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DOI: https://doi.org/10.3897/arphapreprints.e209136

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How many transects make a survey? eDNA-based benchmarks for sampling faunal diversity across a beach–dune ecotone

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Abstract

Sandy beaches and coastal dunes form a narrow ecotone characterised by steep environmental gradients and often intense human use. Efficient biodiversity assessment in these systems requires understanding how community composition varies along the coastline and how much spatial replication is needed for comparable surveys. We used environmental DNA metabarcoding to assess invertebrate diversity from a 55-transect field survey along the North Sea coast. Each transect covered four habitats: lower intertidal, upper intertidal, incipient dunes, and established dunes. We quantified multi-site operational taxonomic unit (OTU) sharing using zeta diversity, examined relationships between OTU detection frequency and transect incidence, and used coverage-based rarefaction and extrapolation to derive habitat-specific benchmarks for sampling effort. Zeta retention declined with increasing order in all habitats, but more slowly in the intertidal than in the dunes, which indicates greater compositional overlap among intertidal transects and greater heterogeneity among dune transects. Detection frequency was positively associated with transect incidence in all habitats, with the steepest slopes in the dunes. Dune assemblages were dominated by low-incidence OTUs, although OTUs detected more consistently across replicate sediment samples also tended to occur in more transects.

Intertidal habitats reached approximately 90% sample coverage with 8–9 transects, whereas established and incipient dunes required 16 and 19 transects, respectively. These findings show that intertidal sediment eDNA assemblages in the study area can be characterised with lower sampling effort, whereas more heterogeneous dune assemblages require denser spatial replication. By linking multi-site sharing, detection–incidence scaling, and sample coverage, this study provides practical sampling targets for sediment eDNA surveys across beach–dune ecosystems.

Key words: beach–dune ecotone; environmental DNA; incidence; sampling completeness; zeta diversity

Introduction

Sandy beaches and dunes form a narrow coastal ecotone where environmental conditions change over short distances (McLachlan and Defeo 2017). From the lower intertidal to the established dunes, inundation, sediment movement, moisture, and vegetation cover vary strongly. These gradients shape the fauna living in and on the sediment. At the same time, many beach–dune systems are heavily used by people. Recreation, beach grooming, nourishment, dune stabilisation, and infrastructure development can modify habitat structure and affect biotic communities (Defeo et al. 2009). This makes beach–dune ecotones important systems for biodiversity assessment and monitoring. For the efficient planning of such assessments, it is important to know how taxa are distributed among sampling locations, and whether taxa detected more often across replicate sediment samples are also likely to occur in more transects (Gaston and Fuller 2008, Legendre and De Cáceres 2013, Rodil and Lastra 2022) .

Most coastal biodiversity studies focus on richness or pairwise dissimilarity. These measures are useful, but they do not show how many taxa are shared across a larger set of sites (Baselga 2013). Zeta diversity provides one way to describe this pattern, because it measures how many taxa are shared among sets of increasing size (Hui and McGeoch 2014, Latombe et al. 2017). If many taxa are shared across transects, additional transects may mostly recover taxa already detected elsewhere. If sharing declines quickly, additional transects are more likely to add different taxa. Detection–incidence relationships and coverage-based accumulation curves provide complementary information. Together, these approaches can show how community assemblage structure affects the number of transects needed for biodiversity surveys.

Environmental DNA metabarcoding makes it possible to survey biodiversity at larger scales and with broad taxonomic coverage (Thomsen and Willerslev 2015, Deiner et al. 2017). In coastal systems, eDNA can detect habitat-specific community patterns across short gradients and across different management settings (O'Donnell et al. 2017, Gold et al. 2021). Rarefaction, extrapolation, and occupancy-based approaches are increasingly used to evaluate sampling effort and survey completeness (Chao and Jost 2012, Dorazio and Erickson 2018). However, practical benchmarks for eDNA sampling effort in beach–dune ecotones are still limited. It remains unclear whether intertidal and dune habitats require similar numbers of transects, or whether sampling effort should be adjusted for each habitat.

We used an eDNA metabarcoding survey along an open North Sea coastline to study faunal community structure across a beach–dune gradient. The survey spanned 55 transects along 35 km of coastline, with both urbanised and more pristine sections. Each transect covered four habitats: lower intertidal, upper intertidal, incipient dunes, and established dunes. Previous work suggests that supralittoral and dune communities can be more affected by urbanisation and management than intertidal communities, and that they can be patchy at short spatial scales (Ruiz-Delgado et al. 2014, Schooler et al. 2017). Beach nourishment and mechanical cleaning can also alter resource input and sediment structure, with effects on macrofaunal and meiofaunal assemblages (Dugan et al. 2003, Fegley et al. 2020).

We expected OTU sharing to differ between intertidal and dune habitats. Intertidal habitats are regularly flooded and form a relatively continuous sandy habitat along the shore. Hydrodynamic transport may also increase connectivity among sites (Defeo and McLachlan 2005, Netto and Fonseca 2017). We therefore expected intertidal habitats to contain more OTUs shared among many transects. Dune habitats are different. They are structured by vegetation mosaics, sand movement, local disturbance, and barriers such as paths or infrastructure. These features may increase compositional heterogeneity and reduce OTU sharing among transects (Bonte et al. 2006, Ittai et al. 2020).

