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Combining eDNA and Visual Surveys Improves Detection of Reef Fishes Across Their Biogeographic Ranges

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ABSTRACT

Aim: Rapid shifts in marine species distributions driven by ocean warming require more effective monitoring across entire ranges to detect emerging ecological change. Traditionally, visual surveys have been used to track these distributional shifts, but they often overlook small-bodied, rare or cryptic species, potentially underestimating range changes. Environmental DNA (eDNA) bypasses these limitations, yet its effectiveness in detecting species near their range limits remains understudied.

Location: Eastern Australia.

Methods: We combined eDNA metabarcoding and visual surveys to assess reef fish communities across nine sites spanning a 2000-km latitudinal gradient within a global warming hotspot encapsulating tropical, subtropical and temperate reefs. Variation in detectability across methods and biogeographic ranges was also assessed at the level of functional traits (trophic guild, thermal guild and water column position).

Results: eDNA and visual surveys revealed different fish species compositions, potentially underestimating the extent of fish biogeographic ranges. eDNA detected 44 more unique tropical species than visual surveys across their range, and was more effective at detecting tropical carnivores, omnivores, invertivores, planktivores, detritivores and all water column positions. In contrast, visual surveys were more effective at detecting temperate carnivores, invertivores and benthic species. For tropical fishes at their cold range edge in temperate ecosystems, eDNA identified 12 unique species, including herbivores and cryptic species not previously recorded by long-term visual surveys. Contrastingly, eDNA detected 20 fewer temperate species than visual surveys across their biogeographic range and was less effective (five unique species) than visual surveys (nine unique species) at detecting temperate species at their warm trailing range in subtropical ecosystems.

Conclusions: Combining eDNA and visual surveys improves the detection of reef fishes near the limits of their known distributions. This approach helps reveal overlooked species, particularly those that are cryptic, rare or low in abundance, and supports more accurate assessments of species distributions across biogeographic gradients.

1 | Introduction

In recent decades, climate change has caused over 12,000 species to shift their biogeographic ranges across terrestrial, freshwater and marine systems (Lenoir et al. 2020). Species shift their biogeographic ranges to escape unfavourable environmental conditions or to explore ecosystems and use resources previously inaccessible (Pecl et al. 2017). Marine organisms have broader thermal tolerances than terrestrial organisms (Pinsky et al. 2013) and shift their geographic ranges faster (~72 km per decade, Poloczanska et al. 2013) than terrestrial organisms (~17 km per decade, Chen et al. 2011), although these responses vary among individual species (Parmesan and Yohe 2003). Biogeographic distributions of species are dynamic and depend on the individual species' environmental tolerance, interactions with other species, habitat associations and dispersal constraints (Lawlor et al. 2024). Consequently, species could face demographic collapse or extinction if they fail to relocate, acclimate or adapt to the changing environment (Berg et al. 2010). Species with high phenotypic plasticity and ecological generalism may perform better under changing conditions (Hayes et al. 2024), whereas species with high specialisation to specific ecosystems and conditions may be more vulnerable to environmental change (Mouillot et al. 2013). Improved detection of species across their biogeographic ranges is therefore crucial for identifying both vulnerable and resilient species under climate change.

In marine ecosystems, climate-driven range expansions occur five times faster than range contractions (Poloczanska et al. 2013), resulting in species gains outpacing species loss. Tropical species live closer to their upper thermal limits and have narrower thermal tolerances compared to temperate species (Comte and Olden 2017). Therefore, warming in subtropical and temperate ecosystems creates a suitable thermal habitat for tropical species expanding their ranges poleward, but not for temperate species (Burrows et al. 2011). Given that the majority of the world's species reside in low-latitude tropical ecosystems, high-latitude temperate ecosystems are likely to experience an influx of tropical species as they expand poleward (Antão et al. 2020), leading to a global reshuffling of ecological communities, altered ecosystem dynamics and functioning at species range edges (Pecl et al. 2017). Accurately detecting shifts in species occurrences along biogeographic gradients is therefore critical for understanding and anticipating ecological responses to climate change.

Tropical fishes are the most diverse group of vertebrates globally and are considered vulnerable to anthropogenic climate change (Comte and Olden 2017). Tropicalisation has increased the dispersal of tropical fishes into subtropical and temperate ecosystems globally (Booth et al. 2011; Yamano et al. 2011; Lloyd et al. 2012; Vergés et al. 2014). Regions associated with tropicalisation are warming ~3–4 times faster than the global average (Ridgway 2007), and the associated major boundary currents are direct dispersal mechanisms of tropical fishes into temperate ecosystems (Vergés et al. 2014). In Australia, over 150 tropical fish species have been observed range-extending along the East Australian coastline annually (Booth et al. 2011; Feary et al. 2014), causing novel species interactions with resident temperate species (Smith et al. 2018; Coni, Booth, et al. 2021). Novel species interactions can both benefit (Smith et al. 2018; Paijmans et al. 2020; Sasaki et al. 2024) and disadvantage (Coni, Booth, et al. 2021; Mitchell et al. 2022) the behavioural and physiological performance of tropical fishes in novel temperate ecosystems. The arrival of tropical fishes into temperate ecosystems introduces novel competitors, prey and predators for both tropical and temperate fishes (Beck et al. 2016; Coni, Booth, et al. 2021), forcing modifications to their behaviours (Coni, et al. 2022; Mitchell et al. 2022; Mitchell et al. 2023b; Mitchell et al. 2025), diet (Kingsbury et al. 2020), microbiome (Hayes et al. 2025), physiology (Mitchell et al. 2023a; Sasaki et al. 2024) and habitat preference (Hayes et al. 2024) to avoid resource competition in the same ecosystem where they co-occur. The increased arrival of tropical fishes, especially ecological generalists, can disrupt temperate ecosystem functionality and stability (Nakamura et al. 2013; Vergés et al. 2016). Therefore, understanding the functional compositions of reef fishes across their biogeographic ranges will enhance our understanding of ecosystem functioning under future climate change.

Visual surveys have been traditionally used to monitor the gradual occurrence of tropical fishes in temperate ecosystems and allow for sampling over large spatial scales (e.g., latitudinal gradients; Booth et al. 2011; Edgar et al. 2020). However, visual surveys are limited by individual taxonomic expertise and species detectability, with bias against small and cryptic species with different morphological and behavioural characteristics which enable them to blend into the environment (Bessey et al. 2020). Failure to detect rare, cryptic or low-abundant species underestimates their presence, abundance and distribution patterns, and will likely affect our understanding of future fish assemblages. Nevertheless, environmental DNA (eDNA) metabarcoding is a modern technique used to profile genetic material naturally released by organisms in an environment (Taberlet et al. 2012) and is emerging as a non-invasive method at detecting rare, low abundant and cryptobenthic species which visual surveys often overlook (Stat et al. 2017; Boussarie et al. 2018). The inability to detect cryptobenthic species can limit our understanding of ecosystem dynamics, since these species play essential roles in maintaining trophic energy pathways and could show different range-shifting strategies compared to more prevalent and well-documented species (Goatley et al. 2016). Therefore, eDNA metabarcoding has the potential to improve the monitoring of species at their range edges and can enhance our understanding of the ecological consequences of these shifts.

