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RECEIVED 29 March 2025

ACCEPTED 04 June 2025

PUBLISHED 02 July 2025

CITATION

Marquardt B, Elder H, Yeoh YK, Heyward A,
Randall CJ, Harrison PL, Logan M and
Howells E (2025) Determining the diversity
and relative abundance of coral taxa in wild
spawning slicks for effective restoration.
Front. Ecol. Evol. 13:1602543.
doi: 10.3389/fevo.2025.1602543

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Determining the diversity and relative abundance of coral taxa in wild spawning slicks for effective restoration

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Coral reefs are increasingly threatened by climate change-induced stressors, including marine heatwaves, which can lead to coral mortality, reduced reproductive output, and compromised natural recovery. Successful coral reef recovery requires the settlement of coral larvae and recruitment in degraded areas, replenishing coral communities and promoting resilience. Some restoration strategies involve utilizing natural spawning slicks, composed of coral gametes and embryos, to produce larvae to reseed reefs. However, verifying the taxonomic composition of these slicks is challenging. Here, we tested the performance of two coral ITS primer sets, CorallITS2 and CorallITS2_acro, on mock communities to evaluate their ability to capture genera composition and relative abundances. Both primer sets demonstrated high accuracy (>97%) in detecting and quantifying coral taxa. Subsequently, these primers were applied to wild-collected spawning slicks from the Great Barrier Reef, revealing variation in scleractinian (reef-building) coral community composition among slicks. For the CorallITS2_acro assay, *Acropora* was consistently the most abundant resolved genus detected across wild slick sample sites, with the exception of samples from the Whitsundays region, where *Platygyra* was dominant. The CorallITS2 assay successfully differentiated reef-building (Scleractinian) corals from other co-occurring spawning taxa, such as soft corals, anemones, and sponges, and revealed that these other co-spawners dominated slicks at two sites. Our findings underscore the potential of eDNA-based monitoring as a scalable tool to confirm the presence and relative abundance of diverse coral assemblages in natural slicks, informing restoration efforts. By enabling the characterization and comparison of slick composition across large spatial and temporal scales, eDNA metabarcoding can support restoration practices that align with the ecological requirements of reef ecosystems, safeguarding biodiversity and promoting resilience against future disturbances.

KEYWORDS

biodiversity, EDNA, ITS2, metabarcoding, great barrier reef, coral seeding, monitoring

1 Introduction

Marine heatwaves and other direct human impacts threaten global biodiversity, as reefs face a combination of disturbances, including coastal development, pollution, and destructive fishing practices, alongside increasingly frequent extreme warming events that affect critical foundation species such as reef-building corals (Fox and Caldwell, 2006; Smale et al., 2019). While marine heatwave events do not always cause mass coral mortality, their increasing severity and frequency are resulting in increased coral losses and reef degradation (Maynard et al., 2008; Weis, 2010; Gadoutsis et al., 2019; Intergovernmental Panel on Climate Change (IPCC), 2023; Yadav et al., 2023). For instance, marine heatwaves have triggered five mass coral bleaching events on the Great Barrier Reef (GBR) in just nine years (2016 to 2024), reducing coral cover on shallow water coral reefs by as much as 50% (Hughes et al., 2017; Dietzel et al., 2021; Henley et al., 2024). The loss of coral, particularly reef-building species, diminishes the structural complexity of these habitats and disrupts ecological functions, compromising the biodiversity and ecosystem services coral reefs provide (Gratwicke and Speight, 2005; Graham and Nash, 2013; Newman et al., 2015; Souter et al., 2020).

The recovery of coral communities following major disturbances depends on successful reproduction and recruitment, in which the dispersive larval phase replenishes populations (Gilmour et al., 2013; Holbrook et al., 2018; Gouezo et al., 2020) and maintains genetic diversity. While coral communities have an inherent capacity for natural recovery after disturbance events (Baker et al., 2008; Sheppard et al., 2008), this process is increasingly hampered by various biological (declines in fecundity of source corals) and environmental challenges (sedimentation, biofilm, and suitable substrates), and can take a significant period of time free from additional disturbances (Ortiz et al., 2018). One critical factor in determining recovery success is the dispersive larval phase, during which coral larvae supply the new recruits needed to rebuild populations (Harrison and Wallace, 1990; Richmond and Hunter, 1990; Connell et al., 1997; Harrison, 2024a); this process requires successful larval settlement in degraded areas for recovery to occur.

Coral bleaching events and other severe disturbances significantly impact larval recruitment by reducing the availability of mature, breeding corals, which are vital for sustaining reproductive output (Hughes et al., 2019). Recruitment levels often plummet to a fraction of historical averages following such disturbances, with the composition of recruits frequently shifting toward more tolerant taxa (Gilmour et al., 2013; Holbrook et al., 2018; Hughes et al., 2019). Adding to this challenge, frequent disturbances subject many surviving adults to sublethal stressors, causing impaired fecundity and reduced gamete quality, diminishing the supply of healthy larvae and undermining the ability of coral populations to sustain themselves (Michalek-Wagner and Willis, 2001; Ward et al., 2002; Cox, 2007; Gilmour et al., 2013; Piñón-González and Banaszak, 2018; Johnston et al., 2020; Briggs et al., 2024). This decline in larval supply, coupled with

altered species composition, hampers natural recovery processes: fewer larvae are available to settle and repopulate degraded reefs, which extends recovery timelines and drives long-term changes in community structure and biodiversity. Moreover, the increasing frequency of mass bleaching events leaves insufficient recovery intervals, often preventing coral communities from regenerating fully before the next disturbance (Babcock et al., 2021). This shortened window for recovery exacerbates the instability of coral populations and contributes to the long-term destabilization of reef ecosystems (Gilmour et al., 2013; van Woesik et al., 2016; Ortiz et al., 2018; Babcock et al., 2021).

Critical gaps exist in monitoring coral reproductive output across assemblages. Current assessment methods are often limited to a few species and rely on resource-intensive techniques such as tissue sampling for fecundity measurements (Howells et al., 2016; Briggs et al., 2024), recruitment data from settlement devices (Mallela and Crabbe, 2009), and benthic surveys (Jonker et al., 2008). These techniques are costly, often restricting their application to small specific sites and target species, and their reliance on morphological identification of recruits can result in low taxonomic resolution; indeed, recruits typically can't be identified beyond the family level for the first several months of life (Babcock et al., 2003). This narrow focus can overlook broader, community-wide reproductive dynamics, such as potential shifts in the taxonomic composition of spawning slicks towards more tolerant taxa. Understanding these shifts is crucial to monitoring and forecasting the response of coral communities to climate change.

