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Comparing beam trawl sampling and eDNA metabarcoding for assessing fish diversity in turbid tropical seagrass habitats

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ABSTRACT

Tropical seagrass habitats support diverse fish assemblages and provide critical nursery habitat for many ecologically and economically important species. However, biodiversity assessments in these systems are challenging due to turbidity, suspended sediments, and dense vegetation, which can restrict both traditional capture- and visual-based surveys, as well as emerging molecular approaches. In this study, we compared fish assemblages detected using beam trawl sampling and multi-assay environmental DNA (eDNA) metabarcoding, including two fish-targeted assays, 16S-Fish/D and MiFish-U, and the broader metazoan COI-Leray assay in a turbid tropical seagrass meadow in Trinity Inlet, north Queensland, Australia.

Environmental DNA metabarcoding detected greater genus-level richness than beam trawling, recovering 112 genera compared with 27 genera detected by beam trawl surveys. Beam trawls detected a smaller subset of taxa associated with local, trawl-accessible fishes, including four taxa not recovered by eDNA metabarcoding. Among the metabarcoding assays, MiFish-U and 16S-Fish/D recovered substantially more fish taxa than COI-Leray, which produced limited fish detections. Univariate analyses indicated that site-level richness patterns varied among sampling approaches, with MiFish-U detecting higher richness at Ellie Point, and beam trawl sampling detecting higher richness at the Esplanade. Multivariate analyses revealed a significant Method × Site interaction, suggesting fish assemblages detected by the two sampling techniques respond to local conditions in different ways. Incomplete overlap in taxonomic detections between MiFish-U and 16S-Fish/D further highlighted the strong influence of marker choice on fish detection.

These findings demonstrate that eDNA metabarcoding and beam trawl surveys capture complementary components of seagrass-associated fish assemblages within the same habitat. Integrating molecular and capture-based methods therefore provides a more comprehensive and reliable assessment of biodiversity in turbid tropical seagrass ecosystems.

1. Introduction

Tropical seagrass meadows are highly productive coastal ecosystems that provide critical habitat, refuge, and nurseries for diverse fish assemblages, including species of ecological and fisheries importance (Unsworth and Cullen-Unsworth, 2014; Jackson et al., 2015; Hyndes et al., 2018; York et al., 2018). In tropical estuarine and coastal environments, seagrass meadows often occur in shallow, turbid waters, characterised by high sediment loads, low visibility, and high catchment runoff, presenting challenges for biodiversity assessment and monitoring (Wolanski et al., 2008; Fabricius et al., 2013; Philpott et al., 2025).

Fish assemblages in seagrass meadows are traditionally assessed using capture-based methods such as beam trawls and nets, or with visual surveys (Guest et al., 2003; French et al., 2021; Henseler and Oesterwind, 2023). These methods provide important demographic and biological information that cannot be derived from molecular methods alone, including individual size structure, age classes, sex

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ratios, and condition, which are critical for understanding population dynamics and nursery function (Philpott et al., 2025). However, these approaches are subject to gear selectivity, behavioural avoidance, and habitat accessibility, and can under-represent cryptic, transient, or structurally-associated taxa (French et al., 2021; Henseler and Oesterwind, 2023).

Turbidity is a defining environmental characteristic of many tropical coastal and estuarine ecosystems and represents a major constraint for both visual and molecular biodiversity surveys (Lunt and Smee, 2019; Kumar et al., 2022a). In the Great Barrier Reef (GBR) region, inshore seagrass meadows, typically occurring within a few kilometres off the coast, are naturally exposed to dynamic turbidity regimes resulting from catchment runoff, tidal resuspension, and episodic disturbance (Wolanski et al., 2008; Orpin and Ridd, 2012; Fabricius et al., 2013). In turbid seagrass habitats, reduced visibility, suspended sediments, and dense vegetation can present challenges for biodiversity surveys, and can result in incomplete assessments of fish assemblages present (Edgar et al., 2004; Zarco-Perello and Enríquez, 2019; Chung et al., 2024). As a result, observed assemblages may not represent actual community composition, highlighting both the value and the limitations of traditional and visual methods and the need for complementary approaches.

Environmental DNA (eDNA) has emerged as a non-destructive approach for monitoring aquatic biodiversity, enabling the detection of a broad range of taxa from a single water sample (Taberlet et al., 2012; Kelly et al., 2014; Oka et al., 2021). Environmental DNA also has potential to complement traditional surveys in complex coastal habitats, particularly where visibility is limited or access is restricted (Taberlet et al., 2012; Thomsen and Willerslev, 2015; Williams et al., 2017; Kumar et al., 2022a). By detecting DNA shed by organisms into the environment, eDNA metabarcoding enhances the detection of rare, cryptic, or transient taxa that may be underrepresented by capture or visual based methods (Jerde et al., 2011; Nester et al., 2020). However, eDNA performance is strongly influenced by environmental conditions, especially in turbid tropical systems where high particulate loads can reduce DNA recovery through filter clogging, co-extraction of PCR inhibitors, and increased DNA degradation or adsorption to particles (Hunter et al., 2019; Kumar et al., 2022a).

Detection outcomes are also influenced by genetic marker choice, as commonly used fish metabarcoding loci embedded in the mitochondrial genes, 12S, 16S, and COI differ in amplification efficiency, taxonomic coverage and resolution, primer-template mismatches, and the completeness of reference databases (Deagle et al., 2014; Kelly et al., 2014; Miya et al., 2015; Alberdi et al., 2018; Zhang et al., 2020). These factors can lead to method- and marker-specific biases in assemblage composition, reinforcing the importance of evaluating multiple assays when interpreting eDNA metabarcoding data alongside traditional capture-based surveys, While numerous studies have used eDNA metabarcoding in coastal environments, most comparative studies with capture- or visual-based surveys have been conducted in relatively low-turbidity systems or have focused on a single genetic marker or assay (Thomsen et al., 2012; Stat et al., 2019; He et al., 2023). As a result, there is limited understanding of how eDNA metabarcoding performs in turbid tropical seagrass systems, which components of seagrass-associated fish assemblages are consistently detected by molecular vs other methods, and how primer choice influences detection patterns under these conditions.

In this study, we conducted a direct comparison of beam trawl sampling and eDNA metabarcoding in a turbid tropical seagrass system in North Queensland, Australia. We expected that eDNA metabarcoding would detect higher overall taxonomic richness than beam trawling, reflecting its capacity to capture DNA from a broader range of taxa than those physically collected at the time of sampling. We also expected that the assemblage structure would differ between methods, as beam trawl sampling provides localised sampling of fish physically present in the seagrass meadow, whereas eDNA can integrate signals over a broader temporal and spatial scale. Finally, we expected that primer choice would influence taxonomic detections using eDNA, with different markers recovering dissimilar but overlapping assemblages due to variation in primer amplification efficiency and reference database coverage. Specifically, we aimed to 1) compare fish taxonomic richness and assemblage detected by beam trawls and eDNA metabarcoding; 2) evaluate how primer choice influences taxonomic detections in a tropical seagrass system; 3) identify shared and unique detections between molecular and capture-based approaches, and 4) assess whether eDNA metabarcoding recovered assemblage patterns consistent with ecological expectations for tropical seagrass habitats. Together, these aims address key knowledge gaps surrounding the applicability and reliability of eDNA metabarcoding in turbid tropical seagrass ecosystems and provide evidence for its use alongside traditional survey methods in coastal and estuarine environments.

2. General methods

2.1. *Study area*

Cairns Harbour, located in tropical North Queensland, Australia, is a shallow estuarine bay at the seaward end of Trinity Inlet (Fig. 1). The system is fringed by extensive mangroves and connects with adjacent seagrass meadows, forming an integrated coastal ecosystem. Freshwater and sediment inputs are from the Barron River catchment, which delivers substantial runoff during the wet season (December – March), when the average annual rainfall exceeds 2000 mm (BOM, 2025).

Seagrass meadows within Trinity Inlet have historically supported diverse fish and prawn assemblages and function as important nursery habitats (Coles et al., 1993; Watson et al., 1993). However, these meadows have undergone substantial spatial and temporal variability over recent decades in response to tropical cyclones, floods, and anthropogenic pressures associated with urban development (Coles et al., 1993; Pollard and Greenway, 2013; McKenna et al., 2015; Reason et al., 2025). Samples were collected from two sites in Trinity Inlet, Cairns: Ellie Point (16°53′05.8″S 145°46′30.4″E) and Esplanade (16°54′25.6″S 145°46′27.4″E) (Fig. 1).

2.2. *Beam trawling*

In February 2025, beam trawl sampling was conducted from a research vessel following established methods previously applied at these sites to assess juvenile fish and prawn assemblages (Coles et al., 1993). The beam trawl measured 1.5 m in width and 0.5 m in height and was fitted with a 2 mm mesh net with a tied cod end. Trawls were towed behind the vessel at a towing speed of approximately 0.75ms^{-1} for 2.5 min, corresponding to a tow distance of 100m, as described by Coles and Lee Long (1985).

At each site, four replicate beam trawl tows were conducted within the seagrass meadow. Each tow was standardised to approximately 100 m by towing between paired GPS start and end points. Replicate tows were positioned within the same general meadow area to sample comparable seagrass habitat, while avoiding overlap among tow paths where possible. These tows were treated as standardised site-level replicate samples of the local fish assemblage. Sites were chosen based on observed seagrass biomass (gDWm^{-2}), area (ha), distribution, and species compositions derived from long-term seagrass monitoring programs and aerial imagery (Reason et al., 2025).

