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Active Versus Passive eDNA Sampling Across Depths at a Tropical Fish-Aggregating Device (FAD)

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ABSTRACT

Environmental DNA (eDNA) is a valuable tool for monitoring fish biodiversity, particularly in pelagic environments, where conventional methods are difficult to implement. Fish aggregating devices (FADs), floating structures widely used by tropical tuna fishers, are known to attract numerous pelagic fish species, making them ideal sampling locations. In this study, we assessed pelagic fish diversity at a FAD off the coast of Bali, Indonesia, using eDNA metabarcoding. We compared different eDNA sampling strategies with the primary aim of evaluating the effectiveness of eDNA metabarcoding for assessing pelagic fish biodiversity, while testing and refining accessible, low-cost protocols suitable for remote and logistically constrained environments. Sampling was conducted over three consecutive days at four depths (1, 10, 40, and 60 m) using two distinct eDNA collection methods: active filtration and a custom-designed passive system consisting of 3D-printed cylinders filled with sterile gauze, mounted on unit holders that allow the simultaneous deployment of triplicate samples at each depth. A total of 66 samples were collected, and metabarcoding was performed using an available primer pair targeting fish 12S mitochondrial DNA, with sequencing performed on an Illumina NovaSeq platform. Across both sampling methods, a total of 39 fish Operational Taxonomic Units (OTUs) were detected, of which 25 were shared between active and passive approaches, and 31 were assigned to the species level. The two sampling methods yield overlapping assemblages dominated by epipelagic taxa commonly reported at FADs, indicating that both approaches are suitable for characterizing fish communities in this environment. Importantly, the integration of passive and active sampling provided a practical balance between deployment flexibility and taxonomic detection. Beyond technical validation, standardized eDNA protocols can be effectively transferred and applied in contexts where logistical constraints are extremely relevant, thereby supporting the development of biodiversity monitoring programs centered around ecologically relevant features such as FADs.

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1 | Introduction

It has long been known that pelagic fish are naturally attracted to floating objects in the open ocean (Hunter and Mitchell 1967; Dempster and Taquet 2004; Fréon and Dagorn 2000; Castro et al. 2002; Rountree 1989). Building on this natural behavior, fishers have developed fish aggregating devices (FADs), such as rafts or buoys deployed to attract pelagic fish species such as tropical tuna. These devices, which can be either anchored or drifting, act as artificial aggregation points in the open ocean, concentrating a variety of pelagic organisms and facilitating harvesting operations (Taquet 2013; Fonteneau et al. 2000). FADs have become particularly common in Southeast Asia, where they are deployed by both artisanal and industrial fisheries to increase catch efficiency (Jani 2020; Hargiyatno et al. 2025).

Beyond their economic role, FADs can also serve as effective platforms for monitoring pelagic fish species, which is particularly valuable in data-poor regions (Moreno et al. 2016; Brehmer et al. 2019). The communities that form around FADs are often diverse and dynamic, influenced by environmental conditions, depth and diel cycles (Doray et al. 2007; Forget et al. 2015; Schneider 2023). Despite growing interest, monitoring the fish communities associated with FADs remains challenging due to the inherent difficulty of accessing the pelagic environment. This highlights the need for alternative, adaptable and accessible monitoring tools that can be deployed across varying depths and operational contexts. To date, our understanding of fish communities associated with FADs has relied almost exclusively on ecological methods based on underwater visual census (Taquet, Sancho, et al. 2007; Gaertner et al. 2008; Forget et al. 2020), underwater videos (Doray et al. 2007; Schneider 2023), echosounders (Doray et al. 2007; Baidai et al. 2024; Mannocci et al. 2021), telemetry (Dagorn et al. 2007; Forget et al. 2015; Tolotti et al. 2020), and catch surveys (Mannocci et al. 2020; Lezama-Ochoa et al. 2017; Mbaru et al. 2018). These approaches have provided valuable insights into community structure and fish behavior. However, such techniques generally present limitations related to depth coverage, species detectability, gear selectivity and logistical constraints.

Metabarcoding of environmental DNA (eDNA) samples has emerged as a powerful tool for monitoring marine biodiversity (Thomsen et al. 2012; Stat et al. 2017; Aglieri et al. 2021), including offshore and pelagic environments (Li et al. 2022; Suter et al. 2021; Dan et al. 2024). Studies across tropical seas have shown that eDNA consistently detects more taxa than traditional methods and can reveal vertical patterns of biodiversity extending from the surface to deep waters (Feng et al. 2022; Monuki et al. 2021).

Given that FADs function as aggregation points for a wide range of taxa, integrating eDNA metabarcoding into their study could significantly expand our understanding of the biodiversity that they support and, more widely, of pelagic fish communities' ecology. However, despite the ecological importance of FADs, to our best knowledge no studies have applied eDNA metabarcoding to directly investigate biodiversity around these structures. This gap reflects the logistical complexity and financial constraints of conducting surveys at offshore FAD locations. In this context, passive eDNA sampling offers a particularly promising alternative (Maiello et al. 2022, 2025), simplifying deployment, reducing costs, and enabling broader spatial replication while maintaining biodiversity detection levels comparable to active sampling approaches (Bessey et al. 2021; Chen et al. 2024). Passive eDNA collection, in fact, uses simpler equipment that allows longer deployments and facilitates sampling in remote or logistically complex environments (Jager et al. 2025).

