately handle unbalanced data and permuted residuals under a reduced model as recommended by Anderson and ter Braak (2003) to ensure valid testing of interactions. Terms included in the PERMANOVA design were region (fixed effect), shore height (fixed effect), site (random effect nested within region), and their interactions. The same two planned contrasts used in the alpha diversity models to determine differences between shore heights were incorporated into the PERMANOVA design. We tested for homogeneity of dispersions using the betadisper() function in *vegan*. Community composition across spatial scales was visualized using non-metric multidimensional scaling (NMDS) based on Jaccard dissimilarity. Ordination was performed with the ordinate() function in *phyloseq*, using three dimensions and other default settings. The first two dimensions were plotted in NMDS plots.

To investigate whether common species observed in the MarClim Project (Mieszkowska et al. 2005) were detected by eDNA in their respective ecological habitats, we categorized their known prevalence at each shore height (i.e., high, low, and open water) and biogeographical realm (i.e., boreal [cold-affinity], lusitanian [warm-affinity], and cosmopolitan). We also categorized those taxa known as invasive to UK waters. Species were classified based on expert opinion and information from the online databases WoRMS (WoRMS Editorial Board 2025) and The Marine Life Information Network (MarLIN [Marine Life Information Network] 2020).

## 3 | Results

### 3.1 | Sequencing Output

The DADA2 pipeline returned 25,709,925 processed reads across 524 samples and 131 controls (field negatives, extraction negatives, PCR negatives, and PCR positives). For 77.3% of ASVs, no taxonomic assignment was possible (COI: 93.5%; 18S: 61.1%). All positive controls contained expected taxa, none of which were found in any other samples. Decontamination steps identified and removed 51 contaminants individually across relevant batches. After decontamination and filtering for target taxa, a total of 9,133,664 reads (mean = 18,086 reads per sample) were obtained across 505 samples, of which 2,697,852 reads (mean = 10,258 reads per sample) were from COI amplicons and 6,435,812 reads (mean = 26,594 reads per sample) from 18S amplicons.

Samples achieved sufficient sequencing depth based on read depth visualization (Figures S1–S4) and rarefaction curves (Figure S5). Although mean dissimilarity remained high across the dataset, technical replicates, both within and across runs, were more like their corresponding samples than non-replicates (Figure S6). Technical replicates also showed a consistent composition between runs (Figure S7) and within runs (Figure S8).

### 3.2 | Taxonomy

The COI marker detected more unique taxa (539) than the 18S marker (420), with 7% (67) of taxa being detected across both primers (Figure 2a; Table S3). More unique taxa were detected in open water samples (233) than in high shore samples (56) or low shore samples (76), with 42% (428) of taxa detected across all three heights (Figure 2b). Considering both unique and shared taxa across markers, we identified a total of 1026 target taxa (62.3% of total detections after decontamination) belonging to 655 genera across 442 families and 19 phyla (Figure 2c; Table S3). In terms of taxonomic resolution, 75.6% of taxa were matched to species-level, 84.2% to at least genus-level, and 91.6% to at least family-level. 737 target invertebrate taxa (71.8% of the filtered data) were identified across 16 target phyla (Porifera, Cnidaria, Ctenophora, Gastrotricha, Annelida, Platyhelminthes, Mollusca, Arthropoda, Bryozoa, Nematoda, Chaetognatha, Echinodermata, Hemichordata, Chordata, Phoronida, and Orthonectida; Figure 2c; Table S3). 289 target algal taxa (28.2% of the filtered data) were identified across three target phyla (Chlorophyta, Ochrophyta, Rhodophyta; Figure 2c; Table S3).

### 3.3 | Alpha Diversity

The random component (site nested within region) of the richness model accounted for 35.8% of the total variance, indicating that the site contributed substantially to the overall variation in richness. We found very strong evidence that richness varied across regions ($\chi^2(4) = 20.4$, $p < 0.001$) and shore heights ($\chi^2(2) = 201.4$, $p < 0.001$; Table S5). Specifically, we found very strong evidence that richness at high shore rockpools was lower compared to low shore rockpools ($z(1) = -12.2$, $p < 0.001$) and that richness at open water was higher compared to rockpools ($z(1) = 8.95$, $p < 0.001$; Figure 3a; Tables S5 and S6). We found very strong evidence that species richness was negatively associated with temperature ($\chi^2(1) = 92.2$, $p < 0.001$; Figure 3c) and pH ($\chi^2(1) = 13.6$, $p < 0.001$; Figure 3e; Table S5).

The random component of the Shannon diversity model accounted for 13.1% of the total variance, indicating that the site contributed little to the overall variation in Shannon diversity. We found no evidence that Shannon diversity varied across regions ($\chi^2(4,30) = 3.4$, $p = 0.494$; Table S5). We found moderate evidence that Shannon diversity varied across shore heights ($\chi^2(2465) = 7.2$, $p = 0.027$; Table S5). Specifically, we found moderate evidence that Shannon diversity at open water was higher compared to rockpools ($t(1,473) = 2.52$, $p = 0.012$; Figure 3b; Tables S5 and S6). However, we found no evidence that Shannon diversity at high shore rockpools differed from low shore rockpools ($t(1,448) = -0.99$, $p = 0.324$; Figure 3b; Tables S5 and S6). We found no evidence that temperature ($\chi^2(1134) = 0.44$, $p = 0.507$; Figure 3d) or pH ($\chi^2$ (1359) = 0.59, $p = 0.442$; Figure 3f) showed a relationship with Shannon diversity (Table S5).