Sampling was conducted in February 2025 over two consecutive nights, with only one site sampled per night to minimise cross-site contamination of associated eDNA samples. All trawls were conducted at night during a high tide on a new moon to maximise capture of juvenile fish.

Catches were processed onboard where feasible. Fish were identified to the lowest taxonomic level and released when humane release was possible. Specimens that could not be released were euthanised using an ice slurry and frozen for laboratory processing. In the laboratory,

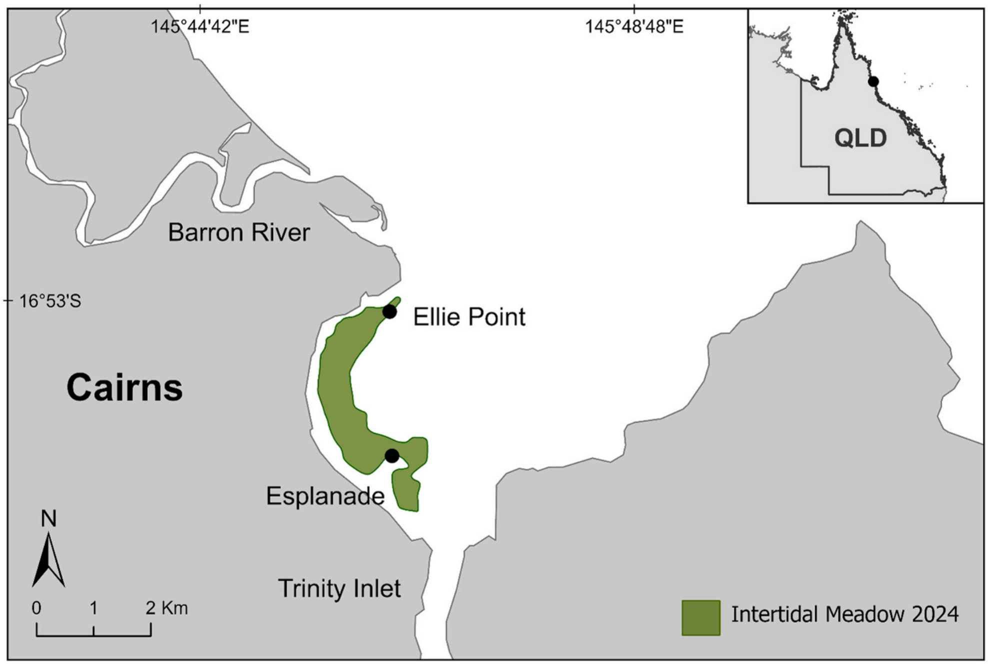

Fig. 1. Location of Ellie Point and Esplanade sampling sites in western Cairns Harbour, tropical north Queensland, Australia. Black points indicate sampling locations where paired beam trawl and eDNA metabarcoding water sampling were conducted. Green polygons show mapped intertidal seagrass meadow extent in 2024 (Reason et al., 2025). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

specimens were identified to lowest taxonomic level based on morphological characteristics. Data recorded included the total number of individuals and number of taxa identified at the species, genus, family, and order levels at each site.

2.3. *Genomic DNA extraction*

Genomic DNA was extracted from a subset of fish specimens collected via beam trawl surveys to support taxonomic verification and reference library development. Tissue samples were preserved in ≥95% ethanol and stored in 2 mL LoBind® microcentrifuge tubes. Approximately 100 mg of tissue (fin clip or muscle) was subsampled from each specimen.

DNA extraction was performed using a cetyltrimethylammonium bromide (CTAB) protocol adapted from Adamkewicz and Harasewych (1996) with minor modifications. The full extraction protocol is provided in Supplementary Methods S1. Briefly, tissue samples were homogenised and incubated in CTAB buffer with proteinase K at 65 °C for 1 h, followed by chloroform–isoamyl alcohol extraction and isopropanol precipitation. DNA pellets were washed with 70% ethanol, air-dried, and eluted in 50 μL of 1 × TE buffer. Extracted DNA quality was assessed by electrophoresis on a 0.8% agarose gel alongside lambda DNA standards, and DNA concentration was quantified using a Qubit Fluorometer (Thermo Fisher Scientific, Victoria, Australia). Extracts were stored at −20 °C until further processing.

A mitochondrial cytochrome *c* oxidase subunit I (COI) fragment (~650 bp) was amplified using endpoint PCR with the primer pair FishF1 (5′-TCAACCAACCACAAAGACATTGGCAC-3′) and FishR1 (5′-TAGACTTCTGGGTGGCCAAAGAATCA-3′) (Ward et al., 2005). PCR amplification and Sanger sequencing were performed by the Australian Genome Research Facility (AGRF; Brisbane, Australia) using standard protocols. Forward and reverse Sanger reads were returned by AGRF and subsequently quality-checked, trimmed, and aligned in Geneious Prime (v2026.0.2 Biomatters Ltd., Auckland, New Zealand) (Kearse et al., 2012). Taxonomic identities were assigned based on the closest BLASTn match, with ≥98% sequence similarity used as a criterion for species-level assignment where possible.

2.4. *Water sample collection*

Water samples for eDNA metabarcoding were collected prior to beam trawl sampling from the same general meadow area as the beam trawl tows. At each site, the vessel was anchored within the seagrass meadow and six replicate 2 L water samples were collected using a 2.5 L Niskin sampler deployed approximately 1 m above the seabed. The Niskin sampler was decontaminated between replicate samples to minimise cross-contamination, and the collected water was transferred into sterile Nalgene bottles. Samples were immediately placed on ice and returned to the laboratory, where filtration occurred within 12 h of collection.

These water samples were treated as field replicates from a fixed sampling station within each site, rather than spatially independent samples distributed across the meadow. All sampling equipment was decontaminated using a 10% bleach solution and rinsed thoroughly in deionised water prior to use. Field collection included a negative filtration control using deionised water at each site, to monitor potential contamination.

2.5. *Filtration and preservation*

Seawater samples were filtered using a Sentino® pump system (Pall Corporation, Port Washington, NY, USA) through sterile 47 mm nylon membrane filters with a pore size of 10 μm (Merck Millipore, Burlington, MA, USA) mounted in Pall MicroFunnel Filtration units (Pall Corporation). A 10 μm pore size was selected as a practical compromise for seawater samples collected from turbid coastal systems, where smaller pore sizes can rapidly clog and reduce the volume of water that can be processed consistently across samples. This filtration strategy was informed by prior workflow optimisation at this study site, which prioritised filtration efficiency, inhibition removal, and consistent DNA recovery from turbid coastal systems (Methods S2). All filtration equipment was decontaminated with a 10% bleach solution and rinsed thoroughly with deionised water prior to use.

One field blank consisting of 1 L of deionised water was handled alongside environmental samples in the field to monitor contamination during field handling and transport. One laboratory filtration blank (1 L deionised water) was filtered in the laboratory under sterile conditions to monitor contamination introduced during laboratory filtration.

Following filtration, membranes were preserved in 2 mL DNA LoBind® microcentrifuge tubes pre-filled with 540 µL ATL buffer (Qiagen, Hilden, Germany) and stored at −20 °C until DNA extraction. Filters were transported on dry ice to the Minderoo OceanOmics Centre (University of Western Australia) for processing.

The eDNA workflow proceeded sequentially from field collection and filtration, through DNA extraction, qPCR amplification and library preparation, sequencing, bioinformatics, taxonomic curation, and downstream genus-level diversity and assemblage analyses.

2.6. Environmental DNA extraction

Preserved filter membranes were pre-washed with 1 mL of diluted phosphate-buffered saline (PBS) to reduce inhibitors prior to extraction. The PBS wash supernatant (Fraction A) was retained, and the filter membrane material (Fraction B) was cut into small fragments. Both fractions were digested overnight at 56 °C in ATL buffer with proteinase K.

After digestion, an inhibitor removal step was performed on the lysate using Solution CD2 from the DNeasy PowerSoil Pro Kit (Qiagen, Hilden, Germany). DNA form both fractions was subsequently extracted using the Maxwell ® RSC PureFood GMO and Authentication Kit (Promega, USA) on a Maxwell® RSC 48 automated extraction platform. One extraction blank was included per extraction batch to monitor potential contamination.

Extracts derived from the filter fraction (Fraction B) were further treated using the Zymo OneStep PCR Inhibitor Removal Kit (Zymo Research, USA) following the manufacturer's instructions, whereas supernatant-derived extracts (Fraction A) did not undergo this additional inhibitor removal step. All extracts were combined and purified using AMPure XP magnetic beads (Beckman Coulter, USA) at a 2:1 bead-to-sample ratio, with final elution in 50 µL of elution buffer (Promega, USA). Purified eDNA was quantified using the Qubit dsDNA HS Assay Kit and a Qubit 4 Fluorometer (Thermo Fisher Scientific).