Here we aim to assess the effectiveness of eDNA metabarcoding compared to visual survey techniques at detecting fishes across their biogeographic ranges within a global warming hotspot. We surveyed nine sites along a ~2000-km latitudinal gradient across tropical, subtropical and temperate shallow-water reefs in eastern Australia. Fish species were categorised into functional guilds based on trophic role, thermal affinity and water column position, and we evaluated differences in detectability between methods across these guilds. This approach aims to identify whether eDNA can detect species that may be overlooked by visual surveys, particularly rare, cryptic or low-abundant species near the edge of their known distributions.

2 | Methods

2.1 | Study Location

Marine ecosystems in eastern Australia are considered a hotspot for ocean warming and tropicalisation (Booth et al. 2011, 2018), facilitated by increased water temperatures (~3–4 times higher than the global average) and strengthening of the East Australian Current (Ridgway 2007; Suthers et al. 2011). This region has been a key area of research on species redistributions, particularly the poleward expansion of tropical fishes into temperate ecosystems (Booth et al. 2007, 2011). However, these diverse ecosystems also include many cryptic, rare or data-deficient species whose known ranges are largely based on visual surveys. As such, eastern Australia provides a unique natural laboratory to evaluate how methods like eDNA can improve detection of species distributions, especially near range limits. Here, we selected nine sites within tropical, subtropical and temperate reefs along a ~2000-km latitudinal gradient of the East coast of Australia during April to July of 2021 to capture the leading range edge of tropical fish species and the trailing range edge of temperate fish species (Figure 1). The tropical reef region (latitude range: 19.1°–23.4° S) represents the core home range for many tropical fish species, and the trailing range for some subtropical species (Booth et al. 2011). The subtropical reef region (latitude range: 28.1°–30.9° S) represents the most southern breeding grounds for some tropical fish species (Figueira et al. 2009), and the warm trailing range edge for temperate fish species which naturally occur at the subtropical region. Both tropical and temperate fish species have co-existed for longer periods of time at the subtropical reefs than at the temperate reefs. The temperate reefs (latitudinal range: 32.8°–36.9° S) are considered a tropicalisation hotspot where tropical fishes have been observed range-extending annually for ~25 years (Booth et al. 2007) but fail to overwinter (although some overwintering individuals have recently been observed, for example, *Thalassoma lunare*, O'Connell et al. 2023). Winter temperatures usually prevent overwintering of range-extending tropical fishes in temperate regions due to thermal physiological constraints (Figueira et al. 2009). This region is the most southern extent for tropical fishes and represents a true temperate rocky reef ecosystem which favours higher abundance of temperate fishes than tropical range-extending fishes (Hayes et al. 2024).

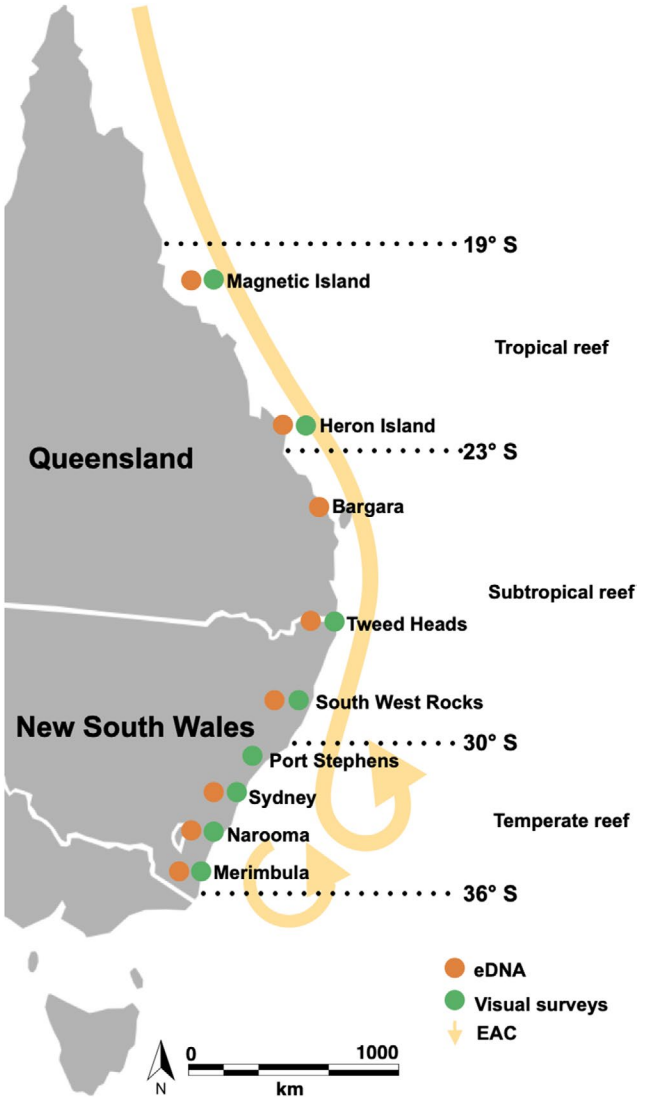

FIGURE 1 | Map showing eDNA (orange) and visual survey (green) sampling sites along the east Australian coastline. Magnetic Island, Heron Island and Bargara are positioned within a tropical reef (latitude range: 19.1°–23.4° S), Tweed Heads and South West Rocks are positioned within a subtropical reef (latitude range: 28.1°–30.9° S), Port Stephens, Sydney, Narooma and Merimbula are positioned within a temperate reef (latitudinal range: 32.8°–36.9° S). The yellow arrows indicate the direction of the East Australian Current (EAC) that disperses tropical fish larvae poleward from the tropical and subtropical reefs into the temperate reefs.

2.2 | Visual Surveys and eDNA Sampling

Visual surveys were used to quantify the presence and abundance of all fish assemblages identified at each site. Snorkellers swam along belt transects (10 m in length) identifying all fish species and counting their abundances within 2 m (width) on each side of the transect tape (40 m^2 per transect, see Table S1 for the number of transect replicates at each site and habitat type). The number of transects conducted per site varied depending on reef size and habitat complexity to ensure adequate representation of fish assemblages across heterogeneous habitats. Transects were conducted at depths of 0.5–3 m to include the shallow habitat range where tropical fishes in temperate ecosystems are most commonly found (Booth et al. 2007, 2011, 2018). Using methods adapted from Edgar et al. (2020), larger pelagic and benthopelagic fish species were identified along the transect first, then an extensive search for the cryptic and small benthic species inside crevices, rocks and under kelp and macroalgae leaves was conducted along the same transect. Transects were randomly placed at each site, with effort stratified across the available hard substrate habitat types (turf, barren, oyster, kelp and coral habitats) to ensure representation of habitat heterogeneity. This approach allowed us to incorporate the relative availability of habitat types at each site while enabling detection of species associated with different substrates. Hence, the sample size of visual surveys is representative of the habitat

availability at each location (Table S1; Figure S1). Habitat types were defined following Coni, Nagelkerken, et al. (2021) and included: turf-forming algae (less than 10 cm in height), barren habitats (characterised by coralline algae-encrusted rock, a consequence of overgrazing by range-extending tropical herbivorous fishes and sea urchins *Centrostephanus rodgersii*), oyster reefs (dominated by *Saccostrea glomerata*), kelp forests (dominated by *Ecklonia radiata*) and coral reefs. At the same reef site and immediately following the visual surveys, 10 replicate 1 L seawater samples were collected randomly via snorkelling across the entire reef area at each site (~1 m depth), ensuring broad spatial coverage of eDNA from the fish assemblages. Seawater samples and field blanks (Milli-Q water) were immediately stored on ice and filtered within ~1 h after collection. Field blanks were filtered before the seawater samples through Whatman GF/C filters (pore size 0.70 μm) following Miya et al. (2020) protocol. Filters were stored in DNA/RNA shield at −20°C during fieldwork, and then transferred to −80°C until eDNA extraction. Since the spatial and temporal signal of eDNA concentration is dependent on the surrounding environment (Harrison et al. 2019), visual surveys and eDNA sampling were conducted from high to low latitudes (temperate to tropical reefs, Figure 1) to avoid false detection of tropical fishes in temperate ecosystems.