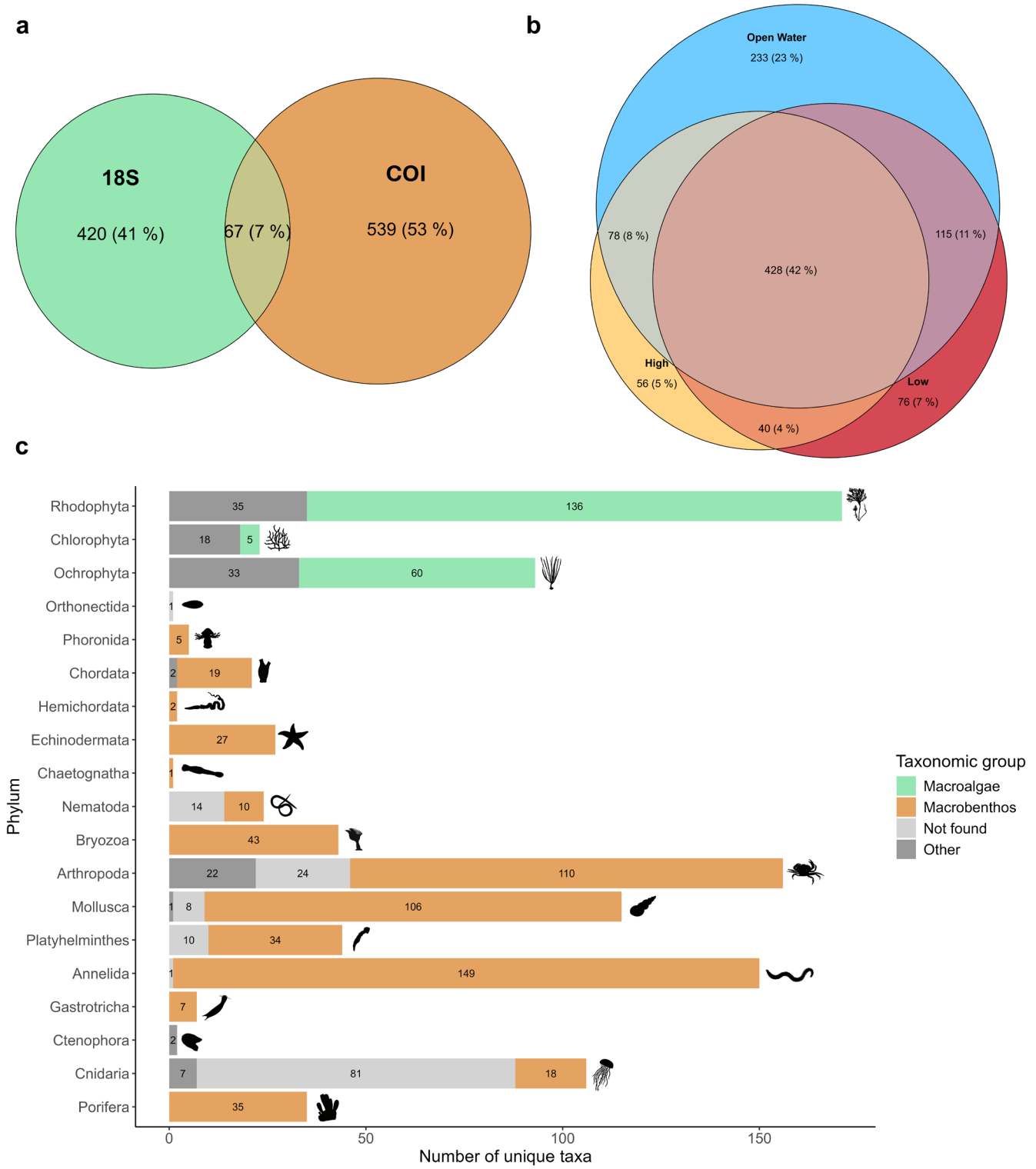


**FIGURE 2** | Target taxa detected across (a) COI and 18S markers, (b) low, high, and open water samples, and (c) phyla and taxonomic groups. 'Not found' indicates that the taxonomic group was not available in the WoRMS database. 'Other' refers to taxonomic groups that were not specifically classified as Macroalgae or Macrobenthos in WoRMS but were still relevant to the study (e.g., epibenthos, endobenthos, meiobenthos).

### 3.4 | Beta Diversity

Beta diversity varied across all spatial scales, as supported by PERMANOVAs, NMDS, and pairwise dissimilarity comparisons.