Comparative analyses of passive and active eDNA metabarcoding have shown that both approaches are generally effective in capturing aquatic biodiversity, but their performance can vary depending on the context and taxa (Cananzi et al. 2025). In fact, while several studies report comparable species richness between methods, particularly for fish communities in both freshwater and marine systems (Chen et al. 2024; Mlinarec et al. 2025; Zhang et al. 2023), passive methods have demonstrated advantages in detecting rare or low-abundance taxa, such as rare mammals (Chen et al. 2024). Other studies highlight that passive collection can yield a higher diversity of amplicon sequence variants (Saltonstall et al. 2024) or broader taxonomic coverage (Jeunen et al. 2024), despite occasional reductions in DNA yield or increased variability (van der Heyde et al. 2023; Jeunen et al. 2022). Active methods, on the other hand, were shown to outperform both passive eDNA and fyke nets in detecting an invasive freshwater fish, with higher detection probabilities, fewer samples required, and greater cost-effectiveness (Morris et al. 2024). These findings suggest that neither approach is superior in all contexts, and the choice between passive and active eDNA should be guided by research goals, target taxa, environmental conditions, and logistical constraints.

In this context, our study investigates the combined use of eDNA metabarcoding to investigate tropical pelagic biodiversity nearby FADs. Specifically, we combined active and passive eDNA sampling methods, focusing on a FAD located in Gondol Bay, Bali, Indonesia. The work was carried out as part of the European-funded MOOBYF project (Monitoring the Open-Ocean BiodiversitY with Fishers), which aims to generate new knowledge on tropical pelagic fish biodiversity by using FADs as monitoring platforms in collaboration with fishers. Accordingly, we pursued three main objectives: (1) to evaluate the feasibility of applying eDNA metabarcoding to study fish assemblages associated with FADs in a tropical offshore environment; (2) to compare fish species richness and composition obtained using active water filtration and custom-designed passive samplers; and (3) to assess how sampling depth influences species detection and overall biodiversity patterns. These objectives are intended to improve the implementation of eDNA approaches in tropical offshore settings, where access and resources are often limited.

2 | Materials and Methods

2.1 | Study Site and Sampling Design

This study was conducted at an experimental FAD located in Gondol Bay, Bali, Indonesia (−8.1012°, 114.7397°; Figure 1).

The FAD consisted in a floating structure (~3 × 2 m size), made of layers of bamboos anchored at the seafloor at approximately

FIGURE 1 | (A) Map of the sampling location in Gondol Bay (northwestern Bali, Indonesia), where the FAD is located (red dot). (B) Fish Aggregating Device (FAD) sampled during the study.

700 m depth, as indicated by local fishers, which is consistent with a >600 m depth detected with scientific echosounder. During the fieldwork activities, the FAD was accessed using a dedicated boat provided by BRIN (Badan Riset dan Inovasi Nasional), the National Research and Innovation Agency of Indonesia. eDNA samples were collected over three consecutive days in August 2024 using two sampling methods: active filtration and passive collection using custom-designed GenoCartridge devices (Acqua proget Ravenna). All samples, both active and passive, were collected in proximity of the same FAD (downcurrent, around 10 m from the floating structure) between 10 AM and 2 PM. Details of each sampling method can be found below.

2.2 | Filtering Water Samples (Active eDNA)

Active eDNA sampling was performed at two depths, the surface (1 m) and mid-water (10 m), with the latter sampled using a Niskin bottle. These two depths were selected to ensure manageable filtration times and feasible water collection under offshore conditions. For each of the three sampling days, five replicates were collected at each depth, for a total of 10 samples per day and 30 active water samples overall. After collection, water samples were transferred into sterile 4 L plastic sampling bags (Twirl'em) and stored in a cool box with ice packs for transport. Samples were then taken to the BRIN laboratories, located approximately 20 min by boat from the sampling site, and filtered within 2–3 h of collection. Filtration was performed using the "Macchinetta", a low-cost home-made portable vacuum pump (Figure 2; protocol details in Section S1), and sterile 0.45 μm PES (polyethersulfone) self-preserving filters (Smith-Root), with an estimated stability of up to six months (www.smith-root.com). For practical reasons, the maximum filtration time was set to 20 min, and the maximum volume to 3.9 L, based on the capacity of the vacuum bottle. This setup allowed for the filtration of 2.6–3.9 L of seawater per sample. One field blank consisting of distilled water was filtered each day at the BRIN laboratory alongside the samples, resulting in three field blanks overall. After filtration, all filters were stored away from heat and direct light and shipped to MARBEC Labs IRD-France. The samples were then sent to the Biology Department of the University of Padua (Italy), where they were analyzed within one month of being collected.

FIGURE 2 | Home-made portable filtration device connected via tubing to a vacuum trap and a filter holder (Smith-Root).

