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eDNA Collected by Custom-Built Unmanned Surface Vehicles (USVs) Reveals Spatio-Temporal Shifts in Tropical Coral Reef Faunal Communities

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ABSTRACT

High-frequency environmental DNA (eDNA) sampling offers new opportunities to reveal fine-scale biodiversity patterns and short-term ecological dynamics, yet its spatial representativity and capacity to capture diel shifts in communities remain poorly understood. Semi-autonomous sampling devices can aid high frequency and standardized collection for such assessments, particularly in remote and low-light conditions, as well as dynamic coastal ecosystems such as tropical coral reefs. Coral reefs are ideal habitats to test such systems, as they support exceptional but rapidly declining biodiversity. We constructed Unmanned Surface Vehicles (USVs) for high-frequency and cost-efficient geolocalized sample retrieval around the break between two reef flats (Hermitage Pass) of the La Saline–Hermitage coral complex on Reunion Island. The USVs were deployed to navigate between eight stations, pumping seawater through filters to capture eDNA. Sampling occurred during nighttime and daytime (“sampling events”), across four days, to assess potential shifts in metazoan communities. Samples underwent DNA metabarcoding, applying a Cytochrome Oxidase I (COI) gene marker, to target faunal assemblages. Analyses revealed significant differences between communities surveyed during the nighttime and the daytime on each collection day, and across differing stations. Although eDNA dispersal was largely confined to each reef flat and limited among some stations, some communities towards Hermitage Pass were not statistically distinct in composition, suggesting that localized environmental conditions may influence eDNA distributions in this area. Altogether, these findings reveal a diverse and dynamic seascape within less than 1 km², and point to key sites for long-term monitoring using low-cost mobile devices for high-frequency eDNA sampling.

1 | Introduction

Dynamic coastal marine ecosystems, such as tropical coral reefs, are ecologically complex, highly biodiverse, and shaped by strong interactions between physical and biological processes (Hatcher 1997; Sala and Knowlton 2006). These systems often undergo overlapping cycles of disturbance and recovery, which can obscure the distinction between natural fluctuations and long-term ecological decline (Hughes

et al. 2010; Emslie et al. 2024), thereby complicating the design of habitat-specific monitoring frameworks. Detecting meaningful change within such evolving contexts requires frequent, fine-scale observations of biological communities and localized environmental conditions, across both spatial and temporal dimensions (Morais et al. 2024; Osland et al. 2025). This approach can contribute to monitoring frameworks capable of capturing differing community states, key inflection points, and fluctuations in seascape heterogeneity tied to

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environmental variation, enabling more accurate interpretation of ecological trajectories (Hughes et al. 2010; Dornelas et al. 2013).

A wide range of methods are commonly implemented for spatiotemporal assessments of coastal marine biodiversity; these include visual (scuba diving and snorkeling inspections, underwater photography, aerial and satellite imagery), retrieval (nettings, traps) and acoustic (hydrophones and echo-sounding) techniques (Costello et al. 2017). Comprehensively accounting for the majority of taxonomic groups within complex systems, however, necessitates incorporating complementary methods capable of detecting elusive, hidden and cryptic organisms (Muenzel et al. 2024). Molecular-based surveys, such as metabarcoding of environmental DNA (eDNA) samples, have proven effective in addressing these constraints, and their consolidation with traditional survey methods can provide additional insights and even improve biodiversity assessments (Keck et al. 2022).

Multiple studies have demonstrated the sensitivity of eDNA-based sampling in detecting spatial differences across a variety of coastal aquatic habitats. For example, Port et al. (2016) conducted an eDNA survey of vertebrate fauna along a 2.5 km transect within a kelp forest ecosystem, reporting distinct assemblages separated by as little as 60 m. Further, O'Donnell et al. (2017) examined a broader spatial area of up to 4 km from shore to identify several distinct communities and concluded that physical proximity was a strong indicator of their similarity. Previous work has also shown the feasibility of eDNA-based temporal monitoring across differing time scales. For instance, Djurhuus et al. (2020) retrieved bimonthly eDNA samples over a period of 18 months, recording fluctuations in marine biological composition with changing seasons and conditions. Over a shorter timescale, Sevellec et al. (2021) examined samples obtained over 4 months to reveal a strong seasonal transition in Arctic faunal assemblages. Additionally, Jensen et al. (2022) surveyed variations in coastal marine communities through hourly water collections over 32 h, observing shifts in taxonomic richness. Each study therefore demonstrated that shorter term replicated sampling is also important for uncovering shifts in coastal biodiversity. Considering spatiotemporal eDNA sampling in a tropical context, Dowell et al. (2024) sampled surface eukaryotic communities every 6 h over 3 days at two coral reef sites, finding that sampling time significantly affected richness and revealed clear diurnal fluctuations among certain planktonic groups. Despite continued progress, however, a recent review of eDNA literature found that temporal replication in eDNA studies remains relatively rare and advised that spatiotemporal sampling should continue to be conducted (Blackman et al. 2024).

The high frequency temporal sampling required to detect rapid shifts in coastal faunal richness and composition further presents a significant challenge, particularly when attempting to pinpoint critical episodes (Dornelas et al. 2013). While eDNA-based surveys may be generally less laborious compared to other approaches, many water collection protocols still require manual sampling. These protocols may be more arduous to conduct at higher frequency, under low light conditions, and in remote or less accessible locations where obtaining collections might be hazardous or even impossible. In these contexts, autonomous eDNA collection devices for “bulk” water sample filtering offer

a means for enhanced biodiversity resolution while ensuring safety and sample standardization (see Yamahara et al. 2025).

Herein, we describe the operation of in-house constructed Unmanned Surface Vehicles (USVs) capable of semi-autonomous (as defined by Yamahara et al. 2025), geolocalized eDNA sampling. The USVs were trial tested through pumping and filtering seawater to capture eDNA at several stations distributed across the La Saline–Hermitage coral reef complex on Reunion Island (Western Indian Ocean, Mascarene archipelago). Reunion Island features short stretches of fringing coral reefs encompassed within back-reef depressions (“lagoons”) that cradle its western coast (Thébaud et al. 2009). The reefs and associated biological assemblages are a significant component of the island's natural, economic and cultural landscape and profoundly shape the surrounding environment by influencing its physical structure, ecological and chemical processes (Tourrand et al. 2013). Reef deterioration, however, has led to changes in organismal abundance and diversity within various lagoons, most notably in terms of percent coral cover (Fari et al. 2022). As such, frequent, comprehensive assessments are essential for identifying short term variations in Reunionese reef communities. Site-based temporal inspections of reef fauna are performed annually or semi-annually with permission granted by the Natural Marine Reserve of Reunion Island (RNMR) that implement traditional survey approaches (Rungassamy et al. 2024). Further, previous eDNA-based surveys conducted within multiple lagoons on the island have shown the applicability of eDNA screening of these habitats (Cowart et al. 2024). For the La Saline–Hermitage reef complex, analyses further revealed distinctive taxonomic profiles nearby the Global Coral Reef Monitoring Network (GCRMN) sites of Toboggan (northern reef flat) and Planch'Alizé (southern reef flat), which are approximately 2 km apart (Cowart et al. 2025). Environmental DNA sampling, however, was limited to single time points, constraining broader assessments of habitat dynamics.

This study therefore focused on the area between the two GCRMN sites, aiming to track spatial and temporal variation in reef-associated faunal assemblages and to identify optimal locations for sustained high-frequency biodiversity monitoring. We employed the self-constructed USVs as cost-efficient platforms for standardized, reproducible sampling across day and night. Collected samples were then processed using eDNA metabarcoding to evaluate biological variation between sampled communities.

2 | Materials and Methods

2.1 | Study Area and Sampling Stations

The reef flats around Hermitage Pass were selected for study (Figure 1). Hermitage Pass is situated between the two subtidal reef flats that encompass the La Saline–Hermitage complex, Reunion's largest reef measuring 230 ha and extending 8–9 km from north to south. The Hermitage Ravine (or “gully”) reaches the shoreline, discharging its stream which flows seaward through Hermitage Pass while interacting with lagoonal currents on their way to the fore reef and open ocean (Tedetti et al. 2011; Kolasinski et al. 2016). This



FIGURE 1 | Location of the study area. The inset indicates the position of Reunion Island, while the central panel presents the island. The main panel shows eight stations around Hermitage Pass, which are represented by the circles: Green circles indicate northern stations, and magenta indicates southern stations. The Fixed North and Fixed South stations were each sampled at the beginning (P1) and end (P5) of each sampling event. Maps were created using QGIS-LTR (QGIS.org, 2025. <http://www.qgis.org>) with the Google Satellite layer accessed September 2025.

area was chosen for sampling due to the frequent monitoring of its benthic communities and currents that incorporate the stream flowing from the gully (Mahabot et al. 2017; Bajjouk et al. 2019; Fari et al. 2022).

Eight stations were selected for sampling north and south of the pass (Figure 1; Tables S1 and S2). Two “fixed” stations were selected as potential representations of faunal assemblages residing on either side of the pass, to determine whether these sites host biological communities similar to stations on the same flat and could therefore be selected as potential long-term monitoring sites. Therefore, the stations selected north of the pass were the fixed station (“Fixed North”), 100 m north of the fixed station (“100 m North”; the GCRMN site Toboggan is approximately 400 m north of this station), and a station at the pass leading out to the open ocean (“Net”). Five stations were chosen on the reef flat south of the pass, including the other fixed station (“Fixed South”), at the edge of a coral bank (“Coral”), 50 m south of the fixed south station (“50 m South”), 100 m south of the fixed south station (“100 m South”) and 400 m south of the fixed south station (“Planch'Alizé”; the GCRMN site Planch'Alizé is approximately 500 m south of this station; Figure 1).

Stations were selected to conduct assessments aimed at (i) examining spatial relationships between coral-reef associated metazoan communities on either side of Hermitage Pass, (ii) determining whether the communities themselves vary over

space and time, and (iii) based on the data, identifying potential locations for longer term monitoring. We hypothesized that communities inhabiting the reef flats would be distinct from one another due to the physical break separating them, and that communities would differ between sampling stations and days collected, in accordance with restricted eDNA dispersal and persistence patterns observed in other coastal marine environments (e.g., Jeunen et al. 2019; Kelly et al. 2018; Murakami et al. 2019).

2.2 | Retrieval of eDNA Using Catamarans as Unmanned Surface Vehicles (USVs)

Unmanned Surface Vehicles (USVs) were constructed in-house and deployed around Hermitage Pass to collect and preserve eDNA through water filtration (Figure 2). Each USV constructed contained a watertight box that sat atop a floating Blue Robotics mini-catamaran base (<https://bluerobotics.com>, St. Torrance, CA, USA). The box housed a Smith-Root® eDNA sampler (ANDe, Vancouver, WA, USA) water pump, which was connected to an array of tubes and solenoid valves controlled by relays to permit or block the flow of water through tubes. These tubes were connected to housing units containing self-preserving 0.45 µm pore polyethersulfone (PES) filter membranes. Housing units have tube extenders which reach < 5 cm below the water surface, allowing the filtration



FIGURE 2 | One of the two in-house built Unmanned Surface Vehicles (USVs) during deployment. The pump and associated equipment are contained within the metal box sitting atop the catamaran base, while the yellow capsules below the white plates are the housing units containing the filter membranes. The housings have tube extenders that reach into the surface of the water to enable filtration. The catamaran base possesses two propellers for locomotion (not shown). Photo credit: Mervyn Ravitchandirane.

of surface water (Figure 2). Only surface sampling was conducted, as a previous eDNA study carried out on Reunion Island's lagoon systems found no significant differences in community composition between surface (< 5 cm) and near-bottom (≤ 2 m) samples (Coward et al. 2024). Once filtering was completed, the filter membranes were preserved through desiccation, whereby the housing units and their tube extenders were removed from the water, yet remained connected to the pump; the pump was then run for approximately 20 s to air dry the membranes prior to storage, in accordance with the manufacturer's instructions. The structural configuration of the USVs prevented water mixing between filter units and stations, as well as backwash into the filter units. Each USV was linked to a graphical interface that enabled remote pump activation and triggering, valve control, video feedback from the pump screen, data streaming from the pump sensors, and controlling the movement of the catamarans.