Active restoration efforts are increasingly recognized as an essential strategy for sustaining coral ecosystems and their services (Bullock et al., 2011; Anthony et al., 2017; Boström-Einarsson et al., 2020; Vaughan, 2021; Banaszak et al., 2023). Among these interventions, two prominent approaches include the use of seeding devices containing settled coral recruits (Chamberland et al., 2017; Randall et al., 2021, 2023; Miller et al., 2022; Whitman et al., 2024) and the collection and deployment of wild coral spawning slicks (Heyward et al., 2002; dela Cruz and Harrison, 2017; 2020; Harrison et al., 2021). Seeding devices often involve controlled breeding and settlement of one or a few species (Chamberland et al., 2017; Randall et al., 2021, 2023; Whitman et al., 2024) to target high-value species, populations, and genotypes of interest, but cannot fully capture the extensive diversity found within broadcast-spawning communities, particularly on Indo-Pacific reefs, including the GBR. In contrast, the use of wild spawning slicks overcomes these limitations by collecting gametes and embryos from diverse coral assemblages and settling them on degraded reefs (Heyward et al., 2002; Doropoulos et al., 2019; Tabalanza et al., 2020). However, the taxonomic composition of these slicks has remained largely uncharacterized, which limits our understanding of the diversity of corals present in the slicks and highlights the risk that other potentially competitive broadcast spawner species (e.g., soft corals, anemones, and sponges) could be inadvertently captured and settled. This underscores the need for monitoring tools to ensure that restoration efforts are inclusive of

diverse taxa and aligned to maintain coral ecosystem resilience, as well as for a better understanding of potential species bias within slicks.

Environmental DNA (eDNA) refers to genetic material obtained directly from the environment. Sequencing of eDNA is widely applied in terrestrial and marine ecosystems for biodiversity assessments (Willerslev et al., 2003; Thomsen et al., 2012; Everett and Park, 2018; Cilleros et al., 2019; Alexander et al., 2020; Dugal et al., 2022) and has been shown to produce coral biodiversity assessments comparable to traditional visual surveys (Dugal et al., 2022). Here, we leveraged coral eDNA sequencing to characterize the taxonomic diversity of corals and their relative abundances in natural spawning slicks. We first generated eDNA profiles of known slick mixes containing larvae of 10 coral species from the GBR to assess the accuracy of existing coral eDNA methodology when applied to coral slicks. Following this, we sequenced eDNA of wild slick samples collected from the Cooktown, Townsville, and Whitsunday Islands sectors of the GBR. We show that eDNA profiles accurately recapitulate the taxonomic composition of coral slicks, thus demonstrating the potential of incorporating eDNA assays into wild spawning slick collection activities to identify the diversity of corals in slicks and target coral species most in need of conservation.

2 Materials and methods

2.1 Creation of a mock coral slick community

To assess the detection sensitivity of existing eDNA assays on coral slicks, a mock slick mix was created using larvae from cultures of 10 known coral species belonging to the four genera *Acropora*, *Dipsastraea* (formerly *Favia*), *Platygyra*, and *Mycedium* from the central GBR (Table 1). Bulk monospecific larval cultures were

TABLE 1 List of Great Barrier Reef scleractinian coral species and quantities used to create known mixes.

Species	Quantity of Larvae per Mix
<i>Acropora loripes</i>	3
<i>Acropora austra</i>	3
<i>Acropora muricata</i>	3
<i>Acropora microphthalma</i>	3
<i>Acropora tenuis</i>	3
<i>Acropora millepora</i>	3
<i>Dipsastraea matthaii</i>	3
<i>Dipsastraea pallida</i>	3
<i>Platygyra daedalea</i>	3
<i>Mycedium elephantotus</i>	3
Total	30

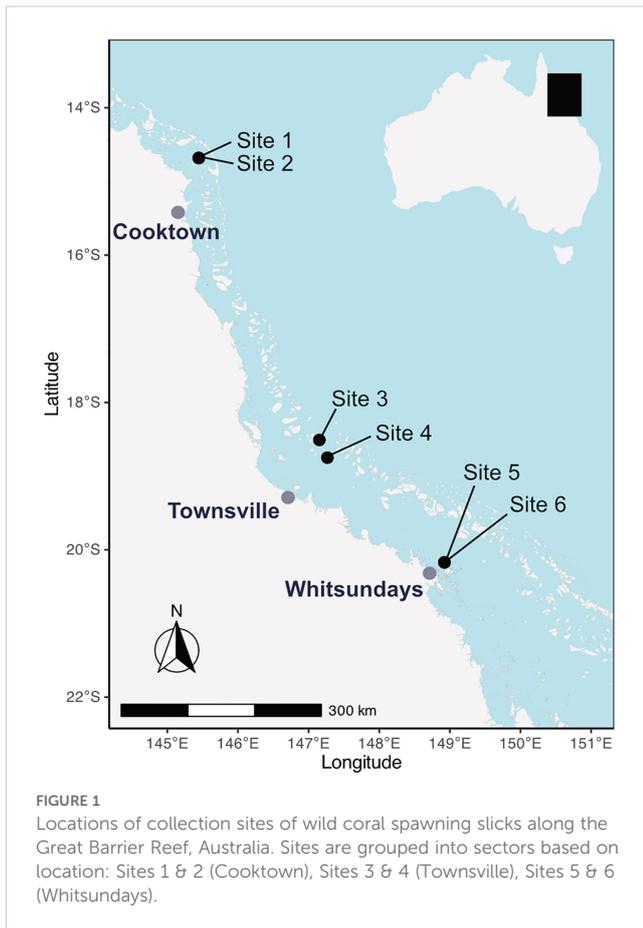
established from colony fragments that were spawned, fertilized, and cultured in November 2018 at the National Sea Simulator Facility (SeaSim) at the Australian Institute of Marine Science (AIMS) in Townsville, Australia (see Randall et al., 2024 for details). Five replicate mocks were created, each containing three larvae of each coral species for a total of 30 larvae. Samples were fixed in 100% ethanol and stored at room temperature until further processing for DNA extraction. Sampling equipment was rinsed with 10% bleach and fully dried between use.