For 18S, we found very strong evidence that variation in community composition was explained by region (11.98%; pseudo-$F(4,29) = 3.50$, $p < 0.001$), site (27.29%; pseudo-$F(27,165) = 5.4$, $p < 0.001$), shore height (2.64%; pseudo-$F(2,35) = 2.78$, $p < 0.001$), and the interaction between shore height and site (16.75%; pseudo-$F(35,165) = 2.57$, $p < 0.001$; Table S7). We found moderate evidence that variation in community composition was explained by the interaction between shore height and region (4.61%; pseudo-$F(8,35) = 1.21$, $p = 0.012$;

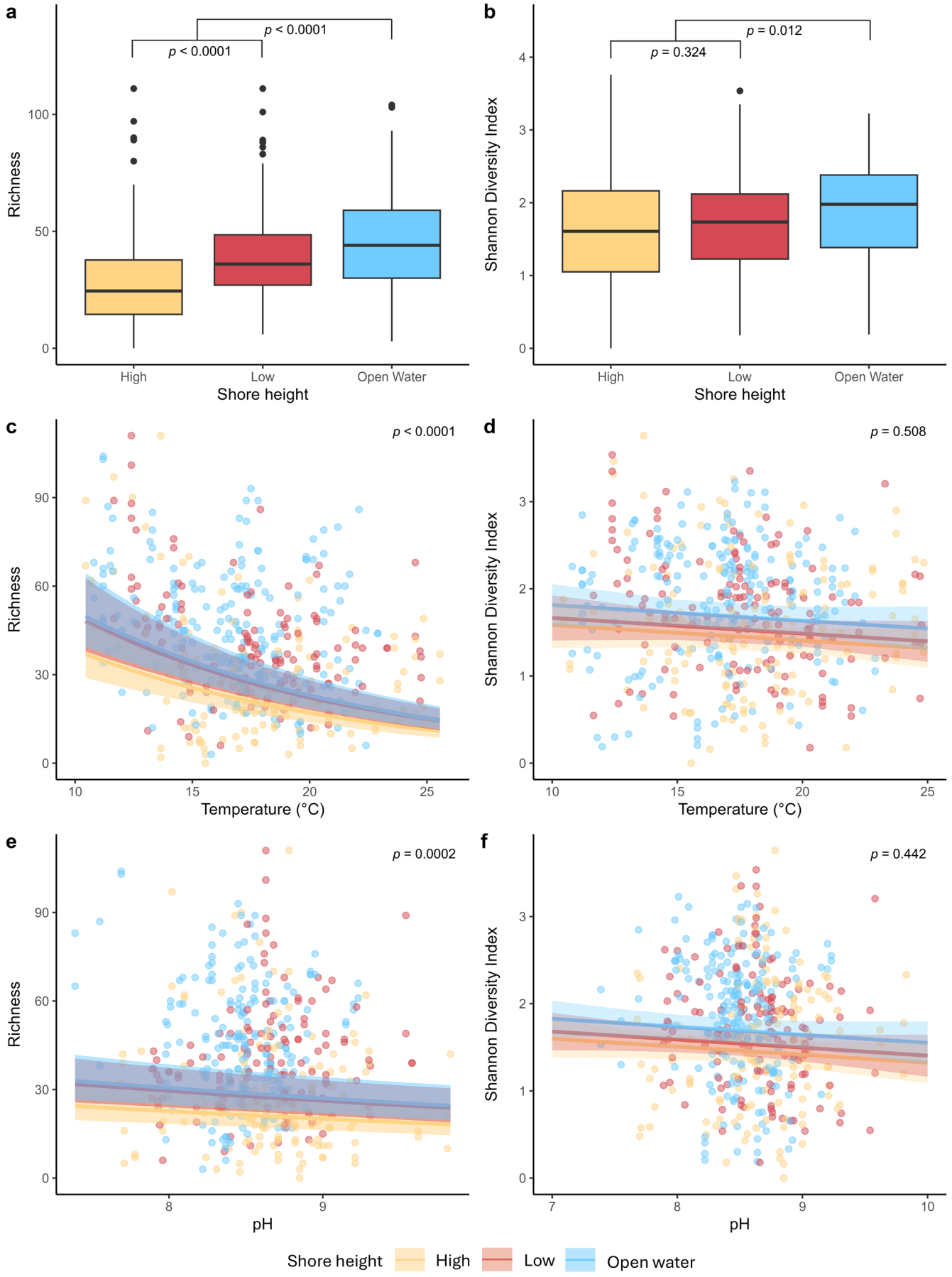


**FIGURE 3** | Legend on next page.

**FIGURE 3** | (a, c, e) Richness and (b, d, f) Shannon diversity across (a, b) shore heights, (c, d) temperature and (e, f) pH. Points in scatter plots (c–f) represent raw diversity estimates from individual samples. Lines of fit in scatter plots (c–f) represent predictions from the generalized linear model (richness) and general linear model (Shannon diversity) at different shore heights, averaging across the random (nested) effects, with bands representing 95% confidence intervals. *p*-values are from mixed models and planned contrasts.

