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A case for multi-method assessments: detection probabilities of nearshore fish with eDNA and seine nets vary by functional traits

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Abstract

Ecological studies aim to understand species distributions, yet the sampling methods affect which species are detected and may be influenced by species traits. Detection of marine fishes based on species traits has been studied using traditional sampling methods, but such studies have generally not extended to environmental DNA (eDNA). Here, we investigated which functional species traits (scale type, schooling behavior, and position-in-water-column) are detected by eDNA metabarcoding and beach seines in nearshore eelgrass, mixed eelgrass, and understory kelp habitats. Using data from 35 sites across southeast Alaska, we applied occupancy modeling to estimate detection of species traits by each method. Detection probability with eDNA was 27 times greater for the species with deciduous scales (*Clupea pallasii*) compared to species with non-deciduous scales, and lower for species with plates (rather than scales). Conversely, species with plates showed greater odds of detection with beach seines. Given the novelty of eDNA sampling, quantifying interactions between detection and functional traits will be important to accurately characterize species distributions across marine habitats.

Key words: environmental DNA (eDNA), quantitative metabarcoding, Bayesian occupancy modeling, indicator species analysis, beach seine, habitat

1. Introduction

Ecological studies sample species communities to understand diversity, ecosystem function, and provide information about species distributions for management and conservation actions (Margules and Pressey 2000; Lefcheck et al. 2015; Oliver et al. 2015). Yet the methods used to sample can influence how effectively particular species are detected. In aquatic ecosystems, nets have traditionally been used to sample the fish community, but comparisons between net sampling and alternative sampling methods show bias in the species of fishes captured (McClanahan and Mangi 2004). More recently, environmental DNA (eDNA) has become a common method for aquatic sampling, with many studies demonstrating increased species richness and diversity based on eDNA samples when compared to nets (Sard et al. 2019; Stoeckle et al. 2021; He et al. 2022). However, in most of these studies, eDNA does not detect all of the same fish species as the nets; thus, complementary sampling methods could be used to maximize sampling efficiency, with eDNA as a complement to traditional capture-based sampling methods (Stat et al. 2019; Andres et al. 2023; Gold et al. 2023).

Generally, species in eDNA samples are identified using metabarcoding, an efficient laboratory procedure that amplifies DNA from a targeted taxonomic group (i.e., all teleost fishes). The amplification process results in species-specific bias originating from the unique characters in the DNA sequence for a given species and whether they perfectly match the primers used to bind to this sequence (Sato et al. 2021; Shelton et al. 2023). When using metabarcoding data as a quantitative metric of relative eDNA abundance, some effort must be made to quantify this amplification bias using either internal DNA standards (Sato et al. 2021; Tsuji et al. 2022) or mock communities comprised of known quantities of DNA for the species community found in field samples (i.e., Shelton et al. 2023). If amplification bias is identified, metabarcoding data can be bias-corrected and then analyzed using standard ecological approaches and compared to traditional sampling methods.

The general observation in comparative studies that eDNA detects some, but not all, of the same fishes as net-based sampling methods (and vice versa), leads to the question of what underpins differences in detection. Put another way, which species traits are being detected by eDNA more or less

than by nets? Patterns of trait-based detection by different sampling methods have been found across aquatic ecosystems (Mbaru et al. 2020; Aglieri et al. 2021). Less common are studies that examine sampling method detection by species functional traits and include eDNA as one of the sampling methods (but see Czeglédi et al. 2021; Pont et al. 2021), although some studies have made general observations about functional traits, even when traits were not explicitly tested. For example, Saunders et al. (2024) found that eDNA detected larger pelagic species that beach seines did not. A key benefit of focusing on functional traits is that traits are more generalizable than individual species, and common traits are identifiable across similar types of aquatic ecosystems around the globe.

Building on the results from previous studies of species detection by sampling method that include eDNA (He et al. 2022; Saunders et al. 2024), our goal is to address the question of why some species are more efficiently detected by different sampling methods. Further, we examine the species traits that underpin differences in detection probability. Here, we define eDNA detection as the ability for eDNA methods to identify the DNA from fish species present in the water column and do not explore other potential aspects of eDNA selectivity (e.g., filter pore size, extraction method, primer choice, etc.). We define detection in beach seines as the presence of a fish in the net (a result that is contingent on the availability of the fish in the environment and the selectivity of the net). We conducted paired sampling using two different nearshore sampling methods, beach seines and eDNA metabarcoding, in three types of nearshore habitats (eelgrass, mixed eelgrass, and understory kelp) in southeast Alaska, USA. We selected nearshore habitats because they are productive ecosystems that maintain complex food webs, confer ecosystem services, and serve as fish nurseries for various species including for commercially important fisheries (Heck Jr. et al. 2003; Beck et al. 2006; Lefcheck et al. 2019). In this study, we (1) compared fish communities across habitats sampled with each sampling method, (2) identified which species are associated with which sampling method, and then (3) quantified the extent to which each of three traits influences detection probabilities by sampling method. The three traits selected for analysis are characteristics that might affect eDNA detection: species' position-in-the-water-column, schooling behavior, and presence of scales/scale-type.

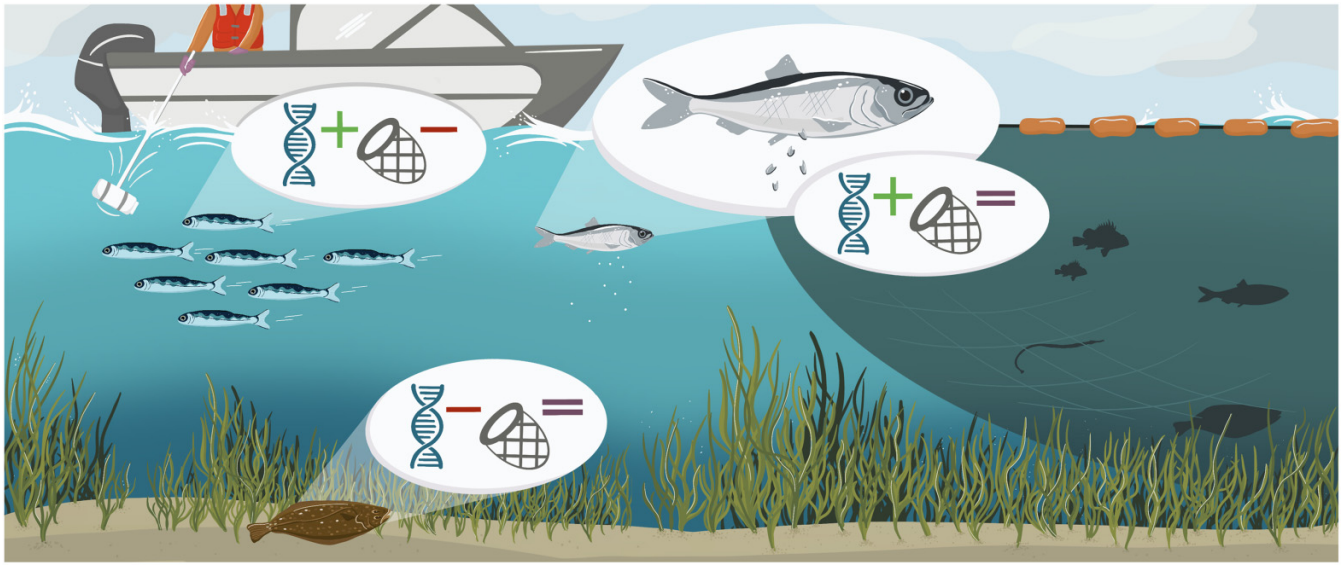
eDNA is released through multiple processes, including the shedding of skin or scales, release of gametes, and excretion of feces (Harrison et al. 2019). Rates of eDNA shedding vary with organismal traits such as body size, age, reproductive status, and form (Maruyama et al. 2014; Klymus et al. 2015; Takeuchi et al. 2019). In particular, different animal forms shed different tissue types (i.e., mucus and scales) and amounts of eDNA, with hard-shelled organisms having lower shedding rates than soft-bodies organisms (Andruszkiewicz Allan et al. 2021). eDNA shedding rates also vary with environmental conditions such as temperature and water stratification (Jo et al. 2019; Jeunen et al. 2020). After release, eDNA undergoes decay from temperature, enzymatic, and microbial processes and UV exposure (Strickler et al. 2015; Jo et al. 2019; Van Bochove et al. 2020), while also being redistributed by physical and biological transport mechanisms. Unlike capture-based methods, eDNA is advected from its source and disperses, such that water can contain DNA from species that are currently, or were recently, present, with detection typically decreasing with increasing distance (e.g., Andruszkiewicz et al. 2019; Baetscher et al. 2024; Brasseale et al. 2025). Consistent with this, higher organism abundance is expected to generate larger amounts of eDNA (e.g., Ledger et al. 2024), which can also increase detection.