Water sampling occurred in mid-February 2024 over 4 days, twice in each 24-h period targeting nighttime (dark) and daytime (light) hours, which were identified as "sampling events" (Table S1). Meteorological conditions across all 4 days were stable and mild with light cloud cover night and day, and water and air temperatures varying between 26°C and 32°C. The USVs were transported to the shore, where the pumps were sterilized by feeding the tubes into a basin that contained a 2.6% bleach solution to allow its circulation through the pump. This was followed by a 10-min rinse with distilled water, in accordance with the manufacturer's cleaning protocols. After sterilization, negative control samples were collected by connecting one tube to a filter unit whose extender was placed in a sterile container filled with ultrapure water dispensed within the previous 24 h. A total of 3 L of ultrapure water was filtered for each USV prior to commencing each sampling event on each day.

The two USVs were transported to the water and launched simultaneously to sample northern and southern stations concurrently. USVs first transited to the "fixed" stations for sampling before the other stations. Once all other stations had been sampled, the fixed stations were re-sampled before terminating the sampling event. At each station, the USVs remained stationary to pump triplicate filters (samples), targeting a total volume of 18 L. Once sampling was completed, the filters were carefully removed from the catamarans, dried as described above, then placed into individual resealable sacs and transported to the lab. All filters remained in dark conditions at 23°C until DNA extraction within 6 months of sampling. Throughout the process, several steps were taken to avoid sample cross-contamination, including careful handling of filters using gloves, thorough cleaning of the catamarans and sterilizing reusable supplies with 10% bleach and/or UV irradiation (Coward et al. 2022).

Sampling occurred at high-tide depths of < 1 m at every station, as the collection period coincided with neap tides and anticyclonic conditions that resulted in lower-than-usual water levels. As a minimum of 20 cm of water is needed for the USVs to operate, sample timing was also linked to high tide. Due to these tidal timing constraints, sampling times occasionally extended beyond strict light and dark hours (Table S1), however, "sampling events" were defined to capture broad diel conditions. Environmental measurements (temperature, dissolved oxygen concentration [O_2 mg/L], and oxygen saturation [$O_2\%$]) were recorded manually by team members at each sampling station at the start of pumping, using an OxyGuard® Polaris C meter (Farum, Denmark) deployed alongside the USV.

2.3 | Sample Processing

Samples were shipped to ADNid (Montferrier sur Lez, France) for processing. The extraction of DNA from every filter was done using the DNeasy Blood and Tissue Kit (Qiagen, Germany), as described in Goldberg et al. (2011) and Pope et al. (2020). Following extraction, samples underwent PCR assaying, targeting a 313-bp fragment of the Cytochrome c Oxidase subunit 1 gene (COI) using forward primer mICOIntF 5'-GGWACW GGWTGAACWGTWTAYCCYCC-3', and reverse primer jgHCO2198 5'-TAIACYTCIGGRTGICCRAARAAYC-3' (Leray et al. 2013; Geller et al. 2013). This COI region was chosen for its broad coverage of metazoan taxa, as well as the availability of publicly-accessible databases for sequence identification (Porter and Hajibabaei 2018; Van Der Loos and Nijland 2021). PCR assays were performed in a total volume of 15 μ L containing 1X Type-it Microsatellite PCR master mix (Qiagen), 0.13 μ M of each primer and 2 μ L of DNA. All assay samples were run on an Eppendorf Mastercycler (Germany) under the following slightly adapted conditions: initial denaturation at 95°C for 10 min, 16 cycles of 95°C for 10 s, 62°C (-1°C per cycle) for 30 s and 68°C for 1 min, followed by 25 cycles of 95°C for 10 s, 46°C for 30 s, 68°C for 1 min, and 68°C for 7 min. The primers were modified to include adapters compatible with the Illumina Nextera XT Index Kit v2, using KAPA HiFi HotStart ReadyMix enzyme (Roche, Switzerland) for amplification, enabling samples to be indexed during a second PCR. PCR amplified products were then pooled and purified on AMPure XP magnetic beads (Beckman Coulter,

USA). Sequencing library quality was assessed using Fragment Analyzer (Agilent Technologies) for PCR product profile validation, and qPCR for accurate quantification. All libraries were sequenced 2 × 250-bp paired end reads using Illumina MiSeq V2.

2.4 | Data Processing and Taxonomic Assignments

Raw sequence data obtained from ADNid was processed through the Standardized and Automated MetaBarcoding Analysis pipeline (SAMBA v3.0.1, Cormier et al. 2021). SAMBA performed sample demultiplexing, followed by primer trimming using cutadapt (Martin 2011), as well as read quality visualization and filtering using DADA2 v. 1.26 (Callahan et al. 2016). Reads were then de-replicated and merged, followed by chimera removal prior to generating a table of amplicon sequence variants (ASVs). The QIIME 2 classifier (Bolyen et al. 2019; v. 2020.2) was implemented for assigning taxonomy to the ASV table, first done using the MIDORI2 reference database (Leray et al. 2022). The entire raw dataset was then re-processed through SAMBA using the same parameters, except taxonomic assignment was instead done against the Barcode of Life Data Systems (BOLD v4, Ratnasingham et al. 2024). Thus, from the raw data, two phyloseq objects (McMurdie and Holmes 2013) were generated from two separate SAMBA runs. Taxonomic assignments from both databases were merged by selecting the most resolved classification at each taxonomic rank. When both databases provided assignments, MIDORI classifications were preferentially selected as this database is adapted for eDNA metabarcoding data. Next, a separate workflow was implemented to improve the taxonomic resolution for ASVs that remained unassigned (Figure S1). ASVs that remained unassigned at the family level were subsequently queried against the NCBI mitochondrial (“mito”) database, followed by the nt database (release 269, December 2025) for those remaining unassigned, using blastn. Hits were filtered to remove those of lower quality (<60% qcovs and <200 len). Although a minimum query coverage threshold of 60% was applied, retained assignments generally exceeded this threshold (median query coverage = 87%), indicating that most accepted matches were based on longer alignments. Next, the best hit for each ASV was selected based on the highest bitscore. Taxonomic assignments were subsequently evaluated using identity thresholds (piden ≥ 85% for family, ≥ 92% for genus, ≥ 97% for species). Assignments falling below these thresholds were considered unreliable and were therefore classified as “Unassigned” at the corresponding rank. ASVs remaining unassigned at these ranks were included in community-level analyses; however, ecological interpretations based on taxonomy were restricted to assigned ASVs, and not below the level of “Family.”

2.5 | Data Analyses

The finalized phyloseq object was further processed and analyzed in R (R Core Team 2025), which included examining the sequencing depth and taxonomic composition of all samples (Figure S2). Due to variation in sequencing depths across samples and the dataset's inherent complexity associated with the hyperdiverse habitat, three normalization methods were applied independently to improve sample comparability and enhance the power of the downstream analyses (see Figure S2).

Following the assessment and removal of negative controls (samples < 5000 reads) and experimental samples with fewer than 20,000 reads, the first method, rarefaction without replacement, was used to equalize sequencing depth across all samples for alpha diversity analyses (McMurdie and Holmes 2013; Weiss et al. 2017). To assess sensitivity to sequencing depth thresholds on taxonomic richness calculations, we initially compared datasets for rarefaction at 20,000 and at 30,000 reads; both produced similar quantitative patterns and therefore, the 30,000-read dataset was retained for downstream analyses as it preserved a similar number of samples.

Next, two additional normalization methods were applied to the full dataset: the Hellinger transformation, appropriate for ordination and beta diversity analyses (see Legendre and Gallagher 2001; Laporte et al. 2021), and the trimmed mean of M-values (TMM) normalization, implemented in *edgeR* (Chen et al. 2025), to standardize count distributions across samples for differential sequence abundance testing. A DGEList object was created from the ASV table, followed by filtering ASVs by a mean count per million (CPM) > 0.1, prior to TMM normalization. Each normalized dataset underwent taxonomic filtering in which ASVs assigned to non-metazoans and metazoans endemic to non-marine habitats, were progressively removed (Figure S2). Filtering included the removal of the fully unassigned ASVs, as well as 35 non-metazoans by Kingdom and Phylum (e.g., Bacteria, Fungi, and Viruses). From the resulting metazoan-only datasets, ASVs assigned to taxa that had only terrestrial or freshwater members in the datasets were discarded (e.g., birds, frogs, and earthworms). Prior to removal, taxonomic verification of these groups was aided by the NCBI taxonomy browser and the World Register of Marine Species (WoRMs, Ah Yong 2024). Finally, the seven ASVs that were present in the experimental samples, yet also found in the negative controls after filtering of non-target taxa, were further examined.

2.5.1 | Taxonomic Richness Analyses

Observed and expected ASVs were calculated using the dataset rarefied at 30,000 reads, to test for the assumption of normality for each factor (e.g., Station, Day, and Sampling Event), conducted using the Shapiro–Wilk test. Significant results (*p*-value < 0.05) led to non-parametric Scheirer-Ray-Hare testing implementing BH corrections (Benjamini and Hochberg 1995), followed by post hoc pairwise Dunn's testing across significant factors. ASV richness and sample coverage, based on incidence data, were calculated using the *iNEXT* (iNterpolation and EXTrapolation, Hsieh et al. 2016) package, whereby accumulation curves were generated to explore sampling efforts and trends. As ASV-richness reflects differences at the sequence-level, these calculations were followed by estimating the numbers of assigned families, genera and species, by excluding unassigned ASVs.

2.5.2 | Comparisons of Community Compositions

Community comparisons were initially conducted using both the Hellinger and TMM datasets, which yielded similar results. Ultimately, the Hellinger dataset was selected for ordination of

spatiotemporal metabarcoding data (see Laporte et al. 2021). An upset plot was generated to visualize and compare the number of ASVs for the top 35 intersections among stations with the aid of the *dplyr* (Wickham et al. 2025) and *ggplot2* (Wickham 2016) packages. Intersection sizes were calculated using presence-absence data; relative abundance of sequence reads (unnormalized) for the top most represented metazoan phyla was calculated within each intersection to characterize taxonomic composition. Mantel testing was also conducted (Spearman's rank, 999 permutations) using the *vegan* package (Oksanen et al. 2025) to assess the association between Bray-Curtis community dissimilarities and geographic distances among stations after removing fixed station samples collected at the end of each sampling event.

Canonical Correspondence Analysis (CCA) ordinations were used to explore community dissimilarities, modeling spatial and temporal factors alongside temperature and oxygen measurements, projected through “envfit” arrows. Permutation Analysis of Variance (PERMANOVA) analysis, also based on Bray-Curtis dissimilarities, was conducted using “adonis2” (*vegan*), and “pairwise.adonis2” (Martinez Arbizu 2020) applied with 999 permutations and BH correction. Marginal tests were first carried out to assess the effect of each main factor and interactions, followed by post hoc pairwise comparisons between significant factors (p -value < 0.05). Subsequent examinations of within group variation between main factors and interactions were performed with the aid of betadisper in *vegan*. Finally, distributions of temperature, O₂ concentration, and O₂ saturation values were assessed for normality, before evaluating differences among significant factors through Kruskal–Wallis rank sum testing.

2.5.3 | Examining Patterns of Relative Sequence Abundance

Differential abundance testing was conducted using the TMM dataset to identify taxonomic families contributing to differences between factors, based on the edgeR pipelines for comparisons between groups (Chen et al. 2025). First, a DGEList object was regenerated from the TMM-normalized phyloseq object, followed by conducting dispersion estimations. Next, design matrices were constructed to model relationships between relative sequence abundance and factors, followed by the generation of contrasts. Negative binomial GLM and quasi-likelihood F -test were subsequently implemented for the contrasts. p -values were adjusted for multiple comparisons using the BH method to control the false discovery rate (FDR). The resulting dataset was filtered to retain ASVs with FDR ≤ 0.001 . Venn and chord plots were then generated to visualize patterns among ASVs with confident family-level assignments.