2.2 Study sites and wild spawn slick sample collection

Six reef sites along the northern and central GBR were sampled opportunistically during the 2018 and 2021 spawning seasons (Figure 1). Coral spawning slick material was collected from Lizard Island (-14.6802, 145.4466) in the Cooktown area, Backnumbers Reef (-18.5087, 147.1529) and Keeper Reef (-18.7492, 147.2656) in the central mid-shelf near Townsville, and Hook Island in the Whitsunday Islands (-20.1705, 148.9225) (Table 2). Coral spawning was visually confirmed by the presence of spawning slicks at the sea surface of each reef during the predicted mass spawning period (Harrison et al., 1984; Baird et al., 2021), and samples were viewed under microscopes and imaged. Slick samples were scooped from the water surface using 1L beakers, and spawn collection nets from Lizard and Hook Island sites (see Harrison, 2024b for details), and 3–5 mL of the slick was transferred using sterile transfer pipettes to 15 mL sterile sample tubes. Samples were fixed in 100% ethanol and stored at room temperature until processing for DNA extraction.

2.3 eDNA extraction and sequencing

DNA was extracted following Liew et al., 2020, with a 2.5–3 hour incubation in preheated buffer (100 mM Tris/EDTA/NaCl, 1% SDS) and RNase treatment. DNA extractions were sent to the Australian Genome Research Facility (AGRF) (Sydney, Australia) for PCR amplicon generation and DNA sequencing. Each PCR reaction mixture (25 μ L) contained 10x PCR Gold buffer, 50 nM MgCl₂, 25 nM dNTPs, 5x SYBR Green, 1 U AmpliTaq PCR buffer, 10 μ M of forward and reverse primer (CoralITS2 and CoralITS2_acro, Supplementary Table S1), 20 μ M of forward and reverse tags, and DNA template. The thermal cycling conditions were as follows: initial denaturation at 95°C for 5 min, followed by 45 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 45 seconds, with a final extension of 72°C for 10 min. Separate DNA libraries were created for the CoralITS2 samples and the CoralITS2_acro samples. The final libraries were size-selected to a range of 160–550 bp (for CoralITS2) and 175–600 bp (for CoralITS2_acro). The cleaned libraries were then quantified, pooled, and sequenced on the Illumina MiSeq platform with 500-cycle V2 chemistry for paired-end sequencing (Illumina, USA).



2.4 Amplicon sequence data processing

Demultiplexed reads provided by AGRF were imported into QIIME2 (v. 2024.5; Bolyen et al., 2019). DADA2 (Callahan et al., 2016) implemented in QIIME2 was used to trim adapter, primer and low-quality sequences, and then denoise, merge, and check the remaining sequences for chimeras, and finally to generate an amplicon sequence variant (ASV) counts table. The LULU algorithm was then used to merge ASVs based on sequence similarity and co-occurrence patterns (Frøslev et al., 2017). To assign taxonomy, the representative sequence of each ASV was compared against the UNITE ITS database (Abarenkov et al., 2024)

TABLE 2 Location and timing of the collection of wild slick samples from the Great Barrier Reef.

GBR Sector	Slick Site	Collection Date	Nights After Full Moon
Cooktown (15° S)	1	Nov-22-2021	3
	2	Nov-24-2021	5
Townsville (19° S)	3	Nov-29-2018	6
	4	Nov-28-2018	5
Whitsundays (20° S)	5	Oct-26-2021	5
	6	Oct-26-2021	5

v9 using BLASTN v2.13.0+ and the alignment outputs imported into MEGAN v6.24.20 (Huson et al., 2007) for assignment using the Lowest Common Ancestor (LCA) algorithm (within top 5% (CoralITS2_acro) and 3% (CoralITS2) based on BLASTN alignment score). Sequences with no matches or taxonomic assignments only at the domain level were discarded.

2.5 Statistical and ecological analysis

Relative abundances were calculated by dividing each feature count by total library size (i.e. total sum scaling). For the mock community samples, average relative abundances were calculated separately for the CoralITS2 and CoralITS2_acro community profiles and the expected versus observed outcomes of average relative abundances were assessed using a hierarchical model in a Bayesian framework (Gelman, 2003). Specifically, a beta-binomial model with weakly informative priors was constructed. The Bayesian model included three chains, each 5,000 interactions, thinned by a factor of ten, with the first 1,000 iterations used for warmup and excluded from the analysis. The model demonstrated good mixing and convergence on a stable posterior (Supplementary Figures S4-S6). Validation was performed using simulated residuals with the DHARMA package (Hartig, 2022; R Foundation for Statistical Computing, 2024). All statistical analyses were performed in the R Statistical and Graphical Environment (version 4.4.1; R Core Team, 2024) via the brms package (Bürkner, 2017). *Post-hoc* comparisons between observed and expected values were made based on the full posteriors before summarizing using draw-level chi-squared values, medians, and highest probability intervals.

To compare the six wild slick samples, a Bray-Curtis distance matrix was calculated for the ASVs detected, and a Principal Coordinate Analysis (PCoA) was performed to visualize the multidimensional relationships between samples in a lower-dimensional space. Each PCoA was created using the 'cmdscale' function in R (R Core Team, 2024) and visualized with Rstudio (v2024.4.2.764; Posit Team, 2025).

3 Results

3.1 Coral slick eDNA metabarcoding recapitulates expected profiles of mock communities

Metabarcoding of the mock communities yielded an average of 31,724 and 24,306 reads per sample for the CoralITS2_acro and CoralITS2 assays, respectively. After processing in DADA2 and merging co-occurring ASVs with high sequence similarity using LULU, this resulted in 54 and 10 ASVs in the CoralITS2_acro and CoralITS2 data sets, respectively. At the ASV level, the CoralITS2_acro and CoralITS2 primer sets were unable to resolve species taxonomies. Of the 10 known coral species present (Table 1), eight including *A. loripes*, *A. austera*, *A.*

microphthalma, *D. mathaii*, *D. pallida*, *M. elephantotus*, and *P. daedalea* were not taxonomically identified in the CoralITS2_acro and CoralITS2 primer sets (Supplementary Figure S1). As such, ASV data were collapsed to genus or next lowest available rank.

In the CoralITS2_acro assay, we identified *Platygyra* ASVs, indicating that *Platygyra* were resolved to the genus level whereas *Dipsastraea* and *Mycedium* were likely only resolved to the suborder level (Vacatina). We therefore combined *Dipsastraea* and *Mycedium* into one expected Vacatina group with an expected 30% relative abundance. Overall, the observed average relative abundances in the known mix were comparable to the expected proportions: *Acropora* (59% observed vs. 60% expected), Vacatina (33% vs. 30%), and *Platygyra* (9% vs. 10%) (Table 3; Figure 2A). Variation between replicate samples was greatest for *Acropora* and Vacatina, resulting in a standard deviation of 18.9% and 15.5%, respectively. *Platygyra* showed average values close to the expected relative abundances and was more consistent between replicates, resulting in a low standard deviation of 3.3%.