Based on this understanding of eDNA production and dispersal, we hypothesized that eDNA detections would be greater for schooling fish that are physically interacting in such a way that they are shedding additional cells compared to non-schooling fish. We expected more eDNA detections for species with deciduous scales that shed easily compared to species with no scales or plated fishes. Finally, we anticipated that eDNA would be less effective at detecting demersal species compared to pelagic species because water samples were collected at the surface (Fig. 1). Conversely, when sampling with beach seines, we hypothesized that detections of mobile schooling fishes would be lower than solitary fishes because schooling fishes may more easily escape the seine net. We also expected that position-in-the-water-column and scale type would not impact detections with beach seines (Fig. 1).

To provide a quantitative estimate of the effect of functional traits on sampling method detection, we applied Bayesian occupancy modeling. Occupancy modeling can be used to determine the detection probability of a species because the models account for both species occurrence (i.e., presence, and which variables (e.g., habitat) influence presence) and detection, which includes an evaluation of whether observation methods are suited to detect the species if it is present at the site. This approach allowed us to model the true occupancy of a species based on observations (Doser et al. 2022*b*). While in many ecological studies, the true species occupancy is the desired outcome, in this study, we apply occupancy modeling to generate a quantitative estimate for the effect of species traits on detection probabilities for both eDNA and beach seines. To our knowledge, the only prior application of occupancy modeling in conjunction with eDNA and sampling method detection is in the context of invasive species, where Pukk et al. (2021) found comparable estimates of detection between eDNA and the most efficient traditional method for two out of the three species investigated. However they did not quantitatively explore why a species was more readily detected in one method over another (Pukk et al. 2021).

Our study quantifies how functional species traits influence detection by eDNA and traditional sampling methods, which is a critical precursor to accurately characterizing species distributions across a spectrum of species traits. This approach provides a framework for quantifying differences in sampling method detection, particularly for eDNA as it quickly becomes a common sampling method for understanding species distributions and fish communities.

Fig. 1. Graphical representation of hypothesized fish species detection based on species traits and sampling method. Hypotheses include: environmental DNA (eDNA) will more readily detect schooling fish compared to non-schooling fish, whereas beach seines are less likely to detect schooling fish compared to non-schooling fish. Position-in-the-water-column will not influence beach seine detections; however, it will influence eDNA detections with demersal species having lower detection compared to pelagic species. Species that possess deciduous, easily shed scales, will have greater detection with eDNA compared to species with plates or no scales. Scale type will not influence detection with beach seines.



2. Materials and methods

2.1. Study area

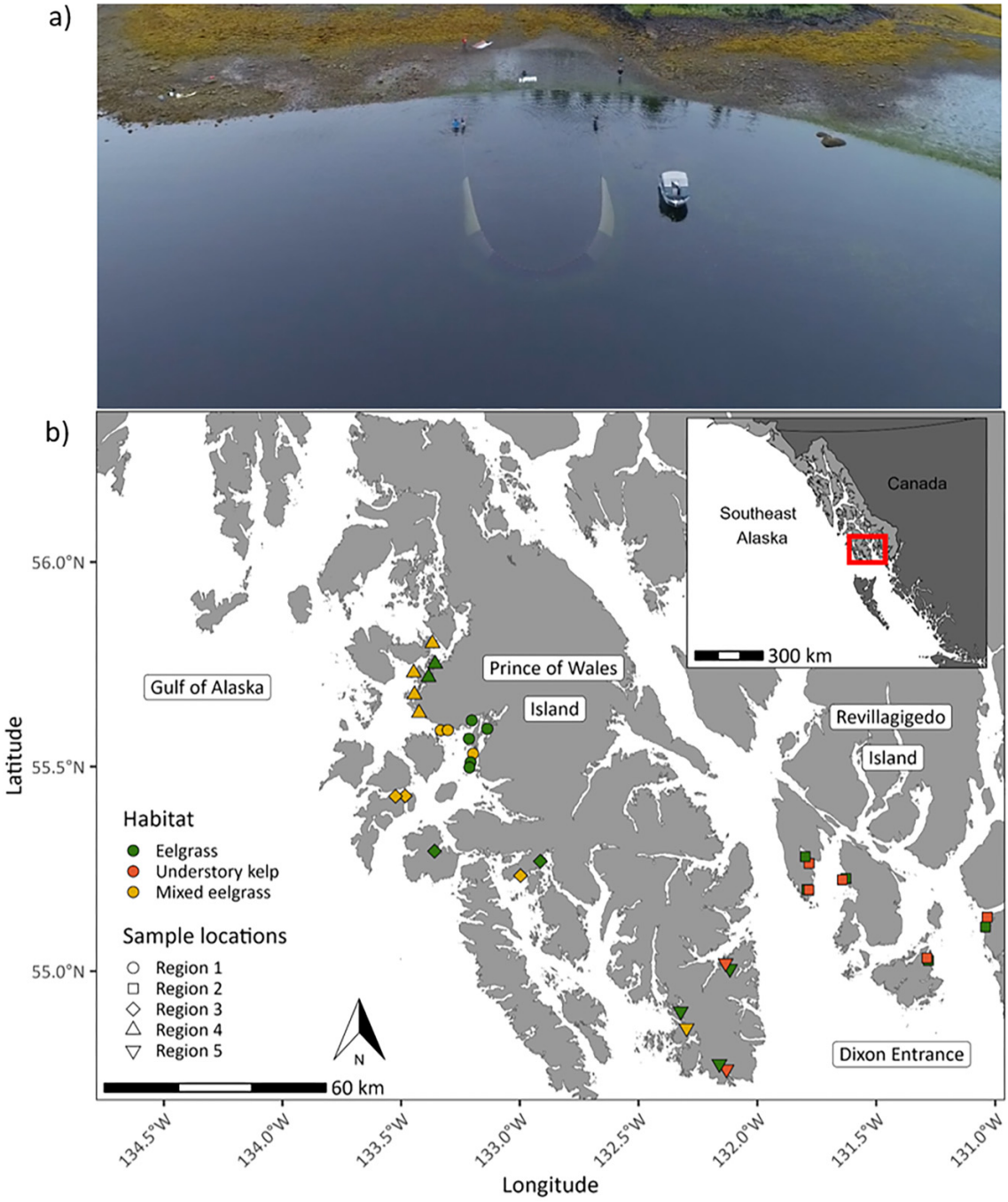
We sampled sites in southern southeast Alaska, around Prince of Wales Island, Revillagigedo Island, and surrounding smaller islands (Fig. 2). This region hosts a diversity of nearshore habitats from low-energy soft-sediment estuaries and protected bays to highly exposed rocky habitats. Low-energy areas and protected bays can have expansive and narrow, fringing eelgrass meadows (*Zostera marina*) in low intertidal and subtidal zones. In cobble and rocky sediment, vegetation shifts to understory kelps (*Laminaria*, *Saccharina*, and *Hedophyllum* spp., among others), surfgrass (*Phyllospadix serrulatus*), and canopy kelps (*Nereocystis luetkeana* and *Macrocystis pyrifera*). We sampled fish assemblages across a variety of these habitats, including on a gradient from expansive eelgrass meadows ($n = 17$) to eelgrass meadows with adjacent habitats (understory kelp or canopy kelp), which we call "mixed eelgrass" ($n = 11$), to understory kelp habitats ($n = 7$). All sites sampled occurred in areas that are semi-protected to wave exposure and with variable diurnal tides (~6 m). Candidate sites were identified using a historical dataset of publicly available aerial imagery and shoreline habitat surveyed between 2004 and 2010 called ShoreZone (Harper and Morris 2014) or from previously sampled locations in the Nearshore Fish Atlas of Alaska (Johnson et al. 2012).