3 | Results

3.1 | Dataset Overview

A total of 256 samples were planned for collection across four sampling days. Due to occasional equipment and filtration issues, such as mechanical obstruction or reduced flow as a result of sediment and algal material accumulated on filters, the

number of samples collected was 207, of which 205 were successfully sequenced. Sample recovery was lower during the first two sampling days, reflecting operational constraints associated with field mobilization and filtration limitations. The metabar-coded dataset consisted of 12,086,990 sequences and 30,852 ASVs, 11% of which were assigned to the level of “Family” using the MIDORI2/BOLD reference databases. The downstream assignment against two additional databases allowed a total of 16.75% of ASVs to be assigned to the family level.

The taxonomic composition of the experimental samples ($n=191$) included 30,816 ASVs assigned to 35 higher taxonomic groups within algae, bacteria, fungi, protists/stramenopiles and viruses, as well as 21 metazoan phyla (Table S3). Metazoans accounted for 32.85% of all ASVs and 62.00% of all sequences. The most prominent metazoans were assigned to Phylum Arthropoda, followed by Mollusca and Cnidaria, in terms of both the number of ASVs and sequences (Figure S3). Lesser represented phyla (“Others”) comprised 0.38% and 0.10% of all metazoan ASVs and sequences, respectively (Figure S3). Fourteen negative control samples produced sequences and contained a total of 104 ASVs consisting of 13,213 sequences, representing 0.33% and 0.11% of all ASVs and sequences, respectively. The ASVs in these controls were assigned to nine known families, most of which were non-target taxa such as fungi and non-marine metazoans (Figure S4). Following non-target taxa filtering, seven ASVs detected in the negative controls also occurred in the experimental samples. A contamination ratio was calculated for each ASV (reads in the controls relative to total reads across the dataset). Only one ASV, matching to an unassigned family, exceeded the contamination threshold of > 5% and was removed from the dataset, while the other ASVs were retained.

Twenty-four samples, including the negative control samples, exhibited low sequencing depth and were removed from the main dataset prior to normalization. This resulted in a dataset consisting of 30,683 ASVs and 181 samples. After normalization through rarefaction at 30,000 sequences, 168 samples and 28,750 ASVs were retained, while the Hellinger normalized dataset consisted of 181 samples and 30,683 ASVs (Table 1). Finally, the TMM normalized dataset consisted of 181 samples and 30,626 ASVs.

3.2 | Taxonomic Richness Was High With Moderate Sample Coverage

Scheirer-Ray-Hare testing identified significant differences among stations for both observed ($H=17.97$; $p=0.035$) and expected richness ($H=18.192$; $p=0.033$), while no other factors were significant. Pairwise post-hoc testing for stations found no significant differences in richness between stations (Figure S5). Accumulation curves displaying ASV richness and sample coverage across the main factors are shown in Figure 3. Steep curves revealed elevated ASV richness, with coverage ranging between 72.70%–76.60% for each sampling event, 58.56%–68.39% for each day, and 44.03%–65.77% for each station. Observed curves continued to rise, indicating that total richness was not yet fully captured, with extrapolated estimates suggesting that plateau could be reached with additional sampling (Figure 3).

TABLE 1 | Normalized dataset variants, including numbers of ASVs and samples retained during each filtering step.

Filtering criteria	Normalized dataset variants					
	Rarefaction at 30 k		Hellinger		TMM	
	# ASVs	# samples	# ASVs	# samples	# ASVs	# samples
Prior to normalization	30,683	181	30,683	181	30,683	181
After normalization	28,750	168	30,683	181	30,626	181
Non-target kingdoms (i.e., bacteria, viruses)	25,708	168	27,350	181	27,303	181
Non-target phyla (e.g., fungal phyla)	9,404	168	9,917	181	9,902	181
Non-target taxa (e.g., non-marine)	6,358	168	6,677	181	6,668	181
Contamination removal	6,357	168	6,676	181	6,667	181

Among metazoan ASVs, only 34.42% were assigned to a known family, 28.00% to a known genus, and 25.15% to a known species (Table S4). The number of assigned families and genera detected was greater during the nighttime sampling event ($n=256$), while both sampling events had an equal number of assigned species detected ($n=377$). With regard to collection day, the highest numbers of assigned families ($n=220$), genera ($n=273$) and species ($n=321$) were detected on Day 3. Finally, station 50m South had the highest number of assigned families ($n=167$) and genera ($n=200$) detected, while the highest number of assigned species was detected at station Coral ($n=223$, Table S4).

3.3 | Unique ASVs Detected Within Each Sampled Station

The upset plot identified station Fixed North–P1 as having the most ASVs detected of the northern stations ($n=1,641$), while 50m South had the most ASVs detected at any single station, north or south ($n=1,689$, Figure 4). For the intersection among northern stations, 44 ASVs were shared, and the relative abundance of reads associated with these shared ASVs was dominated by cnidarians and mollusks (Figure 4, blue dots). Further, the intersection among southern stations consisted of 54 shared ASVs, whose relative read abundance was also dominated by cnidarians and mollusks (Figure 4, orange dots).

3.4 | Community Structure Varied Across Spatial and Temporal Gradients

Visualization of community composition in relation to spatial and temporal factors revealed broad groupings by Sampling Event (“nighttime” and “daytime”), Direction (north or south of Hermitage Pass) and Station, with the first two CCA ordination axes explaining 18.69% of the constrained variation, while additional axes each explained <8% (Figure 5). The observed separation between sampling events remained when samples were examined both north and south of the pass (Figure S6), and on each collection day (Figure S7). Additionally, samples collected from the southern reef flat (Fixed South, 50m South, 100m South, “Planch’Alizé”) formed a tight grouping with station Net, while samples collected from the northern flat (Fixed North and 100m North) clustered with station Coral (Figure 5), although

clustering of samples within stations was distinct when examined by separating directions (Figure S6).

Marginal PERMANOVA testing indicated that community composition varied significantly by Station ($F=1.118$; $p=0.001$) and by the interaction between Day and Sampling Event ($F=1.452$; $p=0.001$), with station explaining a greater proportion of variation in community composition ($R^2=0.095$, Table S5). Subsequent analyses showed that community composition differed significantly between nighttime and daytime samples within each day ($F=2.591$ – 3146 ; $p=0.004$). Pairwise comparisons among stations further indicated significant differences for most station pairs ($F=1.349$ – 3.680 ; $p<0.05$, Figure S8), including a fixed station sampled at both the beginning and end of sampling events. In contrast, several stations located closer to the separation between the flats were not significantly different ($F=0.985$ – 1.266 ; $p>0.05$, Figure S8). When examined separately, communities also differed between northern and southern reef flats ($F=3.212$; $p=0.001$), consistent with the broad spatial clustering observed in the ordination (Figure 5; Table S5). Further, Mantel testing did not detect a significant relationship between geographical distance and community dissimilarity among stations (Mantel $r=0.115$; $p=0.270$), suggesting limited evidence for distance-based structuring. Tests for homogeneity of multivariate dispersion were significant for both Station and the Day $\times$ Sampling Event interaction (permutation tests, $p<0.05$, Table S6), indicating differences in within-group variability. Pairwise comparisons showed few significant differences among stations; however, examination within individual days showed that dispersion differences between sample events were inconsistent (Table S6). These results suggest that differences in community composition are not primarily driven by differences in dispersion among groups.

Environmental measurements represented by “envfit” arrows supported increasing temperatures and O_2 concentrations towards the daytime and southern sampling events (Figure 5), in alignment with higher measurements recorded during these collections (Figure S9). Temperature and O_2 concentration were also significantly associated with variation in community composition ($F=3.831$ – 4.980 ; $p=0.001$, Table S7), indicating that these conditions contributed to community structure. Differences in environmental conditions were also observed across sampling factors. Kruskal–Wallis testing indicated significant variation

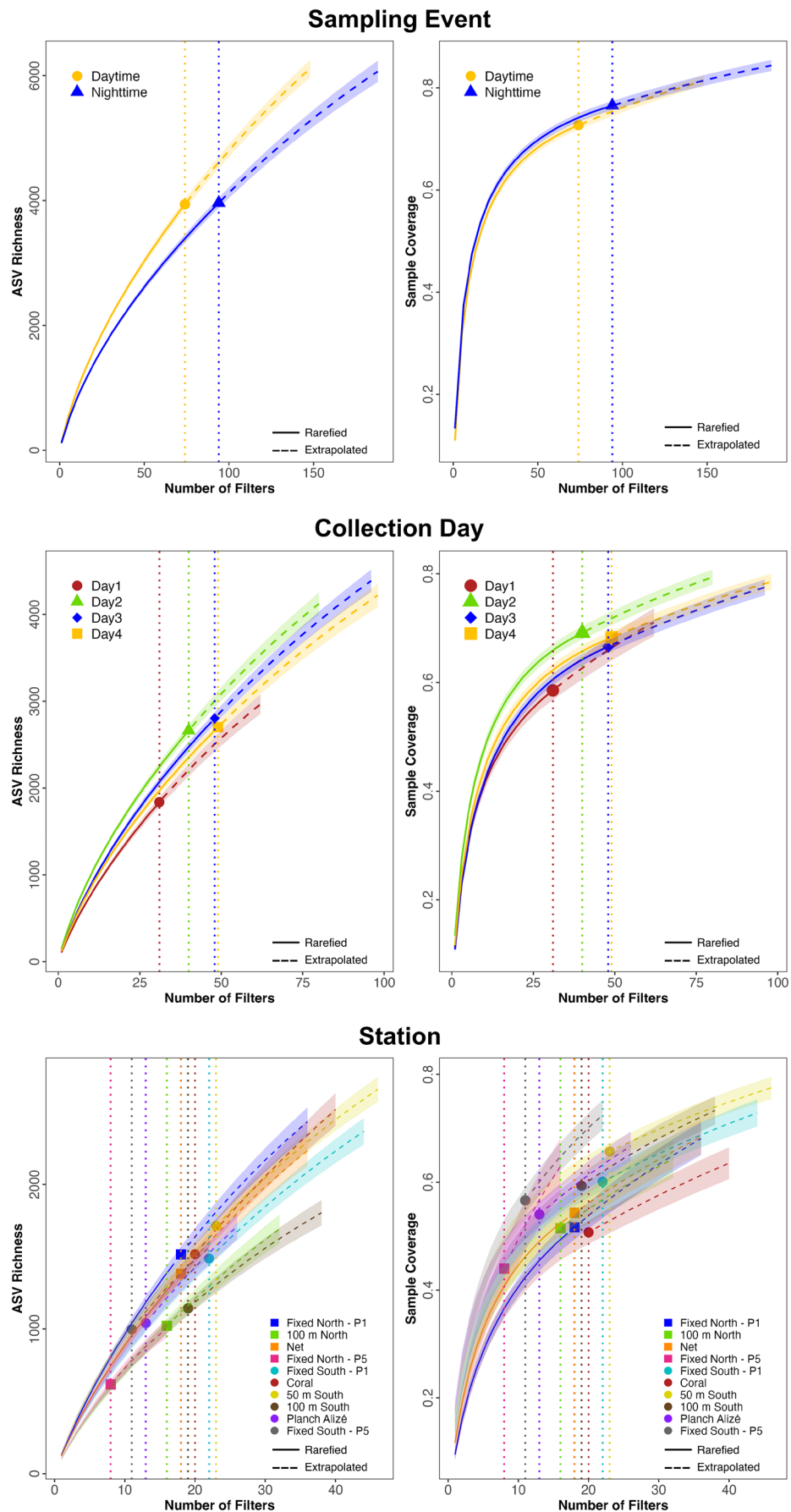


FIGURE 3 | Legend on next page.

FIGURE 3 | Accumulation curves for the number of filters (samples) by ASV richness ($q=0$, left) and sample coverage (right) for Sampling event (top), Collection day (middle) and Station (bottom). Solid curves ending at colored shapes indicate the ASV richness and sample coverage achieved by the number of filters collected, while the dashed curves show the predicted richness and coverage to be potentially obtained through additional filtering (extrapolated). The bottom plots for “Station” denote colored squares for northern stations and circles for southern stations. In every plot, 95% confidence intervals are denoted by shading.