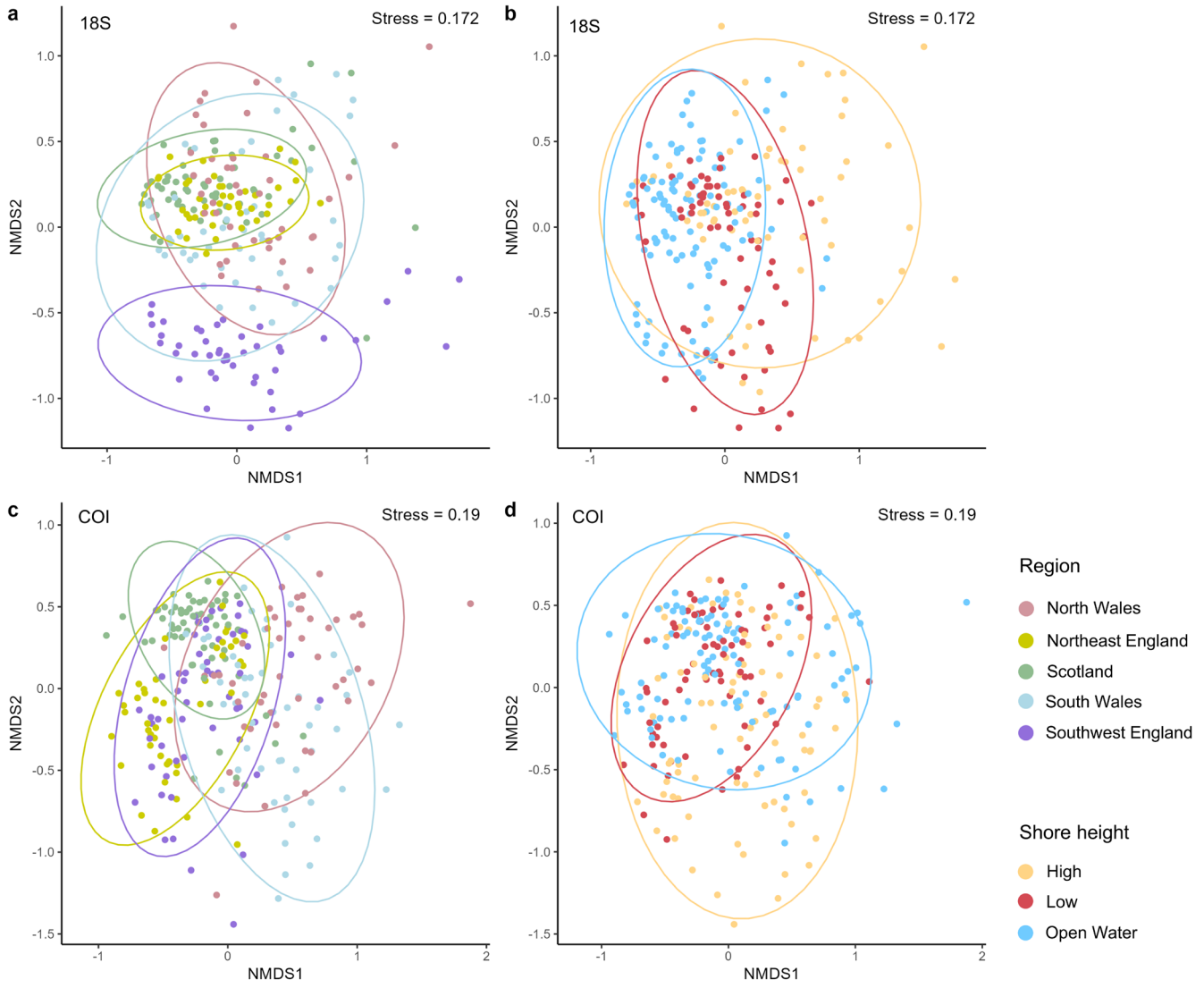


**FIGURE 4,** | Non-metric multidimensional scaling (NMDS) ordination plots based on Jaccard dissimilarities of community compositions for (a, b) 18S and (c, d) COI. Community compositions are grouped by (a, c) region and (b, d) shore height as classification factors. Ellipses represent 95% confidence regions for the centroids of each category, assuming a multivariate t-distribution.

Table S7). The spatial factors explained a total of 76.42% of the observed variability in 18S community composition (Table S7). We found strong evidence that the 18S community composition at high shore was different from low shore (pseudo-$F(1,16) = 2.59$, $p = 0.002$), and very strong evidence that open water was different from rockpools (pseudo-$F(1,19) = 3.01$, $p < 0.001$; Table S7).

For COI, we found very strong evidence that variation in community composition was explained by region (9.24%; pseudo-$F(4,29) = 2.78$, $p < 0.001$), site (25.48%; pseudo-$F(27,186) = 4.47$, $p < 0.001$), shore height (2.22%; pseudo-$F(2,35) = 2.64$, $p < 0.001$), and the interaction between shore height and site (14.77%; pseudo-$F(35,186) = 2.00$, $p < 0.001$; Table S8). There was no evidence of an interaction between shore height and region on COI community composition (pseudo-$F(8,35) = 1.09$, $p = 0.123$; Table S8). The spatial factors explained a total of 71.78% of the observed variability in COI community composition (Table S8). We found strong evidence that the COI community composition at high shore was different from low shore (pseudo-$F(1,16) = 2.92$, $p = 0.001$), and very strong evidence that open water was different from rockpools (pseudo-$F(1,19) = 2.24$, $p < 0.001$; Table S8).

When visualizing dissimilarity using NMDS, regions exhibited clustering with some degree of overlap, which was more pronounced in the 18S marker (Figure 4a,c). There was no obvious