2.2. Field sampling

2.2.1. Beach seine

To characterize nearshore fish communities in these habitats, we sampled fish using beach seines (Fig. 2*a* and as described in Johnson et al. 2012). Sites were sampled in June–July in 2021 and 2022 during negative low-tide cycles (−0.003 to −0.8 m below mean lower low water, MLLW) with a 37 m variable mesh net tapered in width from 10 m in the center to 5 m in the sides. The net has 32 mm square mesh along its sides, decreasing to 6 mm square mesh towards the center with 3.2 mm square mesh in the middle. The seine has a leadline and surface floats such that it is in constant contact with the substrate and floating on the surface while sampling. We modified the net by adding 8 m long ropes on each end to more easily set the net. This resulted in the center of the seine being approximately 26.5 m from shore when fully deployed. We set the net by boat in a round haul by holding one end in waist-deep water and then backing the skiff approximately 18 m away from the start. This procedure swept an area of 260 m^2. Once ashore we identified and counted all fish to the lowest possible taxonomic level using field guides specific to the Pacific or Alaska (Eschmeyer and Herald 1983; Mecklenburg et al. 2002; Johnson et al. 2015). The majority of species were readily identified to species aside from groups of small sculpins that were identified to genus, *Artedius*. We conducted fish sampling with permits from the Alaska Depart-

Fig. 2. Aerial imagery of a beach seine (*a*) in eelgrass taken by Carter J. Johnson. Map of paired environmental DNA and beach seine sampling locations (*b*) throughout southeast Alaska, USA. Habitats sampled include eelgrass (green), understory kelp beds (orange), and "mixed eelgrass" meadows where eelgrass is adjacent to understory or canopy kelp (yellow). Sampling locations are grouped regionally (indicated by shape) for occupancy modelling. Base map from r package rnaturalearth (v 1.0.1) (Massicotte and South 2023).



ment of Fish and Game (#CF-21-062 and CF-22-070) and with approval from the Institutional Animal Use and Care Committee at University of Alaska Fairbanks (project #892147).

2.2.2. Environmental DNA

At the same sites where we collected samples with beach seines, we also collected water samples and processed them for eDNA. At each site, we collected 3 replicate 1 L samples approximately 10 m apart above the target vegetation (i.e., eelgrass or understory kelp) within 0.3 m of the surface in 1.5–2 m of water. Samples and field blanks were collected in bleach-sterilized Nalgene bottles, and collection occurred before beach seining and during the dropping tide. After eDNA sampling, a field blank was collected by filling a 1 L bottle with distilled water. Each sample was transported to the lab

in a bleach-sterilized cooler with ice packs where it was filtered within 3 h through 0.45 μm cellulose nitrate filters and preserved in 5 mL of Longmire's buffer at room temperature until DNA extraction (Renshaw et al. 2015). Prior to reusing Nalgene sample bottles, we cleaned bottles by soaking for 15 min in a 10% bleach solution. We then rinsed the bottles by filling the bottle with tap water, capping it, and shaking the bottle. This rinsing procedure was repeated three times.

2.3. Laboratory methods

2.3.2. eDNA extraction, metabarcoding amplification, and sequencing

Detailed methods for the mock community assembly used to determine species-specific amplification efficiencies, as well as methods for the field eDNA sample extraction, amplification and sequencing are provided in Supplementary Information A. Briefly, eDNA was extracted from filters stored in Longmire's buffer using the Qiagen DNeasy Blood and Tissue kit. A ~186 bp hypervariable region of mitochondrial DNA on the 12S rRNA gene (MiFish; Miya et al. 2015) was amplified using primers from Sales et al. (2019; MiFish-U-F, 5′-GCCGGTAAAACTCGTGCCAGC-3′; MiFish-U-R, 5′-CATAGTGGGGTATCTAATCCCAGTTTG-3′). Each sample was amplified in triplicate, and technical PCR replicates were uniquely barcoded to capture stochastic variation during amplification. Library preparation and Illumina MiSeq sequencing of mock communities were performed separately from environmental samples.

2.4. Bioinformatic analysis

Demultiplexed sequences were processed using the Dadasnake pipeline v0.11.2, which implements cutadapt and DADA2 to generate amplicon sequence variants (Martin 2011; Callahan et al. 2016; Weißbecker et al. 2020). Taxonomic assignments were performed using blastn against the NCBI nucleotide database, followed by custom filtering to exclude ambiguous, non-target, or species outside of the geographic range. ASVs that could not be unambiguously resolved to a single species were consolidated to the lowest possible taxonomic level. Additional taxonomic assignment and quality control steps, including correction for tag-jumping and removal of low-read replicates, are described in Supplementary Information A and Fig. S1.

2.5. Data analyses

2.5.1. Assessing amplification bias in metabarcoding data

Although multiple factors contribute to amplification bias, primer-template mismatches have the most pronounced effect (Shaffer et al. 2025). In addition to assessing amplification bias in metabarcoding data using mock communities (see Supplementary Information A for full analysis), we assessed primer mismatches. We downloaded representative 12S reference sequences for all species detected using beach seine or eDNA sampling. Of these, 54 species had sequences of the MiFish amplicon long enough to include the primer-binding regions. Sequences were trimmed to the primer-binding sites and aligned using Geneious Prime (v. 2025.1.2) to quantify the number of mismatches for each species.