The CoralITS2 assay resulted in 10 ASV matches: eight mapping to known taxonomy in the mocks, one to a genus not present in the mock (*Physophyllia*), and one with no known taxonomic match. *Dipsastraea* was resolved to the genus level (*Dipsastraea*), while *Platygyra* was resolved to the family level (Merulinidae). The remaining ASVs resolved as suborder Vacatina likely represented *Mycedium*. Using this primer set, the observed vs. expected relative abundances were: *Dipsastraea* (49% observed vs. 50% expected), Vacatina (19% vs. 25%), Merulinidae (26% vs. 25%), and *Physophyllia* (6% vs. 0%) (Table 4; Figure 2B). Variation in relative abundances between replicates using this primer set was lower compared to coralITS2_acro, with standard deviations for *Dipsastraea* at 6.5%, Vacatina at 9.4%, Merulinidae at 6.7%, and *Physophyllia* at 2.7% (Table 4).

We applied a Bayesian beta-binomial model to evaluate whether the observed relative abundances deviated from the expected values. The model showed no evidence that the observed relative abundances differed from the expected values for both the CoralITS2_acro assay (median deviation = 0.065, 95% CI [-0.27 – 0.5]; $x < 0 = 0.329$, $x > 0 = 0.671$; Supplementary Figure S2) and the CoralITS2 assay (median deviation = 0.0622, 95% CI [-0.32 – 0.47]; $x < 0 = 0.351$, $x > 0 = 0.649$; Supplementary Figure S3). The variations observed were consistent with database quality, DNA quality, and random fluctuations versus a systematic effect.

TABLE 3 Expected and observed (average and standard deviation) relative abundances of scleractinian coral taxa in known mixes of coral larvae for the CoralITS2 assay.

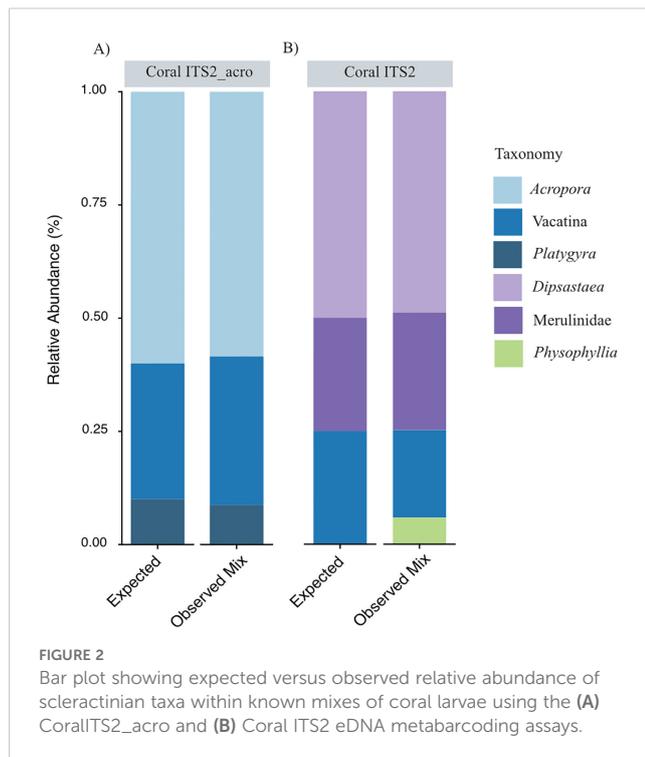
Taxonomy	Expected	Observed (Average \pm SD)
<i>Dipsastraea</i>	0.5	0.489 \pm 0.065
Vacatina	0.25	0.193 \pm 0.094
Merulinidae	0.25	0.259 \pm 0.067
<i>Physophyllia</i>	0.0	0.059 \pm 0.027

3.2 Community composition of wild coral spawning slicks

Metabarcoding of the wild coral spawning slick samples yielded a total of 632,909 and 382,586 demultiplexed sequences, with an average of 37,230 and 23,911 reads per sample for the CoralITS2_acro and CoralITS2 assays, respectively. The CoralITS2_acro assay resulted in a total of 129 ASVs among coral spawning slicks, with an average of 35 ASVs per replicate sample. The CoralITS2 assay resulted in fewer ASVs, 52 in total, with an average of 7 ASVs per sample. In a few instances, one of the three replicates was excluded from downstream analyses of CoralITS2_acro (sites 5, 6) and CoralITS2 (site 4) communities due to failed PCR amplification or low yield with no taxonomic match. A further replicate from site 6 was included in the CoralITS2 relative abundance analysis but removed from the PCoA due to the recovery of a single ASV which made it incomparable with other samples. Taxonomic assignments for the majority of ASVs (89%) were resolved to at least the family level (Figures 3A, B). With the CoralITS2_acro assay, spawning slicks were dominated by Acroporidae at sites 1 (98-99% relative abundance), 3 (37-96%) and 4 (99-100%), and Vacatina (a suborder encompassing Merulinidae and Mussidae) at sites 2 (81-87%), 5 (55-90%) and 6 (98-100%). Poritidae was detected at sites 1 (0- $<$ 1%) and 2 ($<$ 1%) at low relative abundances (Figure 3A). Nearly all assigned taxonomic detections with the CoralITS2_acro assay were reef-building corals (Scleractinia), with the order Zoantharia detected only at site 1 (Cooktown, $<$ 1%). With the CoralITS2 assay, a greater diversity of families was recovered with similar differences in community composition among slicks. Acroporidae remained dominant in the slicks from site 3 (2-53%), while Merulinidae, and Vacatina were prevalent in varying proportions within slicks at sites 2 (2-97%), 5 (0-100%), and 6 (0-100%). However, Alcyoniidae (soft corals) was the dominant family in the slicks at site 1 (75-90%), and Actiniidae (sea anemones) was the dominant family within the slick at site 4 (78-82%). Other scleractinian (Pectiniidae, Poritidae) and non-scleractinian (Aplysinidae, Clionaidae) families were also detected in low quantities ($<$ 1%) in one or more slick samples, except Pectiniidae detection at site 5 (0-18%).