2.3 | GenoCartridge Deployment (Passive eDNA)

Passive eDNA sampling was performed using custom-designed GenoCartridges, consisting of a 3D-printed

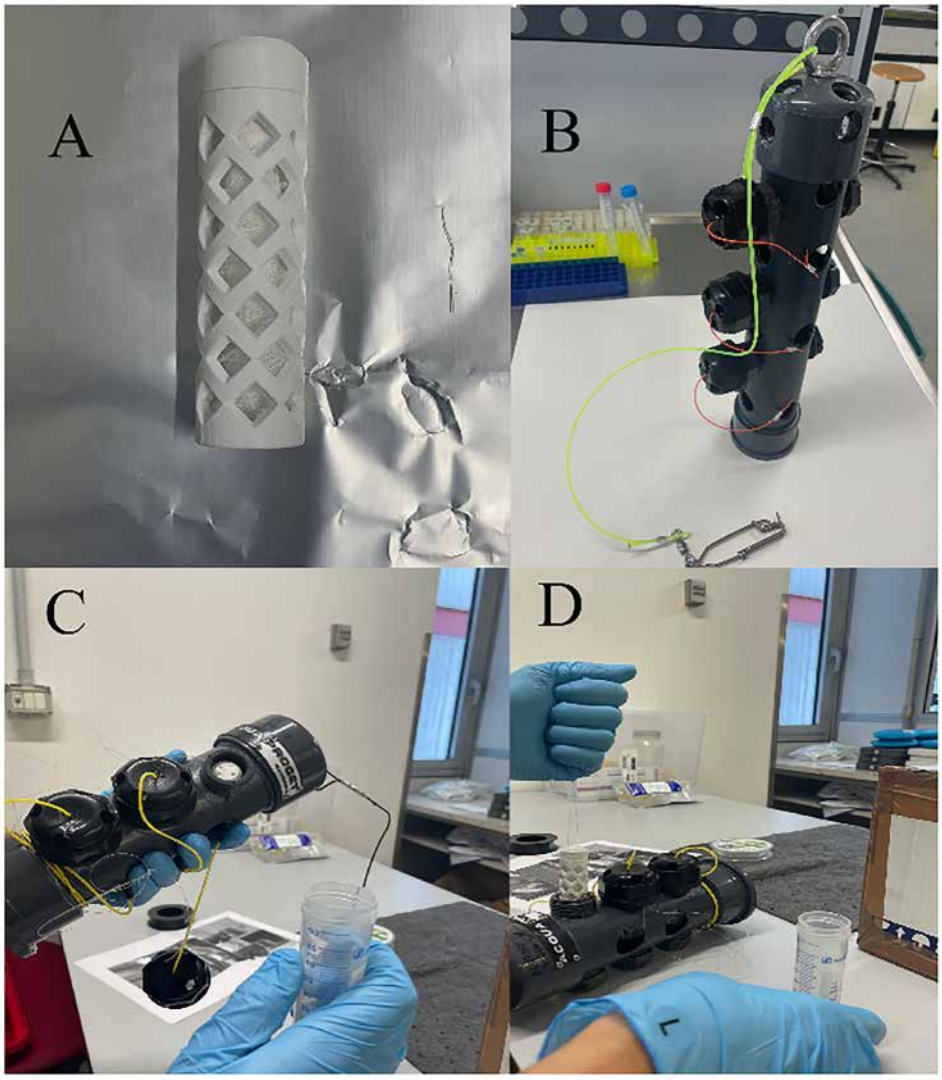

FIGURE 3 | Assembly of the passive eDNA sampling system. (A) A custom-made, 3D-printed GenoCartridge consisting of a perforated, cylindrical housing that is filled with a sterile gauze matrix. (B) A GenoPod holder for deployment at sea that can hold three GenoCartridges. (C) Inserting and securing a GenoCartridge inside the GenoPod. (D) The final setup of the GenoPod with three loaded GenoCartridges.

perforated cylindrical housing enclosing a sterile gauze matrix (see Figure 3A). For deployment at sea, GenoCartridges were inserted into modular GenoPod holders, each capable of accommodating three units (Figure 3B–D). The GenoPod and GenoCartridge systems were designed and manufactured by Acquaproget Ravenna (https://acquaprogetravenna.com/); full technical details are provided in Section S2. At each depth (1, 10, 40, and 60 m), three GenoCartridges were deployed per day, resulting in 12 passive samples per day and 36 across the three-day sampling period. The number of replicates per depth was selected to balance the number of depths sampled while maintaining comparable overall sampling effort between methods. An additional field blank was included each day: in this case, a GenoCartridge was unpacked and kept onboard during the sampling operations. Prior to deployment, the samplers were assembled under clean conditions by inserting sterile GenoCartridges into the designated slots of the GenoPod holder. Each cartridge was sealed securely to ensure water flow across the filter surface during immersion. The GenoPods were then attached to a weighted line and deployed at predetermined depths (1, 10, 40, and 60 m; see Figure 4) at a distance of approximately 10 m from the FAD, and left submerged in the water column for approximately 4 h to passively accumulate eDNA. The choice to extend passive sampling to four depths, rather than the two depths of active sampling, was intentional, reflecting the greater operational flexibility of this method. GenoCartridges can be easily deployed at 40 and 60 m, whereas collecting several Niskin water samples at such depths is often difficult in open-ocean settings due to currents and vessel movement. After retrieval, the devices were opened, and the GenoCartridges were carefully extracted using attached nylon threads to avoid contact with the surface of the cartridge. Each GenoCartridge was immediately transferred into a sterile 50 mL Falcon tube pre-filled with absolute ethanol for preservation; field blanks were processed in the same way at the end of the sampling operation. To minimize ethanol evaporation, the nylon thread was trimmed to prevent interference with the tube cap, and each vial was sealed with parafilm. After retrieval, GenoCartridge samples were similarly kept cool and protected from direct light and heat