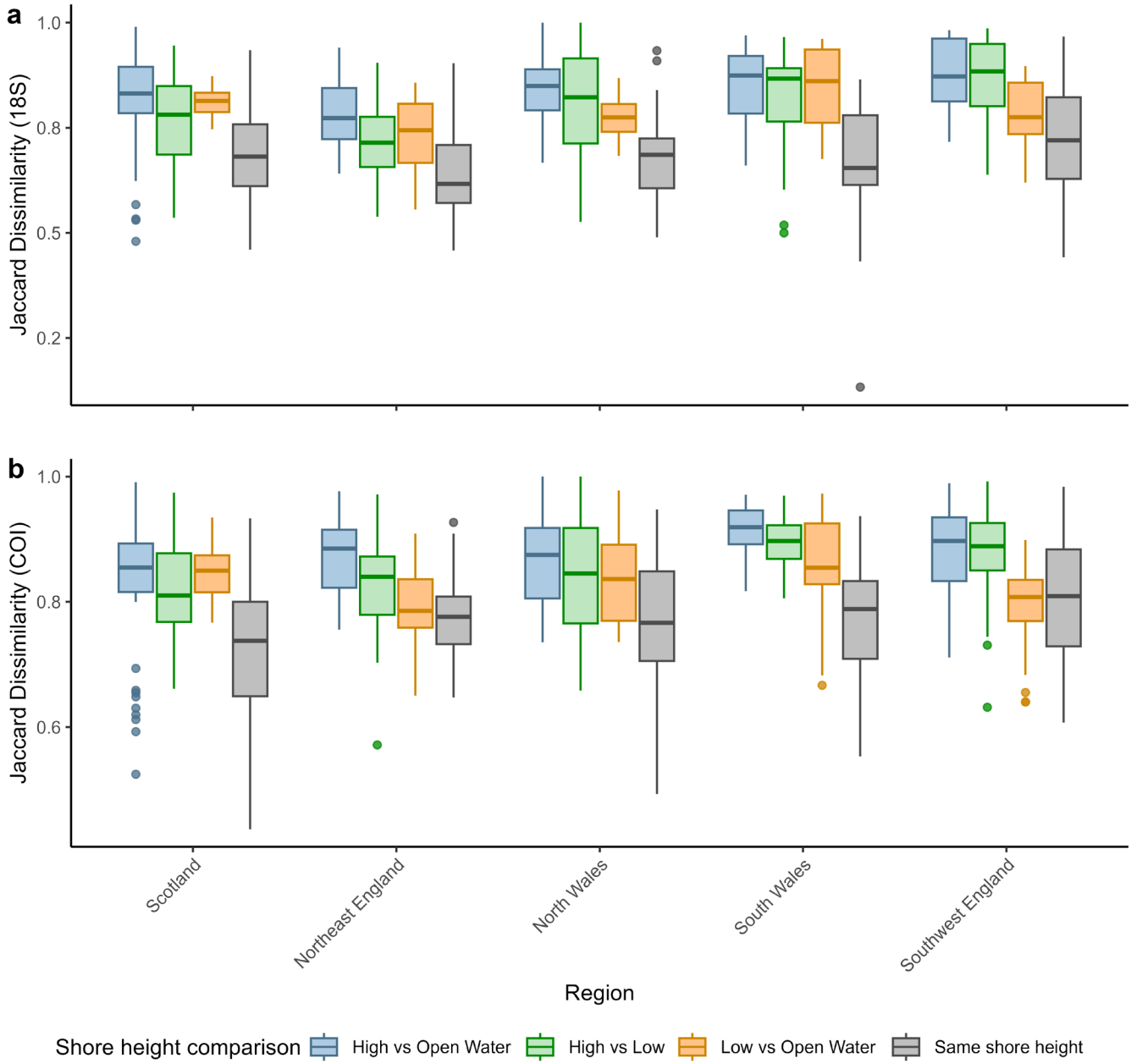


**FIGURE 5** | Shore height pairwise Jaccard dissimilarity comparisons across regions for (a) 18S and (b) COI markers. Comparisons are ordered from least to most predicted similarity based on physical proximity (i.e., high shore and open water samples are the least similar, same shore height samples are the most similar).

clustering when visualizing dissimilarity of shore heights across all samples (Figure 4b,d). Dispersion across regions was not homogenous for the CO1 community ($F(4,257) = 6.51$, $p < 0.001$) or 18S community ($F(4,237) = 7.59$, $p < 0.001$). High shore samples showed the highest dispersion across heights in the CO1 community ($F(2,259) = 14.2$, $p < 0.001$; Figure 4b) and the 18S community ($F(2,239) = 13.3$, $p < 0.001$; Figure 4d).

Pairwise dissimilarity was lower for samples in the same shore height (COI = 0.77 ± 0.004; 18S = 0.68 ± 0.005) than for samples in different heights (Figure 5a,b). Samples from high shore and open water were consistently more dissimilar to each other (COI = 0.87 ± 0.003; 18S = 0.84 ± 0.004) than to other shore heights (Figure 5a,b).

The proportion of typical high shore taxa detected increased from open water (7.7%) to high shore (23.0%; Figure 6a). The proportion of typical low shore taxa and open water taxa detected decreased from open water (10.7%) to high shore (5.0%; Figure 6a). Typical low shore taxa dominated detections across all three positions (Figure 6a).