At the genus level, there was a substantial reduction in explained diversity as 42 ASVs could not be resolved, particularly within the family Merulinidae (3 ASVs, CoralITS2_acro; 6 ASVs, CoralITS2) and suborder Vacatina (10, CoralITS2_acro; 8, CoralITS2). This reduction varies widely from 0% to 100% within replicate samples, with an average decrease of 31% (CoralITS2_acro) and 37% (CoralITS2) (Figures 3C, D). For the CoralITS2_acro assay, *Acropora* was consistently the most abundant resolved genus detected across slicks, except for two samples from sites 5 and 6 (Whitsundays) where *Platygyra* was dominant (Figure 3C). For the CoralITS2 assay, *Dipsastraea* was the most abundant resolved genus at sites 2, 5, and 6, and *Anacropora* and *Montipora* were most abundant at site 3. Soft coral (*Sinularia*) and anemone (*Condylactis*, *Anemonia*) genera were dominant at sites 1 and 4, respectively (Figure 3D).

The relative abundance data revealed notable variability across reef sites regarding taxa composition. At sites 1, 3, and 4, *Acropora* emerged



as the most prevalent group, with the highest variation observed at site 3 (SD = 31.6%), while the other sites showed consistent ratios across replicates (SD < 0.01%). The site 2 spawning slick contained the most diverse taxa, with consistent relative abundance across replicates. While the standard deviation for *Acropora* was comparatively higher at 3.1%, other taxa showed generally low variation (SD < 1%). In contrast, the two Whitsundays sites (5 and 6) exhibited substantial variability, with large standard deviations for *Acropora* (25.1%), *Vacatina* (33.1%), and *Merulinidae* (31.1%) at Whitsundays site 6, as well as a notable detection of *Platygyra* in just one replicate (27.1%). The variability in these sites complicates the interpretation of their community composition.

These patterns of variability and consistency across sites are further supported by the Bray-Curtis dissimilarity analysis, which visualized the relationship between samples based on their taxonomic composition and abundance. The PCoA plot (Figure 4) illustrates that replicate samples from each reef site generally clustered together, suggesting that sites shared similar community compositions. However, certain sites showed closer relationships within and among their slicks. For example, sites 1, 2, 3, and 4 showed greater similarity among their replicate samples,

TABLE 4 Expected and observed (average and standard deviation) relative abundances of scleractinian coral taxa in known mixes of coral larvae for the CoralITS2_acro assay.

Taxonomy	Expected	Observed (Average \pm SD)
<i>Acropora</i>	0.6	0.585 \pm 0.189
<i>Vacatina</i>	0.3	0.328 \pm 0.155
<i>Platygyra</i>	0.1	0.087 \pm 0.033
<i>Physophyllia</i>	0.0	0.059 \pm 0.027

while sites 5 and 6 demonstrated more divergent patterns (Figure 4). This trend is also evident in the CoralITS2 assay (Figure 4B), where site 2 consistently separated from other groups, and site 1 showed divergence within the clustering. Notably, the replicates from Whitsundays sites (5 and 6) were positioned farther apart, aligning with the observed discrepancies in relative abundance, particularly for *Acropora*. Shannon diversity varied across sampling locations within each primer set (Figure 4C). For the ITS2 assay, diversity was lowest at Site 6 (0) and highest at Sites 1, 3, and 4 (1.3-1.5). Similarly, in the ITS2_acro assay, diversity was lowest at Site 6 (0.7) and highest at sites 1, 3, and 4 (2.7-2.8).