2.7. Quantitative PCR and library preparation

Quantitative PCR (qPCR) was performed to assess amplification success and potential PCR inhibition in eDNA extracts. All pre-PCR and post-PCR steps were performed in physically separated laboratories at the Minderoo OceanOmics Centre (University of Western Australia) to minimise contamination risk. Pre-PCR steps, including mastermix preparation and template addition, were automated using a Beckman Coulter Biomek i7 handling system. Three primer sets targeting fish and metazoan assemblages were used: 16S-Fish/D (Berry et al., 2017), MiFish-U (Miya et al., 2015), and COI-Leray (Leray et al., 2013) (Table S1). All primers were synthesised with unique 8-bp combinatorial dual-indexed primers to enable sample-level demultiplexing following sequencing.

Each qPCR reaction was performed in triplicate in a total volume of 15 µL containing 7.5 µL AmpliTaq Gold™ 360 PCR Master Mix (Thermo Fisher Scientific), 0.15 µL Bovine Serum Albumin (20 mg/µL; Thermo Fisher Scientific), 0.375 µL 5x SYBR™ Green I (10,000X concentrate in DMSO, Thermo Fisher Scientific), 0.4 µM each of forward and reversed indexed primers, 2 µL template eDNA, and nuclease-free water to make-up total volume. Reactions were run on a Roche LightCycler 480 instrument, using assay-specific primers and cycling conditions (Table S1). Each qPCR run included no-template controls (NTCs) to detect potential contamination.

Following amplification, technical triplicates were pooled in equal volumes for each sample. Samples were then normalised within each assay plate relative to their endpoint fluorescence (EPF) values to reduce variation in amplicon yield among samples prior to pooling. The resulting assay pools were quantified using a Qubit 4.0 fluorometric (Invitrogen, Thermo Fisher Scientific) and combined equimolarly for library preparation. This normalisation step was implemented to promote balanced sequencing depth across samples.

Replicates exhibiting abnormal amplification curves or melt-curve anomalies were excluded prior to pooling. All negative controls were carried through pooling, library preparation, and sequencing; however, only samples passing qPCR quality control criteria were included in downstream analyses. Pooled amplicons were purified with AMPure XP beads (Beckman Coulter, USA) using a 1.8x bead clean-up and quantified using a Qubit 4.0 Fluorometers (Invitrogen, Thermo Fisher Scientific). Fragment size distributions were verified using an Agilent TapeStation 4200.

Amplicon libraries for 16S-Fish/D and MiFish-U were prepared using the Illumina TruSeq DNA PCR-Free Library Preparation Kit (Illumina) with IDT for Illumina TruSeq DNA UD Indexes v2, following the manufacturer's instructions with minor modifications for eDNA samples. Library preparation used 250 ng DNA input.

Assay pools were diluted to 2 nM based on quantification using Qubit 4.0 Fluorometer (Thermo Fisher Scientific) and Femto Pulse fragment analysis (Agilent Technologies) and were then pooled in equimolar concentrations into the final loading library. The final combined library was loaded at 525 p.m. with 30% PhiX Control v3 (Illumina) to increase sequence diversity. Sequencing was performed on an Illumina NextSeq 2000 platform (Illumina, San Diego, USA) using a NextSeq XLEAP P1 300-cycle kit (2 X 150bp; paired-end) in the dedicated post-PCR sequencing facility at Minderoo OceanOmics Centre (University of Western Australia).

The COI-Leray library was prepared using the same library preparation method but was sequenced separately to allow for the longer amplicon size. For this assay, 100 ng DNA input was used during library preparation. The final pooled library was loaded at 525 pM and spiked with 10% PhiX prior to sequencing on an Illumina NextSeq 2000 using a NextSeq XLEAP P1 600-cycle kit (2 × 300 bp; paired-end).

2.8. Bioinformatics

Raw paired-end FASTQ files from all assays (16S-Fish/D, MiFish-U, and COI-Leray) were processed using the Minderoo Foundation OceanOmics amplicon pipeline, a publicly available NextFlow (v22.10.1) workflow for processing eDNA amplicon data from raw reads to ASV tables, taxonomic assignments, and phyloseq objects (this pipeline is available at: https://github.com/MinderooFoundation/OceanOmics-amplicon-nf). The workflow was built using the nf-core framework (Ewels et al., 2020) and run using Nextflow (Di Tommaso et al., 2017). The key software versions and study-specific parameters used in this analysis are described as follows.

Demultiplexing and primer trimming were performed with Cutadapt (v4.7), using assay-specific forward and reverse primer sequences with zero mismatches permitted. Reads shorter than the minimum expected amplicon length or longer than 1000 bp were discarded. Read quality was assessed using FastQC (v0.11.9) and summarised in MultiQC (v1.20). Amplicon sequence variants (ASVs) were inferred using DADA2 (v1.26.0) (Callahan et al., 2016) in pooled mode, requiring a minimum of 12 bp overlap and no mismatches for merging paired reads, followed by chimera removal. Resulting ASVs were further curated using LULU (Frøslev et al., 2017), which merges highly similar ASVs that consistently co-occur across samples, helping to reduce redundant sequence variants (minimum match = 84%).

Taxonomic assignment was performed by querying ASVs against Australia's National Biodiversity DNA Library Guide (NBDL; research.csiro.au/dnalibrary) using BLASTn (v2.13.0+) applying thresholds of ≥90% sequence identity and 100% query coverage. Ambiguous assignments were resolved using a lowest common ancestor (LCA) approach. A naïve Bayes classifier was run in parallel to support taxonomic assignments, and duplicate hits were removed.

All sequencing runs included extraction blanks, no template PCR controls, and a positive internal control (ITC), to detect contamination and amplification performance. Low-level amplification was detected in some extraction blanks and was addressed using a proportional filtering algorithm implemented within the OceanOmics amplicon pipeline. This algorithm identifies all ASVs present in control samples and proportionally removes the corresponding reads from biological samples according to their relative abundance in controls, while ASVs detected exclusively in controls are removed entirely. Summary statistics describing control-associated ASVs for each metabarcoding marker are provided in Table S2. Following this step, all controls were excluded from downstream analyses, and subsequent rarefaction and diversity analyses were conducted only on filtered biological samples.

Curated ASV tables, assigned taxonomy, and sample metadata were imported into Phyloseq (v1.50.0) (McMurdie and Holmes, 2013) in R for community analyses. To restrict analyses to the target taxa, ASVs were retained only if assigned to Chordata and further classified as teleost fish (Teleostei) or elasmobranchs (Elasmobranchii). For the 16S-Fish/D assay, ASVs classified as Actinopteri were also retained. ASVs assigned to non-target groups (including Aves, Mammalia, Cyanophyceae) or with ambiguous, unclassified, uncertain, or non-informative taxonomic assignments, were excluded.

Following target-taxon filtering, eDNA datasets were further screened to remove taxonomic assignments considered ecologically implausible for shallow tropical seagrass meadows in Cairns Harbour. Exclusions were applied to taxa with no expected occurrence in the study habitat, including primarily freshwater-associated taxa, salmonids, and mesopelagic or deep-water taxa. These decisions were based on known habitat associations, occurrence records from the Global Biodiversity Information Facility (https://www.gbif.org/), and published literature and were applied at the family, genus, or species level depending on the taxonomic resolution of the assignment. Freshwater-associated taxa included Cichlidae, Melanotaeniidae, and Salmonidae, while mesopelagic or deep-water taxa included Myctophidae, Gonostomatidae, Monognathidae, Phosichthyidae, and the deep-water genus *Antimora*. The full list of excluded ASVs is provided in Table S3, with exclusions summarised by taxonomic group and exclusion reason in Table S4. The final retained genus-level eDNA detections used in downstream analyses are provided in Table S5.

To evaluate whether sequencing depth was sufficient for downstream diversity comparisons, rarefaction curves were generated for each assay using the *vegan* package (Oksanen, 2025) (Fig. S1). Rarefaction curves indicated that MiFish-U and 16S-Fish/D datasets approached saturation well before maximum sequencing depth. A conservative minimum read threshold of 10,000 reads per sample was applied, consistent with thresholds adopted in eDNA metabarcoding studies to ensure sufficient coverage while minimising sample loss (Alberdi et al., 2018).

2.9. Data analyses

All statistical analyses were conducted in R (v4.5.1) using the *phyloseq* (v1.52.0) and *vegan* (v2.7) packages (R Core Team, 2025). Environmental DNA metabarcoding and beam trawl datasets were converted to presence–absence matrices, with ASVs scored as detected (1) or not detected (0) in each sample. Sequence read counts were not treated as proxies for organismal abundance due to known biases associated with primer performance, DNA degradation, and transport processes (Elbrecht and Leese, 2015). This detection-based framework enabled direct comparison across molecular assays and capture-based sampling methods. Taxonomic names were standardised across molecular and capture-based datasets to ensure consistency in genus-level comparisons.

Genus-level richness was calculated for each sample or trawl two using presence–absence data. Differences in genus richness among sampling methods and sites were assessed using aligned rank transform analysis of variance (ranked ANOVA), with Method, Site and their interaction included as fixed effects. This approach was used because richness data are discrete counts and the sample sizes were relatively small. Raw mean richness values ± standard error are presented descriptively, while statistical inference was based on aligned ranks. Where significant interactions were detected, post hoc site contrasts were conducted within each sampling method using estimated marginal means from the aligned-rank model.