We also expected the incidence structure of OTUs to differ between dunes and intertidal habitats. Dune habitats are more heterogeneous and can contain many spatially restricted taxa (Webb et al. 2007, Guedo and Lamb 2013). We therefore expected dune assemblages to contain many low-incidence OTUs, meaning OTUs detected in only a small fraction of transects. In addition, we tested whether OTUs detected more

frequently across sediment samples also occurred in more transects, and whether this relationship differed among habitats. Based on these expectations, we tested the following hypotheses:

H1: Taxon sharing among sites declines more rapidly in dunes than in intertidal habitats. If true, taxa shared by many sites should be more common in intertidal habitats, whereas dune assemblages should show lower multi-site sharing and greater compositional heterogeneity among transects.

H2: Dune assemblages are dominated by low-incidence OTUs compared to intertidal habitats. If true, a larger proportion of dune OTUs should occur in only a small fraction of transects.

H3: The relationship between OTU detection frequency and transect incidence differs between dune and intertidal habitats.

Using these analyses, we derive habitat-specific sampling benchmarks for sediment eDNA surveys across a beach–dune gradient. The aim is to estimate how many transects are needed to reach comparable sample coverage in each habitat, and to identify where additional replication is most important.

Methods

The field work, laboratory workflow, and initial bioinformatic processing for this dataset were described previously (Martinez et al. 2025). Here, we use the same eDNA metabarcoding dataset for a different purpose: to derive habitat-specific sampling-effort benchmarks across a beach–dune ecotone. We combined zeta diversity, detection–incidence scaling, and coverage-based rarefaction to assess how sampling effort differed among habitats. In brief, we sampled 55 transects along the North Sea coastline between Scheveningen and Zandvoort, The Netherlands, between July and August 2023. Along each transect, we collected 12 sediment samples, 10 mL each, across four habitat zones spanning the beach-dune gradient: lower intertidal (n = 3), upper intertidal (n = 3), incipient dunes (n = 3), and established dunes (n = 3), resulting in 660 samples. Samples were taken approximately equidistantly within each zone (Figure 1). Sediment was collected with sterile 20-mL plastic cores, transferred to sterile 50-mL tubes, and stored at −20 °C until processing.

DNA was extracted using the SDE protocol (Bollmann-Giolai et al. 2020). We amplified a 313-bp COI fragment with a two-step PCR using LerayXT primers targeting Eukaryota. Cycling conditions were: 96 °C for 3 min; 30 cycles of 96 °C for 15 s, 50 °C for 30 s, and 72 °C for 40 s; and a final extension at 72 °C for 5 min. After the first PCR, we cleaned the PCR products with AMPure beads (Beckman Coulter, Brea, United States) at a 0.9:1 ratio, which removed short fragments and primer dimers. We used a second PCR with individually tagged primers with the same protocol, using 1µl of PCR product from the first PCR as template, and reducing the cycle number to 10. Six extraction/PCR negatives, using Milli-Q water in place of template, were included. Libraries were pooled equimolarly, bead-cleaned, and sequenced on two Illumina NovaSeq 6000 runs.

Raw sequencing reads were processed with APSCALE (v. 1.6.3) (Buchner et al. 2022) using max differences = 10%, minimum overlap = 50 bp, and minimum length = 300 bp, and LULU filtering on default settings. Sequences were clustered into 97% OTUs. OTUs detected in negative controls were subtracted from sample read counts per sample, which is a common approach for reducing potential contaminations (Macher et al. 2023). We removed low sequencing depth samples (< 5% of the median read count) and rare OTUs (< 0.0005% per-sample relative abundance) to limit artefacts (e.g., tag-jumping) (Schnell et al. 2015). Taxonomy was assigned using the NCBI GenBank database using blastn, supplemented with meiofauna COI barcodes generated during taxonomic workshops held in the sampling area (Macher et al. 2024). OTUs that were not assigned to an animal reference sequence with minimum similarity of 90% were excluded.

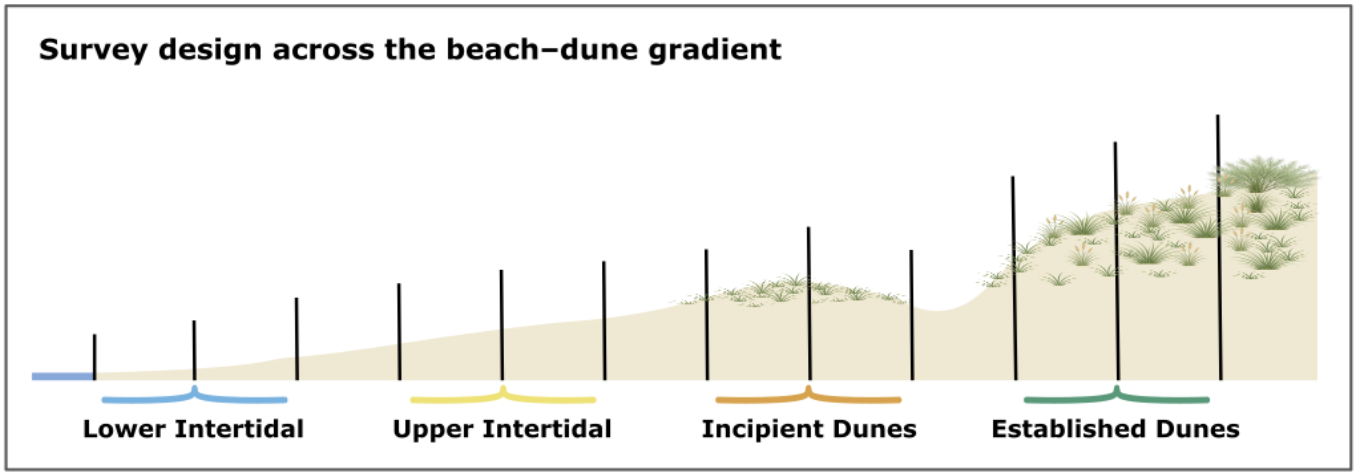

Figure 1: Survey design across the beach-dune gradient. Each transect spans the four habitats lower

intertidal, upper intertidal, incipient dunes, and established dunes, with three sediment samples per habitat and 12 samples per transect. In total, 55 transects were sampled.