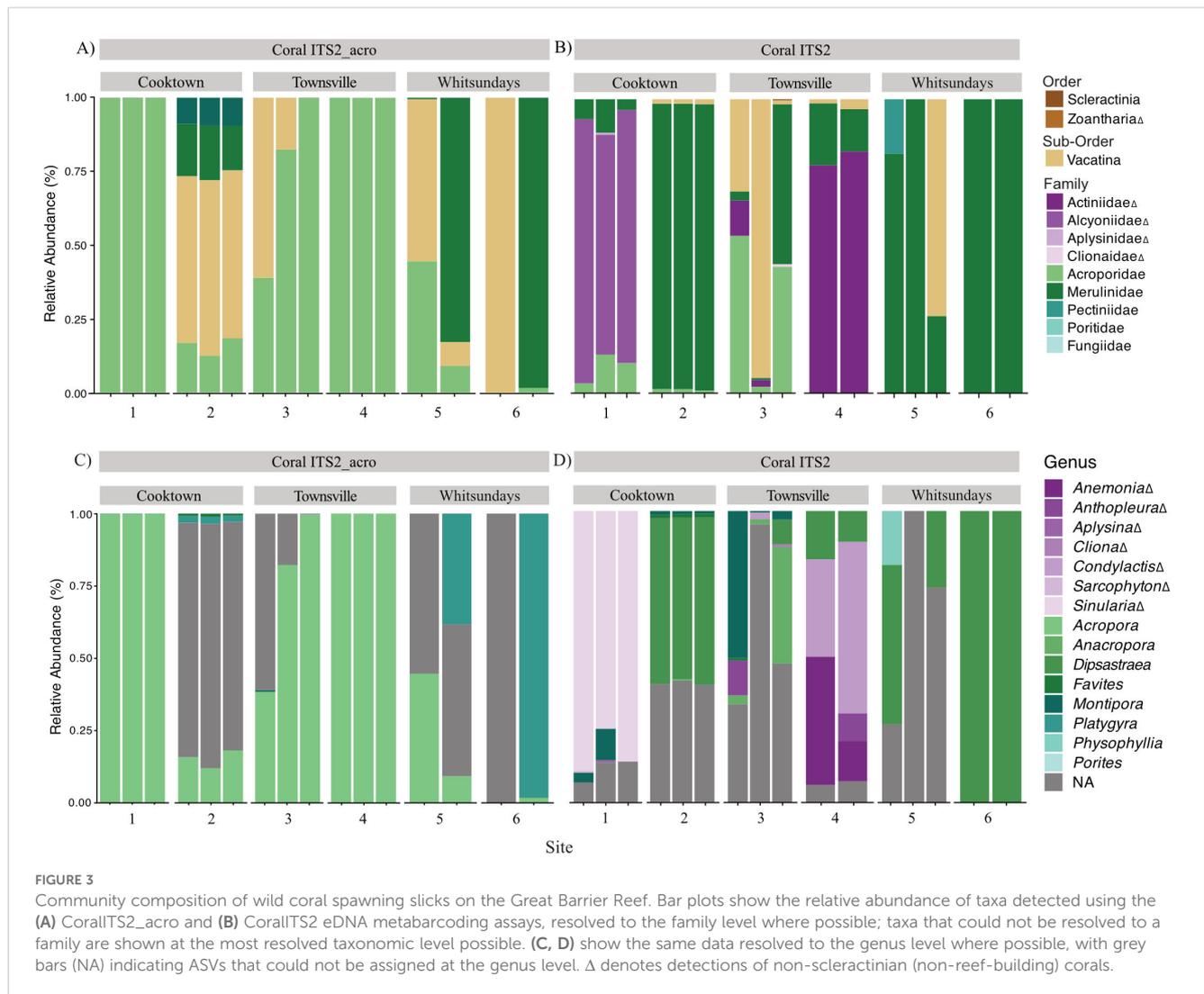
3.3 Assay detection

The assay results showed a variety of identified taxonomic groups, with some taxa not being resolved beyond the family and sub-order level. There was considerable cross-amplification of taxonomic groups across the two assays, and each assay identified unique families and genera (Figure 5). CoralITS2_acro identified a total of five families and six genera, while CoralITS2 identified seven families and 13 genera. As expected, the majority of ASVs recovered by the CoralITS2_acro (135) and CoralITS2 (52) assays were assigned to reef-building coral taxa (order Scleractinia). Several ASVs from the CoralITS2 assay were assigned to non-scleractinian coral taxa, including soft corals (5), anemones (8), sponges (2), and zoanthids (2). While CoralITS2 detected more unique taxonomic groups, of the 13 genera identified by the CoralITS2 assay, five were not corals but rather sea anemones and sponges. Additionally, at the genus level, *Acropora* was exclusively detected with the CoralITS2_acro assay and represented 54% of the ASVs identified in this study, highlighting the importance of this revised primer set in detecting this reef-dominant species. Lastly, 23% of the ASVs identified to the family level could not be resolved to genus, indicating that improved reference database resolution could lead to more precise identification (Figure 5).

4 Discussion

This study revealed the community composition of coral spawning slicks for the first time and demonstrated the applicability of using eDNA metabarcoding technology for this purpose. Analysis using the ITS2 region (CoralITS2 and CoralITS2_acro assays) identified 191 ASVs comprising 9 families and 15 genera from wild-collected slick samples on the Great Barrier Reef. The detection capabilities of both assays were validated with samples of known taxonomic diversity, confirming that observed relative abundance values aligned with expected profiles. These findings support further development of eDNA metabarcoding as a cost-effective approach for surveying the community composition of wild-captured spawning slicks, providing valuable insights for monitoring and restoration efforts.

Across the Cooktown and Townsville wild slick collection sites, *Acropora* was consistently the most abundant genus detected using



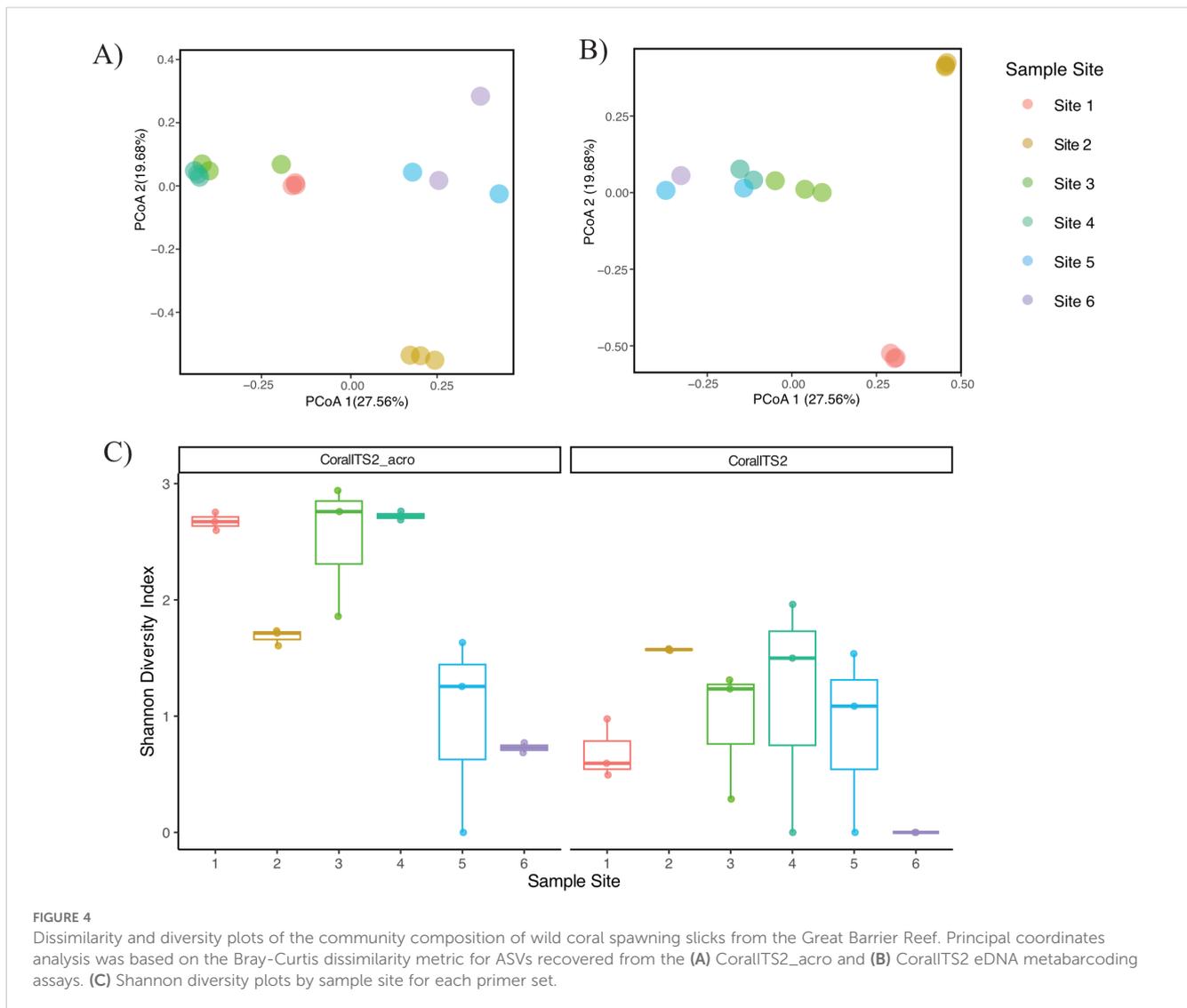
the Coral ITS2_acro assay, aligning with survey data that shows *Acropora* as one of the most, and often the most, abundant broadcast spawning hard coral genera in these regions during the collection years (Australian Institute of Marine Science Long Term Monitoring Program¹). In contrast, slicks from the Whitsundays collection sites were dominated by non-acroporid hard corals, particularly members of the Merulinidae family, which is consistent with benthic hard coral composition in this region during the collection years. These results underscore the ability of eDNA to capture region-specific reproductive outputs that mirror known benthic assemblages.