2.5.2. Influence of sampling method and habitat

To quantify the influence of sampling method and habitat on nearshore fish community composition, we evaluated differences in species composition using multivariate analysis. We calculated the mean proportions across eDNA technical replicates to represent each biological replicate (i.e., each 1 L water sample). Because eDNA metabarcoding data are compositional, we transformed beach seine abundance data to proportional abundance. We applied Wisconsin double standardization on the combined eDNA and beach seine data to minimize differences in absolute abundance across sites and species. Bray–Curtis dissimilarity metrics were then calculated using the vegan package in R (Bray and Curtis 1957; Jongman et al. 1995; Oksanen et al. 2022). We tested the assumption of homogeneity of dispersion using "betadisper" function in the vegan package in R for subsequent permutation-based tests of differences with sampling method and habitat (McArdle and Anderson 2001; Anderson 2006; Oksanen et al. 2022). We evaluated differences in species composition between sampling method, habitat, and the interaction of sampling method and habitat using the vegan "adonis2" function in R, which performs permutation-based Analysis of variance (ANOVA) (PERMANOVA) and included blocks by site to account for the random effect of multiple eDNA samples per site (Oksanen et al. 2022). For each significant difference (alpha = 0.05) in species composition, we used sequential post hoc pairwise tests to determine which combinations of sampling method and habitat were different from each other, focusing only on combinations that represented biological reality. Combinations included a single habitat with different sampling methods (1: eelgrass/eDNA vs. seine, 2: mixed eelgrass/eDNA vs. seine, 3: kelp/eDNA vs. seine) and the same sampling method in different habitats for eDNA (4: eDNA/eelgrass vs. kelp, 5: eDNA/eelgrass vs. mixed eelgrass, 6: eDNA/kelp vs. mixed eelgrass), and beach seines (7: seine/eelgrass vs. kelp, 8: seine/eelgrass vs. mixed eelgrass, 9: seine/kelp vs. mixed eelgrass).

When fish community composition differed significantly by sampling method, habitat, or their interaction, we used indicator species analysis (ISA) to identify which species were associated with eDNA or beach seine, habitat, or combinations of both. ISA identifies how strongly a species associates with a particular group (or combination of groups) by calculating a species relative abundance (or eDNA relative abundance) (specificity) and relative frequency (fidelity) to a particular group (Dufrêne and Legendre 1997). The ISA indicator value ranges from 0 to 1, where 0 indicates a species is absent, and one indicates species that occur only within a specific habitat, sampling method, or habitat/sampling method group. Permutation-based tests were used to determine significance of species and group associations implemented with the "multipatt" function in the indicspecies package (De Cáceres et al. 2010) with a Bonferroni correction for multiple

comparisons. For each species that was significantly associated with a group or combination of groups, we visualized the indicator value magnitude across all logical combinations of sampling method and habitat using a heatmap.

To investigate the role of species functional traits on detection by sampling method, we identified traits for all species identified by either sampling method within each trait-category (scale-type, position-in-the-water-column, and schooling behavior; Table S1) using common reference material (Eschmeyer and Herald 1983; Mecklenburg et al. 2002; ADFG 2008, n.d.). Each trait-category had several levels. For scale-type, the trait-levels were no scales, plates, some scales, scales, or deciduous scales. We had three levels for position-in-the-water-column: demersal, pelagic or both demersal and pelagic; and similarly had three levels for schooling behavior: solitary, facultative schooling, and obligatory schooling. For our multivariate analysis, we created trait groups with all species that shared the same combination of trait-levels within each trait-category. For example, several poachers (*Agonopsis vulsa*, *Pallasina barbata*, and *Podothecus accipenserinus*) and several sculpins (*Blepsias bilobus*, *Blepsias cirrhosis*, *Nautichthys oculofasciatus*, and *Rhamphocottus richardsonii*) represented the trait group of solitary, demersal, and plated fishes. We visualized any patterns in species compositions by sampling method and habitat with a principal coordinate analysis (PCoA) and plotted the combined trait-category groups (described above) that significantly correlated with each axis.

To evaluate the impact of species functional traits on detection probability by sampling method, we used Bayesian integrated occupancy models in the "spOccupancy" R package (Miller et al. 2019; Doser et al. 2022*a*, 2022*b*). First, we transformed our eDNA and beach seine relative-abundance data to binary detection/non-detection data for all 70 species detected by one or both sampling methods. Occupancy models leverage repeated observations of detection/non-detection data to jointly estimate detectability and probability of occurrence (MacKenzie et al. 2002). In our integrated approach, the two sampling methods produced datasets with separate observation process models linked together by a shared site-level occupancy process. Whereas occupancy models are typically used to facilitate inference on occupancy by accounting for imperfect observations, our interest was to use the models to facilitate inference on detection by accounting for heterogeneous patterns of species' presence. In the multi-species context, we assumed species-specific coefficients were random effects that arise from common distributions with community-level mean and variance terms. We hypothesized that species' probabilities of occurrence or use at a site was influenced by habitat and therefore included a site-specific covariate for habitat (based on results from pairwise tests) as well as a site-specific covariate for region (Fig. 2), as sites that were closer together may have had more similar species composition. We assumed a constant probability of detection for both sampling methods and thus fit intercept-only detection submodels for each method. Model convergence was assessed using the Gelman–Rubin diagnostic (Rhat; Brooks and Gelman 1998) and effective sample size of the posterior samples to ensure adequate mixing. We evaluated model fit using posterior predictive checks following Doser et al. (2022*b*), by calculating Bayesian *p*-values based on $\chi 2$ discrepancy statistics calculated for observed and predicted data binned across replicates (Domke and Kachel 2026). Data analyses were performed in R (v. 4.3.3) (R Core Team 2024).

To determine whether species' functional traits influenced detection by sampling method, we fit post hoc secondary linear models (sometimes referred to as a 2-stage analysis), in which the species-specific logit-scale detection parameters from each iteration of the posterior of the occupancy model were treated as response variables (Doser et al. 2022*a*). This was done separately for each sampling method, allowing us to investigate the influence of species functional traits on the probability of sampling method-specific detection. We interpreted effect sizes on the odds-ratio (OR) scale. Odds ratios, calculated as exp(logit-scale coefficient) for each trait, lie on a strictly positive scale, where $OR > 1$ indicated a greater odds of detection relative to the reference trait combination (positive association), $OR < 1$ being a negative association, and an $OR = 1$ being no association between the trait and detection probability. We interpreted the proportion of each posterior distribution that fell above zero on the logit scale (or above or below 1 on the odds-ratio scale), as the probability of a positive association between the corresponding trait and detection probability relative to the reference trait (or the probability of a negative association for the proportion below zero). Thus, if 95% of a logit-scale posterior was >0, that indicated a probability 0.95 of a positive association (and 0.05 for a negative association) between the corresponding trait and detection probability.