2.3 | eDNA Extraction and Metabarcoding

eDNA was extracted from the filters using the ZymoBIOMICS 96 MagBead DNA Kit with modifications to the initial lysis steps. The filters were fragmented and incubated in 1 mL of lysis buffer in 1.5 mL tubes for an hour at room temperature on a tube rotator, then centrifuged at 10,000 *g* for 1 min. 200 μL of the lysate was transferred to a deep-well block, and then the manufacturer's protocol was followed for the remaining extraction steps using the kit components. Metabarcoding of fish was performed using MiFish universal primer pairs: forward 5′–ACA CTC TTT CCC TAC ACG ACG CTC TTC CGA TCT NNN NNN GTC GGT AAA ACT CGT GCC AGC–3′ and reverse 5′–GTG ACT GGA GTT CAG ACG TGT GCT CTT CCG ATC TNN NNN NCA TAG TGG GGT ATC TAA TCC CAG TTT–3′ (Miya et al. 2015; Minamoto et al. 2021) with eight PCR replicates per sample. Each PCR reaction replicate consisted of 2 μL of the eDNA sample combined with 10 μL of master mix (6.0 μL of 2× KAPA HiFi HotStart ReadyMix with a final concentration of 1×), 1.2 μL Milli-Q Direct UltraPure Water, and 2.8 μL of the MiFish-U-F/R primer pair mix at 5 μM (final primer concentration 1.16 μM), giving a total reaction volume of 12 μL. The thermocycler conditions followed Minamoto et al. (2021), with an initial denaturation at 95°C for 3 min, followed by 38 cycles of: (1) denaturation at 98°C for 20 s, (2) annealing at 65°C for 15 s 35 cycles and (3) extension at 72°C for 15 s, followed by final extension at 72°C for 5 min. The eight PCR replicates were pooled together for sequencing. Unique sample indexing was applied during the second-round library preparation step using TruSeq DNA CD Indexes (96 Indexes); however, indexing was not applied during the first-round PCR. We acknowledge this as a limitation, as it prevents tracking of potential low-level cross-contamination between wells. Nevertheless, contamination risks were minimised by performing PCR on batches of sample replicates with strict lab sterilisation protocols. Library preparation and sequencing were performed by the Sequencing Centre (SQC) at the Okinawa Institute of Science and Technology (OIST) on MiSeq with a MiSeq v3 600 cycle cartridge, with pair-end sequencing at 2 × 150 bp for MiFish products.

2.4 | Data Processing and Analyses

The bioinformatics pipeline was run on the high-performance computing clusters of DEIGO at OIST. Forward and reverse eDNA reads were quality filtered and trimmed using CutAdapt (Martin 2011), and merged, denoised and potential chimeras were removed using DADA2 (v.1.28, Callahan et al. 2016) to create Amplicon Sequence Variants (ASVs). The ASVs were then identified through a BLAST search using the 'blastn' command (Basic Local Alignment Search Tool, Altschul et al. 1990) against a globally comprehensive custom database from MitoFish sequences (Sato et al. 2018; Zhu et al. 2023). While MitoFish offers broad global coverage of fishes, it is under-represented for some temperate Australian reef species, which may limit species-level identification in those regions. Species-level identifications were accepted where BLAST matches had ≥ 97% sequence identity and 80% query coverage (although 96% of species-level assignments had 100% query coverage, and 98% had ≥ 99% coverage, respectively). Taxonomic classifications were then selected using the Last Common Ancestor (LCA) script (Mousavi-Derazmahalleh et al. 2021). To minimise the risk of misclassification due to cross-contamination or ambiguous matches, taxa detected in negative controls or taxa known to occur exclusively outside of Australia (e.g., Mediterranean- or Japan-endemic species) were excluded from the dataset. This conservative filtering step was applied given that sequencing was conducted outside of Australia, where the potential for low-level cross-contamination is recognised (see Supporting Information for excluded taxa). Multiple ASVs with the same taxonomic classifications were merged into individual ASVs using the 'tax_glom' function using phyloseq (v.1.44, McMurdie and Holmes 2013). Taxonomic classifications were confirmed through World Register of Marine Species (WoRMS), and species detected by eDNA and visual surveys were split into three functional traits according to their thermal guild, water column position and trophic guild. Functional trait information was obtained from FishBase (Froese and Pauly 2012), and were cross-checked with published literature and the author's knowledge of the species (see Supporting Data for species functional trait information). Thermal guild information was categorised into three guilds based off their historical distribution from FishBase: tropical, subtropical and temperate species. Water column position was described by the position the species spends most of their time: benthic (lives on the substratum), benthopelagic (lives between the substratum and the mid-water column) and pelagic (lives in the open water column). Trophic guild was categorised by the main food items consumed by each species, and was categorised into seven feeding guilds: carnivores (diet dominated by fishes and cephalopods, with the inclusion of macroinvertebrates such as crustaceans and molluscs), omnivores (consumes a combination of plant and animal material), invertivores (diet dominated by benthic invertebrates such as mobile crustaceans,

ascidians and sponges), planktivores (consumes small organisms in the water column, such as planktonic zooplankton and phytoplankton), herbivores (feeds exclusively on plant material such as turf, filamentous algae, fleshy algae and seagrass), corallivores (specialise in consuming sessile organisms such as coral polyps and sea anemones) and detritivores (diet dominated by detritus and undefined organic matter).

The tropical species' core range at tropical and subtropical reefs was categorised by latitudes 19.1°–28.1° S, since breeding adults are known to occur at these latitudes (Figueira et al. 2009). The tropical species' cold leading range in temperate reefs was categorised by latitudes 32.8°–36.9° S, since they have been observed range-extending to these latitudes annually, but not overwintering and forming breeding populations (Booth et al. 2007). For subtropical species, latitudes 19.1°–23.4° S represent their trailing range at tropical reefs, and latitudes 28.1°–36.9° S represent their core range in subtropical and temperate reefs. For temperate species, latitudes 32.7.9°–36.9° S represent their core range in temperate reefs where their abundance is high (Coni, Nagelkerken, et al. 2021), and latitudes 28.1°–30.9° S represent their warm trailing range in subtropical reefs where their abundances are lower than those of tropical species (Miller et al. 2023; Hayes et al. 2024). Species detections outside their core distributional ranges were treated as novel occurrences and interpreted cautiously, without assuming range extensions in the absence of evidence for persistence, reproduction or long-term establishment.