Zeta diversity analyses

We quantified multi-site OTU sharing using zeta diversity (Hui and McGeoch 2014), which measures the number of OTUs shared among sets of *k* sites. For each habitat, we constructed transect-level presence-absence matrices using the complete-replicate dataset. We retained only transect × habitat combinations for which all three replicate sediment samples were available. Replicate samples within each retained transect × habitat combination were collapsed to OTU presence if an OTU was detected in at least one of the three replicate samples. This aggregation ensured that the sampling unit matched the transect-level design, that replicate cores were not treated as independent sites, and that detection opportunity was consistent among retained transects.

Zeta decline curves were calculated across transects using the zetadiv R package (Latombe et al. 2018), with Monte Carlo sampling and 1000 random draws per order up to a maximum of 10 sites. Zeta values were rescaled by first-order zeta, ζ_k / ζ_1, to express the proportion of habitat-level OTU richness retained across sets of *k* transects. We refer to this value as zeta retention. As a sensitivity check, we repeated the analysis using transect × habitat matrices based on all available replicate samples.

Detection-incidence analysis

We quantified the relationship between OTU detection frequency and incidence across transects. To avoid calculating both measures from the same replicate detections, we used different replicate subsets for predictor and response. We retained only transect × habitat combinations with all three replicate sediment samples available. For each split, two replicates were used to calculate the survey-wide detection frequency of each OTU within a habitat, defined as the proportion of held-in sediment samples in which the OTU was detected. The remaining replicate was used to calculate held-out transect incidence, defined as the proportion of transects in which the OTU was detected in the held-out sample. This was repeated for all three possible held-out replicates.

For each replicate split, we fitted a generalized linear model with a quasi-binomial error structure, modelling held-out transect incidence as a function of detection frequency and a habitat × detection-frequency interaction. Habitat-specific slopes on the logit scale were extracted using the emmeans R package and summarised as the mean and range across the three replicate splits. We also tested habitat differences in detection-incidence slopes within each split using pairwise contrasts among habitat-specific trends. For visualisation, fitted relationships were predicted from the split-specific models and averaged across the three held-out replicate splits on the response scale. The analysis describes the association between detection frequency and held-out transect incidence and does not estimate true biological occupancy.

Coverage-based sampling benchmarks

For each habitat, we built transect × OTU incidence matrices using the same complete-replicate sampling frame as in the zeta and detection-incidence analyses. We retained only transect × habitat combinations with all three replicate sediment samples available. Within each retained transect × habitat combination, replicate samples were aggregated to OTU presence if an OTU was detected in at least one of the three samples.

We used iNEXT (Hsieh et al. 2016) to compute incidence-based sample coverage as a function of the number of transects and to estimate OTU richness at the realised sample number. Extrapolation was truncated at twice the number of sampled transects per habitat. Coverage curves were evaluated at every integer transect number from one transect to twice the realised sampling effort. From these curves, we recorded sample coverage and estimated richness at the realised effort, and the first integer number of transects required to reach 90% sample coverage. We used 90% sample coverage as a pragmatic operational benchmark.

Uncertainty in coverage and richness estimates at the realised effort was quantified with 1000 bootstrap resamples in iNEXT to obtain 95% confidence intervals. To estimate uncertainty in the number of transects required to reach the 90% coverage target, we additionally performed a nonparametric transect bootstrap within each habitat. For each bootstrap iteration, transects were resampled with replacement, the incidence-based coverage curve was recalculated, and the first integer number of transects reaching the 90% coverage target was recorded. Bootstrap intervals for the 90% transect benchmarks were calculated from the 2.5% and 97.5% quantiles across 1000 bootstrap iterations. As a sensitivity check, we repeated the coverage analysis using transect × habitat matrices based on all available replicate samples.

Results

We aggregated the 12 samples along each transect into four habitat categories: lower intertidal (levels 1–3), upper intertidal (levels 4–6), incipient dunes (levels 7–9), and established dunes (levels 10–12). For the primary analyses, we retained only transect × habitat combinations with all three replicate sediment samples available. The number of retained transects therefore differed among habitats: 37 in established dunes, 40 in incipient dunes, 49 in the lower intertidal, and 50 in the upper intertidal. The observed OTU richness differed among habitats. Richness was highest in the dune habitats, with 280 OTUs detected in incipient dunes and 266 OTUs in established dunes. The lower and upper intertidal zones contained fewer OTUs, with 107 and 114 OTUs, respectively. We retained a total of 364 OTUs for analysis. Sample-level OTU occurrences and taxonomic assignments are provided in Supplementary Table S1.