Community assemblage was assessed using Jaccard dissimilarities calculated from presence-absence data and visualised using principal coordinates analysis (PCoA). Homogeneity of multivariate dispersion was evaluated using *betadisper*, with significance tested using 999 permutations. Differences in assemblages among sampling methods and sites were tested using permutational multivariate analysis of variance (PERMANOVA) implemented in *adonis2*, including Method (beam trawl, MiFish-U, 16S-Fish/D), Site, and their interaction, with significance assessed using 999 permutations.

Indicator taxa associated with each sampling method were identified by indicator value (IndVal) analysis based on presence–absence data at the genus level, using the *indicspecies* package (v 1.8.0), with significance assessed using 999 permutations (De Cáceres et al., 2011). IndVal combines taxon specificity and fidelity to quantify the strength and specificity of associations between fish families and sampling methods, with statistical significance assessed using permutation tests and p-values adjusted for multiple comparisons where appropriate.

To examine functional patterns across sampling approaches, fish genera were assigned to trophic guilds based on dominant prey type using dietary information from FishBase (Froese and Pauly, 2026). Invertivores were defined as genera with diets that were dominated by invertebrates (e.g. crustaceans, molluscs), and piscivores were assigned to genera feeding predominately on finfish. Herbivores were defined as genera consuming living macroalgal and macrophyte material, while planktivores were defined as genera feeding predominately on zooplankton and/or phytoplankton. Detritivores were defined as taxa whose diets are dominated by detrital organic matter. Genera with mixed feeding strategies, meaning without a single dominant prey category, were classified as omnivores, following FishBase dietary classifications and establishing trophic guild frameworks (Elliott et al., 2007). Differences in trophic guild assemblages among sampling methods were tested using a Pearson chi-square test on the number of unique genera assigned to each trophic guild within each method. Because several expected cell counts were low, significance was assessed using Monte Carlo simulation with 9999 replicates. Cramer's V was calculated as a measure of effect size.

3. Results

A total of eight beam trawl samples and twelve eDNA water samples were collected across Ellie Point and Esplanade. Accordingly, statistical comparisons were based on two sites, with eight beam trawl samples and twelve eDNA samples retained for downstream analyses. Sequencing depth, ASV recovery, sample retention, and genus-level detections varied among metabarcoding assays, with MiFish-U and 16S-Fish/D retained for downstream fish assemblage analyses, while COI-Leray produced limited fish detections and was excluded from comparative assemblage analyses (Table 1).

3.1. Sequencing output and marker performance

Across the fish-targeted MiFish-U and 16S-Fish/D, assays, eDNA metabarcoding generated a total of 17,844,625 quality-filtered reads from seawater samples collected at Ellie Point and Esplanade in Trinity Inlet. After quality filtering, chimera removal, control-informed ASV removal, and assay-specific taxonomic curation all 12 samples were retained for both the MiFish-U and 16S-Fish assays. In contrast, only three samples met minimum read-depth thresholds for the COI-LeRay

Table 1
Summary of sampling effort and methodological outputs across beam trawling and eDNA metabarcoding assays. Samples retained refer to samples passing quality-control, taxonomic filtering, and minimum read-depth thresholds for downstream genus-level analyses. Read depth values are shown for the final retained eDNA datasets used in analyses.

Method	Samples retained	Total reads retained	Median reads per sample	Read depth range	ASVs retained	Families detected	Genera detected
Beam Trawl	8	NA	NA	NA	NA	24	27
MiFish-U	12	6,430,090	516,916	214,375–982,726	258	53	91
16S-Fish/D	12	11,414,535	990,400	285,612–1,584,825	186	47	63

assay, which produced limited fish detections and was excluded from further comparative assemblage analysis.

The MiFish-U assay generated a total of 6,430,090 reads, with a mean sequencing depth of 535,841 ± 68,049 reads per sample (median = 516,916, n = 12). The 16S-Fish/D assay produced 11,414,535 total reads, with a higher mean sequencing depth of 951,211 ± 122,912 reads per sample (median = 990,400, n = 12).

Across all markers, amplicon sequence variant (ASV) richness varied among assays. The COI-Leray assay generated the highest overall ASV richness (2142 ASVs), reflecting broad amplification of metazoan DNA. However, taxonomic assignment was extremely limited, with >99.9% of COI reads remaining unassigned at the phylum or class level. Only three COI ASVs were assigned to fish taxa, corresponding to <0.01% of total COI reads.

In contrast, the fish-specific MiFish-U and 16S-Fish/D assays produced fewer total ASVs but a substantially higher proportion of fish detections. These results highlight the limited suitability of COI-Leray for fish community detection in environmental water samples from turbid tropical seagrass systems at this study site, compared with targeted fish metabarcoding markers.

3.2. Taxonomic summary of eDNA detections

Following fish-only filtering and removal of unresolved assignments, eDNA metabarcoding recovered 112 fish genera across MiFish-U and 16S-Fish/D assays, spanning 64 families and 27 orders. The MiFish-U dataset comprised 258 ASVs, assigned to Teleostei, whereas the 16S-Fish/D dataset comprised 186 ASVs, of which 95.6 % were assigned to Actinopteri, with a small proportion assigned to Elasmobranchii (4.4%).

While analyses in this study focused primarily on genus-level patterns, the most genus rich and frequently detected families across both assays included Gobiidae (gobies), Engraulidae (anchovies), Leiognathidae (ponyfish), Mugilidae (mullet), and Terapontidae (grunters), which are characteristic of tropical estuarine and coastal seagrass associated fish assemblages in north Queensland (Coles et al., 1993; Froese and Pauly, 2026).

Several detected genera were of potential conservation or management relevance. These detections included elasmobranch genera such as *Carcharhinus* (reef shark), *Aetobatus* (eagle ray) and *Glaucostegus* (guitarfish), which contain species listed under threatened or near-threatened categories on the IUCN Red List. The eDNA datasets also detected coastal and pelagic fish genera with recognised conservation or fisheries concern, including *Scomberomorus* (mackerel) and *Trichiurus* (hairtail), highlighting the value of metabarcoding for detecting taxa of fisheries importance (ABRS, 2026).

3.3. Comparison of beam trawling and eDNA metabarcoding

Beam trawling and eDNA metabarcoding combined detected fish taxa across 29 orders, 67 families, and 116 genera. Environmental DNA metabarcoding recovered 27 orders, 64 families, and 112 genera, while beam trawl surveys detected 8 orders, 24 families, and 27 genera.

At the genus level, a total of 116 unique genera were detected across all methods. Environmental DNA metabarcoding recovered 112 genera in total, compared with the 27 genera detected by beam trawl surveys. Of these, 89 genera were detected by eDNA metabarcoding, four genera were detected only by beam trawling, and 23 genera were detected by both approaches.

Comparison of genus-level detections across sampling methods revealed both overlap and clear method-specific differences in taxa detected (Fig. 2). MiFish-U recovered the greatest number of genera overall, detecting 91 genera, while the 16S-Fish/D assay detected 63 genera (Figs. 2 and 3). The proportion of replicate detections (Fig. 3) further highlights genera that were consistently detected across methods. Beam trawl surveys additionally identified taxa not detected by eDNA metabarcoding including *Trixiphichthys* (tripodfish), *Nibea* (croaker), and *Monocanthus* (filefish), *Dussumieria* (rainbow sardine), reflecting differences in detectability between capture-based and molecular approaches (Fig. 3).

These results demonstrate that eDNA metabarcoding detects higher taxonomic richness overall, while beam trawl surveys contributed a small number of unique detections absent from the eDNA datasets. Together, these results demonstrate that eDNA metabarcoding and beam trawl surveys provide complementary approaches to fish assemblages in turbid tropical seagrass systems.

3.4. Taxonomic richness across sampling methods and sites

Across samples, genus richness varied among sampling methods

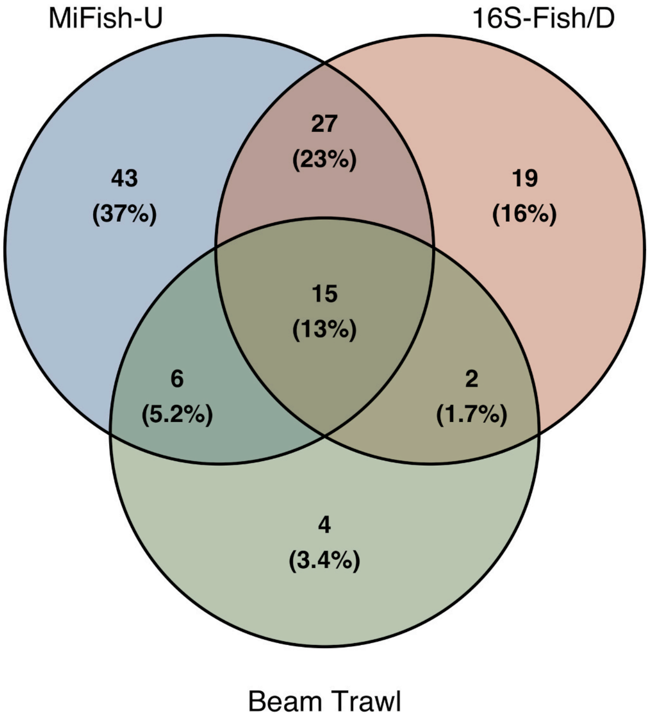

Fig. 2. Overlap in genus-level fish detections among MiFish-U and 16S-Fish/D assays and beam trawl sampling in a tropical seagrass meadow in Cairns Harbour. Detections were converted to presence-absence and pooled across all biological samples from Ellie Point and Esplanade. Numbers indicate genera detected uniquely by each method or shared among methods. Percentages indicate the proportion of unique genera detected by the method.