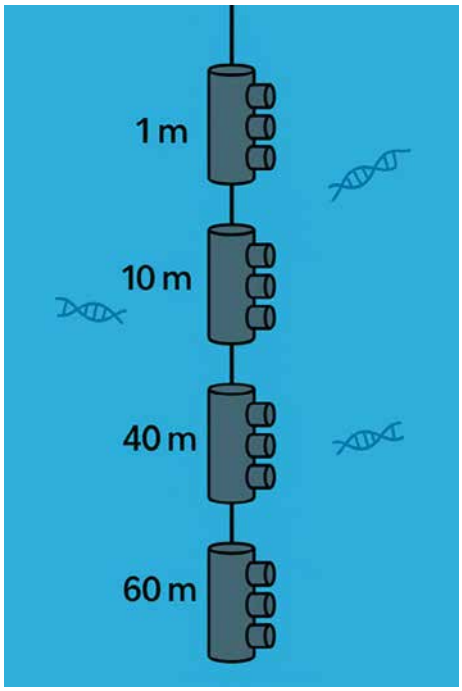

FIGURE 4 | Schematic representation of the GenoPod deployment at the four sampling depths (1, 10, 40, and 60 m) used in the study.

sources. After transport to the laboratory, the ethanol in each vial was replaced with fresh ethanol for long-term storage. All the equipment was thoroughly cleaned after each deployment using a 10% bleach solution followed by a freshwater rinse to prevent cross-contamination between samples. Prior to air transport, ethanol was removed from the cartridges to comply with shipping regulations and replenished with fresh ethanol upon arrival. As with the filter samples, transportation was first directed to the MARBEC Labs (IRD-France) for ethanol refill and subsequently to the Department of Biology, University of Padua, for storage and analysis.

2.4 | DNA Extraction

All DNA extractions were performed under a laminar flow hood in a dedicated clean laboratory. Before extraction, work surfaces and instruments were wiped with a 10% bleach solution, rinsed with distilled water, and exposed to ultraviolet (UV) light to minimize the risk of cross-contamination.

For active eDNA samples, one half of each filter was used for DNA extraction, while the other half was stored at −80°C as a backup. Filters were processed using the DNeasy Blood & Tissue Kit (Qiagen), with modifications to reagent volumes to ensure that the shredded filter material was fully covered during the first incubation (ATL buffer 567 μL, Proteinase K 50 μL). Extraction was performed according to the manufacturer's protocol with proportional adjustments to reagent volumes where necessary. Each half filter was cut into small pieces using sterile scissors and forceps before extraction. Between samples, all instruments were cleaned by immersion in denatured ethanol and rinsed with distilled water to avoid cross-contamination.

For passive eDNA samples, each GenoCartridge was opened under a laminar flow hood previously cleaned with 10% bleach and exposed to UV light; all instruments were likewise UV-irradiated. Three gauze strips were cut from different, nonadjacent sides of each GenoCartridge using sterile scissors and forceps. This approach was intended to represent all surfaces exposed to the surrounding water and to obtain a more representative subsample of the eDNA captured within the cartridge. These strips were air-dried under the laminar flow hood to remove residual ethanol prior to extraction. DNA was then extracted using the same Qiagen DNeasy Blood & Tissue Kit, with modified reagent volumes to accommodate the high absorbency of the gauze material (ATL buffer 700 μL, Proteinase K 80 μL).

2.5 | Library Preparation

The primer pair Tele02 from Taberlet et al. (2018), targeting a short fragment of about 130–209 bp of the mitochondrial 12S rRNA gene specific to teleost fishes, was used for metabarcoding analysis (Taberlet et al. 2018). eDNA samples were amplified using a single-step PCR protocol. To enable sample demultiplexing after sequencing, both the forward and reverse primers were tagged at the 5′ end with a combination of an 8-base sample-specific barcode and up to four degenerate bases (5′-NNNN-Barcode-Primer-3′). This strategy allowed for unique identification of each amplicon through double-tagging and introduced base diversity, which improves cluster recognition during Illumina sequencing (Naik et al. 2023). PCR reactions were performed using a thermocycler (SimpliAmp, Applied Biosystems) in three technical replicates to reduce amplification stochasticity. Each reaction had a final volume of 10 μL and included AmpliTaq Gold 360 MasterMix 1X (Life Technologies), both primers at a final concentration of 0.5 μM, and 1 μL of template DNA (sample or blank). The thermal profile for amplification consisted of an initial denaturation at 95°C for 10 min, followed by 35 cycles of 30 s at 95°C, 1 min at 50°C, and 1 min at 72°C, concluding with a final elongation step at 72°C for 7 min. PCR blanks, using molecular-grade water as templates, were included. All reactions were set up in a dedicated pre-PCR clean room using only filter tips and sterile reagents. In addition to field, extraction, and PCR blanks, sequencing blanks were also included in the library preparation. These consisted of unique barcode combinations (both forward and reverse) not physically used in the sample library, allowing detection and quantification of possible tag-jumping artifacts (Schnell et al. 2015).

Amplicon presence was confirmed by running 1.8% agarose gels under UV light using a transilluminator (Gel Doc XR+, Bio-Rad). Successful PCR products were then pooled across up to three replicates for each sample and purified using the MinElute PCR Purification Kit (Qiagen), following the manufacturer's protocol with final elution in 16 μL of elution buffer. Six purification replicates were performed to reduce stochasticity. The final amplicon pool was quantified using both Nanodrop 2000c and Qubit 4 Fluorometer (ThermoFisher). A volume of 20 μL of the purified amplicon library was then sent to an external sequencing service (Fasteris) for sequencing library preparation with Illumina adapters and paired-end

sequencing (2 × 150 bp) on an Illumina NovaSeq platform, with the service guaranteeing approximately 50 million usable reads after PhiX removal.