Zeta retention

Zeta retention declined with order in all habitats (Fig. 2, Supplementary Table S2), but the decline was slower in the intertidal habitats. In the lower intertidal, OTU retention decreased from 0.54 ± 0.14 at k = 2 to 0.11 ± 0.06 at k = 10, indicating that a subset of OTUs was still shared across larger sets of transects. In the upper intertidal, retention decreased from 0.39 ± 0.20 at k = 2 to 0.01 ± 0.02 at k = 10. Dune habitats showed a more rapid decline in shared OTUs. In established dunes, retention decreased from 0.24 ± 0.14 at k = 2 to 0.03 ± 0.03 at k = 5 and <0.01 by k = 10. In incipient dunes, retention decreased from 0.19 ± 0.20 at k = 2 to <0.01 by k = 5 and effectively zero by k = 10. These patterns indicate that multi-site OTU sharing was more persistent in the intertidal habitats, especially the lower intertidal, whereas dune assemblages showed very low sharing among larger sets of transects.

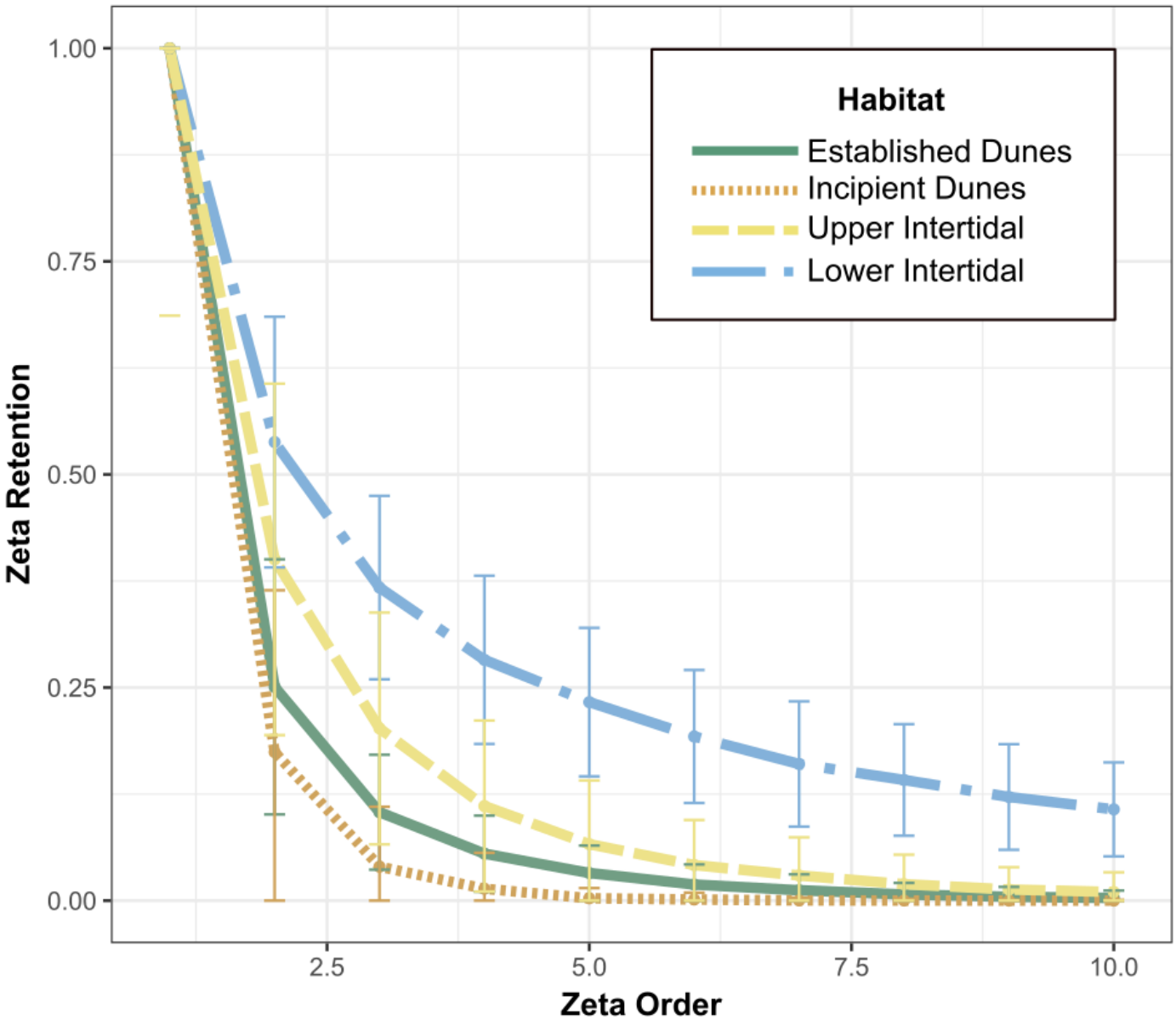

Figure 2: Zeta retention curves showing multi-site OTU sharing across the beach-dune gradient. Curves are based on transect × habitat units with all three replicate sediment samples available. The x-axis shows zeta order, meaning the number of transects; the y-axis shows zeta retention, calculated as ζ_k / ζ_1, or the proportion of habitat-level OTU richness retained across sets of *k* transects. Lines and points show mean retention, and vertical error bars show ±1 standard deviation across 1,000 Monte Carlo draws. Colours indicate habitat.