The proportion of boreal taxa detected increased from regions with lower latitudes (i.e., Southwest England; 53.3%) to regions with high latitudes (i.e., Scotland; 89.9%; Figure 6b). The proportion of lusitanian taxa detected approximately increased from regions with higher latitudes (i.e., Scotland; 6.7%) to regions with lower latitudes, although South Wales had the highest proportion of lusitanian taxa (28.5%; Figure 6b). The highest proportion of invasive

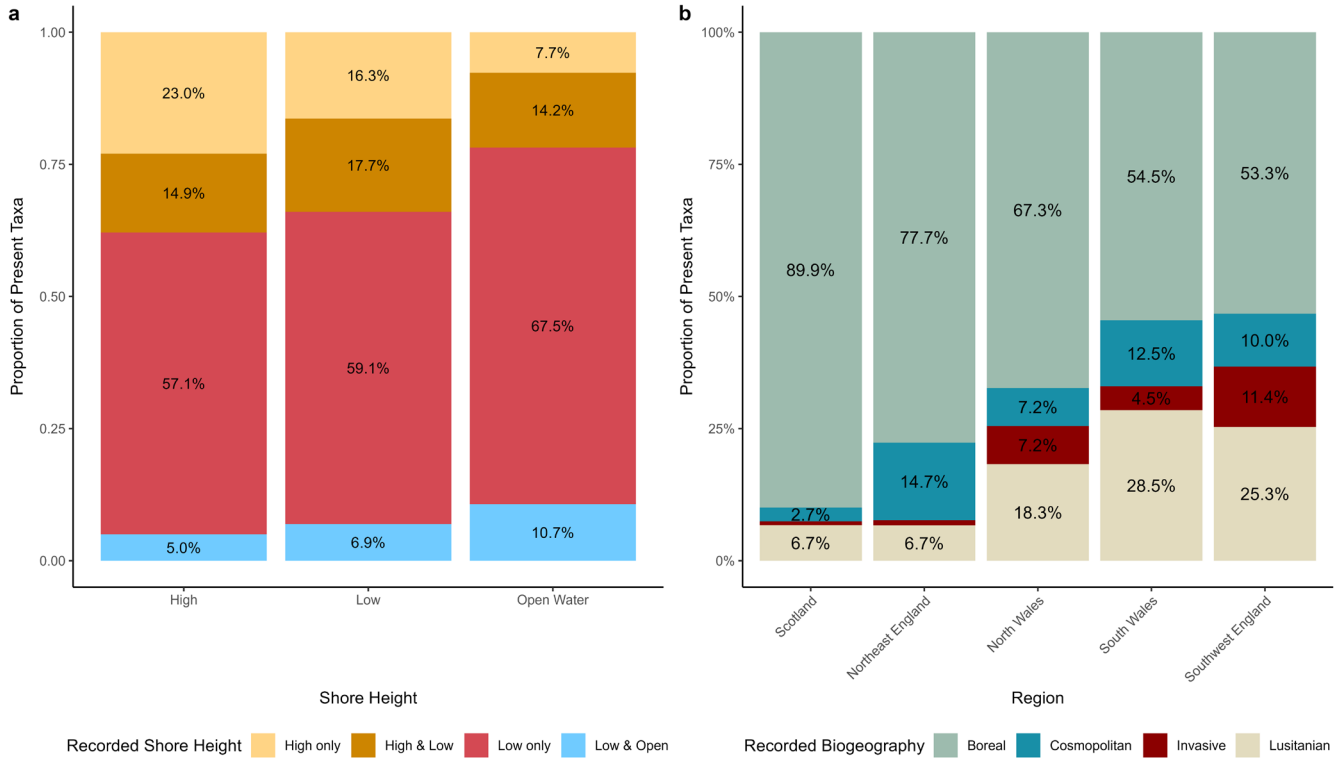


**FIGURE 6** | Proportion of subsetted detected taxa across (a) shore positions and (b) regions, categorized by their typical intertidal zone (high only, low only, open water only, or a combination) and biogeographical realm (boreal, Lusitanian, cosmopolitan, or invasive) based on available ecological information. Shore heights are ordered to reflect their true position on the shore (as shown in Figure 1b). Regions are ordered by descending latitude, with Scotland at the highest latitude and Southwest England at the lowest latitude.

taxa was detected in Southwest England (11.4%), and the lowest proportion was detected in Scotland and Northeast England (< 1%; Figure 6b). Proportions of cosmopolitan species showed no relationship with changing latitude (Figure 6b).

## 4 | Discussion

This study examined the ability of eDNA metabarcoding to detect rocky intertidal communities at a national scale (hundreds of kilometers apart), a regional scale (tens of kilometers apart), and a local scale (metres apart). We detected a broad range of marine taxa and observed discrete ecological communities at all spatial scales, with differences in eDNA signals at larger scales (i.e., between regions) more discrete than at smaller scales (i.e., within sites). Our methods effectively captured well-established ecological zonation and biogeographical patterns. We suggest that observed detection patterns could be driven by differences in marker performance, a higher degree of DNA homogenization at small scales compared to large scales, and local abiotic gradients in rocky shores.

### 4.1 | Performance and Variability of eDNA Metabarcoding

A broad range of marine taxa was detected across target and non-target taxonomic groups, with minimal overlap in communities derived from the two markers. Differences in marker detections may be attributed to variations in taxonomic coverage, sequence availability in reference databases, and the resolution of taxonomic assignments. Previous work has shown that the 18S gene can target a broader range of taxa with lower taxonomic resolution, while COI can provide a higher taxonomic resolution but for limited taxonomic coverage (Casey et al. 2021). A substantial proportion of assigned taxa (37.7%) consisted of non-target amplifications, highlighting the limited specificity of the chosen universal barcodes. This finding demonstrates that the approach can detect a broader range of taxa than initially targeted. Notably, many micro planktonic organisms comprised a large proportion of non-target marine species in this study (Table S4). Although the primers were not specifically designed for plankton and such detections may be sub-optimal, the ability to effectively monitor planktonic groups through shore-based surveys could prove highly valuable, especially considering metabarcoding has been previously applied to monitor plankton (Chen et al. 2024). The distinct and broad detections by each marker highlight the already well-established need for a multigene approach in metabarcoding to overcome marker-specific limitations and achieve a comprehensive representation of biodiversity (Casey et al. 2021; Mashar et al. 2025).