2.6 | Sequences Analysis

Raw Illumina reads were demultiplexed using Cutadapt (Martin 2011; version 4.4), which removed primer and barcode sequences and assigned reads to their corresponding samples based on unique tag combinations. Paired-end reads were merged, denoised, and chimera-filtered using DADA2 (Callahan et al. 2016), which enables high-resolution reconstruction of Amplicon Sequence Variants (ASVs). Chimera detection and removal were carried out using the "consensus" method integrated in DADA2.

All downstream analyses were performed using the QIIME2 framework (Bolyen et al. 2019; version 2024.10).

The ASVs were first taxonomically assigned individually. Fish taxonomic assignment was carried out at 97% identity using the Mitohelper reference database (Lim and Thompson 2021), version updated in January 2025, which was filtered to retain only reference sequences assigned to marine fish species reported from Indonesian waters based on a FishBase-derived species list. This biogeography-aware filtering of the reference database was adopted to reduce false positives from contamination that could affect methodological comparisons (active vs. passive sampling, depth, and sampling day effects). However, although this choice is pragmatic given that the primary objective of the study was to evaluate the feasibility of applying eDNA metabarcoding to fish assemblages associated with FADs, restricting the reference database to taxa expected in the study area could mask genuine range extensions or novel detections and is therefore not appropriate in general for eDNA studies, particularly when the focus is on invasive, unexpected, or newly arriving taxa.

Subsequently, ASVs sharing the same taxonomic label (species, or higher taxonomic rank when species level identification was not available) were collapsed by summing their read counts into single Operational Taxonomic Units (OTUs) for downstream ecological analyses, following the approach described by Martino et al. (2025). A version-pinned, runnable workflow of bioinformatic scripts is provided in Section S3. Total read counts for all samples, including controls, together with their associated metadata, are provided in Section S4. As a quality-control step, sequencing depth per sample was evaluated using rarefaction curves computed with the *vegan* package (Oksanen et al. 2025; version 2.6-10) and sample coverage estimates computed with *iNEXT* (Hsieh et al. 2016; version 3.0.2) (Section S4). The OTU table was then curated using a two-step decontamination protocol. First, to estimate and correct for tag-jumping artifacts, sequencing blanks were analyzed. For each OTU, the tag-jump ratio was calculated as the proportion of reads detected in these sequencing blanks relative to the total number of reads across all samples. This proportion was then subtracted from the corresponding counts in each sample. Second, experimental blanks—including field, extraction, and PCR blanks—were used to detect and correct potential cross-contamination. For each OTU, the distribution of reads among all blanks was evaluated, and a conservative correction was applied by subtracting from each sample the number of reads equivalent to the maximum of blank values for that OTU. Summaries showing the effect of QC are reported in Section S4.

2.7 | Statistical Analysis

Number of OTUs was calculated overall and by sample type, depth and sampling days. Statistical differences in OTU richness among samples were evaluated using nonparametric tests: Mann–Whitney *U* tests (Mann and Whitney 1947) were used for two-group comparisons, Kruskal-Wallis tests (Vargha and Delaney 1998) were used for multi-group comparisons followed, in case of significance, by pairwise Dunn tests with Benjamini-Hochberg correction (Dunn 1964; Benjamini and Hochberg 1995). All tests were implemented using the R package *rstatix* (Kassambara 2025).

Heatmaps were produced for samples collected at 1 and 10 m to compare sequence count distribution between sampling methods as follows. First, read counts were summed across replicates for each combination of sampling day, depth and sample type. Then, the 20 taxa that occurred in the largest number of these combined samples were retained. For these taxa, nonzero read counts were log10 transformed across all groups from both methods combined, and the 33rd and 66th percentiles of this distribution were used as global thresholds. Cells with zero reads were classified as "Absent", and nonzero values with log10 counts at or below the 33rd percentile, between the 33rd and 66th percentiles, and above the 66th percentile were classified as "Low," "Medium," and "High," respectively.

Community dissimilarity, on which all the subsequent analyses have been performed, was calculated from a converted presence-absence OTUs table, using the Sørensen-Dice index with the vegdist function (*vegan* R package; version 2.6-10). Principal Coordinates Analysis (PCoA) was performed on the resulting distance matrices using cmdscale (base R). Ordinations were visualized using the first three axes in all possible pairwise combinations (PCoA1 vs. PCoA2, PCoA1 vs. PCoA3 and PCoA2 vs. PCoA3), with convex hulls highlighting depth, sampling day or sample type. Species vectors were derived using weighted-average scores (wascores; *vegan* R package) for the 10 most frequently detected OTUs within the analyzed dataset, and plots were generated with *ggplot2* (Wickham 2011), using ggforce for convex hulls and ggrepel for species labels. To formally test which factors explained variation along each PCoA axis, we fitted linear models using sample coordinates on the first three PCoA axes as response variables and depth, sampling day, and sampling type as predictors (R function lm). Finally, PERMANOVA (Anderson 2017) was performed in R using the function adonis2 (vegan v2.6-10) with 999 permutations on Sørensen-Dice dissimilarities computed with the R package *proxy* (Meyer and Buchta 2025), with *proxy*::dist (method = "Dice"). Homogeneity of multivariate dispersion among groups was tested with *vegan*::betadisper followed by permutation tests (*vegan*::permutest), which showed no significant differences among groups. Depth, sampling day, and sample type were used as explanatory factors, testing both the full dataset and a subset including only 1 and 10 m samples. Since, for practical reasons, five replicates were

performed for active sampling and three for GenoCartridges, unequal replication could influence the comparison between sampling methods. To estimate this potential bias, we implemented a subsampling approach. Using a custom R script, we randomly extracted (without replacement) only three filters for each combination of sampling day and depth. Subsampled filters were added to the original GenoCartridges allowing to produce balanced (3 vs. 3) dataset. Subsampling was repeated 200 times to obtain different possible combinations of three filter replicates among the five available and each balanced subset was analyzed independently using PERMANOVA on Dice dissimilarities.