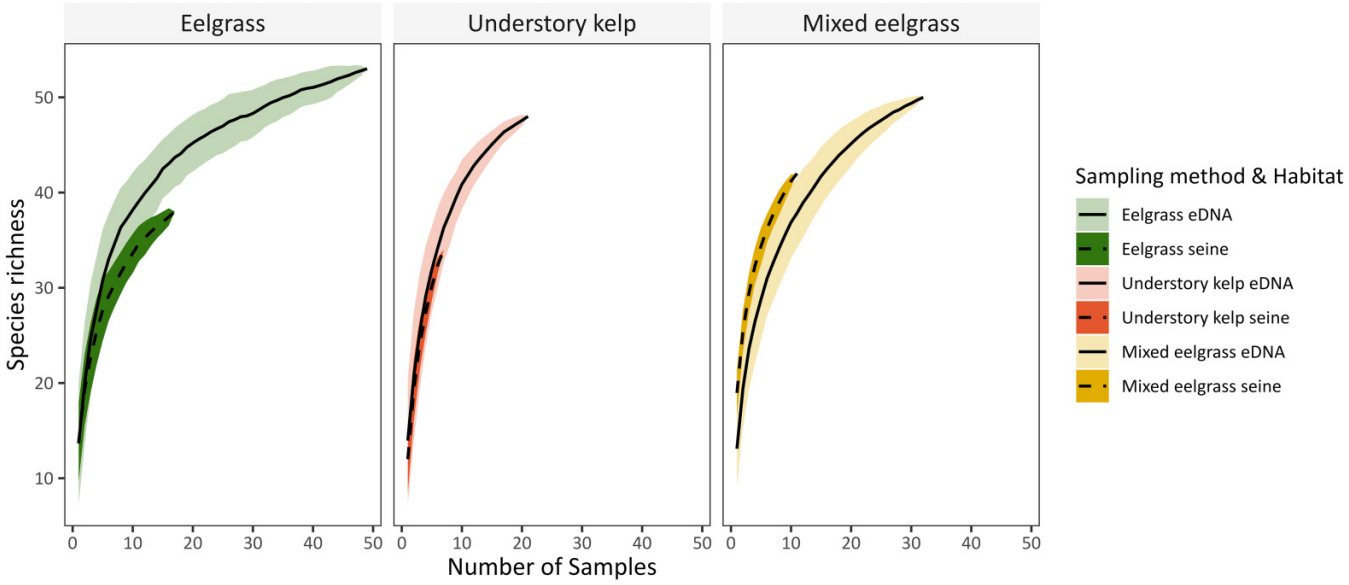
3. Results

3.1. eDNA metabarcoding data and mock communities

After performing quality control on sequence reads from environmental and mock community samples, the final dataset included 4.83 million reads from 576 ASVs representing 66 taxa in field samples, and 1.46 million reads from 50 ASVs representing 24 taxa in mock communities (see Table S2 and Supplementary Information A for details). Analysis of mock community data showed no consistent amplification bias across species (see Supplementary Information A for full results and Figs. S2–S3).

As a potential source of amplification bias, we examined primer-template mismatches. Of the 54 species with reference sequences spanning the primer sites, three taxa had a single mismatch and two taxa had two mismatches. No species in the mock communities contained primer-template mismatches (Fig. S4). Given that estimated amplification efficiencies across species in the mock communities were relatively similar and few species contained primer mismatches, we did not apply the quantitative metabarcoding model to field samples. Because our dataset did not require using the quantitative metabarcoding model, we could expand the number of species analyzed beyond those in the mock communities to include all detected taxa and calculated means

Fig. 3. Species accumulation curves in Eelgrass (left, green), Understory Kelp (middle, orange), and Mixed Eelgrass Meadows (right, yellow) for sites sampled with eDNA (solid lines, light ribbon) and beach seines (dashed lines, dark ribbon).



of metabarcoding read proportions from the technical replicates to represent each biological replicate.

3.2. Sampling method and habitat influence observed fish community composition

Overall, eDNA and beach seines identified 70 species (eDNA = 66, seine = 49) with four species only detected with seines and 21 species only detected with eDNA. Across the three habitat types eDNA identified more species than beach seines in each habitat (Fig. 3; eDNA: 53 species [eelgrass], 50 [mixed eelgrass], 48 [understory kelp]; seine: 38 [eelgrass], 42 [mixed eelgrass], 34 [understory kelp]).

Fish community composition differed significantly with the interaction of sampling method and habitat (PERMANOVA, Fig. 4, Table 1). We detected no within-group dispersion for sampling method ($F\text{-statistic}_{1,135} = 1.91$, p-value = 0.169) or habitat ($F\text{-statistic}_{2,134} = 1.89$, p-value = 0.154). We found strong evidence for differences in species composition in each of the nine logical pairwise combinations of sampling method and habitat (Table S3, p-values < 0.05). We visualized species composition with principal coordinates analysis (PCoA) with the interaction of sampling method and habitat type representing 40.4% of the total variability in species composition with the first two axes (Fig. 4).

3.3. Indicator taxa associated with sampling method and/or habitat

ISA identified significant associations of 1–5 species with eight sampling method-habitat combinations, with more indicator species with beach seines compared to eDNA (eDNA mean = 0.7 species/habitat combination, seine mean = 1.7 species/habitat combination; Fig. 5). ISA identified three species with significant indicator values across all habitats sampled with eDNA (*Anoplarchus* sp., *Clupea pallasii*, and *Oligocottus maculosus*; Fig. 5). Other indicator species for habitats sampled with eDNA included *Xiphister* in understory kelp.

Indicator species varied by sampling method. Five species were indicator species across all beach-seined habitats (*Artedius* spp., *Hexagrammos* spp., *Syngnathus leptorhynchus*, *Aulorhynchus flavidus*, and *Apodichthys flavidus*). Indicator species for understory kelp and mixed habitats included *Sebastes* 1 (see Table S4) and *B. cirrhosus*. *Lumpenus sagitta* was the sole indicator species for eelgrass and mixed eelgrass. For beach seining in understory kelp sites, we identified two indicator species: *Brachyistius frenatus* and *Oxylebius pictus*, and in mixed eelgrass we identified two indicator species, *Ophiodon elongatus* and *Hemilepidotus* spp. The only indicator species significant in both sampling methods was the group with the genera *Cottus*/*Leptocottus* in eelgrass habitats (Fig. 5).

3.4. Species traits influence fish community sampled

3.4.1. Trait-based assemblages across sampling methods and habitats

Seven trait group vectors were correlated with the PCoA axes (p-value < 0.01) with the length and direction of the lines indicating the strength and direction of the relationship (Fig. 4). These significant trait groups included (1) obligatory schooling species with plates rather than scales that inhabit either demersal or pelagic positions-in-the-water-column, (2) facultative schooling species that are pelagic and have scales, (3) solitary, pelagic species with plates, (4) solitary, demersal species with plates, (5) solitary demersal species with scales, (6) obligatory schooling pelagic species with scales, and (7) obligatory schooling pelagic species with deciduous scales.