Detection-incidence relationships

To characterise how regional incidence was distributed among OTUs, we binned incidence into 10% classes using the full three-replicate transect-level data from the complete-replicate dataset (Supplementary Table

S3). In both dune habitats, most OTUs occurred in the two lowest incidence classes, 0–10% and 10–20% of transects. In established dunes, 38.3% of OTUs occurred in the 0–10% bin and 41.7% in the 10–20% bin. In incipient dunes, the corresponding values were 57.1% and 22.1%. Only 2 OTUs in established dunes and none in incipient dunes occurred in at least 70% of transects. Intertidal assemblages also contained many low-incidence OTUs, with 56.1% of lower-intertidal OTUs and 58.8% of upper-intertidal OTUs occurring in the 0–20% incidence classes. However, the lower intertidal contained a clearer widespread set: 13 OTUs (12.1%) occurred in ≥70% of transects, compared to 5 OTUs (4.4%) in the upper intertidal.

The split-replicate detection-incidence analysis showed positive relationships in all habitats (Figure 3; Supplementary Table S4). OTUs detected more frequently across the two held-in replicate samples per transect also tended to occur in a larger proportion of transects in the held-out replicate sample. Mean slopes across the three held-out replicate splits were highest in incipient dunes (8.60, range: 7.26–10.82), followed by established dunes (7.50, range: 6.83–8.68). Slopes were lower and similar in the lower intertidal (5.95, range: 5.09–6.39) and upper intertidal (6.02, range: 4.78–7.12). Habitat differences in detection-incidence slopes varied among replicate splits: the habitat × detection-frequency interaction was detected in two of the three split-specific models, while pairwise slope contrasts were not significant for all habitat comparisons in all splits. Overall, these results indicate that detection frequency was positively associated with held-out transect incidence in all habitats, with the strongest mean scaling in dune assemblages.

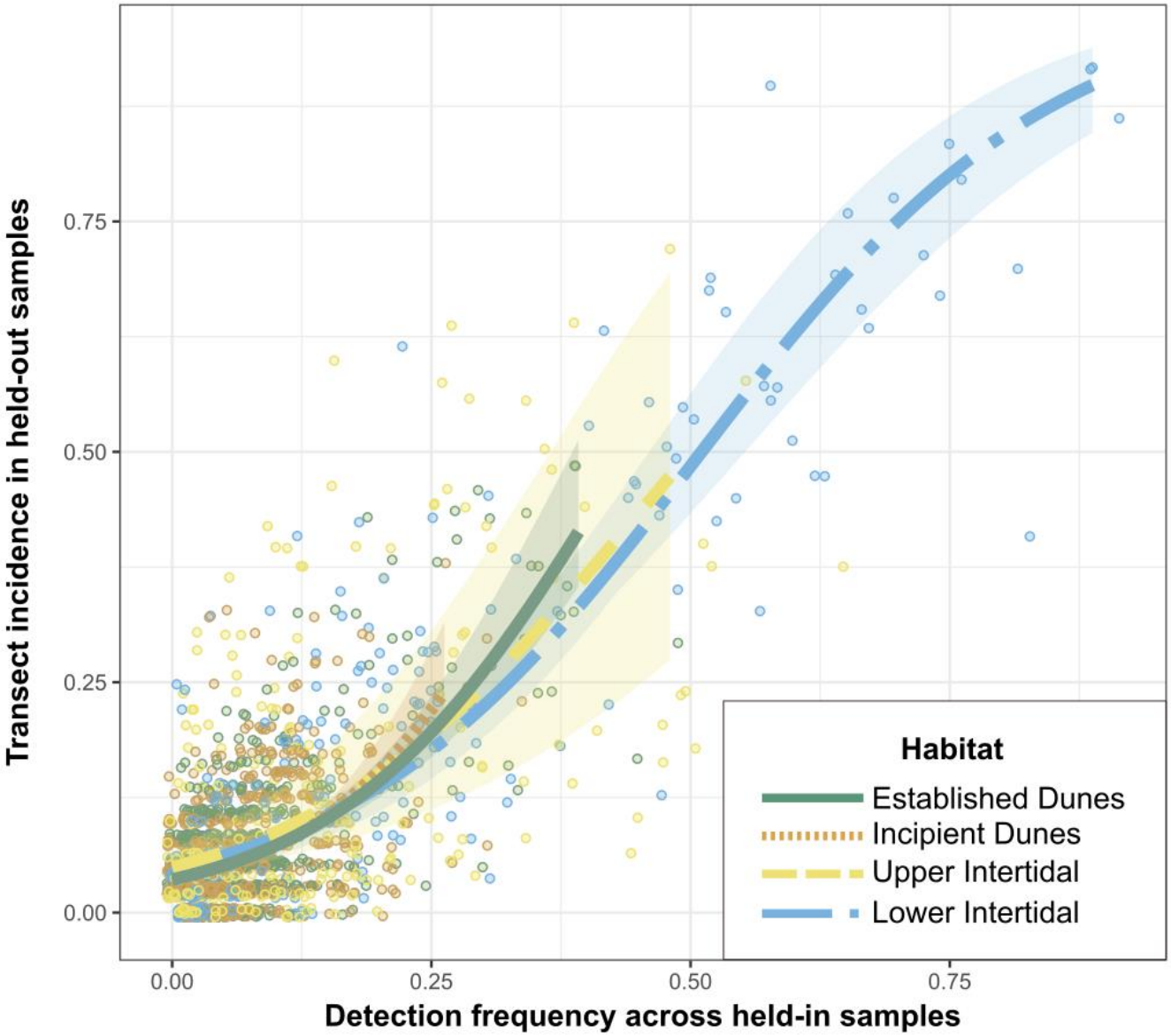

Figure 3: Detection-incidence relationships across the beach-dune gradient. Each point represents an OTU within a habitat and one replicate split. Detection frequency was calculated from two held-in replicate samples per transect, while held-out transect incidence was calculated from the remaining replicate sample across transects. Lines show fitted relationships averaged across the three split-specific quasi-binomial models on the response scale; shaded bands show the range of fitted relationships across the three held-out replicate splits. Reported habitat-specific slope estimates were derived separately for each replicate split and are summarised as mean and range across splits.