Technical replicates, both within and across sequencing runs, resembled their corresponding samples, as evidenced by similar taxa compositions and lower average dissimilarity compared to non-corresponding samples. However, overall dissimilarity was high across all technical replicates, which has been observed in other marine metabarcoding studies (Shea and Boehm 2024; Stauffer et al. 2021). Field replicates (i.e., samples collected from the same site and shore height) exhibited high dissimilarity, which could be driven by two mechanisms. Firstly, high

biological variation can be found between different rockpools, such that not all taxa suited to a particular shore height can inhabit every available rockpool due to site-specific environmental factors (Martins et al. 2007; Hawkins et al. 2019). This is supported by how shore height influenced community composition differently across sites in our multivariate analyses. Secondly, the limited biological and technical replication in our study due to resource constraints may have reduced the probability of detecting all taxa present. Therefore, we interpret any absence of taxa with caution when assessing spatial differences. Future work should conduct direct comparisons with visual observations to ground-truth eDNA detections, which would help understand the probability of detecting certain taxa with eDNA. Nevertheless, prioritizing resources on site-level eDNA sample replication was appropriate for our multiscale experimental design and rigorous quality control checks confirmed our methods were robust for providing a representation of biodiversity.

### 4.2 | Greater Differentiation of eDNA Signals at Larger Spatial Scales

The three spatial factors investigated in this study explained a large proportion of community variation, demonstrating that eDNA methods can effectively capture spatial influences on rocky shores. Nevertheless, we proceed with caution when assessing spatial differences due to observed dispersion effects, which could be driving significant results in PERMANOVAs (Anderson 2017).

At a local scale, detections and community composition showed high overlap across shore heights, with shore height having a minor influence on community composition compared to regional and site effects. These findings suggest that eDNA signals of intertidal communities are more strongly structured by geographic location than by vertical shore height. This is a probable consequence of greater water mixing and DNA homogenization at a local scale over the tidal cycle, when rockpools are physically connected, than at a regional or national scale. DNA transport and diffusion over small distances within rocky shore sites have been demonstrated by Ely et al. (2021), who showed that foreign DNA could move across an entire 35 m transect within 1.5 h of introduction. The effect of shore height also varied across sites, which accounted for the largest proportion of variation among all spatial variables. Thus, site-specific environmental features could be driving local community differences by enabling species typically confined to particular areas to expand their shore height range. For example, site exposure (i.e., the degree to which a site is subject to wave action and prevailing wind) is a prominent driving force for structuring intertidal communities (Williams 1993) and varies substantially across UK sites. Our work supports the concept that communities detected by eDNA decrease in similarity as they become further apart (O'Donnell et al. 2017) and eDNA signals, while not necessarily endogenous to a shore height, are probably endogenous to the site (Kelly et al. 2018).

Although less pronounced, unique eDNA signals were nevertheless found at the local scale, with greater similarity among samples from the same shore height and the highest dissimilarity between the extremes of the shore (i.e., between open water and high shore rockpools). These findings demonstrate that unique eDNA signals can develop at different areas of the same shore—a pattern also observed by Shea and Boehm (2024)—although we find elements of similarity persist over very small distances (e.g., approximately 10 m apart). This rationale is further supported by the consistent proportional decline in taxa found outside their expected shore height with distance. In addition, few taxa were shared exclusively between high and low shore samples without also being detected in the open water, suggesting that shore-wide similarity is primarily driven by the persistence of DNA from tidal wash-in during high tide, rather than by direct transfer between rockpools. Thus, unique signals in rockpools may persist even after they become physically connected to the rest of the shore, as also found in Shea and Boehm (2024). Our work contributes to the growing body of evidence indicating that eDNA signals can exhibit spatial discreteness across sites in dynamic coastal environments (Jeunen et al. 2019; Larson et al. 2022; Scriver et al. 2024; Shea and Boehm 2024; West et al. 2020). However, community discreteness is less pronounced at short distances within sites, particularly in the context of a multisite study such as ours.

### 4.3 | eDNA Signals Reflect Known Rocky Shore Ecology

eDNA signals correspond with established patterns in rocky shore ecology at both local and national scales. At the local scale, species richness declined from the open water toward the high shore and with increasing temperature. This finding could reflect changes in species richness driven by the abiotic stress gradient commonly observed in intertidal environments, a key factor in shaping local biodiversity (Menge et al. 2002). For example, high shore areas are exposed to desiccation and extreme temperature fluctuations due to prolonged exposure at low tide, which limit the survival of less heat-tolerant species (Scrosati et al. 2011). Temperature is known to accelerate eDNA degradation in aquatic systems (Strickler et al. 2015); however, DNA has been shown to persist for at least 24 h after shedding (Holman et al. 2022), even at temperatures above 20°C (McCartin et al. 2022). Furthermore, considering most rocky shore macroinvertebrates and macroalgae are sessile, it is unlikely that individuals inhabiting a rockpool would have relocated within a tidal cycle period, and hence are expected to continually shed DNA over the detection period. Thus, we propose that the decline in richness with increasing temperature reflects the increased stress of warmer areas, which are less favorable to a broader range of species (Satyam and Thiruchitrambalam 2018). We suggest the absence of patterns observed by the Shannon diversity index reflects the lack of concordance between sequencing reads and true abundance. Therefore, caution is needed when using abundance-based diversity indices to interpret metabarcoding data, unless techniques are applied to account for discrepancies between amplicon counts and true taxon abundance, such as PCR-mechanistic statistical models (Shelton et al. 2023).