Leveraging eDNA methods for monitoring the community composition of coral spawning slicks offers several significant advantages for understanding coral spawning patterns and coral restoration applications. Spawning slicks represent a critical life-history stage for corals, acting as a source of larval dispersal and

recruitment that ultimately shapes reef population dynamics (Harrison and Wallace, 1990; Jones et al., 2009). Understanding the taxonomic composition of these slicks allows for improved monitoring of reproductive success, assessment of species-specific contributions, and evaluation of how environmental stressors may influence reproductive output. This study makes key advancements in this area by providing the first taxonomic characterization of wild-collected spawning slicks, validating the use of eDNA for slick monitoring, and highlighting the potential for applying this approach in restoration efforts.

eDNA enhances detection capabilities by allowing for the simultaneous identification of multiple taxa, including those that are difficult to observe directly, such as cryptic or rare species. This approach also enables differentiation between reef-building corals and other marine mass synchronized spawners—such as soft corals, anemones, worms, and sponges (see Babcock et al., 1986, 1992) that may have contributed to the spawning slick and might otherwise be mistaken for corals based on visual observations alone. Moreover, eDNA reduces the need for specialized training and extensive night diving to accurately record observations of coral spawning on the reef during nocturnal

¹ <https://apps.aims.gov.au/reef-monitoring/reefs>

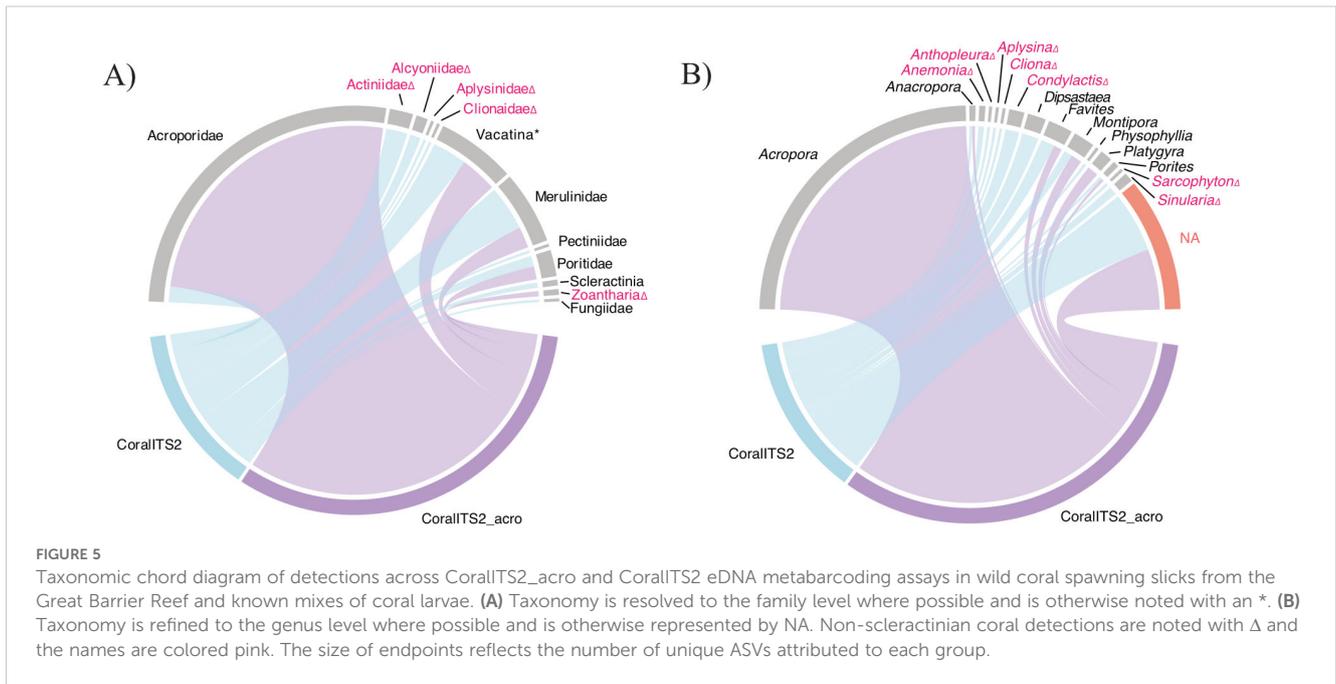


spawning periods (Harrison et al., 1984; Babcock et al., 1986). This makes it an accessible and scalable option for monitoring coral reefs, especially for sites that are expensive and/or difficult to reach, facilitating frequent and widespread surveys at a lower cost. Additionally, eDNA-based methods provide a valuable tool for tracking changes in spawning patterns in response to environmental stressors, such as bleaching events, and offer insights into the health and reproductive output of coral spawning communities over time. Furthermore, emerging eRNA approaches, such as those described by Ye et al. (2025), may offer additional benefits by providing more immediate assessments of metabolically active organisms. These methods could enhance monitoring efforts during the culture of sticks for restoration, offering insights into coral larval viability and early-stage development.

Despite its advantages, the resolution of eDNA-based identification is currently constrained by inherent challenges in taxonomic assignment. The ITS2 region captures both inter- and intra-specific variation, describing diversity within and across coral species. Historical and contemporary hybridization events contribute to the intragenomic variation observed in the ITS2 region (Coleman and Van Oppen, 2008; Chan et al., 2018, 2019;

Lamb et al., 2024) and can lead to ambiguous or incorrect taxonomic assignments, resulting in errors in sequence-based identification (Willis et al., 2006). Additionally, multiple potential matches may arise, leading to lower resolution in taxonomic assignments. Here we observed a reduction in the overall explained diversity when resolving taxa from the family to the genus level, likely due to these complexities.

Taxonomic resolution is further constrained by limitations in available sequence databases, which may lack comprehensive or up-to-date references for coral taxa (Dugal et al., 2022). These database gaps introduce uncertainties in eDNA-based identifications, increase the likelihood of unresolved classifications, and result in trade-offs between detailed resolution (genus-level) and the completeness of diversity representation (family-level). To address these issues, future work could enhance reference databases with better-curated and updated taxonomy and/or include internal spiked datasets containing known samples to improve detection depth and classification accuracy (Dugal et al., 2022). Moreover, differences in the coral taxa recovered between the CorallITS2 and CorallITS2_acro assays underscore the necessity of integrating



multiple or alternative DNA markers, especially to capture *Acropora* and non-*Acropora* taxa.