Fig. 4. Association of sampling methods with traits. Principal coordinate analysis (PCoA) of species composition sampled by environmental DNA (eDNA) (purple) and beach seine (red) in eelgrass (circle), mixed eelgrass (square), and understory kelp (diamond). Each point represents the composition of fish species for a single replicate at a given site, with unfilled larger points representing the average position of points for one sampling method-habitat combination. Vectors indicate trait combinations (e.g., schooling behavior, position-in-water-column, and scale type) that are significantly correlated with PCoA axes (p-value < 0.01), with the length and direction of the lines indicating the strength and direction of the relationship. For example, obligatory schooling species that are pelagic with scales or deciduous scales are more associated with the lower left quadrant of the PCoA where fish communities sampled with eDNA are most prevalent. Biological replicates for eDNA samples taken at the same site are shown, whereas only one sample was collected at each site by the beach seine.

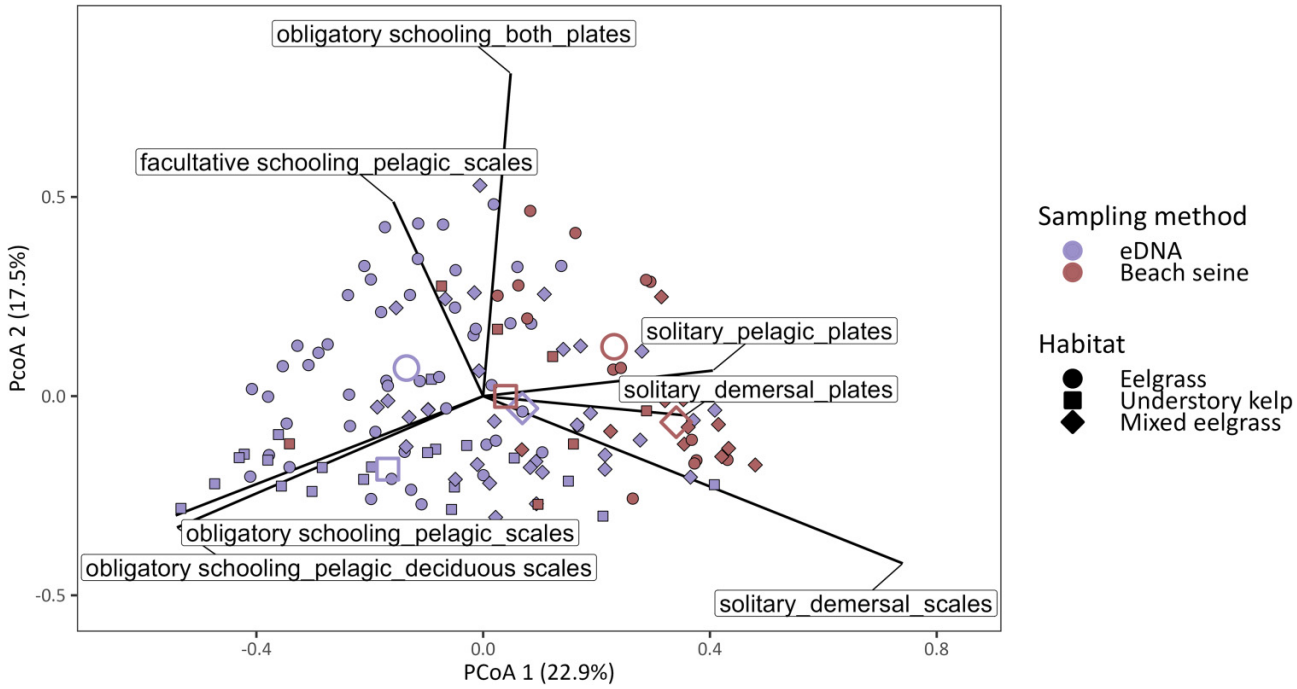


Table 1. Permutation-based ANOVA (PERMANOVA) of the interaction between habitat type (eelgrass, mixed eelgrass, and understory kelp) and sampling method (environmental DNA and beach seine) for fish community composition.

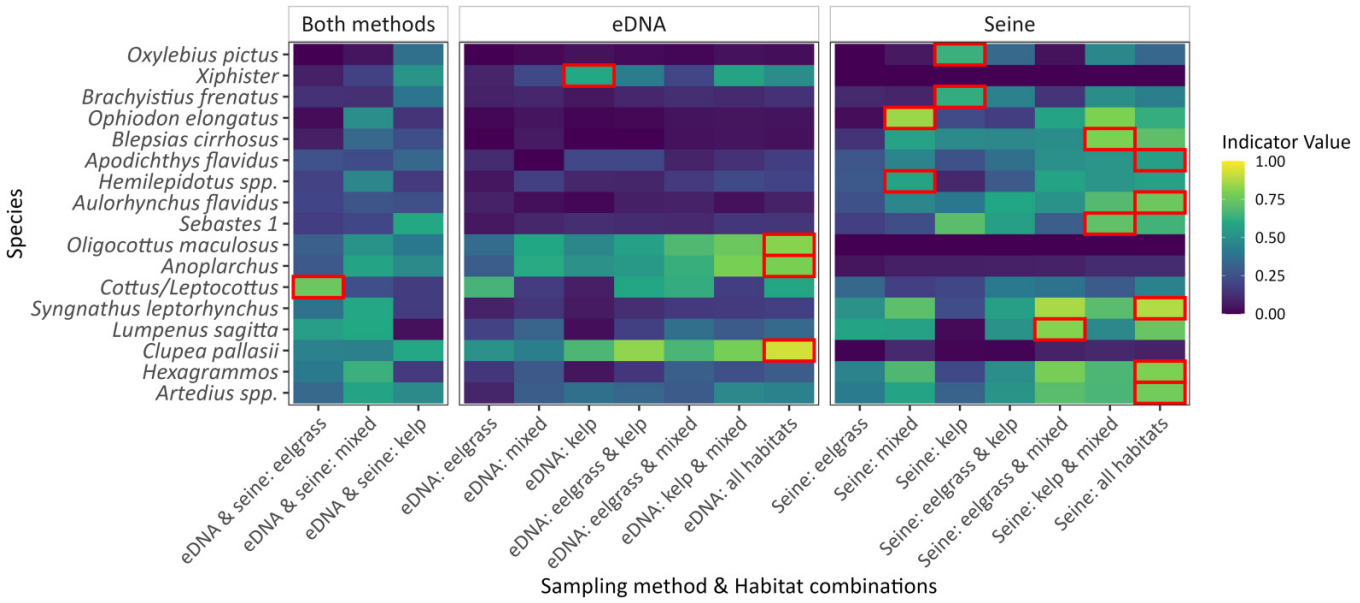
	DF	Marginal R^2	Sum of squares	Pseudo-F statistic	p-value
Habitat × sampling method	2	0.026	1.207	1.996	0.002
Residual	131	0.845	39.586		
Total	136	1.0	46.870		

Note: Permutations were performed with blocks to account for within site variability across multiple eDNA water samples collected at each site.