Coverage-based sampling benchmarks

Sampling completeness was high across all habitats (Fig. 4, Supplementary Table S5), with coverage values ranging from 0.973 to 0.993 at the realised sampling effort. In established dunes, coverage was 0.987 at 37 transects (richness: 266 OTUs, 95% CI: 259–273), and the 90% coverage target was reached after 16 transects, with a bootstrap interval of 13–19 transects. In incipient dunes, coverage was 0.973 at 40 transects (richness: 280 OTUs, 95% CI: 271–289), with 90% coverage reached after 19 transects, with a bootstrap interval of 14–21 transects.

Intertidal habitats required fewer transects to reach comparable completeness. In the lower intertidal, coverage was 0.993 at 49 transects (richness: 107 OTUs, 95% CI: 102–112), and 90% coverage was reached after 8 transects, with a bootstrap interval of 6–8 transects. In the upper intertidal, coverage was 0.988 at 50 transects (richness: 114 OTUs, 95% CI: 108–120), with 90% coverage reached after 9 transects, with a bootstrap interval of 8–10 transects. Thus, dune habitats required roughly twice as many transects as intertidal habitats to reach the same 90% coverage benchmark.

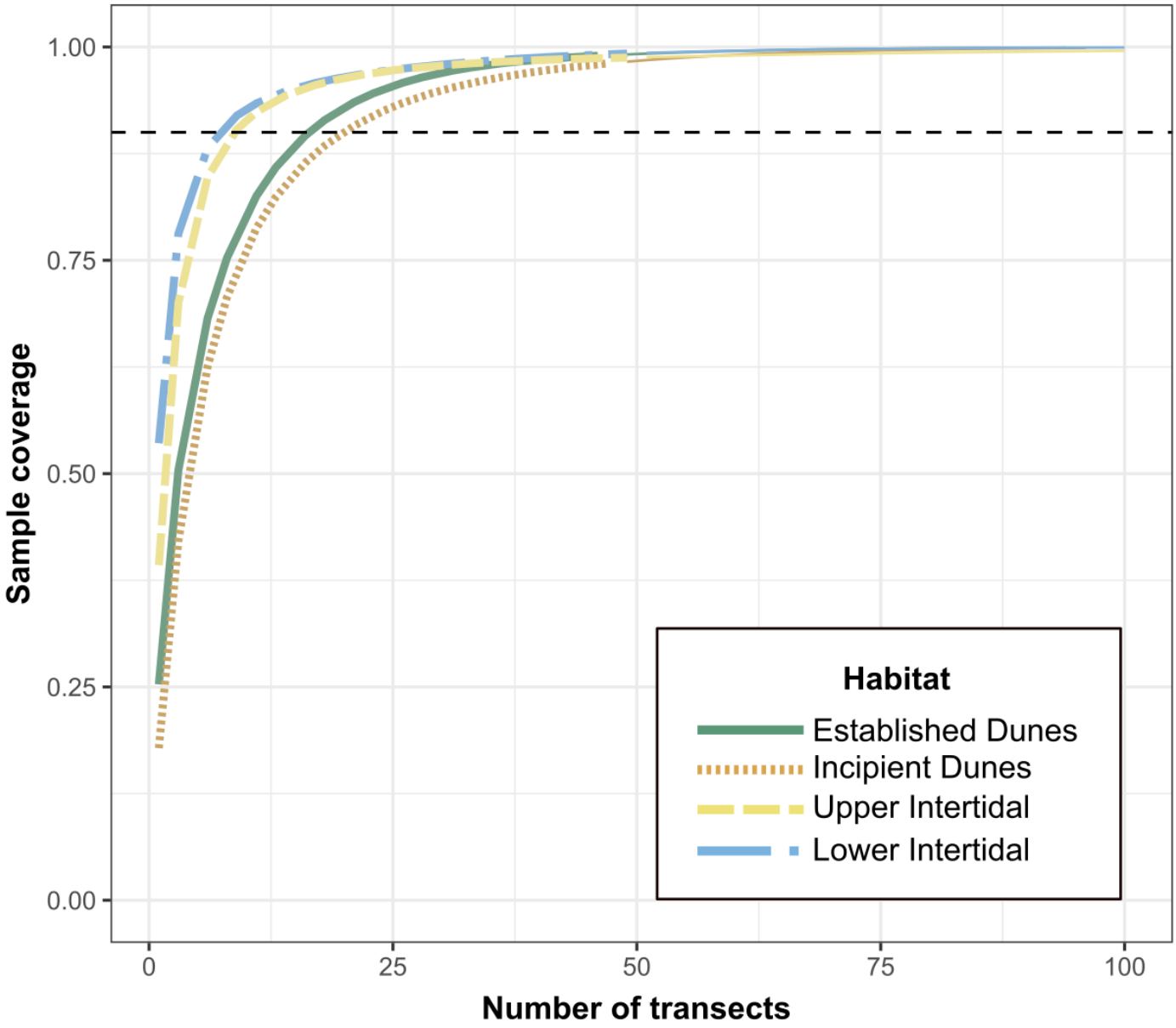

Figure 4. Incidence-based sample coverage curves across the four beach-dune habitats, with transects as sampling units. Curves are based on transect × habitat units with all three replicate sediment samples available. Coverage was evaluated at every integer transect number from one transect to twice the realised sampling effort. Thick lines show rarefaction, thin lines show extrapolation, and the horizontal dashed line marks the main operational benchmark of 90% sample coverage.