Data were first presence/absence transformed (0 = absent, 1 = present) at the species level, then species richness for each functional trait (thermal guild, trophic guild and water column position) was calculated per eDNA sample or visual survey transect, providing a count-based measure of species richness (see Figure S1 for species accumulation curves and sample sizes). Generalised linear models (GLM) with Poisson distribution and log-link function were first fit for each functional trait, using richness as the response variable and latitude and method as the predictor variables. The weight of Akaike's information criteria (AIC_c) was then used to rank models, and the dispersion statistic was fit to the top-ranked model. If large dispersion was detected in top-ranked models, we developed GLM with negative binomial distribution and log-link function using the glm.nb function (from the package MASS, Venables and Ripley 2002), and AIC_c was used again to rank the final best fit GLM with negative binomial distribution model. Top-ranked models with low dispersion remained as GLM with Poisson distribution. For the top-ranked models where best fit AIC_c supported the null model, the results are shown in Supporting Information but not used in results interpretation (see Tables S2–S4). R^2 for GLM with Poisson distribution was calculated using the percentage of deviance (likelihood ratio between the top-ranked model and the null model), and for GLM with binomial distribution and log-link function was calculated using McFadden's R^2 formula (log-likelihood ratio between top-ranked model and the null model). Heatmaps were developed using presence/absence data for eDNA, and abundance 10 m^2 for visual surveys to show the detection frequency and distribution patterns for each species. All analyses and graphs were generated in R Studio (v.4.3.1, R Core Team 2023).

3 | Results

3.1 | eDNA Sequencing Results

Across all samples, a total of 44,280,382 raw reads were obtained, with an average of 365,954 ± 25,485 reads per sample (mean ± SE). After quality filtering, denoising and merging, 35,633,809 high-quality reads remained (80.5% retention of initial reads), with an average of 296,948 ± 22,374 reads per sample.

3.2 | Species Detections Along Biogeographic Ranges

Irrespective of thermal affinity and biogeographic ranges, eDNA detected 25 more fish species than visual surveys (eDNA: 144 species, visual survey: 119 species, see Table S5 for taxa classified to genus level), but only 33 species were detected by both methods. For tropical species, both methods showed a decrease in species richness from their core distributional range at tropical reefs towards their leading range edge at cold temperate reefs (Figure 2a, GLM: $p < 0.001$, Table S6), with eDNA detecting 44 more tropical species than visual surveys across their entire range (eDNA: 102 species, visual survey: 58 species, shared: 14 species, Figure 2a, GLM: $p < 0.001$, Table S6). At the tropical species' leading range edge in temperate latitudes (latitudes 32.8°–36.9° S), eDNA and visual surveys detected different species compositions, with minimal overlap in species detections (Figure 3). eDNA detected thirteen unique species at the tropical species' leading range edge in temperate latitudes (*Acanthurus lineatus, Ctenochaetus striatus, Diodon liturosus, Scarus psittacus, Stethojulis interrupta, Pempheris schwenkii, Enneapterygius philippinus, Parablennius thysanius, Planiliza macrolepis, Sargocentron punctatissimum, Spratelloides delicatulus, Spratelloides gracilis* and *Stenatherina panatela*, Table S9). Visual surveys detected 12 unique tropical species at their leading range edge in temperate ecosystems (*Abudefduf sexfasciatus, Abudefduf whitleyi, Acanthurus olivaceus, Antennarius pictus, Chaetodon auriga, Chaetodon guentheri, Dascyllus trimaculatus, Halichoeres chrysus, Pomacentrus coelestis, Priolepis cincta, Pteragogus enneacanthus* and *Pycnochromis margaritifer*). While both methods shared four detections of tropical species at their leading range edge in temperate ecosystems (*Abudefduf bengalensis, Abudefduf vaigiensis, Acanthurus nigrofuscus* and *Acanthurus triostegus*). At their core distributional range in the tropical reefs (latitudes 19.1°–28.1° S), eDNA detected 46 more unique tropical species than visual surveys (eDNA: 83 species, visual survey: 37 species, shared: 10 species, Figure 3). For both eDNA and visual surveys, tropical species richness was negatively correlated with increasing latitude across all trophic guilds (Figure 4, GLM: $p < 0.05$, Table S7) and water column positions (Figure S2, GLM: $p < 0.004$, Table S8). eDNA detected significantly higher tropical species richness than visual surveys for carnivores ($p < 0.001$; Figure 4, Table S7), omnivores ($p = 0.022$), invertivores ($p < 0.001$), planktivores ($p < 0.001$) and detritivores ($p = 0.002$), but not for herbivores ($p = 0.147$) or corallivores ($p = 0.144$). For water column position, eDNA also detected significantly higher tropical species richness than visual surveys for benthic ($p < 0.001$; Figure S2, Table S8), benthopelagic ($p = 0.035$) and pelagic ($p < 0.001$) species.

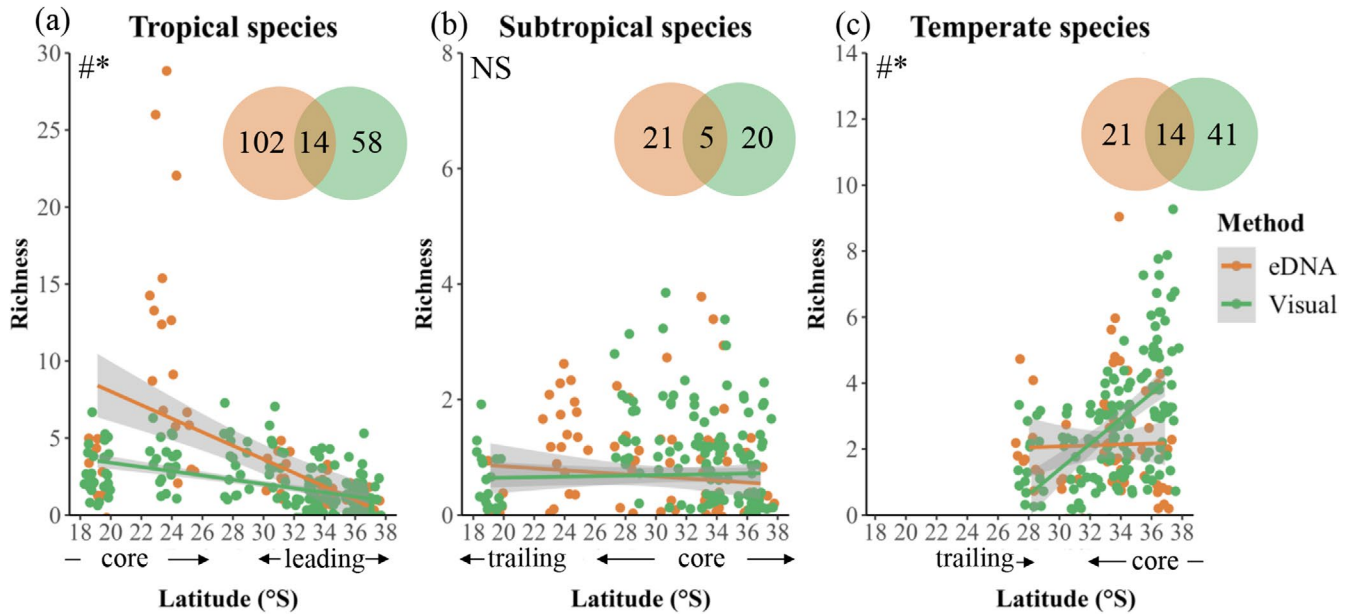

FIGURE 2 | Species richness (number of species per eDNA sample or visual transect) along a latitudinal gradient for methods: EDNA (orange) and visual surveys (green). For tropical species (a), latitudes 19.1°–28.1° S represent their core range at tropical and subtropical reefs, and latitudes 32.8°–36.9° S represent their leading range edge in novel temperate reefs. For subtropical species (b), latitudes 19.1°–23.4° S represent their trailing range at tropical reefs, and latitudes 28.1°–36.9° S represent their core range in subtropical and temperate reefs. For temperate species (c), latitudes 28.1°–30.9° S represent their trailing range edge at warming subtropical reefs, and latitudes 32.8°–36.9° S represent their core range in temperate reefs. Points along the *x*-axis have been jittered to view any overlapping data points and grey shaded area represents the confidence interval. Venn diagrams show the total number of unique fish species detected for each thermal guild for eDNA and visual surveys, and the number of species detected by both methods across their entire range. # indicates a significant effect of latitude, * indicates a significant effect of method, and NS denotes non-significant results.