3.4.2. (Some) functional traits drive variation in detection probability by sampling method

We observed different detection probability by sampling method and species' functional traits. Species with a combination of scales, solitary, and demersal traits (represented by the intercept) had similar detection probability with both eDNA and beach seines (mean and 95% credible interval for detection probability = 0.216 [0.12–0.34; eDNA] and 0.211 [0.09–0.40; seines]; Fig. 6). We found evidence of associations between three trait-level and sampling method-detection combinations. With eDNA, detection probability for a species with deciduous scales was greater than that of otherwise similar species that shared all other traits: a species with deciduous scales had 27 times greater odds of detection compared to a similar species with scales (p(OR > 1) = 0.97). Conversely, a fish with plates rather than scales had lower odds of detection with eDNA (0.31 times lower compared to a similar fish with scales; p(OR > 1) = 0.04), but showed some indication of greater odds of detection with the beach seine (again, relative to scaled fish) with 2.4 times greater odds (p(OR > 1) = 0.81). Fish with no scales had reduced detection probability with beach seines compared to fish with scales (p(OR > 1) = 0.05), while this trait was not associated with any difference in detection with eDNA (p(OR > 1) = 0.53). Fishes that were both demersal and pelagic compared to just demersal had increased detection probability with both eDNA and beach seines, with 3.9 and 19.8 times greater odds of detection, respectively (p(OR > 1) = 0.77 [eDNA] and p(OR > 1) = 0.86 [seines]). For several traits there was no clear evidence of difference in detection probability by trait-levels in either sam-

Fig. 5. Indicator species are associated with sampling methods and habitat combinations. Indicator values for species with a significant association for a given combination of sampling method (environmental DNA (eDNA), seine, or both) and habitats (eelgrass, kelp, mixed) are boxed in red (Bonferroni corrected p-value < 0.05). Indicator value ranges from 0 (dark blue), where a species is absent, to 1 (yellow), where a species occurs exclusively with a sampling method/habitat combination. For example, *Cottus/Leptocottus* (a group of sculpins that are taxonomically indistinguishable with the 12S MiFish metabarcoding marker) is an indicator species for eelgrass sampled with eDNA or seine because both sampling methods captured relatively high abundance and fidelity of this species in eelgrass, and not in other habitats.



pling method, including presence of some scales, obligatory and facultative schooling traits, and pelagic species.

4. Discussion

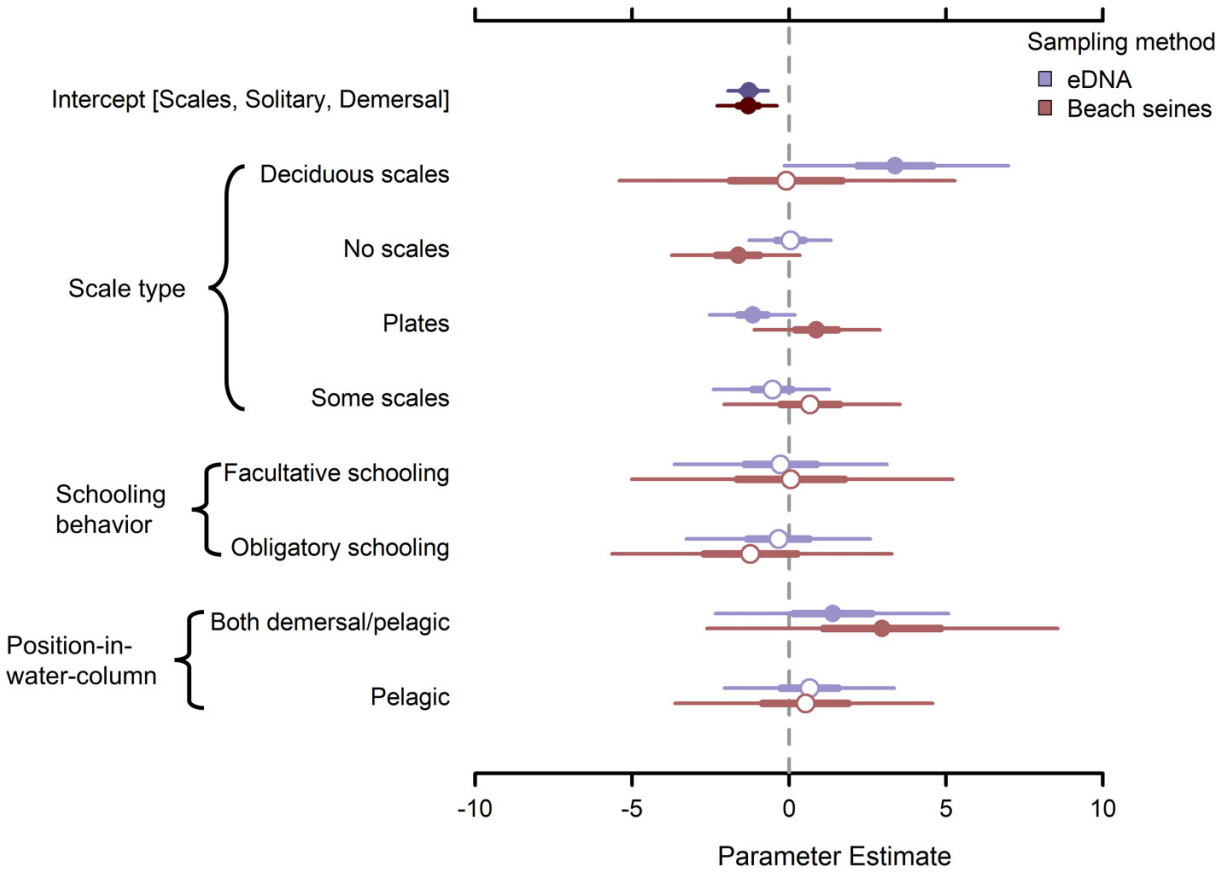
Studies often make ecological inferences based on sampling community composition, but each sampling method has biases that lead to some species being more readily detected. In this study, we identified the functional traits underlying detection of fishes across three nearshore habitats in southeast Alaska using eDNA and beach seines. We found significant differences in the fish community across eelgrass meadows, understory kelp, and mixed eelgrass habitats when sampled with eDNA and beach seines. Fewer than 20 species out of the 70 detected were identified as indicator species for combinations of habitat and sampling method. Functional traits hypothesized to influence differences in detection between eDNA and seines included a species' position-in-the-water-column, schooling behavior, and scale-type/presence of scales. Of these traits, scale-type had the greatest impact on probability of detection by either sampling method. Although detection of specific functional traits has been qualitatively inferred in previous studies, our use of occupancy models to quantify differences in detection probabilities by sampling method is a novel extension of previous analyses.

Of the 70 species detected by either eDNA or beach seine, 17 species (or species groups) were identified in the indicator analysis as being associated with one or more sampling methods and habitats (Fig. 5). Only one taxonomic group, *Cottus/Leptocottus* (a group of two genera of sculpins that are taxonomically indistinguishable with the MiFish metabarcoding marker) was significantly associated with both sampling methods, and only in eelgrass meadows. The strongest indicator value for any species across all sampling method/habitat combinations was *Clupea pallasii*, Pacific Herring, which was significantly associated with eDNA sampled in all habitats. This result was consistent with our hypotheses about the ability for eDNA to detect schooling species with deciduous scales more readily than seines (Fig. 1). Otherwise, patterns of association were difficult to disentangle between functional traits, species, and habitats.