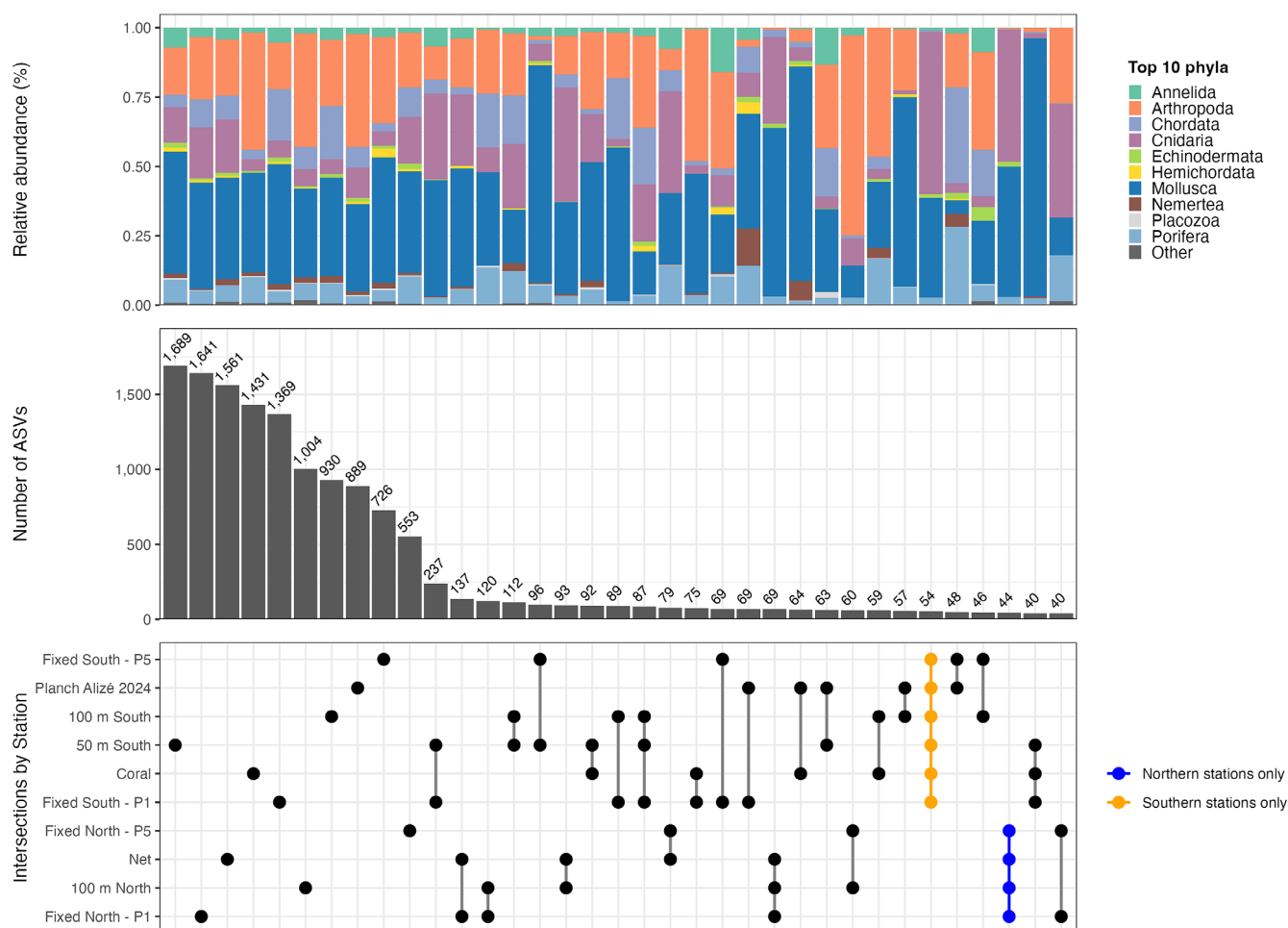


FIGURE 4 | Upset plot for all stations. The bottom panel shows the dot matrix representing the top 35 intersections among stations, defined as the highest number of ASVs. The connected dots indicate shared occurrences. Blue dots denote the intersection among northern stations, while orange dots denote the intersection among southern stations. The middle panel displays the number of ASVs associated with each intersection. The top panel shows the relative abundance of sequence reads (based on unnormalized read counts) assigned to the 10 most represented metazoan phyla for each intersection indicated by the dot matrix.

in temperature among stations and the Day $\times$ Sampling Event interaction ($p < 0.05$), while O_2 concentration and saturation did not differ significantly among stations ($p > 0.05$) but varied when temporal factors were considered ($p < 0.05$, Table S8).

3.5 | Differences in Sampling Event Communities Reflected Shifts in Assigned Taxonomic Groups

The separation between nighttime and daytime samples in ordination space (Figure 5) prompted further examination into the taxonomic differences between these sampling events. Differential sequence abundance analysis identified numerous ASVs associated with daytime and nighttime sampling events. When aggregated at

the assigned family level, these ASVs corresponded to 270 families, many of which were represented in both sampling events. Of these families, a subset ($n=149$) was exclusively associated with either sampling event. To refine understanding of the most represented families detected exclusively during each sampling event, the most relatively abundant were further selected from the 149 families, using a threshold of sequence abundance values expressed as log-CPM ≥ 7 . Of these 49 families remaining, 29 were identified during the nighttime sampling events, matching to assigned families such as Belonidae (Needlefish), Chanida (e.g., milkfish), Tetraliidae (crabs), Pseudocerotidae (flatworms), Euphylliidae and Fungiidae (corals), as well as Thorectidae (sponges) (Figure 6). Further, 20 families were identified as highly relatively abundant across daytime collections, such as Hipponicidae (snails), Calappidae (crabs),

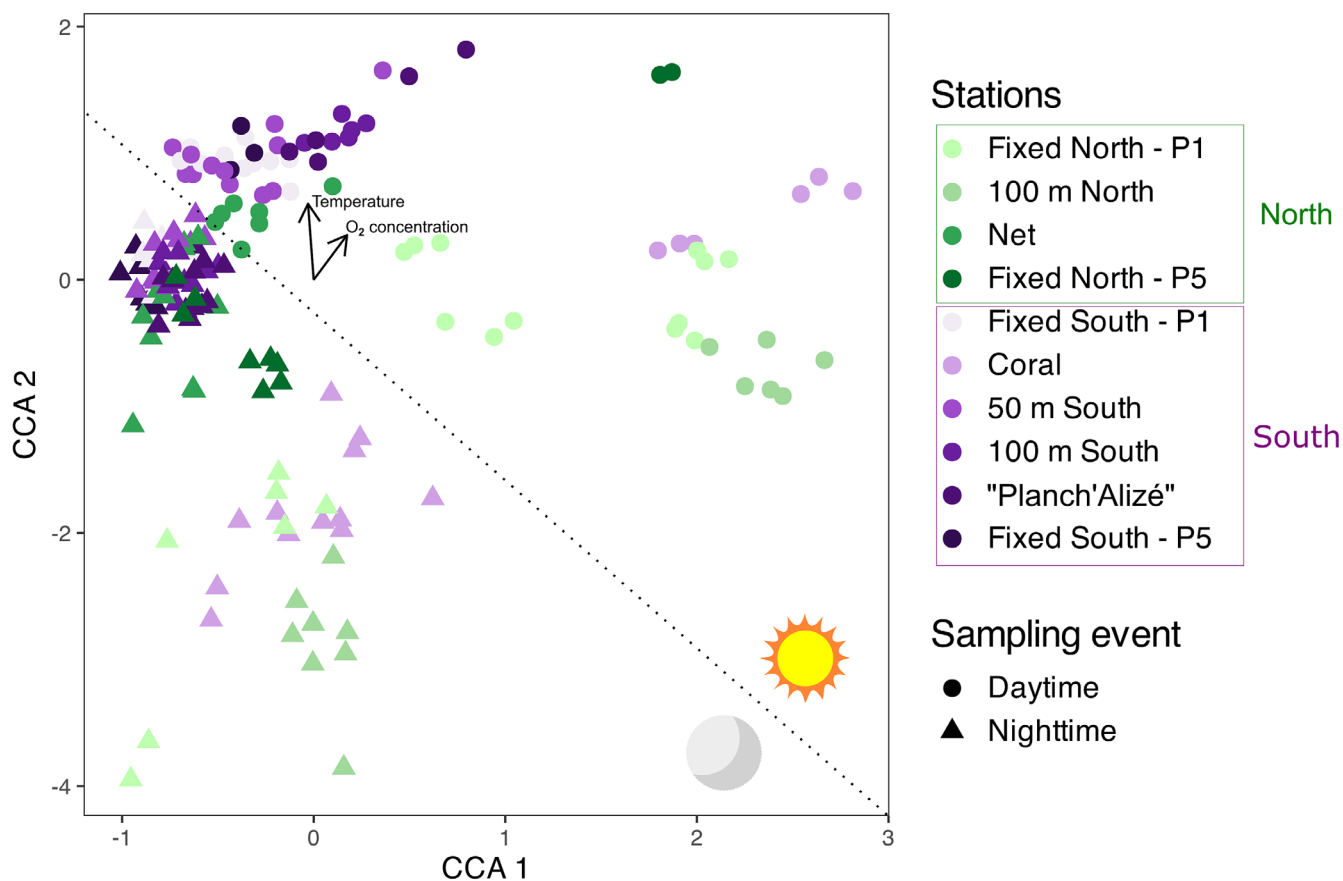


FIGURE 5 | CCA plot modeled as a function of spatial (Station) and temporal (Day and Sampling Event) factors with abiotic measurements placed as “envfit” projection arrows. Only temperature and O_2 concentration were plotted, as O_2 saturation showed high colinearity with O_2 concentration. Each point represents a sample, which is shape coded; stations north of Hermitage Pass are colored green while those south of the Pass are colored purple. The dotted diagonal line was added to emphasize the separation between sampling events.

Alpheidae (shrimp), Actinocyclusidae (sea slugs), and Oceaniidae (hydrozoans) (Figure 6).

To assess patterns across collection days, analyses were also conducted separately for each day using all significant features ($FDR \leq 0.001$), without additional filtering by relative sequence abundance. Across the nighttime events, the number of assigned families represented among differentially abundant ASVs was highest on Day 3 ($n = 46$) and Day 4 ($n = 31$), with 36 families shared across Day 2, Day 3, and Day 4 (Figure S10). Across daytime events, the number of assigned families was highest on Day 3 ($n = 26$) and Day 4 ($n = 29$), followed by Day 2 ($n = 19$). Overlap in differentially abundant families across days showed that relatively few assigned families were consistently associated with either nighttime or daytime sampling events. Two families (Acanthuridae, Trochidae) were significantly more relatively abundant, in terms of sequence representation, in nighttime samples across all 4 days, while 13 families were significantly more relatively abundant in daytime samples across the collection period (Figure S10).

4 | Discussion

We followed the spatiotemporal evolution of faunal communities inhabiting the La Saline–Hermitage coral reef complex distributed across a geographic area of approximately 600 m², through

the collection of eDNA samples obtained by self-constructed and user-friendly USVs. The resulting DNA metabarcoding data underwent rigorous processing and taxonomic assignment, using bioinformatic workflows applied to leverage multiple reference databases for more improved assignments, and to maximize the analytical potential of the dataset. Several patterns emerged from the study data, with one of the most prominent being the differentiation between sampling events on each collection day, suggesting diel shifts in community composition around Hermitage Pass.