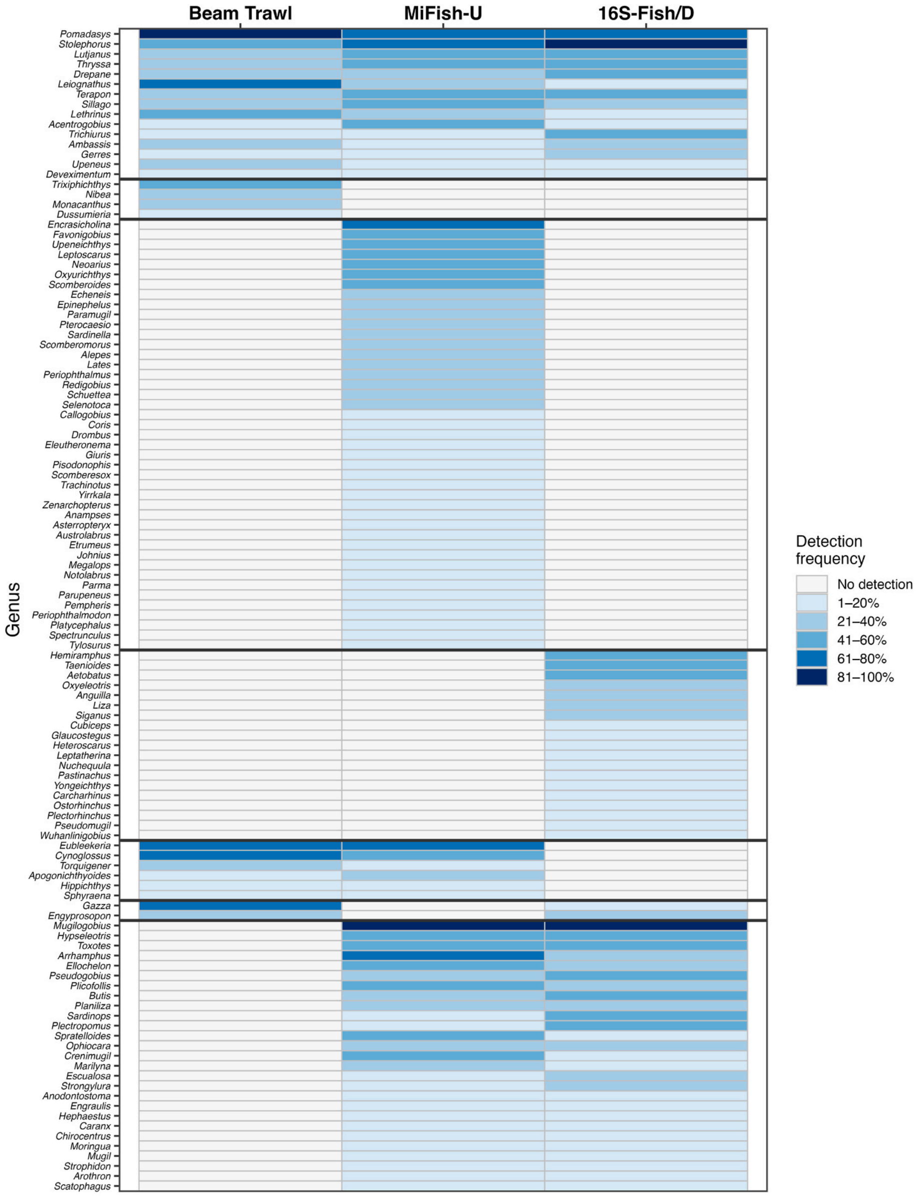

Fig. 3. Genus-level heatmap showing detection frequencies across beam trawl surveys and two eDNA metabarcoding assays (MiFish-U and 16S-Fish/D). Colour intensity indicates the proportion of biological replicates within each method in which a genus was detected, ranging from 0 (not detected) to 1 (detected in all replicates). Darker colours therefore indicate more consistent detection within a method. Genera are grouped according to their method-overlap pattern, separating taxa detected by all three methods, taxa detected exclusively by a single method, and taxa detected by pairs of methods. Within each group, genera are ordered by detection frequency to highlight differences in consistency among methods. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

(Fig. 4). MiFish-U recovered the highest mean genus richness (24.7 ± 3.20 SE, n = 12), followed by the 16S-Fish/D assay (17.6 ± 1.64 SE, n = 12), while beam trawl surveys detected fewer genera per tow (9.9 ± 1.16 SE, n = 8). Genus richness differed among sampling methods and sites, with a significant Method × Site interaction (aligned rank transform ANOVA $F_{2,26} = 22.68$, $p < 0.001$; Table S6).

Site-level patterns differed among sampling methods. MiFish-U recovered significantly higher mean genus richness at Ellie Point (33.0 ± 3.65 SE) than at the Esplanade (16.3 ± 1.96 SE; post hoc contrast, p = 0.0078). The 16S-Fish/D assay also showed higher mean genus richness at Ellie Point (20.7 ± 2.06 SE) than at the Esplanade (14.5 ± 1.96 SE), although this site difference was not statistically significant (p = 0.7843). In contrast, beam trawl surveys showed the opposite pattern, with significantly higher genus richness at the Esplanade (12.0 ± 1.73 SE) than at Ellie Point (7.8 ± 0.48 SE; p = 0.0005). These contrasting site-level patterns were reflected in the significant Method × Site interaction.

3.5. *Community assemblage*

Principal coordinates analysis (PCoA) ordination revealed clear separation of fish assemblages by sampling method at genus levels, with beam trawl samples forming distinct clusters from eDNA metabarcoding samples (Fig. 5).

Homogeneity of multivariate dispersion tests indicated that dispersion differed significantly among sampling methods (F = 3.71, df = 2, 29, $p = 0.046$), but not between sites (F = 1.17, df = 1, 30, $p = 0.301$), suggesting that method-related differences may partly reflect variation in within-method heterogeneity. When examined within individual sampling methods, site significantly influenced genus-level assemblage

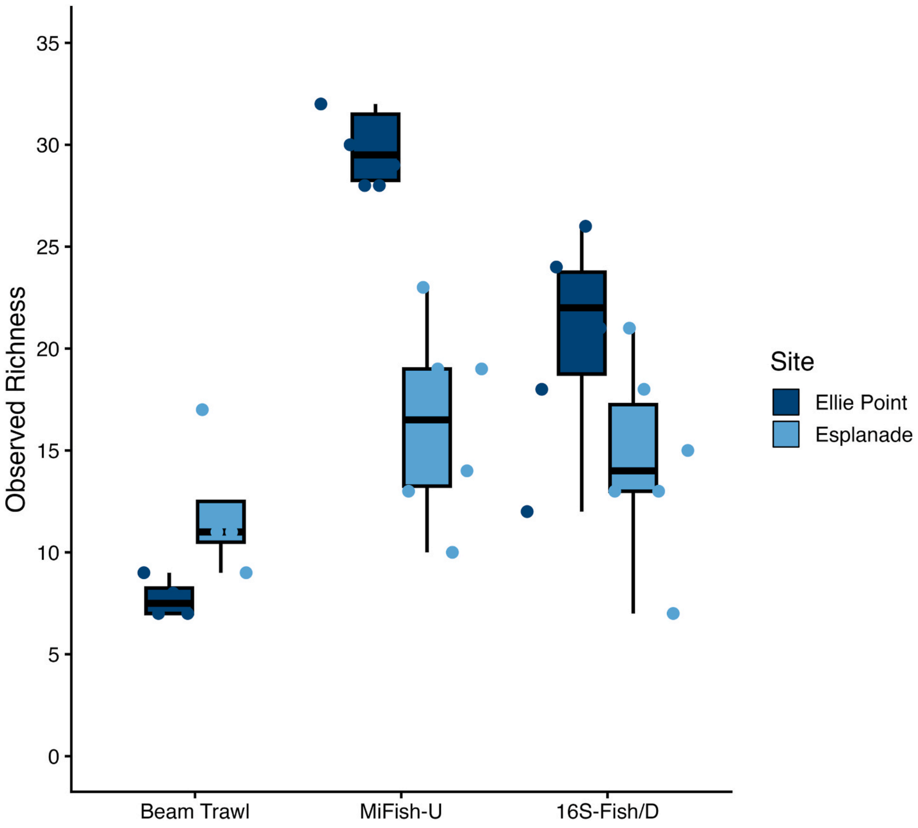

Fig. 4. Genus-level fish richness detected by beam trawling and eDNA metabarcoding (MiFish-U and 16S-Fish/D) at Ellie Point and Esplanade. Richness represents the number of fish genera detected per biological replicate for each sampling method. Beam trawl surveys included four trawl replicates per site, while MiFish-U and 16S-Fish/D each included six biological eDNA replicates per site. Boxes show the interquartile range, horizontal lines show median richness; whiskers indicate 1.5 × the interquartile range, and points represent individual samples. Colours denote site, with darker blue representing Ellie Point and lighter blue representing Esplanade. Both eDNA assays generally detected higher genus richness than beam trawling, although richness varied between sites and markers. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

composition for beam trawl surveys ($F = 4.14$, df = 1, 6, $R^2 = 0.408$, $p = 0.027$), MiFish-U ($F = 2.69$, df = 1, 10, $R^2 = 0.212$, $p = 0.004$), and 16S-Fish/D ($F = 3.76$ df = 1,10, $R^2 = 0.273$, $p = 0.008$; Table S8).