3 | Results

3.1 | OTU Richness and Distribution by Sample Type and Depth

Rarefaction curves indicated that sequencing depth was sufficient for all samples, reaching a clear plateau (Section S4). In addition, *iNEXT* analyses confirmed complete sample coverage for all samples (sample completeness = 1), supporting the use of the dataset for downstream analyses. A total of 39 OTUs, 31 of which were assigned at the species level, was detected across all the 66 eDNA samples processed (Table 1). Active filtration recovered 32 OTUs, and the same number was found with GenoCartridge, with an overlap of 25 OTUs between methods (Figure 5). When OTU presence was stratified by sampling depth, surface samples at 1 m contained 31 different OTUs, whereas samples collected at 10 m detected 35 OTUs across all replicates. Deeper samples showed reduced richness: at 40 m depth, 18 OTUs were recorded, and at 60 m that number decreased further to 14. Filters showed OTU richness distributions comparable to those of GenoCartridges (Figure 6A), with no evidence of a difference between methods (Mann–Whitney U test, $p = 0.706$). Within each method, richness varied across depths (Figure 6B), but these differences were not statistically supported (Filters: Kruskal–Wallis, $p = 0.900$; GenoCartridges: $p = 0.252$). When all depths were considered, richness differed across sampling days for both methods (Figure 6C; Filters: Kruskal-Wallis, $p = 0.0257$; GenoCartridges: $p = 0.0185$). Post hoc tests identified a significant Day1-Day2 contrast for Filters (Dunn test with Benjamini-Hochberg correction, $p.adj = 0.021$) and a significant Day1-Day3 contrast for GenoCartridges ($p.adj = 0.019$), while the remaining pairwise comparisons were not significant after BH adjustment.

A heatmap of the 20 most abundant OTUs detected across all samples (Figure 7) illustrated how relative abundance varied among taxa and sample types.

In each "sampling day-depth" group within a given "sample type", these 20 OTUs accounted for a substantial fraction of total reads, with a median of 91.1% in Filter samples and 99.4% in GenoCartridge samples. Across the top 20 OTUs, the taxa detected most repeatedly across day-depth groups also showed the strongest signals, including *Selar crumenophthalmus*, *Coryphaena hippurus*, and *Abudefduf* sp. (Figure 7A,B). Within the 1–10 m range, *Auxis* sp. was detected across all three sampling days in Filters, whereas it was not detected in GenoCartridges at 1–10 m, despite being detected at 40 and 60 m. For GenoCartridges, Day1 showed lower heterogeneity than the other days, with the three most frequent OTUs displaying high read categories while the remaining OTUs in the top-20 set were not detected, except for *Decapterus macrosoma*, which showed a low signal.

3.2 | Multivariate Ordination and Drivers of Taxonomic Variation

In the full dataset ordination, no clear clustering of samples was visually apparent across the considered variables (Section S4). To examine patterns under a more comparable sampling design, ordinations were therefore repeated on the 1–10 m subset, restricting the analysis to depths shared by both sample types (Figure 8).

Within the 1–10 m subset, samples showed a separation by depth (Figure 8A–C), most clearly along PCoA1. Among the taxa displayed as vectors (top 10 by occurrence within the subset), *Abudefduf* sp. was aligned with the positive direction of PCoA1, consistent with the region of the ordination space occupied predominantly by 1 m samples. Temporal structure (Figure 8D–F) was expressed mainly along PCoA3 with vectors for *Aluterus monoceros, Hemiramphus far*, and *Canthidermis maculata* oriented toward the sector where Day 3 samples were more represented. In contrast, separation by sample type (Figure 8G–I) remained limited, with substantial overlap between Filter and GenoCartridge samples across all projections. Although *Auxis* sp. was detected in the 1–10 m subset only in Filter samples, its vector did not show a strong alignment with the main ordination structure.

Linear models applied to the PCoA scores of the first three axes revealed that the ordination axes captured the effects of a combination of depth, sampling day, and sampling method (see Table 2).

In the full dataset, PCoA1 was significantly associated with both Depth and Sample type ($F = 4.788$, $p = 0.005$; $F = 4.834$, $p = 0.032$; adjusted $R^2 = 0.208$), whereas Sampling day was not significant ($p = 0.201$). For PCoA2, significant associations were detected for Depth ($F = 4.765$, $p = 0.005$) and Sampling day ($F = 5.858$, $p = 0.005$), while Sample type showed no effect ($p = 0.305$; adjusted $R^2 = 0.115$). PCoA3 was significantly associated with Depth ($F = 6.516$, $p < 0.001$) and Sample type ($F = 6.707$, $p = 0.012$), with no detectable contribution of Sampling day ($p = 0.971$; adjusted $R^2 = 0.386$).