4.1 | Broad Spatiotemporal Flux Around Hermitage Pass as Revealed by eDNA Sampling

The La Saline–Hermitage complex has been the subject of numerous surveys, identifying site differences in coral reef community composition and distinct physico-chemical characteristics that shape this ecosystem (Tourrand et al. 2013; Bajjouk et al. 2019; Tedetti et al. 2020). In particular, recent eDNA-based analyses identified distinct communities near the GCRMN sites of Toboggan and Planch'Alizé, separated by 2 km (Cowart et al. 2025). We focused on the area between the two sites to assess the spatial scale at which assemblages begin to diverge (Figure 1). At the finer spatial scale, community composition varied between the stations on the two reef flats, supporting

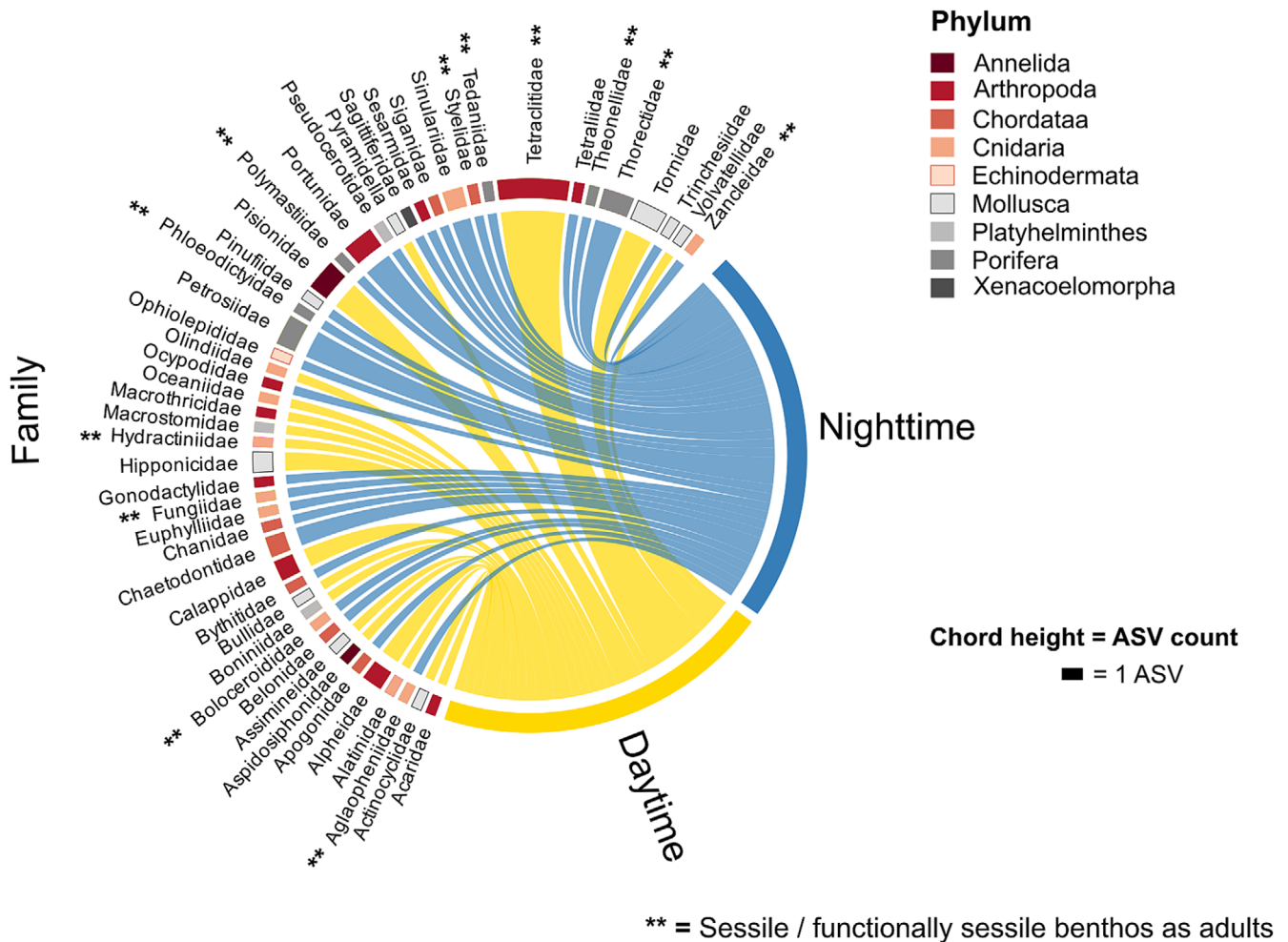


FIGURE 6 | Chord diagram illustrating assigned families ($n=49$) identified as being highly differentially abundant, in terms of sequence representation, between sampling events, at a threshold of ≥ 7 mean $\log_2$ counts-per-million (CpM). These families were represented exclusively by ASVs detected either during the nighttime or daytime collections. Chord widths reflect the number of ASVs assigned to each family, with wider chords indicating multiple ASVs. Families marked with ** denote taxa that are sessile or functionally sessile as adults, as some have planktonic larval stages.

the hypothesis that the physical break between flats contributes to structuring metazoan assemblages (Figure 5; Table S5). However, substantial variation was also observed among the stations within each reef flat, indicating that community composition was not homogeneous at this scale. Notably, the two “fixed” stations, initially selected as representatives of each flat despite being separated by only 35.6 m across Hermitage pass, did not fully capture the range of communities observed across stations on the same flat (Figure S8). In addition to spatial variation, differences in community composition were observed between sampling events within collection days (Table S5). Together, these results indicate that both spatial structure and short-term temporal variability contribute to patterns in eDNA-detected assemblages within the study area, while also highlighting the value of localized, high frequency eDNA sampling to capture these dynamics.

While most samples broadly grouped according to their positioning either north or south of the pass, exceptions were observed. In particular, station Net and station Coral did not consistently cluster with other stations from their respective reef flats; Net samples largely grouped with southern stations,

while Coral samples were most closely associated with northern stations in ordination space (Figure 5). Pairwise statistical comparisons supported similar community compositions between Net and some southern stations (Fixed South and 50 m South), and between Coral and one northern station (Fixed North–P1), although these relationships were not consistent across all comparisons (Figure S8). Notably, Net and Coral remained compositionally distinct from one another, despite their similar benthic composition and close geographic proximity (51.5 m, Table S2). These patterns coincided with differences in environmental conditions at the reef flats, with southern stations and Net exhibiting more distinct separation in temperature, O_2 concentration and O_2 saturation between sampling events, whereas northern stations and Coral showed substantial overlap in these measurements between sampling events (Figure S9). This suggests that environmental conditions were more consistent across sampling events on the northern reef flat and Coral. Overall, the alignment between environmental similarity and ordination patterns indicates that communities at Net and Coral may be influenced by conditions more similar to those on the opposite reef flat. This emphasizes the role of localized environmental conditions combined with habitat heterogeneity near the

boundary between flats, resulting in mixed community signals in this area.

With respect to taxonomic composition, relative sequence read abundances of phyla were broadly consistent among stations (Figure 4, top), suggesting that higher-level taxonomic structure was relatively stable across the study area. In contrast, ASV richness did not differ significantly among stations (Figure S5). Further, samples recovered approximately 44%–65% of the present ASVs across stations, indicating that increasing sampling effort would likely identify additional ASVs and taxa. The moderate coverage may also reflect methodological constraints. First, some samples were not retained due to low sequencing depth, while dataset normalization using a rarefaction threshold further excluded samples (Table 1), suggesting that improved sequencing depth and increasing samples may aid in continued taxonomic characterization at these stations.

4.2 | Diel Variation in Community Composition

One key finding was the difference between communities surveyed between sampling events, a pattern which persisted when examining each day (Figure 5, Table S5). Differences between sampling events are unlikely to be strictly attributable to light versus dark conditions, as sampling events spanned 4 h on average and sometimes overlapped with crepuscular periods (Table S1). On Day 1, for instance, sampling began within the 03h00h and finished within the 08h00h, with sunrise occurring at 06h07. Additionally, the time difference between when one sampling event ended and the next event began (i.e., the period in which no sampling occurred) ranged from 5 to 8 h. This is in accordance with a previous study recording rapid diel changes in tropical eukaryotic communities of the Chagos Archipelago (western Indo-Pacific), over a period as short as 6 h (Dowell et al. 2024). Although samples were grouped into nighttime and daytime sampling events based on study design, some collections occurred near transitions in light conditions and as such, “nighttime” and “daytime” groupings reflect broad diel periods rather than strict light-defined categories. Therefore, observed differences between sampling events likely capture both light-related and short-term temporal variation in eDNA signals. Additionally, there was no identified consistent relationship between sampling time and community similarity. Samples collected within the same hourly window did not necessarily cluster together in ordination space (Figure 5). For instance, samples collected within the same hour on Day 3 were compositionally distinct, while samples collected near transitions in light conditions did not consistently cluster together (Figure 5, Table S1). Altogether, this suggests that timing and light transitions alone did not drive the patterns in community composition.

4.3 | Taxonomic Drivers of Diel Differences

Several assigned metazoan families were found to have relatively higher sequence read abundances either during nighttime or daytime sampling events (Figure 6). Although these analyses highlight taxa contributing most strongly to differences observed between these samples, these results are to be interpreted with caution. Taxonomic assignment remains challenging due

to limitations in genetic reference databases for the island's coral reef-associated species, and therefore, we examined taxonomic patterns at the family level (see Kelly et al. 2018; Dowell et al. 2024), despite ecological interpretations being constrained at this resolution (Bevilacqua et al. 2012). Furthermore, the detection of an assigned family during nighttime or daytime sampling does not necessarily indicate that they are strictly nocturnal, diurnal or crepuscular in activity or lifestyle, but reflects that their eDNA had been released and persisted at the time sampling (Barnes et al. 2014).

Nocturnal communities are of specific interest as organismal activity is more difficult to observe due to low light, resulting in fewer data on nocturnal marine organisms compared to those active during the light hours (Hammerschlag et al. 2017). Among the hundreds of families shared across multiple days in both nighttime and daytime collections, only two families, Acanthuridae and Trochidae, were consistently detected across all four collection days (Figure S10). Although the fish family Acanthuridae (surgeonfishes, tangs and unicornfishes) is primarily composed of tropical coral reef species that are broadly diurnal, some species, such as *Naso unicornis*, sometimes exhibit nocturnal activity, namely commuting and foraging (Meyer and Holland 2005; Collins et al. 2022), which may explain why eDNA assigned to this family was detected during the nighttime sampling events. Further, algal grazing has been observed in at least one species of the family Trochidae (top snails) during dark periods (Evans et al. 2011), though behavioral data for this family and related taxa remain scarce, particularly for those inhabiting the island's lagoons.

Several families such as Calappidae box crabs, Hipponicidae and Tornidae snails, Actinocyclusidae sea slugs (nudibranchs), and Alpheidae snapping shrimp had relatively more abundant sequence reads in samples collected during the daytime sampling events (Figure 6). Calappidae box crabs, such as the species *Calappa hepatica*, have been observed actively foraging, burrowing, and re-emerging during the daytime (Betti and Hoeksema 2024). Further, some members of the Alpheidae shrimp exhibit diel variation in snapping activity, which is associated with behaviors such as burrowing, foraging, and competitive interactions, and is influenced by light conditions (Song et al. 2023). The detection of these taxa within daytime samples may therefore partly reflect daytime biological activity, although detection is also likely influenced by other environmental processes (discussed below).

eDNA matching to sessile or functionally sessile animals, such as sponges (e.g., Thorectidae, Tedaniidae, Phloeodictyidae), were more highly represented in nighttime collections, though barnacles (e.g., Tetraclitidae) were also detected during daytime sampling events (Figure 6). Signals from these sessile organisms suggest that eDNA detection could be due to their presence as well as diel activities influencing DNA release. For instance, certain sponges filter algae during light and dark periods, with clearance efficiency varying according to the algae and the time of day (Varamogianni-Mamatsi et al. 2021), while others can increase pumping and oxygen uptake at night (Moskovich et al. 2023), all of which could account for eDNA being released into the surrounding environment. Similarly, some barnacle species exhibit context-dependent feeding activity, with feeding

rates varying across diel cycles in response to changes in food availability and predation risk (Pasternak and Achituv 2007). Notably, several families assigned to sequences detected in the samples are not currently reported from Reunion Island (e.g., Alatinidae box jellies, Boloceroididae anemones, Pisionidae worms; Global Biodiversity Information Facility [GBIF] 2025, <https://www.gbif.org/>). Reference sequence coverage remains limited for many of the island's taxa (Couëdel et al. 2024), consistent with the wider underrepresentation of tropical species in genetic databases (Huerlimann et al. 2020). As a result, sequences may be assigned to the closest available match (Keck et al. 2023; Mugnai et al. 2023), and some detections may reflect cryptic or misidentified species whose sequences cluster more closely with taxa described elsewhere. Altogether, this highlights the need for customized regional reference databases to enhance the accuracy of eDNA-based biodiversity assessments for the island's coral reef assemblages.