3.6. Indicator species

Indicator genus analysis identified several genera associated with individual sampling methods based on presence-absence detections across replicates. Two genera remained significant after Benjamini-Hochberg correction: *Encrasicholina*, which was associated with MiFish-U detections, and *Mugilogobius*, which was associated with 16S-Fish/D detections. Additional genera were significant at the unadjusted $p \leq 0.05$ threshold and are reported in Table S6. These included beam trawl-associated genera such as *Trixiphichthys, Gazza, Nibea* and *Leiognathus*, 16S-Fish/D-associated genera such as *Hemiramphus, Taenioides, Sardinops*, *Aetobatus* and *Oxyeleotris*, and several MiFish-U-associated genera spanning multiple trophic guilds.

3.7. Trophic guild

Functional composition of fish assemblages was assessed by assigning detected genera to trophic guilds and comparing their representation across the two assays (MiFish-U and 16S-Fish/D) and beam trawling (Fig. 6). Trophic guild composition did not differ significantly among sampling methods based on a Pearson chi-square test with Monte Carlo simulation ($\chi^2 = 10.69$, simulated $p = 0.564$). The association between sampling method and trophic guild composition was weak (Cramer's $V = 0.17$). Across methods, detected genera were dominated by invertivores and planktivores, although eDNA metabarcoding detected genera across a broader range of trophic guilds, including guilds such as detritivore, omnivore, and herbivore that were absent from beam trawl sampling.

4. Discussion

This study compared eDNA metabarcoding and beam trawl surveys for assessing fish assemblages in a turbid tropical seagrass system, including two eDNA assays that specifically target fish (MiFish-U and 16S-Fish/D). Across both taxonomic and community assemblage analyses, eDNA metabarcoding detected higher fish diversity per sample than beam trawl surveys, with the MiFish-U assay detecting the greatest number of genera. However, differences in fish richness and assemblage among sampling approaches were site dependent, with a significant Method × Site interaction, emphasising that spatial patterns varied according to the sampling method used, with eDNA detections showing stronger separation between sites compared to beam trawl detections. Importantly, neither method alone captured the full diversity of fish at each site, with each approach detecting unique taxa not recovered by the other.

Multivariate analyses further demonstrated clear separation of assemblages by sampling method, with method choice explaining more variation than site location. However, site effects were evident within individual methods, indicating that the spatial difference between Ellie Point and Esplanade (2.5 km) was sufficient to influence detected assemblage structure despite their proximity.

Notably, eDNA metabarcoding, when including the modified extraction steps, enabled consistent genus-level detections despite challenging environmental conditions characterised by turbidity,

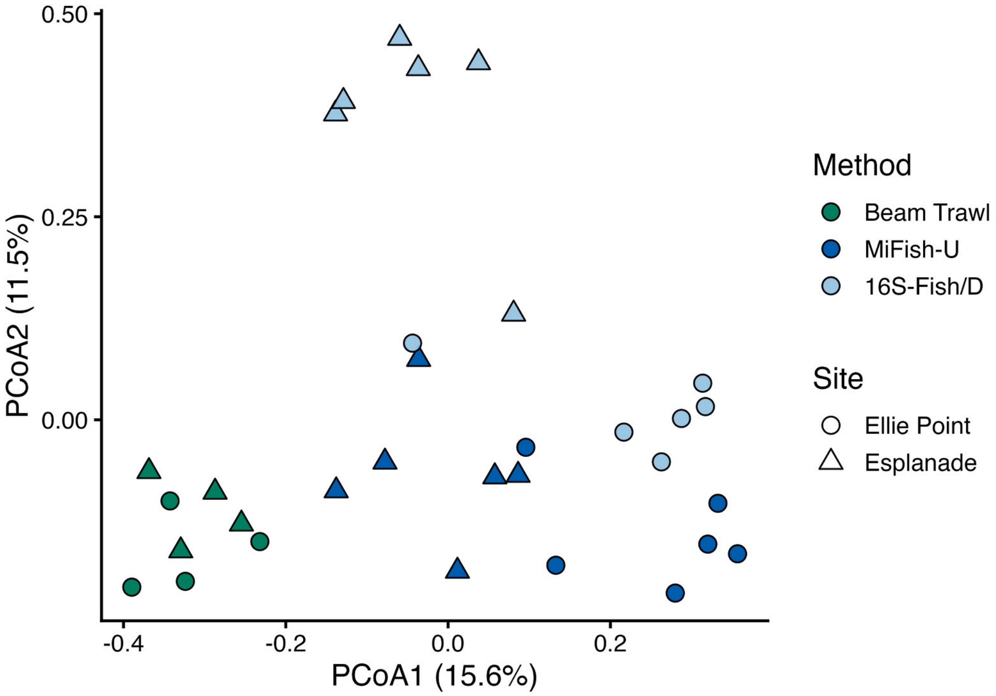

Fig. 5. Principal coordinates analysis (PCoA) of fish assemblage based on Jaccard presence–absence dissimilarities for genus-level detections. Beam trawl surveys included four trawl replicates per site, while each eDNA assay included six biological replicates per site. Colours denote sampling method (Beam Trawl, MiFish-U, 16S-Fish/D), and shapes denote site (Ellie Point and Esplanade). Axis labels show the percentage of variation explained by each PCoA axis. Samples show clustering by sampling method and site, indicating differences in assemblage composition detected by beam trawling and eDNA metabarcoding. Patterns observed in the PCoA ordination were supported by PERMANOVA based on Jaccard dissimilarities of presence–absence data. Fish assemblages differed significantly among sampling methods ($F = 5.08$, $df = 2{,}26$, $R^2 = 0.221$, $p = 0.001$) and between sites ($F = 4.04$, $df = 1{,}26$, $R^2 = 0.088$, $p = 0.001$), with a significant Method × Site interaction ($F = 2.95$, $df = 2{,}26$ $R^2 = 0.128$, $p = 0.001$; Table S7). These results indicate that differences among sampling approaches were site dependent, with method-specific assemblage patterns varying between Ellie Point and Esplanade. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

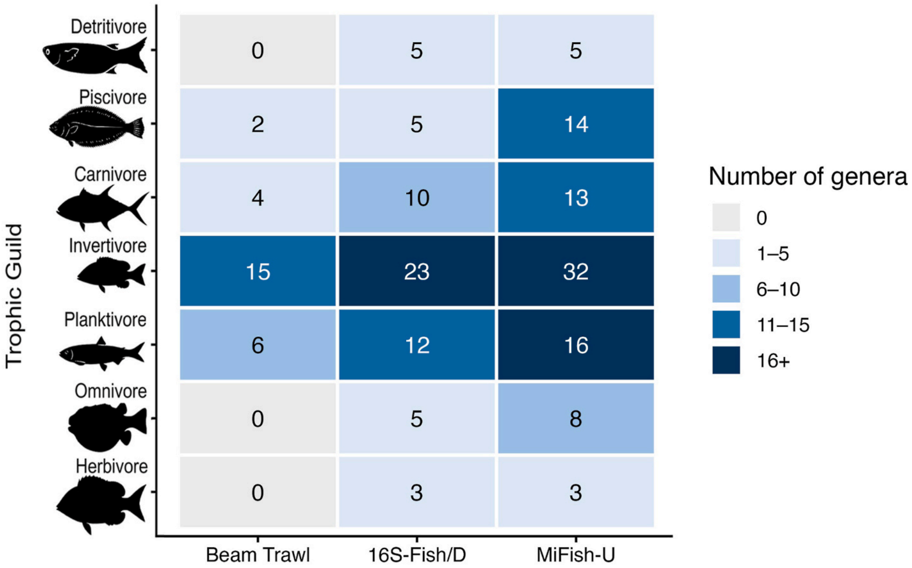

Fig. 6. Trophic guild composition of fish genera detected by beam trawling and eDNA metabarcoding (MiFish-U and 16S-Fish/D) in Cairns Harbour seagrass meadow. Genera detected by each method were assigned to a primary trophic guild, and numbers within tiles indicate the total number of genera detected in each guild by each method. Colours indicate richness categories, with darker blue representing a greater number of genera. Rows represent trophic guilds and columns represent sampling methods. MiFish-U and 16S-Fish/D detected genera across all trophic guilds, whereas beam trawling detected fewer genera overall and no detritivore, omnivore or herbivore genera in this dataset. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

suspended sediments, and potential PCR inhibitors typical of tropical estuarine seagrass habitats. Trinity Inlet contains elevated suspended sediments and organic particulates, such as humic acids and tannins (Ports, 2014). These factors can reduce filtration efficiency, lower DNA recovery, and inhibit qPCR amplification (Sanches and Schreier, 2020; Kumar et al., 2022a). By incorporating modified extraction steps including a pre-wash, inhibitor removal, and bead-based DNA concentration informed by prior optimisation conducted within this system, we achieved consistent recovery of diverse fish assemblages. These findings demonstrate that appropriately adapted workflows enable reliable eDNA metabarcoding in turbid tropical coastal and estuarine systems and supports the integration of molecular and capture-based methods for comprehensive biodiversity assessments of a tropical seagrass system.