Discussion

Beach-dune ecotones are important for coastal protection, connect sea and land, and support diverse invertebrate communities under high natural variability and often intense human use. Because natural processes and management reshape these habitats, biodiversity assessments need baselines for beach

biodiversity (Martínez et al. 2025) and a better understanding of how communities are distributed locally and regionally (Defeo et al. 2009). Using eDNA metabarcoding from 55 transects of North Sea coastline, we compared four habitats across the beach–dune gradient and tested three hypotheses: H1, that multi-site sharing declines faster in dunes than in intertidal areas; H2, that dune assemblages are dominated by low-incidence OTUs; and H3, that detection–incidence relationships differ between dune and intertidal habitats.

Our results broadly supported H1 and were consistent with H2. For H3, detection–incidence slopes differed among habitats, with the steepest mean slopes in dunes, but pairwise contrasts were not significant for all habitat comparisons in all replicate splits. Zeta retention declined more rapidly in incipient and established dunes than in the intertidal habitats. This means that OTUs were shared among larger sets of transects more often in the intertidal, especially in the lower intertidal, whereas dune assemblages showed much lower multi-transect sharing. This pattern fits with the idea that intertidal habitats form a more continuous and connected sandy environment along the shore, while dune habitats are more patchy in vegetation structure and local conditions (Defeo and McLachlan 2005, van Puijenbroek et al. 2021).

The detection-incidence results show that dune assemblages were dominated by low-incidence OTUs, so most dune OTUs occurred in only a small fraction of transects. At the same time, detection-incidence slopes were steepest in the dune habitats. This does not mean that dune OTUs were generally widespread. Rather, it suggests that dune OTUs detected more frequently across the held-in replicate samples within a habitat were also more likely to occur in more transects in the held-out sample. Thus, dune assemblages appear to combine a large pool of rarely detected, low-incidence OTUs with a smaller subset of more consistently detected OTUs. In contrast, intertidal assemblages showed more coherent OTU composition among transects.

Zeta retention

Retention curves show how fast cross-site overlap of OTUs declines as more transects are added. In the lower intertidal, retention across higher orders means that additional transects often recovered some of the same OTUs. In the upper intertidal, the decline was steeper but still gradual. In both dune habitats, retention

dropped rapidly and approached zero at low to intermediate orders. This shows that shared OTUs across many transects were uncommon in the dunes.

These contrasts are consistent with differences in habitat structure across the ecotone. Hydrodynamic connectivity and continuous substrate may increase overlap among intertidal transects (Defeo and McLachlan 2005, Hyndes et al. 2022). In contrast, vegetation mosaics, sand movement, and local disturbances may contribute to more patchy dune assemblages and lower overlap among transects (Bonte et al. 2006, Maes et al. 2006). Similar patterns have been observed in other coastal systems, where shoreline communities show higher spatial coherence (Jaramillo et al. 2001, Lagos et al. 2007, Lara et al. 2019) while supralittoral and dune assemblages are structured by patchy resources and disturbances (Comor et al. 2008, Ruiz-Delgado et al. 2014, Van De Walle et al. 2025). Our findings therefore support H1 and show that this contrast in multi-site sharing is detectable from eDNA-inferred assemblages along a coastline. For biodiversity assessments, this means that intertidal assemblages can be characterised with fewer transects because additional transects often recover OTUs already detected elsewhere, whereas dune assemblages require denser replication because additional transects are more likely to add different OTUs.

Detection-incidence

The incidence distributions were consistent with H2. Dune assemblages were dominated by low-incidence OTUs, with most dune OTUs occurring in the two lowest incidence classes. This type of uneven incidence structure is common in ecological assemblages, where many taxa are rare or locally restricted and fewer taxa are widespread (Hanski 1982, Gibson et al. 1999, Gaston et al. 2002).

The detection–incidence analysis also addressed H3. Slopes were steepest on average in the dune habitats, meaning that dune OTUs detected more frequently across the held-in replicate samples also tended to occur in more transects in the held-out sample. This does not mean that dune OTUs were generally widespread. Instead, it suggests that dune assemblages contain both a large pool of low-incidence OTUs and a smaller subset of OTUs that are detected more consistently and occur across more transects.

This result should still be interpreted cautiously. Predictor and response were calculated from different replicate subsets, so the pattern is not caused by directly reusing the same replicate detections. However, pairwise slope contrasts were not significant for all habitat comparisons in all splits. The slope results

306 therefore indicate a directional pattern rather than clear pairwise separation among all habitats. They should
307 also not be interpreted as a direct test of dispersal, abundance, or true occupancy, because eDNA
308 metabarcoding detections are affected by imperfect detection and by variation introduced during field
309 sampling, molecular processing, and sequencing (Lahoz-Monfort et al. 2016, McClenaghan et al. 2020).