4.4 | Environmental Influences on eDNA Distribution

In the study system, currents move parallel to shore on the northern and southern reef flats, converging at the break between the two flats, where they join the stream flowing from the gully before being funneled through Hermitage Pass towards the fore reef and open ocean (Tedetti et al. 2011; Lagoutte et al. 2024). These hydrodynamic and geomorphological features may influence the movement of eDNA within this reef system. Communities from multiple stations closest to the fore reef and Hermitage pass (e.g., Net and adjacent stations such as Fixed South, 50m South) were not consistently distinct when compared (Figure S8), suggesting localized similarity in communities across shorter spatial scales. In contrast, stations located farther from this boundary (e.g., 100m North, 100m South and "Planch'Alizé") showed more distinct community compositions, with fewer to no non-significant pairwise comparisons (Figure S8). Taken together, these patterns suggest that community similarity may decrease with distance from the reef break and among more isolated stations (i.e., "Planch'Alizé"). While localized water movement may contribute to these patterns, the observed similarity could also reflect relatively homogenous habitat conditions among nearby sites. Overall, the limited spatial dispersal of eDNA signals seen at some stations is consistent with observations in other marine systems (Port et al. 2016; O'Donnell et al. 2017; Jeunen et al. 2019; Kelly et al. 2018; Murakami et al. 2019; Baetscher et al. 2024).

Altogether, these patterns support our initial hypothesis that the physical break at Hermitage Pass contributes to structuring community composition, while also suggesting a potential influence of localized hydrodynamics and environmental variation. For example, features such as the gully stream may help to act as a partial boundary that influences water movement between the flats. In addition, clear diel variability of environmental conditions was observed, with temperatures fluctuating by as much as 5°C within 24-h periods. Southern stations experienced consistently higher daytime temperatures and elevated O₂ concentration and O₂ saturation, likely reflecting greater solar exposure and higher photosynthetic activity at Hermitage (Clavier et al. 2008; Lagoutte et al. 2024). Such patterns typically result

from photosynthetic oxygen production and respiration, water mixing, and elevated metabolic activity under tropical conditions (Nelson and Altieri 2019). Despite the spatial structure observed, Mantel testing did not detect a relationship between geographic distance and community similarity, as has been reported elsewhere (O'Donnell et al. 2017; Dugal et al. 2023). This suggests that community patterns are influenced more by spatial organization and environmental variation than by distance alone.

The distribution of eDNA reflects an interaction between its release, degradation time and transport that is influenced by environmental conditions and water movement (Barnes and Turner 2016). Elevated temperatures and solar exposure are known to accelerate eDNA decay and detectability (Huerlimann et al. 2020; Lamb et al. 2022), particularly in tropical systems, while hydrodynamic conditions can influence its redistribution (Xiong et al. 2025), complicating the interpretation of spatial patterns. In this tropical coral reef system, elevated temperatures, particularly during the daytime sampling events on the southern reef, along with hydrodynamic conditions, may contribute to faster eDNA degradation and reduced dispersal between stations, potentially resulting in largely station-specific signals observed. Observed diel differences further suggest that biological activity likely contributes to temporal variation in eDNA signals, possibly reflecting shifts in organismal presence or behavior between sampling events. More refined experimentation, as well as environmental sampling that includes rigorous measurements of local currents will be essential for resolving the mechanisms shaping eDNA transport and distribution in this area.

These relationships between stations and their positioning suggest three prospective stations for longer term monitoring: one location on the northern flat (e.g., 100m North), one on the southern flat (e.g., 100m South), and another at station Net. On the northern reef flat, Fixed North and 100m North exhibited similar community composition, suggesting that they capture eDNA signals representative of that area. Station 100m North, however, was more compositionally distinct from southern stations and station Net, indicating that it may better represent the northern assemblages with less influence from the pass and the fore reef. On the southern reef flat, Fixed South and 100m South shared similar community composition, but 100m South showed greater dissimilarity from other stations overall, suggesting that it may capture a broader component of southern assemblages farther from the pass. Station Net, located near the fore reef, exhibited similarities with several southern stations, indicating that it may represent a transitional zone where eDNA-based community signals may converge. Altogether, these three stations span separate and transitional zones of the study region, and their combined use for longer term monitoring could provide effective coverage of spatial variability, while aiding the detection of rapid temporal changes in metazoan communities.

4.5 | Insights and Areas for Advancement

eDNA-based surveys, paired with low-cost, easily constructed surface vehicles, offer a valuable approach for monitoring changes in biodiversity at dynamic coastal habitats, particularly

under rapidly changing conditions. Events such as the passage of Cyclone Garance in February 2025 highlight the need for high-frequency sampling approaches that can capture both short-term ecological dynamics and recovery trajectories following extreme weather events, improving our understanding of ecosystem resilience.

The self-constructed USVs proved effective for repeated eDNA sampling across multiple days, operating through the collection period. Nonetheless, all biodiversity survey approaches have inherent limitations in resolution, labor and taxonomic scope. Some constraints were encountered, particularly due to low water levels during February 2024. This required that sampling events were planned around high tides where at least 20 cm of water was available for operation, sometimes shifting timing across dawn and dusk periods. Additionally, shifts in local lagoonal conditions, such as increased sedimentation and algal presence, clogged filters, leading to inability to collect some samples. Filter clogging may be somewhat alleviated by using filters with larger pore sizes (e.g., 1.2 μm), depending on the biological targets of interest. Ultimately, the USVs enabled safe sampling, particularly at night when visibility was low, as well as at station Net, where swimming and boating access is prohibited due to hazardous currents. Moving forward, the USV prototype will likely require more robust mechanical parts for longer-term use, and integrating environmental sensors on these devices would allow automated, precise and consistent measurements of local environmental conditions during sampling. Mounted cameras would additionally pair eDNA surveys with visual surveys, to provide complementary validation of detected fauna.

In the context of continued research questions, a key priority will be to gain a deeper understanding of eDNA transport dynamics, which requires additional study of DNA degradation under tropical environmental conditions, as proposed by Huerlimann et al. (2020). This will help to more accurately link hydrodynamics with eDNA persistence and dispersal around Hermitage Pass. Concurrently, additional steps should focus on identifying specific species driving diel and spatial differences in community composition, to be addressed with simultaneous visual censuses and eDNA surveys, the application of multiple genetic markers, and continued contribution to regional genetic databases.

Author Contributions

Dominique A. Cowart, Anne-Elise Nieblas, Magali Duval, Serge Bernard, and Sylvain Bonhommeau were involved in the conceptualization of the study, while Dominique A. Cowart, Anne-Elise Nieblas, and Sylvain Bonhommeau were responsible for the study design. Jérémie Chanut, Mervyn Ravitchandirane, Victor Illien, and Serge Bernard were responsible for building, maintaining and operating the USVs. All authors were involved in data acquisition, while Dominique A. Cowart and Thomas Chevrier were responsible for data analyses. All authors aided in the interpretation of the data. Dominique A. Cowart wrote the manuscript and all authors provided manuscript revisions and approved the final draft.

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Ethics Statement

The Réserve Naturelle Marine de La Réunion (RNMR) was notified prior to collecting water samples. The RNMR scientific committee (2023-021_DCM 2023-138) was notified prior to beginning the project.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Raw sequence data for this study is available at the European Nucleotide Archive (ENA) at <https://www.ebi.ac.uk/ena/browser/home> under accession PRJEB93966 (study: ERP176845) and can also be found on <https://zenodo.org/> under DOI [10.5281/zenodo.19060142](https://doi.org/10.5281/zenodo.19060142). Custom R scripts used for data processing and analyses are available from the corresponding author, upon reasonable request.

References

- Ahyong, e. a. 2024. "World Register of Marine Species." <https://www.marinespecies.org>.
- Baetscher, D. S., M. R. Pochardt, P. D. Barry, and W. A. Larson. 2024. "Tide Impacts the Dispersion of eDNA From Nearshore Net Pens in a Dynamic High-Latitude Marine Environment." *Environmental DNA* 6, no. 2: e533. <https://doi.org/10.1002/edn3.533>.
- Bajjouk, T., P. Mouquet, M. Ropert, et al. 2019. "Detection of Changes in Shallow Coral Reefs Status: Towards a Spatial Approach Using Hyperspectral and Multispectral Data." *Ecological Indicators* 96: 174–191. <https://doi.org/10.1016/j.ecolind.2018.08.052>.
- Barnes, M. A., and C. R. Turner. 2016. "The Ecology of Environmental DNA and Implications for Conservation Genetics." *Conservation Genetics* 17, no. 1: 1–17. <https://doi.org/10.1007/s10592-015-0775-4>.
- Barnes, M. A., C. R. Turner, C. L. Jerde, M. A. Renshaw, W. L. Chadderton, and D. M. Lodge. 2014. "Environmental Conditions Influence eDNA Persistence in Aquatic Systems." *Environmental Science & Technology* 48, no. 3: 1819–1827. <https://doi.org/10.1021/es404734p>.
- Benjamini, Y., and Y. Hochberg. 1995. "Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing." *Journal of the Royal Statistical Society: Series B: Methodological* 57, no. 1: 289–300. <https://doi.org/10.1111/j.2517-6161.1995.tb02031.x>.
- Betti, F., and B. W. Hoeksema. 2024. "The Box Crab *Calappa hepatica* as a Nuclear Species for the Opportunistic Foraging Behaviour of the Flowery Flounder, *Bothus mancus*, in the Indo-Pacific." *Diversity* 16, no. 11: 662. <https://doi.org/10.3390/d16110662>.
- Bevilacqua, S., A. Terlizzi, J. Claudet, S. Frascchetti, and F. Boero. 2012. "Taxonomic Relatedness Does Not Matter for Species Surrogacy in the Assessment of Community Responses to Environmental Drivers." *Journal of Applied Ecology* 49, no. 2: 357–366. <https://doi.org/10.1111/j.1365-2664.2011.02096.x>.