4.1. Primer choice shapes ecological inference

Primer choice strongly influenced detections and taxonomic resolution in this study, with clear differences in detections between the MiFish-U and 16S-Fish/D assays. MiFish-U recovered a greater number of ASVs (258) and detected a broader range of families overall, whereas 16S-Fish/D detected fewer ASVs (186) but recovered elasmobranch taxa that were absent from the MiFish-U dataset. Only 27 genera were shared between the two assays, highlighting that primer selection can strongly determine the inferred fish assemblages in eDNA metabarcoding studies (Polanco et al., 2021).

The contrasting fish detections between the two assays are consistent with known differences in marker properties and primer-specific amplification biases (Kelly et al., 2014; Miya et al., 2015; Deiner et al., 2017). MiFish-U targets a short fragment (~170 bp) of the mitochondrial 12S rRNA gene, which was designed for broad fish detection (Miya et al., 2015). The 16S-Fish/D assay targets a region of the mitochondrial 16S rRNA fragment that has also been widely applied in marine fish eDNA studies (Berry et al., 2017). Previous studies have similarly demonstrated that primer choice can influence both taxonomic recovery and ecological inference (Zhang et al., 2020; Kumar et al., 2022b; Xu et al., 2024).

The limited performance of the COI-Leray assay for fish detections in this study, further illustrates the importance of marker selection in turbid tropical estuarine systems. Although COI is widely used for metazoan metabarcoding (Leray et al., 2013; Andújar et al., 2018; Baetscher et al., 2023), it yielded extremely limited fish detections in Trinity Inlet, with <0.01% of reads assigned to Chordata. The longer amplicon length and greater variability of COI primer-binding regions may reduce amplification success from degraded or low-concentration eDNA (Deagle et al., 2014). Consistent with other studies reporting reduced performance of COI for vertebrate eDNA in complex systems (Alberdi et al., 2018; Collins et al., 2019a; Casey et al., 2021), these findings indicate that COI-Leray is unlikely to be suitable as a standalone marker for fish-focused studies in turbid coastal environments. Fish-targeted mitochondrial assays, such as MiFish-U and 16S-Fish/D, may therefore provide a more reliable taxonomic recovery for fish assemblage assessment, whereas COI may be more appropriate as a complementary marker, where broader metazoan diversity is of interest (Collins et al., 2019b; Kumar et al., 2022b).

Consistent with findings from other aquatic systems, the results from this study demonstrate that no single fish assay provides a complete representation of fish assemblages in a coastal system (Thomsen et al., 2012; Kumar et al., 2022b; Zhu and Iwasaki, 2023). Primer-specific biases in amplification and taxonomic resolution inevitably influence ecological inference, and combining complementary assays, such as MiFish-U and 16S-Fish/D, improves taxonomic coverage and reduces detection bias. While these factors apply broadly across environments, they are particularly relevant in dynamic and species-rich tropical estuarine systems, such as Trinity Inlet.

4.2. Environmental DNA vs beam trawling

Environmental DNA metabarcoding detected a greater cumulative number of fish genera than beam trawling across Trinity Inlet, recovering 112 genera compared with 27 genera for beam trawl surveys. This result is consistent with previous comparative studies demonstrating higher taxonomic recovery using eDNA metabarcoding relative to capture-based methods in aquatic systems (Afzali et al., 2021; Gibson et al., 2023; He et al., 2023; Salter et al., 2026). However, genus-level richness differences at the sample level were less pronounced, and richness patterns varied among sites depending on the method used, as indicated by the significant Method × Site interaction. Specifically, MiFish-U detected higher richness at Ellie Point, whereas beam trawls detected higher richness at the Esplanade. These contrasting site-level responses demonstrate that spatial patterns in diversity can be method-dependent (Thomsen et al., 2016).

Due to beam trawling and eDNA metabarcoding operating across different spatial and temporal scales, direct comparisons between methods should be interpreted cautiously. Beam trawls provide a localised, time-specific sample of individuals physically present during collection (~100 m), whereas eDNA integrates biological signals across broader and more variable spatial (hundreds of metres to several kilometres) and temporal scales, ranging from hours to weeks depending on environmental conditions (Rotherham et al., 2012; Collins et al., 2018; Du et al., 2018; Beentjes et al., 2019). Despite these methodological differences, 23 genera were consistently detected by both methods, representing a core assemblage of dominant taxa in seagrass systems. These dominant taxa included gobies, ponyfish, anchovies, pufferfish, grunters and goatfish (e.g. *Acentrogobius*, *Deveximentum, Stolephorus*, *Terapon, Torquigener, Upeneus*), which are characteristic of assemblages associated with tropical seagrass nursery habitats at this site (Coles et al., 1993), and northern Australia in general (Froese and Pauly, 2026).

Despite this overlap in dominant seagrass-associated genera, assemblage patterns remained strongly method and site-dependent. The significant Method × Site interaction in the multivariate analyses, indicated that eDNA metabarcoding and beam trawl surveys captured different components of the genus-level fish assemblage within Trinity Inlet. These method and site-dependent patterns likely arise from differences in how taxa are detected by each approach. Beam trawl surveys rely on the physical capture of individuals and are influenced by gear selectivity, fish behaviour (such as net avoidance), and habitat accessibility (Henseler and Oesterwind, 2023; Hammerl et al., 2024; Philpott et al., 2025). In contrast, eDNA metabarcoding detects genetic material shed into the environment across a broader spatial and temporal scale through water movement and DNA transport (Barnes et al., 2014; Strickler et al., 2015), enabling detection of cryptic, elusive, or less abundant species that evade physical capture during trawling events (Thomsen et al., 2012; Valentini et al., 2016). However, this broader detection window also means that eDNA assemblages may include signals from taxa that were not physically present within the sampled meadow at the exact time of collection (Hinlo et al., 2018; Jeunen et al., 2019). In shallow estuarine systems, tidal exchange, currents, and sediment resuspension can transport DNA, potentially contributing to detections of mobile, pelagic, or reef-associated taxa that move through or occur adjacent to seagrass habitats (Turner et al., 2015). In addition, eDNA persistence is influenced by degradation processes associated with temperature, UV exposure, microbial activity, and suspended sediments (Strickler et al., 2015; Barnes and Turner, 2016). Our DNA detections may therefore reflect a combination of local presence and DNA transported from adjacent habitats, affected by differential DNA persistence across taxa.

The 89 genera detected exclusively by eDNA metabarcoding likely include taxa that are either cryptic, rare, large, mobile, or otherwise poorly sampled by beam trawl tows and taxa whose DNA may have been transported into the sampled meadow from adjacent habitats. Pelagic and fast-swimming taxa (e.g. *Caranx*, *Alepes,* and *Scomberomorus)* may contribute detectable DNA while transiting through seagrass meadows and actively avoiding slow beam trawl nets. Large mobile reef-associated predators and elasmobranch genera (e.g. *Epinephelus, Plectropomus, Aetobatus, Carcharhinus)* can avoid trawling gear and likely occur at low densities in shallow seagrass habitats. In contrast, the four genera detected exclusively by beam trawls including *Trixiphichthys* (tripodfish), *Nibea* (croaker), *Monocanthus* (filefish) and *Dussumieria* (rainbow sardine), highlight the capacity of capture-based methods to detect trawl-accessible taxa that may be under-represented in eDNA samples.

However, the small number of genus-level detections only obtained with beam trawls, and thus absent with eDNA metabarcoding, should not be interpreted as true absence from the water samples collected in this study. Metabarcoding non-detections may arise from primer-template mismatch, low template concentration, PCR stochasticity, limited DNA shedding or variation in the particle-size fraction captured during sampling, particularly for taxa occurring at low abundances or contributing limited DNA (Ficetola et al., 2008; Deiner et al., 2017; Barnes et al., 2021). Filtration strategy is therefore an important consideration in turbid seagrass systems (Kumar et al., 2022a; Gómez-Repollés et al., 2026). The use of 10 μm filters in this study was selected as a practical compromise to improve filtration efficiency and allow consistent processing of 2 L water samples from a turbid seagrass meadow. Previous research has shown that fish eDNA can occur across a range of particle-size fractions, from 1 to 10 μm, and that larger pore sizes can remain effective for capturing particle-associated eDNA while improving filtration in challenging environments (Turner et al., 2014; Cooper et al., 2022; Kumar et al., 2022a). However, this strategy may have under-represented extracellular, free, or highly fragmented DNA that may be more efficiently captured by smaller pore sizes, and therefore some taxa may have gone undetected (Verdier et al., 2024).