Subtropical species richness was not correlated with latitude for either eDNA or visual surveys (null model outperformed predictor variables, Figure 2b, Table S2, Table S7), except for carnivores, where richness increased with latitude (Figure 4, GLM: $p = 0.007$, Table S7). Across their distributional range, eDNA detected 21 unique subtropical species, whereas visual surveys detected 20 unique species (Figure 2b, Figure S3). eDNA detected significantly higher richness than visual surveys only for planktivores (Figure 4, $p = 0.029$, Table S7), while visual surveys detected higher richness for benthic species (Figure S2, $p = 0.017$, Table S8). No significant differences between methods were observed for carnivores ($p = 0.135$), herbivores ($p = 0.995$), pelagic species ($p = 0.991$) or for omnivores, invertivores and benthopelagic species where the null model outperformed predictor variables.

Temperate species richness decreased from their core distributional range at temperate reefs towards their warm-trailing range edge at the subtropical region for visual surveys, but not for eDNA (Figure 2c, GLM: $p < 0.001$, Table S6). Across their entire distributional range, visual surveys detected 20 more unique temperate species than eDNA (eDNA: 21 species, visual surveys: 41 species, shared: 14, Figure 2c, GLM: $p = 0.017$, Table S6). At their warm-trailing range edge in subtropical reefs (latitudes 28.1°–30.9° S), visual surveys were more effective at detecting temperate species than eDNA (Figure S4). At these latitudes, visual surveys detected nine unique temperate species (*Acanthopagrus australis, Centropogon australis, Chromis hypsilepis, Girella elevata, Iso rhothophilus, Latropiscis purpurissatus, Ophthalmolepis lineolate, Pseudocaranx georgianus* and *Schuettea scalaripinnis*), whereas eDNA detected five unique temperate species (*Enoplosus armatus, Hyperlophus vittatus, Scobinichthys granulatus, Sillago flindersi* and *Torquigener pleurogramma*). Both methods shared four temperate species detections in this area (*Atypichthys strigatu Girella tricuspidate, Scorpis lineolata* and *Tetractenos glaber*). At their core distributional range in temperate reefs (latitudes 32.8°–36.9° S), visual surveys detected 25 more unique species than eDNA (eDNA: 5 species, visual survey: 30 species, shared: 9 species, Figure S4). For both methods, temperate species richness was positively correlated with latitude for the trophic guilds of omnivores, invertivores and herbivores (Figure 4, GLM: $p < 0.001$, Table S7), and for the water column positions of benthic and benthopelagic (Figure S2, GLM: $p < 0.026$, Table S8). Visual surveys detected significantly higher temperate species richness than eDNA for carnivores (Figure 4, $p < 0.001$, Table S7) and invertivores ($p = 0.001$), but not for omnivores ($p = 0.040$), planktivores or herbivores, where method was not retained in the best-fit model. For water column position, visual surveys also detected significantly higher richness than eDNA for benthic fishes (Figure S2, $p = 0.017$, Table S8), but not for benthopelagic ($p = 0.065$) or pelagic species ($p = 0.252$).

4 | Discussion

Visual surveys and eDNA metabarcoding techniques show considerable differences in their species detections across various functional traits (thermal guild, trophic guild and water column position) for tropical and temperate fish species.

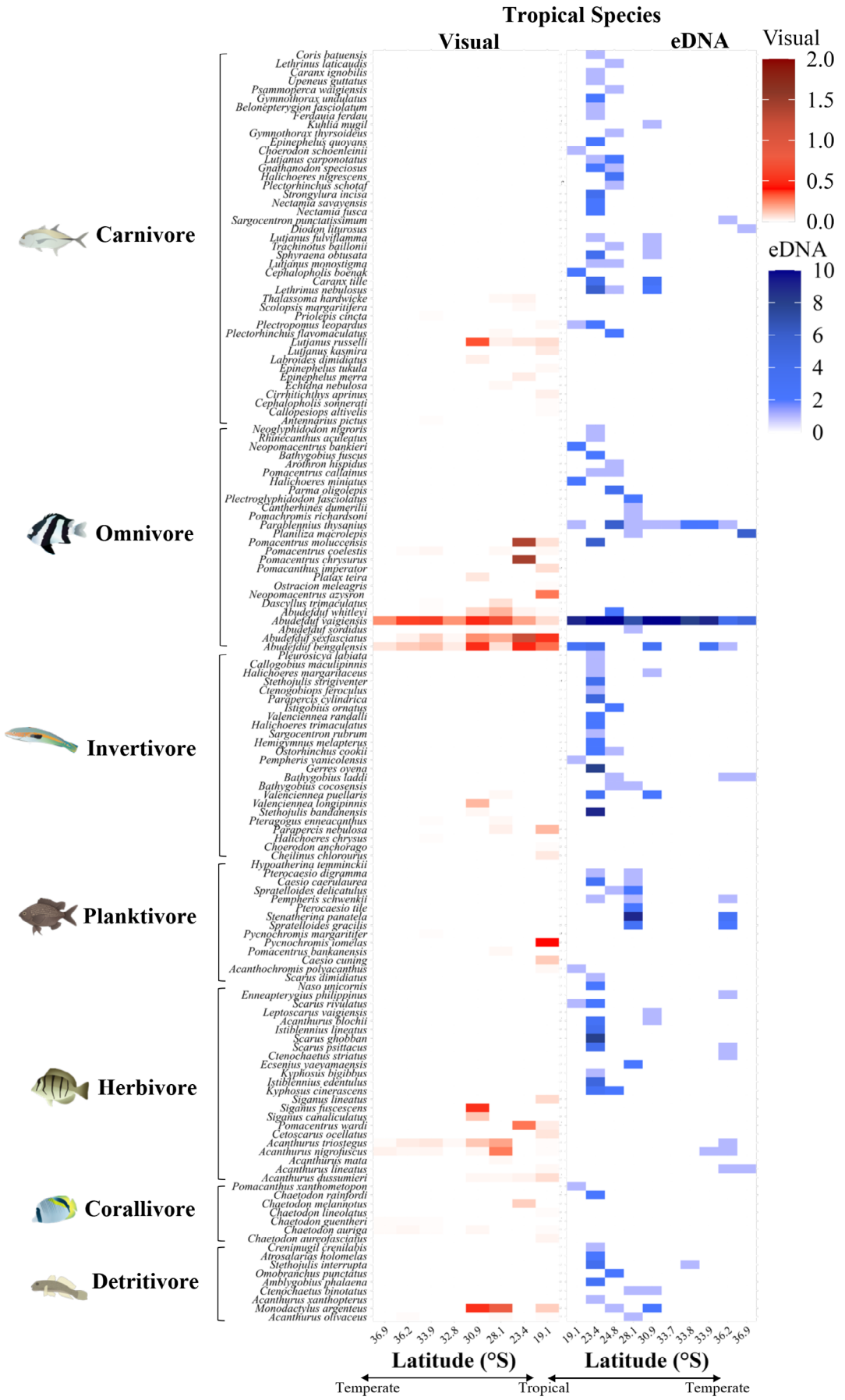

FIGURE 3 | Legend on next page.