- Blackman, R., M. Couton, F. Keck, et al. 2024. "Environmental DNA: The Next Chapter." *Molecular Ecology* 33, no. 11: e17355. <https://doi.org/10.1111/mec.17355>.
- Bolyen, E., J. R. Rideout, M. R. Dillon, et al. 2019. "Reproducible, Interactive, Scalable and Extensible Microbiome Data Science Using QIIME 2." *Nature Biotechnology* 37, no. 8: 852–857. <https://doi.org/10.1038/s41587-019-0252-6>.
- Callahan, B. J., P. J. McMurdie, M. J. Rosen, A. W. Han, A. J. A. Johnson, and S. P. Holmes. 2016. "DADA2: High-Resolution Sample Inference From Illumina Amplicon Data." *Nature Methods* 13, no. 7: 581–583. <https://doi.org/10.1038/nmeth.3869>.
- Chen, Y., L. Chen, A. T. Lun, P. L. Baldoni, and G. K. Smyth. 2025. "edgeR v4: Powerful Differential Analysis of Sequencing Data With Expanded Functionality and Improved Support for Small Counts and Larger Datasets." *Nucleic Acids Research* 53, no. 2: gkaf018. <https://doi.org/10.1093/nar/gkaf018>.
- Clavier, J., L. Chauvaud, P. Cuet, et al. 2008. "Diel Variation of Benthic Respiration in a Coral Reef Sediment (Reunion Island, Indian Ocean)." *Estuarine, Coastal and Shelf Science* 76, no. 2: 369–377. <https://doi.org/10.1016/j.ecss.2007.07.028>.
- Collins, W. P., D. R. Bellwood, and R. A. Morais. 2022. "The Role of Nocturnal Fishes on Coral Reefs: A Quantitative Functional Evaluation." *Ecology and Evolution* 12, no. 8: e9249. <https://doi.org/10.1002/ece3.9249>.
- Cormier, A., P. Durand, C. Noel, and L. Leroi. 2021. "SAMBA: Standardized and Automated MetaBarcoding Analyses Workflow." <https://gitlab.ifremer.fr/bioinfo/workflows/samba>.
- Costello, M. J., Z. Basher, L. McLeod, et al. 2017. "Methods for the Study of Marine Biodiversity." In *The GEO Handbook on Biodiversity Observation Networks*, edited by M. Walters and R. J. Scholes, Springer Open. https://doi.org/10.1007/978-3-319-27288-7_6.
- Couëdel, M., A. Dettai, M. M. Guillaume, C. Bonillo, B. Frattini, and J. H. Bruggemann. 2024. "Settlement Patterns and Temporal Successions of Coral Reef Cryptic Communities Affect Diversity Assessments Using Autonomous Reef Monitoring Structures (ARMS)." *Scientific Reports* 14, no. 1: 27,061. <https://doi.org/10.1038/s41598-024-76834-8>.
- Cowart, D. A., T. Chevrier, A. E. Nieblas, J. Chanut, S. Bernard, and S. Bonhommeau. 2024. "Detecting Local Variations Across Metazoan Communities in Back-Reef Depressions of Reunion Island (Mascarene Archipelago) Through Environmental DNA Survey." *Frontiers in Marine Science* 11: 1423676. <https://doi.org/10.3389/fmars.2024.1423676>.
- Cowart, D. A., T. Chevrier, A. E. Nieblas, et al. 2025. "Environmental DNA Data Provides Taxa-Specific Insights Into Community Differences Across Reunion Island Back-Reef Depressions." *Regional Studies in Marine Science* 93: 104642. <https://doi.org/10.1016/j.rsma.2025.104642>.
- Cowart, D. A., K. R. Murphy, and C. H. C. Cheng. 2022. "Environmental DNA From Marine Waters and Substrates: Protocols for Sampling and eDNA Extraction." In *Marine Genomics: Methods and Protocols*, 225–251. Springer US.
- Djurhuus, A., C. J. Closek, R. P. Kelly, et al. 2020. "Environmental DNA Reveals Seasonal Shifts and Potential Interactions in a Marine Community." *Nature Communications* 11, no. 1: 254. <https://doi.org/10.1038/s41467-019-14105-1>.
- Dornelas, M., A. E. Magurran, S. T. Buckland, et al. 2013. "Quantifying Temporal Change in Biodiversity: Challenges and Opportunities." *Proceedings of the Royal Society B: Biological Sciences* 280, no. 1750: 20121931. <https://doi.org/10.1098/rspb.2012.1931>.
- Dowell, R., N. Dunn, C. Head, C. Yesson, J. Williams, and E. Ransome. 2024. "Environmental DNA Captures Diurnal Fluctuations of Surface Eukaryotes on a Tropical Coral Reef." *Environmental DNA* 6, no. 1: e512. <https://doi.org/10.1002/edn3.512>.
- Dugal, L., L. Thomas, A. Meenakshisundaram, et al. 2023. "Distinct Coral Reef Habitat Communities Characterized by Environmental DNA Metabarcoding." *Coral Reefs* 42, no. 1: 17–30. <https://doi.org/10.1007/s00338-022-02301-3>.
- Emslie, M. J., M. Logan, P. Bray, et al. 2024. "Increasing Disturbance Frequency Undermines Coral Reef Recovery." *Ecological Monographs* 94, no. 3: e1619. <https://doi.org/10.1002/ecm.1619>.
- Evans, J., J. A. Borg, and P. J. Schembri. 2011. "Distribution, Habitat Preferences and Behaviour of the Critically Endangered Maltese Top-Shell *Gibbula Nivosa* (Gastropoda: Trochidae)." *Marine Biology* 158, no. 3: 603–611. <https://doi.org/10.1007/s00227-010-1584-4>.
- Fari, C., E. Grondin, C. Treguier, and M. Duval. 2022. "Compilation Des Fiches Masses d'eau DCE de La Réunion: Évaluation "Annuelle" de l'état au 31/12/2021." Fiches par masse d'eau. R.RBE/DOI/2022–021. <https://doi.org/10.13155/90337>.
- Geller, J., C. Meyer, M. Parker, and H. Hawk. 2013. "Redesign of PCR Primers for Mitochondrial Cytochrome c Oxidase Subunit I for Marine Invertebrates and Application in All-Taxa Biotic Surveys." *Molecular Ecology Resources* 13: 851–861. <https://doi.org/10.1111/1755-0998.12138>.
- Global Biodiversity Information Facility (GBIF). 2025. "Occurrence Data for Multiple Taxa." Accessed August 19, 2025. <https://www.gbif.org>.
- Goldberg, C. S., D. S. Pilliod, R. S. Arkle, and L. P. Waits. 2011. "Molecular Detection of Vertebrates in Stream Water: A Demonstration Using Rocky Mountain Tailed Frogs and Idaho Giant Salamanders." *PLoS One* 6, no. 7: e22746. <https://doi.org/10.1371/journal.pone.0022746>.
- Hammerschlag, N., C. G. Meyer, M. S. Grace, et al. 2017. "Shining a Light on Fish at Night: An Overview of Fish and Fisheries in the Dark of Night, and in Deep and Polar Seas." *Bulletin of Marine Science* 93, no. 2: 253–284. <https://doi.org/10.5343/bms.2016.1082>.
- Hatcher, B. G. 1997. "Coral Reef Ecosystems: How Much Greater Is the Whole Than the Sum of the Parts?" *Coral Reefs* 16, no. Suppl 1: S77–S91. <https://doi.org/10.1007/s003380050244>.
- Hsieh, T. C., K. Ma, and A. Chao. 2016. "iNEXT: An R Package for Rarefaction and Extrapolation of Species Diversity (Hill Numbers)." *Methods in Ecology and Evolution* 7, no. 12: 1451–1456. <https://doi.org/10.1111/2041-210X.12613>.
- Huerlimann, R., M. K. Cooper, R. C. Edmunds, et al. 2020. "Enhancing Tropical Conservation and Ecology Research With Aquatic Environmental DNA Methods: An Introduction for Non-Environmental DNA Specialists." *Animal Conservation* 23, no. 6: 632–645. <https://doi.org/10.1111/acv.12583>.
- Hughes, T. P., N. A. Graham, J. B. Jackson, P. J. Mumby, and R. S. Steneck. 2010. "Rising to the Challenge of Sustaining Coral Reef Resilience." *Trends in Ecology & Evolution* 25, no. 11: 633–642. <https://doi.org/10.1016/j.tree.2010.07.011>.
- Jensen, M. R., E. E. Sigsgaard, M. d. P. Ávila, et al. 2022. "Short-Term Temporal Variation of Coastal Marine eDNA." *Environmental DNA* 4, no. 4: 747–762. <https://doi.org/10.1002/edn3.285>.
- Jeunen, G. J., M. Knapp, H. G. Spencer, et al. 2019. "Environmental DNA (eDNA) Metabarcoding Reveals Strong Discrimination Among Diverse Marine Habitats Connected by Water Movement." *Molecular Ecology Resources* 19, no. 2: 426–438. <https://doi.org/10.1111/1755-0998.12982>.
- Keck, F., R. C. Blackman, R. Bossart, et al. 2022. "Meta-Analysis Shows Both Congruence and Complementarity of DNA and eDNA Metabarcoding to Traditional Methods for Biological Community Assessment." *Molecular Ecology* 31, no. 6: 1820–1835. <https://doi.org/10.1111/mec.16364>.
- Keck, F., M. Couton, and F. Altermatt. 2023. "Navigating the Seven Challenges of Taxonomic Reference Databases in Metabarcoding