In the 1–10 m subset, PCoA1 was strongly associated with Depth ($F = 15.699$, $p < 0.001$; adjusted $R^2 = 0.305$), and Sample type remained significant ($F = 4.465$, $p = 0.041$), whereas Sampling day was not ($p = 0.148$). No predictors were significant for PCoA2 (Depth $p = 0.463$, Sample type $p = 0.118$, Sampling day $p = 0.095$; adjusted $R^2 = 0.082$). In contrast, PCoA3 was primarily associated with Sampling day ($F = 13.354$, $p < 0.001$; adjusted $R^2 = 0.335$), with no significant effects of Depth ($p = 0.708$) or Sample type ($p = 0.547$). The adjusted R^2 of these models was 0.305 for PCoA1 and 0.335 for PCoA3, indicating that a substantial fraction of variation along these axes is accounted for by the tested factors. Permutational multivariate analysis of variance (PERMANOVA) on Dice dissimilarities indicated significant

TABLE 1 | List of fish taxa detected across sampling methods, depths, and days. Columns report the taxonomic classification (Order, Family, Genus, Species) and the detection method: "Filter/Geno" indicates whether each taxon was detected only by filtration ("F"), only by GenoCartridges ("G"), or by both ("Both"). Columns 1, 10, 40, and 60 m indicate detections at the corresponding depths. Columns Day1, Day2, and Day3 indicate detections across the three consecutive sampling days, with "X" marking presence in a given day.

Order	Family	Genus	Species	Filter/Geno	1 m	10 m	40 m	60 m	Day1	Day2	Day3
Beloniformes	Belonidae	*Tylosurus*	*Tylosurus crocodilus*	Filter		F			X		
Beloniformes	Exocoetidae			Filter	F	F			X		
Beloniformes	Hemiramphidae	*Hemiramphus*	*Hemiramphus far*	Both	F	F		G	X		X
Beloniformes	Hemiramphidae	*Hemiramphus*		Both	FG	F		G		X	X
Blenniiformes	Blenniidae	*Petroscirtes*	*Petroscirtes breviceps*	Both	FG	F			X	X	
Carangiformes	Carangidae	*Caranx*	*Caranx sexfasciatus*	Geno				G	X		X
Carangiformes	Carangidae	*Decapterus*	*Decapterus macrosoma*	Both	G	FG	G		X		
Carangiformes	Carangidae	*Decapterus*		Both	FG	FG	G		X	X	X
Carangiformes	Carangidae	*Elagatis*	*Elagatis bipinnulata*	Both	FG	G	G		X	X	X
Carangiformes	Carangidae	*Selar*	*Selar crumenophthalmus*	Both	FG	FG	G	G	X	X	X
Carangiformes	Coryphaenidae	*Coryphaena*	*Coryphaena equiselis*	Both	FG	G	G	G		X	X
Carangiformes	Coryphaenidae	*Coryphaena*	*Coryphaena hippurus*	Both	FG	FG	G	G	X	X	X
Carangiformes	Sphyraenidae	*Sphyraena*	*Sphyraena putnamae*	Both	FG	G	G		X	X	X
Centrarchiformes	Kyphosidae	*Kyphosus*	*Kyphosus cinerascens*	Both	FG	FG				X	X
Centrarchiformes	Kyphosidae	*Kyphosus*	*Kyphosus vaigiensis*	Geno	G	G	G			X	X
Centrarchiformes	Kyphosidae	*Kyphosus*		Geno		G				X	
Clupeiformes	Clupeidae	*Amblygaster*	*Amblygaster leiogaster*	Both		F		G	X		X
Clupeiformes	Clupeidae	*Amblygaster*	*Amblygaster sirm*	Both		FG			X	X	
Clupeiformes	Clupeidae	*Spratelloides*	*Spratelloides delicatulus*	Filter	F	F			X		
Elopiformes	Elopidae	*Elops*	*Elops hawaiensis*	Filter	F	F				X	
Lophiiformes	Ceratiidae	*Cryptopsaras*	*Cryptopsaras couesii*	Geno			G			X	
Myctophiformes	Myctophidae	*Myctophum*	*Myctophum brachygnathum*	Both	FG				X	X	
Ophidiiformes	Carapidae	*Carapus*	*Carapus mourlani*	Both	G	FG	G		X	X	
Perciformes	Pomacentridae	*Abudefduf*		Both	FG	FG		G	X	X	X
Perciformes	Serranidae	*Epinephelus*	*Epinephelus lanceolatus*	Filter		F			X	X	
Perciformes	Serranidae	*Epinephelus*		Both	FG	F			X	X	X

(Continues)

TABLE 1 | (Continued)

Order	Family	Genus	Species	Filter/Geno	1 m	10 m	40 m	60 m	Day1	Day2	Day3
Priacanthiformes	Priacanthidae	*Priacanthus*	*Priacanthus sagittarius*	Geno	G	G	G			X	X
Scombriformes	Gempylidae	*Nealotus*	*Nealotus tripes*	Both	G	FG	G		X	X	
Scombriformes	Nomeidae	*Cubiceps*	*Cubiceps pauciradiatus*	Both	FG	F			X	X	
Scombriformes	Nomeidae	*Cubiceps*	*Cubiceps whiteleggii*	Both	FG	FG	G	G	X	X	
Scombriformes	Scombridae	*Auxis*		Both	F	F	G	G	X	X	X
Scombriformes	Scombridae	*Rastrelliger*	*Rastrelliger kanagurta*	Geno				G	X		
Scombriformes	Scombridae	*Rastrelliger*		Geno	G	G		G	X		
Spariformes	Lethrinidae	*Lethrinus*	*Lethrinus erythracanthus*	Filter	F	F			X		
Spariformes	Lethrinidae	*Lethrinus*	*Lethrinus nebulosus*	Filter	F	F			X		
Stomiiformes	Stomiidae	*Photonectes*	*Photonectes albipennis*	Both	FG	FG	G		X	X	
Tetraodontiformes	Balistidae	*Canthidermis*	*Canthidermis maculata*	Both	FG	FG	G	G		X	X
Tetraodontiformes	Monacanthidae	*Aluterus*	*Aluterus monoceros*	Both	FG	FG	G	G			X
Tetraodontiformes	Monacanthidae	*Aluterus*	*Aluterus scriptus*	Both	FG	G	G		X	X	X