FIGURE 3 | Heatmap comparison of tropical reef fish species detections using visual survey (red) and eDNA (blue) methods across a latitudinal gradient. The left column shows the average abundance ($10\,m^{-2}$) of each species detected by visual surveys, while the right column shows the number of times a species was detected at each site (maximum detection value of 10 at a site). Each row represents tropical coral reef fish species, which are ordered by trophic guild (carnivore, omnivore, invertivore, planktivore, herbivore, corallivore and detritivore). The symbols and illustrations are courtesy of the Integration and Application Network, University of Maryland Centre for Environmental Science (ian.umces.edu/symbols/).

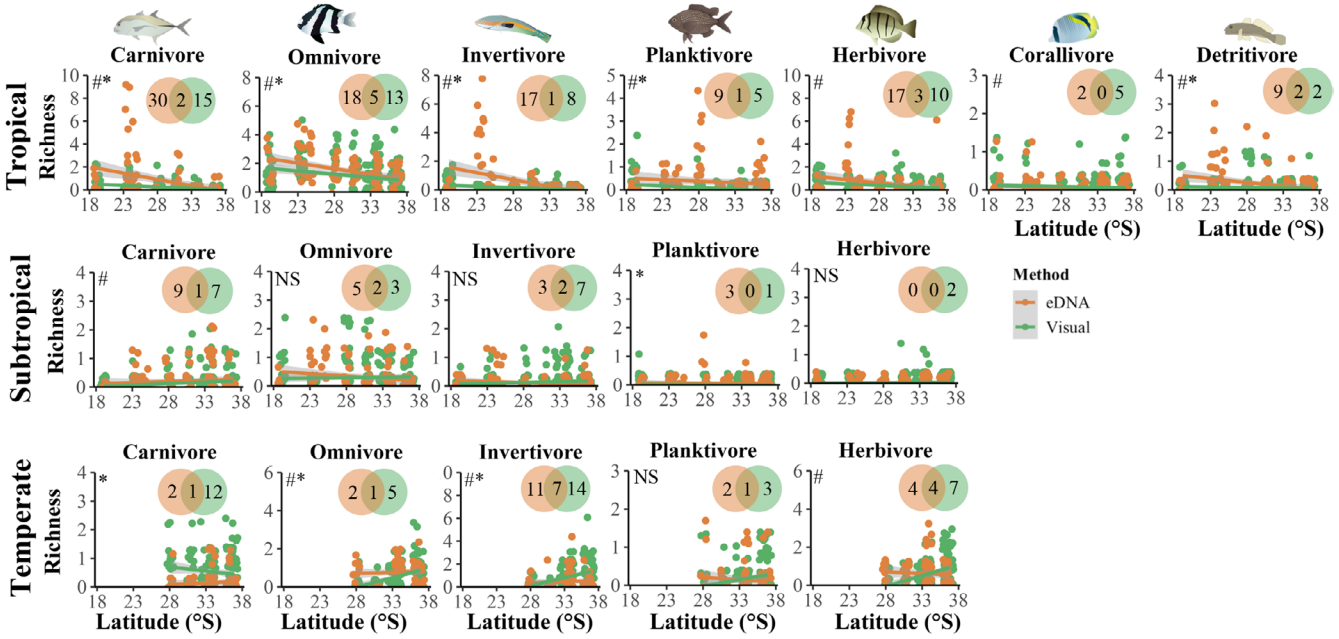

FIGURE 4 | Species richness (number of species) detected across a latitudinal gradient for the seven trophic guilds (carnivore, omnivore, invertivore, planktivore, herbivore, corallivore and detritivore) within three thermal guilds (tropical, subtropical and temperate). Orange datapoints denote eDNA methods, green datapoints denote visual survey methods and grey shaded area represents the confidence interval. Venn diagrams show the total number of unique species detected for eDNA (orange), visual surveys (green) and the number of overlapping species detected by both methods. The symbols and illustrations are courtesy of the Integration and Application Network, University of Maryland Centre for Environmental Science (ian.umces.edu/symbols/). # indicates a significant effect of latitude, * indicates a significant effect of method, and NS denotes nonsignificant results.

eDNA identified 13 unique tropical fish species in temperate ecosystems (Table S9), with 12 of those species detected outside of their known southernmost latitude (see Atlas of Living Australia; Booth et al. 2011; DiBattista et al. 2022, Table S9), including three tropical herbivorous fishes (*Acanthurus lineatus*, *Ctenochaetus striatus* and *Scarus psittacus*), two cryptobenthic fishes (*Enneapterygius philippinus* and *Parablennius thysanius*), three nocturnal fish with cryptic behaviours that hide in caves during the day (*Diodon liturosus, Pempheris schwenkii* and *Sargocentron punctatissimum*) and four pelagic fish (*Spratelloides delicatulus*, *Spratelloides gracilis*, *Stenatherina panatela* and *Planiliza macrolepis*). Failure to detect species within key functional traits limits our understanding of ecosystem structure and functionality, particularly for ecosystem engineers such as tropical herbivorous fishes that overgraze temperate habitat-forming kelp forests and mediate phase shifts to barren-dominated ecosystems (Vergés et al. 2014, 2016), which can facilitate the establishment of other range-extending fishes (Coni, Nagelkerken, et al. 2021). Cryptobenthic species are also often overlooked in biodiversity assessments (Pearman et al. 2016; Stat et al. 2017), despite playing important ecological roles in maintaining trophic energy flows (Goatley et al. 2016), such as the transfer of energy along carnivorous trophic pathways (predators of small crustaceans and prey for piscivores) and detrital pathways (recycling of primary productivity) (Depczynski and Bellwood 2003). Identifying which tropical fishes are present in temperate ecosystems is important for understanding shifts in community composition and ecological function. Some tropical fishes range-extending into temperate ecosystems can modify their ecological niches (e.g., diet and behaviour) to reduce competition with resident temperate fishes (Kingsbury et al. 2020; Coni et al. 2022). Cryptobenthic species may show different range-shifting strategies, by occupying cryptic habitats and niche space to enhance their success in novel ecosystems, although this remains unknown. Since eDNA metabarcoding and visual surveys detected different tropical fish assemblages, we conclude that multiple methods are necessary to fully capture the presence of ecologically important tropical species in temperate ecosystems, especially ecosystem engineers and cryptobenthic species.