- Analyses." *Molecular Ecology Resources* 23, no. 4: 742–755. <https://doi.org/10.1111/1755-0998.13746>.
- Kelly, R. P., R. Gallego, and E. Jacobs-Palmer. 2018. "The Effect of Tides on Nearshore Environmental DNA." *PeerJ* 6: e4521. <https://doi.org/10.7717/peerj.4521>.
- Kolasinski, J., S. Nahon, K. Rogers, A. Chauvin, L. Bigot, and P. Frouin. 2016. "Stable Isotopes Reveal Spatial Variability in the Trophic Structure of a Macro-Benthic Invertebrate Community in a Tropical Coral Reef." *Rapid Communications in Mass Spectrometry* 30, no. 3: 433–446. <https://doi.org/10.1002/rcm.7443>.
- Lagoutte, E., A. Tribollet, S. Bureau, et al. 2024. "Biogeochemical Evidence of Flow Re-Entrainment on the Main Fringing Reef of La Reunion Island." *Marine Chemistry* 259: 104,352. <https://doi.org/10.1016/j.marchem.2024.104352>.
- Lamb, P. D., V. G. Fonseca, D. L. Maxwell, and C. C. Nnanatu. 2022. "Systematic Review and Meta-Analysis: Water Type and Temperature Affect Environmental DNA Decay." *Molecular Ecology Resources* 22, no. 7: 2494–2505. <https://doi.org/10.1111/1755-0998.13627>.
- Laporte, M., E. Reny-Nolin, V. Chouinard, et al. 2021. "Proper Environmental DNA Metabarcoding Data Transformation Reveals Temporal Stability of Fish Communities in a Dendritic River System." *Environmental DNA* 3, no. 5: 1007–1022. <https://doi.org/10.1002/edn3.224>.
- Legendre, P., and E. D. Gallagher. 2001. "Ecologically Meaningful Transformations for Ordination of Species Data." *Oecologia* 129, no. 2: 271–280. <https://doi.org/10.1007/s004420100716>.
- Leray, M., N. Knowlton, and R. J. Machida. 2022. "MIDORI2: A Collection of Quality Controlled, Preformatted, and Regularly Updated Reference Databases for Taxonomic Assignment of Eukaryotic Mitochondrial Sequences." *Environmental DNA* 4, no. 4: 894–907. <https://doi.org/10.1002/edn3.303>.
- Leray, M., J. Y. Yang, C. P. Meyer, et al. 2013. "A New Versatile Primer Set Targeting a Short Fragment of the Mitochondrial COI Region for Metabarcoding Metazoan Diversity: Application for Characterizing Coral Reef Fish Gut Contents." *Frontiers in Zoology* 10: 34. <https://doi.org/10.1186/1742-9994-10-34>.
- Mahabot, M. M., M. Jaud, G. Pennober, et al. 2017. "The Basics for a Permanent Observatory of Shoreline Evolution in Tropical Environments; Lessons From Back-Reef Beaches in La Reunion Island." *Comptes Rendus Geoscience* 349, no. 6–7: 330–340. <https://doi.org/10.1016/j.crte.2017.09.010>.
- Martin, M. 2011. "Cutadapt Removes Adapter Sequences From High-Throughput Sequencing Reads." *EMBnet. Journal* 17, no. 1: 10–12. <https://doi.org/10.14806/ej.17.1.200>.
- Martinez Arbizu, P. 2020. "pairwiseAdonis: Pairwise Multilevel Comparison Using Adonis (R Package Version 0.4)." <https://github.com/pmartinezarbizu/pairwiseAdonis>.
- McMurdie, P. J., and S. Holmes. 2013. "Phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data." *PLoS One* 8, no. 4: e61217. <https://doi.org/10.1371/journal.pone.0061217>.
- Meyer, C. G., and K. N. Holland. 2005. "Movement Patterns, Home Range Size and Habitat Utilization of the Bluespine Unicornfish, *Naso unicornis* (Acanthuridae) in a Hawaiian Marine Reserve." *Environmental Biology of Fishes* 73, no. 2: 201–210. <https://doi.org/10.1007/s10641-005-0559-7>.
- Morais, J., S. B. Tebbett, R. A. Morais, and D. R. Bellwood. 2024. "Natural Recovery of Corals After Severe Disturbance." *Ecology Letters* 27, no. 1: e14332. <https://doi.org/10.1111/ele.14332>.
- Moskovich, R., R. Diga, M. Ilan, and G. Yahel. 2023. "High Variability and Enhanced Nocturnal Oxygen Uptake in Coral Reef Sponges." *Limnology and Oceanography* 68, no. 7: 1517–1529. <https://doi.org/10.1002/lno.12361>.
- Muenzel, D., A. Bani, M. De Brauwier, et al. 2024. "Combining Environmental DNA and Visual Surveys Can Inform Conservation Planning for Coral Reefs." *Proceedings of the National Academy of Sciences of the United States of America* 121, no. 17: e2307214121. <https://doi.org/10.1073/pnas.2307214121>.
- Mugnai, F., F. Costantini, A. Chenuil, M. Leduc, J. M. Gutiérrez Ortega, and E. Megléc. 2023. "Be Positive: Customized Reference Databases and New, Local Barcodes Balance False Taxonomic Assignments in Metabarcoding Studies." *PeerJ* 11: e14616. <https://doi.org/10.7717/peerj.14616>.
- Murakami, H., S. Yoon, A. Kasai, et al. 2019. "Dispersion and Degradation of Environmental DNA From Caged Fish in a Marine Environment." *Fisheries Science* 85, no. 2: 327–337. <https://doi.org/10.1007/s12562-018-1282-6>.
- Nelson, H. R., and A. H. Altieri. 2019. "Oxygen: The Universal Currency on Coral Reefs." *Coral Reefs* 38, no. 2: 177–198. <https://doi.org/10.1007/s00338-019-01765-0>.
- O'Donnell, J. L., R. P. Kelly, A. O. Shelton, J. F. Samhour, N. C. Lowell, and G. D. Williams. 2017. "Spatial Distribution of Environmental DNA in a Nearshore Marine Habitat." *PeerJ* 5: e3044. <https://doi.org/10.7717/peerj.3044>.
- Oksanen, J., G. Simpson, F. Blanchet, et al. 2025. "Vegan: Community Ecology Package." R Package Version 2.6–10. <https://CRAN.R-project.org/package=vegan>.
- Osland, M. J., J. B. Bradford, L. T. Toth, et al. 2025. "Ecological Thresholds and Transformations due to Climate Change: The Role of Abiotic Stress." *Ecosphere* 16, no. 4: e70229. <https://doi.org/10.1002/ecs2.70229>.
- Pasternak, Z., and Y. Achituv. 2007. "Feeding Behavior of Shallow-Water Barnacles From the Mediterranean and Red Seas." *Journal of Crustacean Biology* 27, no. 4: 543–547. <https://doi.org/10.1651/S-2785.1>.
- Pope, K. L., C. S. Goldberg, N. L. Nelson, A. K. Cummings, T. Seaborn, and J. Piovio-Scott. 2020. "Designing Environmental DNA Surveys in Complex Aquatic Systems: Backpack Sampling for Rare Amphibians in Sierra Nevada Meadows." *Aquatic Conservation: Marine and Freshwater Ecosystems* 30, no. 10: 1975–1987. <https://doi.org/10.1002/aqc.3444>.
- Port, J. A., J. L. O'Donnell, O. C. Romero-Maraccini, et al. 2016. "Assessing Vertebrate Biodiversity in a Kelp Forest Ecosystem Using Environmental DNA." *Molecular Ecology* 25, no. 2: 527–541. <https://doi.org/10.1111/mec.13481>.
- Porter, T. M., and M. Hajibabaei. 2018. "Over 2.5 Million COI Sequences in GenBank and Growing." *PLoS One* 13, no. 9: e0200177. <https://doi.org/10.1371/journal.pone.0200177>.
- R Core Team. 2025. "A Language and Environment for Statistical Computing." R Foundation for Statistical Computing.
- Ratnasingham, S., C. Wei, D. Chan, et al. 2024. "BOLD v4: A Centralized Bioinformatics Platform for DNA-Based Biodiversity Data." In *DNA Barcoding: Methods and Protocols*, 403–441. Springer US. https://doi.org/10.1007/978-1-0716-3581-0_26.
- Rungassamy, T., B. Cauvin, and L. Maurel. 2024. "Benthic Categories Analyzed by the Photoquadrat/CPCe Method—Impact of the Marine Reserve of Reunion Island (RNMR) on Biodiversity." *RNMR (Réserve Naturelle Marine de la Réunion), UMR Entropie, Kart'eau*. SEANO. <https://doi.org/10.17882/99788>.
- Sala, E., and N. Knowlton. 2006. "Global Marine Biodiversity Trends." *Annual Review of Environment and Resources* 31, no. 2006: 93–122. <https://doi.org/10.1146/annurev.energy.31.020105.100235>.
- Sevellec, M., A. Lacoursière-Roussel, L. Bernatchez, et al. 2021. "Detecting Community Change in Arctic Marine Ecosystems Using the Temporal Dynamics of Environmental DNA." *Environmental DNA* 3, no. 3: 573–590. <https://doi.org/10.1002/edn3.155>.

- Song, Z., W. Ou, Y. Su, et al. 2023. "Sounds of Snapping Shrimp (Alpheidae) as Important Input to the Soundscape in the Southeast China Coastal Sea." *Frontiers in Marine Science* 10: 1,029,003. <https://doi.org/10.3389/fmars.2023.1029003>.
- Tedetti, M., L. Bigot, J. Turquet, et al. 2020. "Influence of Freshwater Discharges on Biogeochemistry and Benthic Communities of a Coral Reef Ecosystem (La Réunion Island, Indian Ocean)." *Frontiers in Marine Science* 7: 596,165. <https://doi.org/10.3389/fmars.2020.596165>.
- Tedetti, M., P. Cuet, C. Guigue, and M. Goutx. 2011. "Characterization of Dissolved Organic Matter in a Coral Reef Ecosystem Subjected to Anthropogenic Pressures (La Réunion Island, Indian Ocean) Using Multi-Dimensional Fluorescence Spectroscopy." *Science of the Total Environment* 409, no. 11: 2198–2210. <https://doi.org/10.1016/j.scitotenv.2011.01.058>.
- Thébaud, C., B. H. Warren, D. Strasberg, and A. Cheke. 2009. "Mascarene Islands, Biology." *Atoll Research Bulletin* 127: 1–216.
- Tourrand, C., O. Naim, L. Bigot, et al. 2013. "Fringing Reefs of Reunion Island and Eutrophication Effects-Part 1: Long-Term Monitoring of Two Shallow Coral Reef Communities." *Atoll Research Bulletin* 596: 1–35. <https://doi.org/10.5479/si.00775630.596>.
- Van Der Loos, L. M., and R. Nijland. 2021. "Biases in Bulk: DNA Metabarcoding of Marine Communities and the Methodology Involved." *Molecular Ecology* 30, no. 13: 3270–3288. <https://doi.org/10.1111/mec.15592>.
- Varamogianni-Mamatsi, D., T. I. Anastasiou, E. Vernadou, et al. 2021. "A Multi-Species Investigation of Sponges' Filtering Activity Towards Marine Microalgae." *Marine Drugs* 20, no. 1: 24. <https://doi.org/10.3390/md20010024>.
- Weiss, S., Z. Z. Xu, S. Peddada, et al. 2017. "Normalization and Microbial Differential Abundance Strategies Depend Upon Data Characteristics." *Microbiome* 5: 1–18. <https://doi.org/10.1186/s40168-017-0237-y>.
- Wickham, H. 2016. *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York. <https://ggplot2.tidyverse.org>.
- Wickham, H., R. François, L. Henry, K. Müller, and D. Vaughan. 2025. "Dplyr: A Grammar of Data Manipulation." R Package Version 1.2.1. <https://dplyr.tidyverse.org>.
- Xiong, J., P. MacCready, E. Brasseale, et al. 2025. "Advective Transport Drives Environmental Dna Dispersal in an Estuary." *Environmental Science & Technology* 59, no. 15: 7506–7516. <https://doi.org/10.1021/acs.est.5c01286>.
- Yamahara, K. M., E. A. Allan, J. Robidart, et al. 2025. "A State-Of-The-Art Review of Aquatic eDNA Sampling Technologies and Instrumentation: Advancements, Challenges, and Future Prospects." *Environmental DNA* 7, no. 4: e70170. <https://doi.org/10.1002/edn3.70170>.

Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** Taxonomic assignment workflow for Amplicon Sequence Variants (ASVs) following DADA2 processing in the SAMBA workflow. Green boxes denote the number of ASVs assigned using reference databases (yellow boxes), while number of ASVs in the red boxes denote the number remaining unassigned. **Figure S2:** Post SAMBA workflow for data processing and statistical analyses. **Figure S3:** Proportion of ASVs (left) and sequences (right) assigned to the 15 most prominent metazoan phyla. "Others" included Chaetognatha, Ctenophora, Entoprocta, Nematoda, Priapulida, Tardigrada, and Xenacoelomorpha. **Figure S4:** Relative abundance of sequences assigned to 104 ASVs detected across 14 negative field controls. Taxonomic labels are reported at the family level for ASVs with $\geq 85\%$ sequence identity, while those not meeting this threshold are classified as "Unassigned". **Figure S5:** Observed (top) and expected (Chao1, bottom) ASV richness by Station. Results from Scheirer-Ray-Hare testing were significant ($p < 0.05$) for Station, while post-hoc Dunn

comparisons were not ($p > 0.05$). **Figure S6:** CCA plot by Direction (North and South) modeled as a function of spatial (Station), temporal (Day and Sampling event) and abiotic (Temperature and O_2 concentration) factors, for the Hellinger transformed dataset. O_2 saturation showed high colinearity with O_2 concentration and was therefore not included. Each point represents a sample, which are color and shape coded. **Figure S7:** CCA plot by Day modeled as a function of spatial (Station), temporal (Day and Sampling event) and abiotic (Temperature and O_2 concentration) factors, for the Hellinger transformed dataset. O_2 saturation showed high colinearity with O_2 concentration and was therefore not included. Each point represents a sample, which are color and shape coded. **Figure S8:** Grid of pairwise PERMANOVA results between each Station. Blue cells indicate BH corrected p -values below the significance threshold of ≤ 0.05 , while light grey cells indicate non-significant p -values. F -values are shown in each cell. **Figure S9:** Plots of (A) temperature ($^{\circ}C$), (B) O_2 concentration (mg/L) and (C) O_2 saturation (%) by Station, for each Sampling event. Station names on the x-axis were organized by their broad groupings north or south of Hermitage Pass based on CCA ordination analyses. **Figure S10:** Diagrams illustrating numbers and percentages of families assigned to the ASVs identified as significantly abundant during the nighttime (top) and daytime (bottom) sampling events, on each collection day. The color gradient intensity illustrates the count of families across sampling days. Families not meeting the $\geq 85\%$ sequence identity threshold are classified as "Unassigned" and were not included in the counts. **Table S1:** Metadata for all samples collected. Stations are organized by their location north or south of Hermitage Pass. Latitude and longitude are reported in decimal degrees, and times are in hh:mm format. The "Time" column reports sampling time in hourly bins. **Table S2:** Pairwise distance matrix for sampled stations, in meters, and observed benthic composition. **Table S3:** List of broad taxonomic groups and associated number of ASVs and number of sequences. Grouping refers to whether the taxa is metazoan or non-metazoan. **Table S4:** Numbers of assigned metazoan families, genera and species by station, collection day and sampling event. "Percent ASVs assigned" refers to the percentage of total ASVs assigned at each respective taxonomic level. **Table S5:** Multivariate testing strategy and results. PERMANOVA tests were conducted to identify whether community composition differed among spatial (station) and temporal (day, sampling event) factors. **Table S6:** Permutation test results (at 999 permutations) for differences between groups (Station and Day $\times$ Sampling_event interaction), based on multivariate homogeneity of groups dispersions assessed using betadisper on Bray–Curtis dissimilarity distances. **Table S7:** Multivariate testing strategy and results. PERMANOVA tests were conducted to assess whether variation in community composition was associated with abiotic variables (temperature, oxygen concentration). **Table S8:** Kruskal–Wallis Rank Sum test results for examining temperature and oxygen measurement differences in the distributions among factors.