Non-detection can also reflect limitations in taxonomic assignment due to incomplete reference libraries (Schenekar et al., 2020). Taxonomic assignments in this study used the recently developed CSIRO DNA Reference Library (NBDL), which expands sequence coverage for Australian marine taxa (NBDL; research.csiro.au/dnalibrary), suggesting that the absence of these taxa from the metabarcoding datasets is more likely due to methodological and ecological differences in DNA capture and organism detectability, rather than limitations in the reference databases used for taxonomic assignment. Targeted, taxon-specific assays applied to DNA extracts post-hoc could help confirm whether taxa detected only by beam trawling were present in the environmental samples, but not recovered by eDNA metabarcoding (Stoeckle et al., 2018). Although such validation was beyond the scope of this biodiversity-focused comparison, it represents a useful future step for distinguishing true ecological absence from metabarcoding. We also acknowledge that proving absence with this approach would require a significant increase in eDNA sampling effort and water filtration volume to meet the requirements of a robust statistical framework, which includes occupancy modelling and detection probability estimations (Ficetola et al., 2008; Hunter et al., 2017; Ruppert et al., 2019).

Beam trawl surveys also provide direct physical evidence of species presence and enable the collection of demographic, morphological and physiological data that cannot be obtained from eDNA alone (Pierce et al., 1990; Philpott et al., 2025). Capture-based methods also enable taxonomic identification, which can validate eDNA detections and resolve taxonomic uncertainties arising from incomplete reference databases (Pierce et al., 1990; French et al., 2021). If the specimens are retained and accessioned in natural history museums, this can increase the capacity of biodiversity data repositories and DNA reference libraries (Schmid et al., 2025; Norris and Tweddle, 2026). Importantly, while eDNA metabarcoding captured more genera overall, beam trawling recovered a small number of genera not detected by eDNA and provided direct ecological information unavailable from molecular data alone.

4.3. *Indicator taxa and trophic guild patterns*

Indicator taxa analysis revealed a small number of method-specific genus-level associations, reflecting differences in how beam trawling and eDNA metabarcoding sample fish assemblages in turbid seagrass habitats. Beam trawl-associated indicator genera were few and included genera such as *Gazza* (ponyfish) and *Trixiphichthys* (tripodfish), which were more consistently associated with physical capture than with eDNA detections. In contrast, indicator genera associated with eDNA metabarcoding spanned a broader range of water-column taxa, feeding guilds and habitat associations. MiFish-U indicator genera included *Encrasicholina* (anchovies), *Sardinella* (sardines), and *Arrhamphus* (garfish/halfbeaks), and the 16S-Fish/D assay identified indicator genera including *Sardinops* (sardine) and *Hemiramphus* (garfish/halfbeaks). These associations suggest that eDNA metabarcoding detected a wider range of trophic and ecological groups than beam trawling, including taxa that may be more mobile, patchily distributed, or less consistently captured by beam trawl surveys.

Trophic guild comparison further reinforced these method-specific differences (Fig. 6). Beam trawl detections were strongly dominated by invertivore genera, reflecting the efficiency of trawling for taxa that forage on benthic and epiphytic invertebrates, which are abundant in seagrass habitats (Burchmore et al., 1984; Edgar and Shaw, 1995; Hindell et al., 2000; Nakamura and Sano, 2005). In contrast, eDNA metabarcoding recovered a broader range of trophic guilds, including carnivore, piscivore, and planktivore taxa. The absence of herbivorous families and limited representation of planktivores and higher trophic levels in beam trawl surveys suggests that capture-based methods may under-represent more mobile feeding groups, a result consistent with other capture-based studies (Harmelin-Vivien and Francour, 1992).

Differences in the life stage and biomass of the organism may contribute to the differences in fish detection between methods. The small (1.5 m wide, 2 mm mesh) and slow-moving beam trawl directly targeted larval and juvenile fishes, providing information on size structure and early life stages. In contrast, eDNA metabarcoding detects DNA shed by individuals across life stages and cannot distinguish ontogenetic stage (Maruyama et al., 2014; Sassoubre et al., 2016). Although eDNA shedding rates can vary with the species, body size, life stage, physiology, skin-type, and mucus production, detection is often more closely linked to the total biomass of the species in the sampling area (Maruyama et al., 2014; Klymus et al., 2015; Sassoubre et al., 2016). As a result, some taxa may have high abundances of small or juvenile individuals, making them easier to target with beam trawling; however, their low overall biomass may limit their detectability by eDNA metabarcoding, despite being present in the system (Rice et al., 2018; He et al., 2022).

Collectively, indicator taxa and trophic guild analyses demonstrate that beam trawl surveys and eDNA metabarcoding capture different functional and ecological components of seagrass-associated fish assemblages. Beam trawls provide critical life-history and demographic information necessary for assessing nursery function, while eDNA metabarcoding captures broader assemblage presence across spatial and temporal scales. Reliance on a single approach may therefore under-represent assessments of seagrass ecosystem function.

5. Implications for monitoring and conservation in turbid seagrass systems

This study demonstrates that methodological choice strongly influences inferred fish assemblages, which has important implications for biodiversity monitoring and ecological interpretations in turbid seagrass systems. Environmental DNA metabarcoding offers a scalable, non-invasive approach for detecting a broad range of fish genera including rare, cryptic, and mobile taxa that are difficult to sample using capture-based methods (Taberlet et al., 2012; Thomsen and Willerslev, 2015; Deiner et al., 2017). In contrast, beam trawl surveys remain essential for obtaining demographic, size-structure, and life-stage information that cannot be inferred from eDNA metabarcoding alone.

The interpretation of these patterns should be considered within the spatial and temporal scope of the study. Sampling was restricted to two seagrass sites within Trinity Inlet and was conducted over a limited temporal window (two days), which constrains the extent to which method-specific patterns can be generalised across broader tropical seagrass systems or seasons. This is particularly relevant for eDNA metabarcoding, as spatial heterogeneity, temporal variation, and biological replication can influence taxon detection and estimates of community composition (Beentjes et al., 2019; Mathieu et al., 2020; Courtaillac et al., 2024). The observed Method × Site interaction further suggests that differences among sampling approaches may vary over relatively small spatial scales.

The analytical framework also influences our interpretation of the results. Presence–absence data were used for comparisons between sequence and capture-based detections, but this approach does not account for variation in read abundance, organism abundance, or species-level biomass. Although PERMANOVA identified significant assemblage differences among methods and sites, these results should be interpreted alongside the multivariate dispersion tests, which indicated significant differences in dispersion among methods. This indicates that method-related differences may reflect both differences in the taxa detected and differences in the variability of detections within each method. These findings provide a focused comparison of method performance within a turbid tropical estuarine seagrass system, while highlighting the need for broader spatial and temporal replication in future monitoring applications.

Monitoring design should therefore be guided by the ecological objectives of the study. Where the objective is to characterise broad assemblage diversity, detect mobile or rare taxa, or resolve subtle site differences, eDNA metabarcoding may provide the most effective approach. Where the objective is to assess population structure, recruitment, nursery function, or life-stage composition, capture-based methods are more appropriate because they provide direct physical and demographic information that cannot be inferred from eDNA alone (Philpott et al., 2025). In dynamic turbid seagrass systems, integrated approaches that combine complementary methods across spatial and temporal scales are likely to provide the most robust and informative assessments of fish communities (Beentjes et al., 2019; Mathieu et al., 2020; Courtaillac et al., 2024). Future molecular monitoring could also explore environmental RNA (eRNA) as a complementary approach, as its more rapid degradation relative to DNA may provide a closer indication of recent biological activity and distinguish localised signals from DNA (Kitahashi et al., 2020; Giroux et al., 2022; Zhang et al., 2024). However, further validation is needed before eRNA can be routinely applied in turbid tropical estuarine systems, as its greater sensitivity to degradation requires more stringent sample preservation, handling and laboratory processing than eDNA (Zaiko et al., 2018; Littlefair et al., 2022).

6. Conclusion

This study demonstrates that eDNA metabarcoding and beam trawl surveys provide complementary approaches for sampling fish assemblages in turbid tropical seagrass systems. Environmental DNA metabarcoding provided broader taxonomic detection, particularly for mobile and cryptic taxa, while beam trawl surveys provided direct physical evidence of local occurrence and information on demographic data that cannot be inferred from eDNA alone. Although beam trawling contributed fewer unique genera, its capacity to validate local presence and capture life-stage and size-structure information remains important for ecological interpretation. Together, these findings demonstrate that the sampling method strongly influences how seagrass-associated fish assemblages are characterised, and that integrating molecular and capture-based approaches can provide a more complete ecological assessment of fish diversity than either method alone.

Ethics and permit approvals

All sampling procedures were conducted in accordance with relevant regulatory approvals, operating under the Queensland Department of Agriculture and Fisheries (DAF) General Fisheries Permit (#259152), the Great Barrier Reef Marine Park Authority (GBRMPA; permit G23/48334.1), and James Cook University Animal Ethics approval (A2896).

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CRediT authorship contribution statement

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Darcy Philpott reports financial support was provided by Holsworth Wildlife Research Endowment Equity Trustees Charitable Foundation. Michael Rasheed, Paul York reports financial support was provided by North Queensland Bulk Ports Corporation. Michael Rasheed, Paul York reports financial support was provided by Australian Research Council Grant. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecss.2026.109968.

Data availability

All survey data needed to replicate the study, and statistical analyses are available as supplementary material. Raw sequence data are also available as mtDNA COI haplotype sequences deposited on NCBI GenBank (accession numbers: PZ100018–100029) to support fish species identification from beam trawls. Raw sequence reads for individual eDNA samples have been deposited in the European Nucleotide Archive (ENA) under project accession number PRJEB113069 to support eDNA detections.

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