Although eDNA was effective at detecting tropical fishes in temperate ecosystems, it was less effective than visual surveys at detecting temperate species at their warm trailing range edge, where these species may be more vulnerable to range contractions under future ocean warming. Nevertheless, eDNA detected five temperate species not observed by visual surveys at their warm trailing range edge, although these species are already known

to occur at these latitudes (based on Reef Life Surveys and Atlas of Living Australia records). Survey methodology can influence biodiversity assessments in marine systems, with eDNA usually being more effective at detecting species across different functional traits (Aglieri et al. 2020). For example, eDNA identified pelagic temperate fishes (e.g., *Hyperlophus vittatu*) that morphologically resemble tropical pelagic species that were detected in temperate ecosystems (e.g., *Spratelloides delicatulus, S. gracilis* and *Stenatherina panatela*) which are difficult to distinguish in visual surveys. Differences in detection between methods likely reflect ecological traits of temperate fishes, which typically have larger home ranges, greater mobility and lower local densities at their warm trailing edges; therefore, reducing the likelihood of localised eDNA detection compared to more site-attached tropical species (Yates et al. 2019). Primer and reference database limitations may also contribute to reduced eDNA detection of temperate species, particularly in under-represented regions. While visual surveys could suggest potential range contractions of temperate species at their warm trailing range edge, eDNA does not support this, highlighting that each method in isolation could lead to contrasting interpretations of temperate fish biogeography.

Species detectability differed among functional guilds and water column positions. eDNA was more effective than visual surveys at detecting tropical carnivores, omnivores, invertivores, planktivores and detritivores, and across benthic, benthopelagic and pelagic water column positions. This likely reflects that eDNA captures DNA shed over time and across habitats, which increases detections of mobile, schooling, cryptic and low-density taxa, making eDNA more effective at capturing functional diversity (Aglieri et al. 2020; Rourke et al. 2021). Highly mobile pelagic species were also favoured by eDNA but were underrepresented in visual surveys, probably due to evasive fish behaviour in response to divers and the restricted area of transects (Prato et al. 2017). Visual surveys are less suitable for cryptobenthic species, consistent with evidence that substrate-blending colouration and inconspicuous behaviour reduce visual detections, whereas conspicuous or curious benthopelagic species are more easily recorded visually (Willis 2001). In contrast, visual surveys detected more temperate benthic carnivores and invertivores because these groups are conspicuous, diurnal and site-attached along the seafloor where observers conduct visual counts. Together, these mechanisms explain why eDNA better reflects functional diversity in tropical assemblages, while visual surveys emphasise benthic temperate fishes.

Although we highlight that visual surveys and eDNA are complementary for assessing biogeographic ranges of fishes, each methodology may reveal different species compositions through distinct limitations and biases. Traditional methods can be limited in detecting small and cryptic species, may rely on bait (e.g., baited remote underwater video stations), involve invasive techniques (e.g., lethal sampling), and are influenced by diver presence, water visibility in inclement weather and observer taxonomic bias (Bessey et al. 2020). In contrast, eDNA metabarcoding bypasses many of these limitations but is constrained by incomplete reference databases, potential PCR biases and environmental factors affecting DNA degradation. eDNA is also non-quantitative and based on presence/absence data without disclosing information on population abundance and size class, whereas visual surveys can quantify both. This could result in eDNA detections reflecting a species' transient presence rather than an established population. Methodological differences may also explain variation in detection across functional groups; for instance, eDNA may be more effective at detecting highly mobile or pelagic species, which are often missed in visual surveys that are focused on hard substrate habitats. Incomplete reference databases for underrepresented taxa and regions, such as the temperate ecosystems and species here, may also limit the ability of eDNA to detect abundant species observed in visual surveys. More abundant species shed greater overall quantities of DNA into the environment, whereas those at lower densities near their trailing range edges may fall below the detection threshold of eDNA metabarcoding (Rourke et al. 2021). Although we did not explicitly test the relationship between species abundance and eDNA detection frequency, this mechanism could have contributed to the patterns observed here. We acknowledge that some species-level identifications are uncertain and should be interpreted cautiously; therefore, temporal monitoring is required to confirm their occurrences in novel ecosystems. For example, the detection of *Stenatherina panatela* at the southernmost sites represents a substantial apparent range extension and should be interpreted with caution, as it may reflect a misassignment due to taxonomic similarity or incomplete reference coverage. Similarly, detections of tropical species *Parablennius thysanius* and *Planiliza macrolepis* could be incorrect taxonomic assignments to closely related temperate species such as *Parablennius tasmanianus* or other mullet species, respectively, known to reside in the region. For the cryptobenthic species *Enneapterygius philippinus*, limited distributional information prevents us from determining its presence in the region, and it is possible that the eDNA signals reflect a misidentification of the closely related species *Enneapterygius rufopileus*, which also occurs in the region (Booth, unpublished data). eDNA could not reliably distinguish between closely related species, such as *Abudefduf vaigiensis* and *A. sexfasciatus*, resulting in taxonomic classifications being collapsed at the genus level (Table S5). While these uncertainties affect a small number of individual records, they do not undermine broader patterns in species richness, functional trait comparisons or the comparative detectability between methods. To assess the plausibility of species-level assignments, we applied conservative filtering and manually cross-checked species distributions against authoritative sources (FishBase, WoRMS, Reef Life Survey, Atlas of Living Australia and published literature). Although some limitations remain, particularly in the taxonomic resolution of closely related or underrepresented taxa, these reflect broader challenges in eDNA reference coverage which are expected to improve over time. Despite these challenges, eDNA metabarcoding remains a valuable tool for detecting species that may be missed by traditional visual surveys (Stat et al. 2018), especially cryptic or pelagic fishes that are difficult to observe visually.

In conclusion, combining visual surveys with eDNA metabarcoding provides a more comprehensive and less biased approach to assessing fish communities across biogeographic ranges. Each method detected distinct species compositions, highlighting the risk of underestimating biodiversity when relying on a single approach. Visual surveys can overlook low-abundance or cryptobenthic species and underrepresent some key functional groups that are more readily detected by eDNA. Our findings

suggest that combining both methods improves the detection of species that may be missed when using either approach alone, especially cryptic tropical taxa. This combined framework can strengthen biodiversity assessments and improve our understanding of how ecosystems are responding to environmental change across latitudinal gradients.

Author Contributions

C.H. and I.N. conceived and designed the study. C.H., A.M., I.N. and D.J.B. conducted the fieldwork. A.H.O.A. extracted the DNA, prepared for sequencing and performed bioinformatics. T.R. contributed funding, reagents and supplies. C.H. conducted statistics, data analysis, visualisation and wrote the manuscript. All authors reviewed the manuscript and contributed to the final version.

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Ethics Statement

This experiment was conducted according to The University of Adelaide Animal Ethics and University of Technology Sydney guidelines and permits: S-2020-13 and 2017-1117, and under New South Wales DPI Scientific Collection Permit: F94/696(A)-9.0 and Great Barrier Reef Marine Park Permits: G20/43958.1 and G21/45557.1.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are openly available in figshare at DOI: 10.25909/27312888. Sequence data have been deposited in the NCBI Sequence Read Archive (BioProject ID: PRJNA1290874).