Because ISA takes into account both relative abundance and fidelity, species that are not abundant, but have high fidelity, may still be significant. *Oxylebius pictus* (Painted Greenling), for example, were only significant in kelp habitat sampled by seines. Since *O. pictus* has scales (but not deciduous scales), it is possible that low relative species or DNA abundance (Fig. S5), coupled with solitary and demersal traits, kept the species from receiving a higher proportion of eDNA sequencing reads. Seines may have yielded more indicator species than eDNA because their method of capture provides a precise link between species and habitats. In contrast, the dispersal of eDNA creates a "smoothing effect" on species detections which may make it a less precise indicator of fine-scale habitat use (Jo et al. 2025; McCartin et al. 2025). Further, multiple eDNA water samples were collected at each site, potentially increasing the likelihood of detecting nearby species (Murakami et al. 2019; Baetscher et al. 2024).

The primary axis of variation in the PcoA appeared to separate communities detected by eDNA from those de-

Fig. 6. Species functional traits influence detection by sampling method. Logit-scale median parameter estimates from the post hoc secondary linear models from the occupancy model indicate the influence of functional trait on detection probability for each sampling method: environmental DNA (eDNA) (purple) or beach seines (red) relative to the reference trait combination (i.e., the intercept, represented by a fish that has scales, is solitary, and demersal). Thick and thin bars correspond to 50% and 95% credible intervals (CI); filled circles indicate that the 50% CI does not overlap 0. Each sampling method was modeled independently. Positive parameter estimates represent increased detection relative to the intercept and negative parameter estimates indicate decreased detection. For example, a fish with plates that is solitary and demersal will have decreased detection with eDNA and increased detection with beach seines relative to a fish that has scales and is solitary and demersal.



tected by seines (Fig. 4). This axis also separated trait groups that included schooling behavior, suggesting an association between eDNA and schooling behavior. No clear pattern was found linking species' position-in-the-water column to the PCoA axes, potentially related to the well-mixed nearshore waters. Ultimately, the relationship between sampling method detection and functional trait is potentially confounded by multiple interactions involving habitat and species distribution (Fig. 4).

Trait-based quantitative occupancy modeling allowed us to disentangle the influence of multiple interacting traits on detection probability. A prime example of this phenomenon is that increased detection probability of *C. pallasii* when sampled with eDNA could have been caused by deciduous scales, schooling behavior, or an unattributed trait. Based on results from the Bayesian trait analysis, species with deciduous scales were more likely to be detected by eDNA than species with non-deciduous scales (Fig. 6). In comparison, schooling behavior did not appear to impact detection probabilities for either sampling method. The impact of scale type on detections can be observed in comparing detections of *C. pallasii* (deciduous scales) and *S. leptorhynchus* (plated). We observed *C. pallasii* in nearly all our sites with eDNA and rarely with beach seines and the opposite pattern with *S. leptorhynchus*, which was caught in most sites with beach seines, but rarely detected with eDNA (Fig. S5). Given that *C. pallasii* was the only species with deciduous scales in our study, additional data from species with this scale trait-level would be valuable for testing the generalizability of increased probability of detection with eDNA for species with deciduous scales such as sardines, anchovies, and other baitfishes.

Traits other than the ones we evaluated (impact of schooling behavior, position-in-the-water-column, and scale type), could influence detection. Previous studies of detection by sampling method in nearshore habitats have examined traits including diet, body size, depth, swimming speed, and period of activity, in addition to schooling behavior and position-in-the-water-column (e.g., Mbaru et al. 2020; Henseler and Oesterwind 2023). While many studies identify differences between functional traits detected by different sampling

methods, most of these studies use a combination of traditional fishing and sampling gears (e.g., Mbaru et al. 2020; Carvalho et al. 2021, but see Czeglédi et al. 2021 for a comparison between eDNA, electrofishing, and gillnetting). Consequently, some traits analyzed in other studies may be less likely to impact detection probabilities for eDNA sampling (e.g., feeding behaviors; Franco et al. 2012; Henseler and Oesterwind 2023). Investigating certain traits can impact how effective a sampling method appears. For example, Aglieri et al. (2021) found that eDNA identified the full spectrum of functional traits considered in their study; however, untested traits, like scale type, could have a confounding effect on detection with eDNA. Likely additional ecological and functional traits are relevant to evaluating nearshore fish communities, in particular with eDNA, which may not be captured in the present study. Future studies evaluating traits influencing eDNA detections may consider including attributes related to DNA shedding, metabolic, and/or movement rates (Ely et al. 2021).

Using metabarcoding data as a reflection of relative eDNA composition requires understanding the impact of amplification bias on the dataset (Shelton et al. 2023). The primary conclusion from our mock community data was that none of the species that we tested exhibited consistent and significant amplification bias. Given the substantial amount of effort required to construct mock communities, finding a method to test for potential amplification issues prior to beginning lab work would be valuable. For example, if amplification bias is primarily driven by primer-template mismatches (i.e., Shaffer et al. 2025), then in silico sequence alignments could provide valuable information as to whether mock communities are required to correct amplification biases in metabarcoding data (e.g., Fig. S4).

Taxonomic assignment is a major challenge in eDNA metabarcoding studies (Locatelli et al. 2020; Blackman et al. 2023). Here, we aggregate uncertain species-level assignments to multi-species groups within a single genus (e.g., *Sebastes* groups 1–3) or, in some cases, a family (e.g., Pleuronectidae). The same taxonomic uncertainty can also occur in beach seine sampling based on potential misidentification, especially of small and hard to distinguish taxa (e.g., *Artedius* spp. sculpins). Despite the potential for misidentification, we doubt the aggregation of species to genus or family impacts the analysis of detection by sampling method given the similarity of the functional traits that we chose within taxonomic groups.

Increasingly, multi-method assessments could mitigate sampling biases of individual sampling methods rather than benchmarking one method (typically eDNA) against another (most often traditional sampling; i.e., nets). Imperfect detection of species using traditional sampling methods may be influenced by size and/or habitat characteristics (e.g., vegetation) which can change ecological conclusions depending on which species from the community are sampled (Parsley et al. 1989; Rozas and Minello 1997; McClanahan and Mangi 2004). Rather than single method sampling, multiple studies found that a combination of two sampling methods can adequately sample fish communities (French et al. 2021; Henseler and Oesterwind 2023) assuming the sampling methods are complementary (Stat et al. 2019; Andres et al. 2023). In our study, the clearest example of this was the increased detection of deciduous scales with eDNA that allowed for *C. pallasii* to be identified across habitats where it was entirely absent in the beach seines. The framework we describe for analyzing the effect of functional traits on sampling methods detection probabilities provides a model for how similar quantitative assessments could be performed in future studies. Additionally, data from multi-method assessments could be used to build joint models, which can integrate information from multiple sampling methods in a spatially explicit framework (e.g., Guri et al. 2024; Keller and Kelly 2025 to estimate abundance/biomass or Doser et al. 2022*a* for occupancy presence/absence). Study design and choice of sampling method(s) should align with which species (and which functional traits) are being targeted. eDNA represents a particularly valuable addition to sampling methods because it is relatively non-invasive, can be used in areas restricted to other types of sampling (i.e., marine reserves), and likely has reduced impacts to species and ecosystems when compared to nets.

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Data availability

Data are available through Github for analyses and beach seine data (Domke and Kachel 2026) and processed eDNA data (Ledger 2026). Raw metabarcoding data are available through NCBI SRA BioProject ID PRJNA1437145.

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Competing interests

The authors declare no conflict of interest.

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Supplementary material

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