Ecological expectations were also met at a regional scale, with detections of cold-affinity boreal species increasing with latitude, whilst warm-affinity lusitanian species declined with latitude. The high proportion of boreal species in Scotland is consistent with its unique geological history and more northern latitude relative to

other UK Regional Seas (Hall et al. 2019). In addition, the detection of a high proportion of invasive species in southwest England aligns with current understanding of invasive species distributions in the UK (Eno et al. 1997), detecting the presence of non-native species such as the red alga *Asparagopsis armata* (Harpoon weed), the arthropod *Austrominius modestus* (Modest barnacle), the brown alga *Colpomenia peregrina* (Oyster thief), and the red alga *Grateloupia turuturu* (Devil's tongue weed). While moderate overlap of detections was found using eDNA across regions, ubiquitous taxa are common within UK rocky shore communities. Hence, it is likely the observed patterns reflect the true ecology of the system, though direct comparisons to visual observations at the same spatial scales are needed for validation.

### 4.4 | Implications for Monitoring Intertidal Biodiversity Using eDNA

We offer recommendations for optimizing sampling practices to monitor intertidal biodiversity using eDNA-based methods. Due to the evidence presented that eDNA-derived communities are less discrete at small spatial scales (i.e., within sites), scale-appropriate sampling protocols should be tailored to the specific monitoring objectives. For example, when investigating changes in small-scale biodiversity (e.g., microhabitats across a single shore), sampling at the point of greatest physical isolation (i.e., low tide) will help identify unique ecological signals across different areas. To capture all species present at a given site (i.e., conducting a BioBlitz as described in Robinson et al. 2013), we recommend increasing local-scale replication, such as by taking multiple samples from the same rockpool, to reduce the likelihood of false negatives. Sampling from various media such as rock scrapes in addition to seawater could enhance detection rates and capture different communities, as shown in other marine studies (Brandt et al. 2021).

Whilst eDNA-based methods have utility for fine-scale monitoring, our findings suggest that sampling at larger scales is more effective at capturing biogeographical shifts in intertidal communities. For example, when assessing the overall biodiversity at a site without the need to differentiate between specific local areas, sampling at high tide from open water will increase the likelihood of capturing a broader range of DNA and improve sampling efficiency. This is based on our findings that show open water samples detected the most unique taxa and showed considerable overlap in detections with other shore heights. Increasing site-wide replication or employing larger-scale filters (e.g., autosamplers) over a tidal cycle will improve sampling effort and DNA capture. When targeting a particular species, such as an invasive species, the use of more specific primers and increased site-level replication may be necessary. Moreover, when interest extends beyond the site itself, sampling in open water could offer broader applicability for marine surveys, as rocky shores are more accessible and cost-effective for sampling compared to offshore environments.

## 5 | Conclusion

Our broad geographic study demonstrates the effectiveness of a multi-marker eDNA metabarcoding approach to characterize coastal biodiversity and its potential for multiscale monitoring in highly connected marine environments. Observed differences in communities across shore heights and regions were consistent with known ecological patterns, suggesting that eDNA methods can accurately capture true community structure. eDNA signals were more strongly structured by geographic location than by vertical shore height, reflecting greater DNA homogenization at smaller spatial scales, with potential implications for application in ecological studies. Thus, we recommend the adoption of scale-appropriate sampling protocols tailored to specific monitoring objectives. For large-scale biodiversity assessments, prioritizing open water sampling at high tide could optimize broad site-level detections. Alternatively, sampling at a rockpool scale at low tide could be more effective at capturing local patterns. Overall, our findings suggest that onshore eDNA-based approaches could be utilized in multiscale ecological studies to monitor broad marine biodiversity beyond the specific target taxa of this study, although future work should further validate detections through direct comparisons with visual surveys.

#### Author Contributions

D.-L.S. conceived the study and led its design with assistance from N.M., H.H., T.J.W., and M.S. Field collection was conducted by D.-L.S. with assistance from N.M. Laboratory work was conducted by D.-L.S. with support from G.J.H. Bioinformatics was conducted by D.-L.S. with assistance from H.H. D.-L.S. led the statistical analyses and interpretation of the results, with the advice of T.J.W. and M.S. D.-L.S. led the writing of the manuscript with feedback from N.M., H.H., T.J.W., and M.S.

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#### Ethics Statement

The authors have nothing to report.

#### Conflicts of Interest

The authors declare no conflicts of interest.

#### Data Availability Statement

Data, scripts, and figures associated with this manuscript are openly available in the following Git repositories: Simons (2025) [https://github.com/dinaleighsimons/intertidal-eDNA-formatting-and-quality-control] for raw data, formatting, filtering, and quality control; and Simons et al. (2025) [https://github.com/dinaleighsimons/intertidal-eDNA-spatial-analyses] for processed data and spatial analyses.

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## Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Data S1:** edn370203-sup-0001-Supinfo.docx.