Peer Review

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/ddi.70089.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Table S1:** Summary of site locations, survey dates, and sampling effort for visual fish surveys and eDNA sampling. For visual surveys, each number represents the count of independent 10 m × 4 m belt transects conducted per habitat type at each site. Habitat types include turf, barren, oyster, kelp, coral, and coral rubble. The total number of transects reflects the sum across all habitats at a site. The number of transects varied among sites due to differences in reef size and habitat heterogeneity. For eDNA surveys, a single 10 L water sample was collected per replicate, with ten replicates per site. **Figure S1:** Mean species accumulation curves for each site using eDNA (left) and visual survey (right) methods. Line colours indicate sites within regions: tropical (orange), subtropical (green), and temperate (blue). Replicates were randomly permuted 100 times to estimate mean species richness and associated confidence intervals. Replicates refer to independent eDNA water samples (1 L) or visual belt transects (10 × 4 m). On the eDNA panel, asterisks (*) indicate 10 replicates per line and are used for visual clarity due to overlapping curves. On the visual survey panel, numbers indicate the total number of transects per site (see Table S1). **Table S2:** Summary of the best fit generalised linear models (GLMs) when predicting species richness (number of species) for the thermal guilds tropical, subtropical, and temperate species. Model predictors include latitudinal gradient (latitude) and sampling method (method: eDNA or visual surveys). k shows the number of model parameters, LL shows the log-likelihood, AIC_c shows the corrected weight given by Akaike's information criterion, and Δ AIC_c shows the difference between the ranked model. The top-ranked models for each scenario are shown in bold. GLM method is reported to account for dispersion which can be observed in fish abundance or count data (GLM with negative binomial distribution with log-link function was used when dispersion statistic is > 1.5, and GLM with Poisson distribution is used if dispersion statistic is < 1.5). Model for the subtropical species resulted in the best fit AIC_c in support of the null model; results are shown but not used in results interpretation. **Table S3:** Summary of the best fit generalised linear models (GLMs) when predicting species richness (number of species) for trophic guilds (carnivore, omnivore, invertivore, planktivore, herbivore, corallivore, detritivore) within thermal guilds tropical, subtropical, and temperate species. Model predictors include latitudinal gradient (latitude) and sampling method (method: eDNA or visual surveys). k shows the number of model parameters, LL shows the log-likelihood, AIC_c shows the corrected weight given by Akaike's information criterion, and Δ AIC_c shows the difference between the ranked model. The top ranked models for each scenario are shown in bold. GLM method is reported to account for dispersion which can be observed in fish abundance or count data (GLM with negative binomial distribution with log-link function was used when dispersion statistic is > 1.5, and GLM with Poisson distribution is used if dispersion statistic is < 1). Models for the subtropical omnivore and invertivore resulted in the best fit AIC_c in support of the null model; results are shown but not used in results interpretation. **Table S4:** Summary of the best fit generalised linear models (GLMs) when predicting species richness (number of species) for water column positions (benthic, benthopelagic and pelagic) within thermal guilds tropical, subtropical, and temperate. Model predictors include latitudinal gradient (latitude) and sampling

method (method: eDNA or visual surveys). k shows the number of model parameters, LL shows the log-likelihood, AIC_c shows the corrected weight given by Akaike's information criterion, and Δ AIC_c shows the difference between the ranked model. The top ranked models for each scenario are shown in bold. GLM method is reported to account for dispersion which can be observed in fish abundance or count data (GLM with negative binomial distribution with log-link function was used when dispersion statistic is >1.5, and GLM with Poisson distribution is used if dispersion statistic is <1). **Table S5:** Fish taxonomic classifications that were unidentified at species level and assessed to genus or family level for both eDNA and visual surveys. **Table S6:** Generalised linear model (GLM) summary for top-ranked models (see Table S2 for AIC_c best-fit models) for thermal guilds tropical, subtropical, and temperate. Model predictors include latitudinal gradient (latitude) and sampling method (method: eDNA or visual surveys). R^2 for GLM with Poisson distribution were calculated using the percentage of deviance (likelihood ratio between the best-fit model and the null model), and R^2 for GLM with negative binomial distribution was calculated using McFadden's formula (based on the log-likelihood values). Model for the subtropical species resulted in the best fit AIC_c in support of the null model; results are shown but not used in results interpretation. **Table S7:** Generalised linear model (GLM) summary for top ranked models (see Table S3 for AIC_c best-fit models) for feeding guilds (carnivore, omnivore, invertivore, planktivore, herbivore, corallivore and detritivore) within the thermal guilds tropical, subtropical, and temperate. Model predictors include latitudinal gradient (latitude) and sampling method (method: eDNA or visual surveys). R^2 for GLM with Poisson distribution were calculated using the percentage of deviance (likelihood ratio between the best-fit model and the null model), and R^2 for GLM with negative binomial distribution was calculated using McFadden's formula (based on the log-likelihood values). Models for the subtropical omnivore and invertivore resulted in the best fit AIC_c in support of the null model; results are shown but not used in interpretation. **Figure S2:** Species richness (number of species) detected across a latitudinal gradient for water column positions (benthic, benthopelagic and pelagic) within three thermal guilds (tropical, subtropical, and temperate). Orange datapoints denote eDNA methods, green datapoints denote visual survey methods, and grey shaded area represents the confidence interval. Venn diagrams show the total number of unique species detected for eDNA (orange), visual surveys (green), and the number of overlapping species detected by both methods. The symbols and illustrations are courtesy of the Integration and Application Network, University of Maryland Centre for Environmental Science (ian.umces.edu/symbols/). **Table S8:** Generalised linear model (GLM) summary for top ranked models (see Table S4 for AIC_c best-fit models) for water column positions (benthic, benthopelagic and pelagic) within the thermal guilds tropical, subtropical, and temperate. Model predictors include latitudinal gradient (latitude) and sampling method (method: eDNA or visual surveys). R^2 for GLM with Poisson distribution were calculated using the percentage of deviance (likelihood ratio between the best-fit model and the null model), and R^2 for GLM with negative binomial distribution was calculated using McFadden's formula (based on the log-likelihood values). **Figure S3:** Heatmap comparison of subtropical reef fish species detections using visual survey (red) and eDNA (blue) methods across a latitudinal gradient. The left column shows the average abundance ($10\,m^{-2}$) of each species detected by visual surveys, while the right column shows the number of times a species was detected at each site (maximum detection value of 10 at a site). Each row represents subtropical reef fish species, which are ordered by feeding guild (carnivore, omnivore, invertivore, planktivore, and herbivore). The symbols and illustrations are courtesy of the Integration and Application Network, University of Maryland Centre for Environmental Science (ian.umces.edu/symbols/). **Figure S4:** Heatmap comparison of temperate reef fish species detections using visual survey (red) and eDNA (blue) methods across a latitudinal gradient. The left column shows the average abundance ($10\,m^{-2}$) of each species detected by visual surveys, while the right column shows the number of times a species was detected at each site (maximum detection value of 10 at a site). Each row represents temperate reef fish species, which are ordered by feeding guild (carnivore, omnivore, invertivore, planktivore, and herbivore). The symbols and illustrations are courtesy of the Integration and Application Network, University of Maryland Centre for Environmental Science (ian.umces.edu/symbols/). **Table S9:**: Tropical fish species uniquely detected by eDNA at temperate reef sites. The table includes the southernmost known latitude of each species and year of the most recent record based on visual observations from the Atlas of Living Australia (ALA), the southernmost latitude where each species was detected in this study, BLAST match percentage, query coverage, and the ASV sequence.