ITS2 community profiling could be biased by variation in DNA copy number and fragment length, with shorter amplicons preferentially amplified during PCR, and species with more ITS2 copies potentially overestimated. To account for this, mock communities composed of known coral taxa were included in this study. A Bayesian beta-binomial model was applied to compare observed and expected taxonomic compositions within these mock communities. The model showed no evidence that expected and recovered relative abundances differed. This quality control step lends support that the taxonomic profiles recovered from wild slick samples reflect true biological community composition rather than technical artifacts introduced during amplification or sequencing.

Here we used three technical replicates per sample of briefly mixed samples. This recommended approach (Ficetola et al., 2015; Fonseca, 2018) produced consistent averages in our curated mix, however, more variable relative abundance ratios were observed in the wild slick samples. We suspect this is due to biological sample heterogeneity as gametes and larvae may clump together, particularly for slicks collected shortly after spawning when not all gamete bundles have dissociated. Yet, we cannot exclude the inconsistencies in DNA extraction efficiency and PCR amplification as contributors to variation among technical replicates. In future studies, we recommend implementing a more thorough mixing process to fully homogenize samples and exploring the effect of various levels of replication in quantifying the abundance of taxa in wild samples.

To further enhance the robustness of future analyses, we suggest that biological replicates from the same spawning slick are also collected to provide more precise estimates of community composition. Pairing this information with comprehensive environmental data such as hydrodynamic models (Gouezo et al.,

2025), water temperature, and other relevant conditions during spawning (Keith et al., 2016; Lin and Nozawa, 2023; Nozawa, 2012; van Woesik, 2010) and with the context of disturbance history (e.g. bleaching events) would enable a deeper understanding of how environmental factors influence community-level reproductive output. Resampling the same regions over multiple spawning seasons can also shed light on the trajectories of community recovery following bleaching events, where surviving corals are expected to experience reduced fecundity (Ward et al., 2002). Once temporal variability of taxa in slicks at a given reef site has been characterized, shifts in the relative abundance of taxa within spawning slicks can be linked to changes on the reef benthos; these data would provide important insights into the long-term dynamics of coral populations and assemblages.

Importantly, sampling limitations arise from the timing of surface-collected spawning slick material, which may not capture the full diversity of coral taxa. For example, certain groups, such as taxa that release negatively or neutrally buoyant gamete bundles, and species that spawn during the day rather than at night, and at different times after sunset or on different lunar phases may be missed (Babcock et al., 1986; Harrison and Wallace, 1990; Harrison, 2024a). The timing of sampling relative to the full moon also influences what species may be captured due to differences in reproductive timing across lunar phases and months (Baird et al., 2021). Furthermore, brooding coral species, which release fully formed swimming larvae will not be captured in spawning slicks. Improving the temporal resolution of eDNA sampling around the timing of spawning to collect over multiple days and nights around the full moon would increase our understanding of the diversity of species reproducing during these periods (Ip et al., 2023).

Lastly, in the context of restoration activities that use wild slicks as material for restoring degraded reefs (Heyward et al., 2002;

Harrison et al., 2021; Banaszak et al., 2023; Harrison, 2024a), the current processing times for PCR and sequencing of eDNA samples can exceed the time available before settlement begins. Understanding the species composition and diversity within larval cohorts is essential for predicting settlement and recruitment dynamics. More importantly, identifying potential contamination by soft corals, which are major competitors for reef-building corals, is crucial before larvae are introduced to target reef sites. A potential solution to this challenge is the use of lateral flow assay (LFA) dipstick methods, as demonstrated by Doyle and Uthicke (2020). LFA enables rapid, field-based detection of eDNA within minutes, rather than the days required for traditional sequencing-based workflows. Incorporating LFA into spawning slick monitoring could facilitate real-time taxa verification, ensuring that the collected spawn produces larval cohorts that are appropriate for larval restoration, including target taxa before larval enhancement occurs.

This study provides significant advances in our understanding of the taxonomic composition of wild coral spawning slicks and demonstrates the feasibility of using eDNA metabarcoding for their characterization. While current limitations in taxonomic resolution and database completeness remain challenges, the integration of additional markers, database improvements, and methodological refinements can further enhance eDNA-based monitoring. The successful validation of this approach in both mock communities and wild slicks establishes eDNA as a valuable tool for assessing coral community reproductive output, monitoring ecosystem health, and informing restoration strategies.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: <https://www.ncbi.nlm.nih.gov/>, PRJNA1230602.

Ethics statement

Ethical approval was not required for the study involving animals in accordance with the local legislation and institutional requirements because Proper permits obtained (see manuscript), no other ethical approval required.

Author contributions

BM: Visualization, Formal analysis, Writing – review & editing, Writing – original draft. HE: Supervision, Writing – review & editing. YY: Data curation, Writing – review & editing, Supervision. CR: Resources, Writing – review & editing. AH: Resources, Writing – review & editing. PH: Funding acquisition, Resources, Writing – review & editing. ML: Formal analysis, Writing – review & editing. EH: Conceptualization, Methodology, Writing – review & editing, Investigation, Supervision, Funding acquisition.

Funding

The author(s) declare that financial support was received for the research and/or publication of this article. This study was funded by The Reef Restoration and Adaptation Program (RRAP), a partnership between the Australian Governments Reef Trust and the Great Barrier Reef Foundation, and a Paul G Allen Family Foundation Grant to PH.

Acknowledgments

We acknowledge the Dinggaal, Bindal, and Ngaro Peoples as the Traditional Custodians of the sea Countries where this research took place, and we acknowledge their Elder's past, present, and emerging, and their continuing spiritual connection to sea Country. We thank Christina Langley for collecting the Whitsundays samples and the SCU and CSIRO coral larval restoration team members for field support at Lizard Island and in the Whitsundays. This study was funded by The Reef Restoration and Adaptation Program (RRAP), a partnership between the Australian Governments Reef Trust and the Great Barrier Reef Foundation, and a Paul G Allen Family Foundation Grant to PH. Broodstock coral colonies for mock slick creation were collected under permits G12/35236.1 and G19/43024.1, and spawn slick samples were collected under permit G21/46077.1, issued by the Great Barrier Reef Marine Park Authority (GBRMPA).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that Generative AI was used in the creation of this manuscript. AI tool ChatGPT 4o was utilized to improve the manuscript flow.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fevo.2025.1602543/full#supplementary-material>

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