310 Fine-scale habitat heterogeneity due to vegetation structure, wrack availability, and local disturbance may
311 contribute to the high number of low-incidence OTUs in supralittoral and dune habitats (McLachlan 1991,
312 Hyndes et al. 2022). The more consistently detected OTUs may represent taxa associated with recurring
313 dune microhabitats, broader habitat tolerance, or stronger dispersal abilities, but we cannot separate these
314 mechanisms with the present data. For monitoring, the key point is that steep dune slopes do not mean that
315 dune biodiversity is easy to sample. The large pool of low-incidence OTUs still requires dense spatial
316 replication.

317 **Coverage-based sampling benchmarks**

318 Coverage-based accumulation showed a clear habitat contrast. Intertidal habitats reached about 90% sample
319 coverage with fewer transects than dune habitats. The lower and upper intertidal reached the 90% coverage
320 target after 8 and 9 transects, respectively. In contrast, established and incipient dunes required 16 and 19
321 transects, with bootstrap intervals of 13–19 and 14–21 transects. Thus, dune habitats required roughly twice
322 as many transects as intertidal habitats to reach the same coverage benchmark.

323 This pattern agrees with the zeta results. Higher OTU retention in the intertidal habitats means that additional
324 intertidal transects often re-encounter OTUs already detected elsewhere. In dunes, retention declined more
325 rapidly, so additional transects were more likely to add different OTUs. The coverage analysis translates this
326 assemblage structure into practical sampling benchmarks. Intertidal sediment eDNA assemblages could be
327 characterised with modest effort, whereas dune assemblages required higher sampling intensity.

328 We use 90% sample coverage as a pragmatic benchmark that balances representativeness and effort, similar
329 to other eDNA studies that define sampling thresholds for biodiversity assessments (Cantera et al. 2019).
330 However, sample coverage estimates the completeness of the incidence-based assemblage in this dataset.
331 It should not be interpreted as the proportion of all regional OTUs recovered, especially for low-incidence
332 taxa.

The benchmarks are also method- and design-dependent. Inferred richness and sampling targets depend on the molecular pipeline, including primer choice and bioinformatic processing (Alberdi et al. 2018, Hajibabaei et al. 2019). Our results should therefore be interpreted as within-method benchmarks for this sediment eDNA workflow. They should also be interpreted for transects distributed across a coastline comparable to the present study, rather than as fixed targets for a spatially restricted area such as a single small beach. Comparisons across datasets, markers, seasons, and coastlines will be needed once more eDNA datasets for beach and dune habitats are available.

Future directions

The eDNA-based analyses of OTU sharing, detection-incidence scaling, and sample coverage provide guidance for biodiversity assessments in beach and dune ecosystems, but limitations remain. Presence-absence data from eDNA metabarcoding do not represent abundance directly. Taxon detection can vary with primer bias and reference database completeness (Elbrecht and Leese 2015, Weigand et al. 2019); and eDNA persistence can differ among sediment types (Freeman et al. 2020). These caveats are common in metabarcoding studies and show the need to test whether patterns are stable under different methodological and environmental settings (Zinger et al. 2019, Pawlowski et al. 2022).

Future work should test the stability of these baselines across seasons, years, and additional coastal habitats. It should also examine how the spatial arrangement of transects affects coverage targets, because clustered and coastline-scale sampling designs may require different levels of replication. Experimental approaches would be useful to assess how natural and human disturbances, such as storms, beach cleaning, nourishment, or trampling, influence biodiversity patterns. Expanding reference libraries (Weigand et al. 2019, Macher et al. 2024), improving primer coverage (Elbrecht et al. 2019), and incorporating ecological traits (Boyé et al. 2019, Daché et al. 2025) would further help to understand differences among taxonomic groups and how they are distributed locally and regionally. Applying this workflow to additional coastlines could support more comparable reporting of sampling effort across regions and help integrate sediment eDNA into monitoring frameworks such as the Water Framework Directive, the Marine Strategy Framework Directive, and the Habitats Directive.

Conclusion

Intertidal sediment eDNA assemblages showed higher multi-site OTU sharing than dune assemblages, whereas dune habitats showed greater compositional heterogeneity and required denser sampling. Dune assemblages also contained many low-incidence OTUs, but OTUs detected more frequently across replicate sediment samples also tended to occur in more transects. By analysing zeta diversity, detection-incidence relationships, and coverage-based rarefaction together, we show that sediment eDNA sampling effort should be matched to habitat structure. In this beach–dune system, intertidal habitats reached about 90% sample coverage with 8–9 transects, while dune habitats required 16–19 transects. These benchmarks should be interpreted as within-method estimates for this COI sediment eDNA workflow and for similarly distributed coastline-scale sampling, but they provide a practical basis for designing comparable biodiversity surveys across beach–dune habitats.

Data Availability Statement

All raw data are available in the NCBI Sequence Read Archive (SRA), under BioProject number PRJNA1426151. The R script and required input tables are available in figshare, DOI: 10.6084/m9.figshare.33049562.

Acknowledgements

We would like to thank the Naturalis lab team for their support, and Rijkswaterstaat, Staatsbosbeheer, Dunea, and Waternet for facilitating access to the study area. This study is part of the MeioMon grant to JNM, funded by the Hollmann Foundation.

Conflict of Interest Statement

The authors have no conflict of interest to declare.

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