effects of all three factors on community composition. In the full dataset, depth explained 12.2% of the variance ($R^2 = 0.122$, $p = 0.001$), sampling day explained 10.1% ($R^2 = 0.101$, $p = 0.001$), and sample type explained 3.4% ($R^2 = 0.0338$, $p = 0.006$). In the restricted 1–10 m subset, sampling day accounted for the largest share of variation ($R^2 = 0.121$, $p = 0.001$), followed by depth ($R^2 = 0.0747$, $p = 0.002$) and sample type ($R^2 = 0.0502$, $p = 0.010$), with all terms remaining significant. When the analysis was repeated using 200 balanced 3 vs. 3 samples subsets (see Section 2) obtained by considering the GenoCartridge samples with random combinations of three filter samples out of the five available, the overall picture did not change. Differences in community composition were still mainly associated with depth (mean $R^2 = 0.13$, $p < 0.05$ in 100% of the balanced dataset) and sampling day (mean $R^2 = 0.12$, 100% significant as before). Whereas, the sampling method explained only a small proportion of the variation and was not significant in 16% of the simulation (mean $R^2 = 0.038$, 84% of the simulations significant).

4 | Discussion

This study provides novel insights into the use of active and passive eDNA sampling in pelagic marine environments in proximity of FADs, demonstrating that both approaches can yield valuable information on tropical pelagic teleost fish communities. Although eDNA has been widely applied and validated in coastal, transitional, and demersal environments (Aglieri et al. 2021; Cananzi et al. 2022; Martino et al. 2025; Thomsen et al. 2016), its application in the pelagic realm has remained limited due to the difficulty of accessing these remote areas (Li et al. 2022; West et al. 2020). Moreover, the relatively low density of pelagic species compared to other environments, together with the operational challenges associated with eDNA sampling in the open ocean, pose additional constraints on the effective use of this approach and highlight the need for a clear assessment of the performance of active and passive eDNA sampling methods.

Globally, we found an overlap (64%) between the species collected through active and passive eDNA sampling. At the scale of individual samples, richness levels were broadly comparable, with no clear tendency for either method to consistently outperform the other across depths or sampling days. These results suggest that active filtration provides an operationally robust baseline for biodiversity assessment, whereas passive samplers achieve comparable coverage and occasionally contribute additional taxa, albeit with greater variability in detection among replicates. Several recent studies have compared the practical advantages and limitations of passive versus active eDNA sampling in aquatic systems (Bessey et al. 2021; Saltonstall et al. 2024; Morris et al. 2024). Passive samplers can match or even exceed conventional water filtration in terms of detected diversity, while avoiding the need to pump and filter large water volumes, which is often the main logistical bottleneck, especially in productive or turbid waters and in remote locations.

In this study, the passive approach was operationally convenient because attaching the devices to the FAD buoy enabled them to be deployed without the vessel having to remain on station throughout the entire submersion period, thus freeing up time

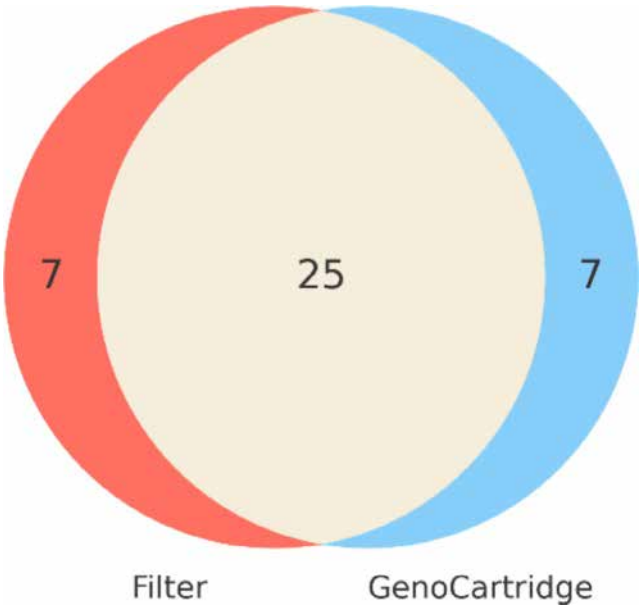

FIGURE 5 | Venn diagram showing OTUs detected by active water filtration (7) and passive GenoCartridge deployment (7), with 25 OTUs shared between methods.

for the ship to carry out other activities; in addition, passive samplers allowed an expanded sampling depth (40 and 60 m) without a significant increase in effort. By contrast, active sampling required dedicated time at each depth and additional gear for samples at 10 m (e.g., Niskin bottles), which can represent a constraint for small-vessel operations or opportunistic surveys and made unpractical sampling at deepest waters.

Overall, the differences between active filtration and passive GenoCartridges in estimating fish eDNA diversity around the FAD were small, indicating that in this context passive samplers may be logistically preferable, particularly at greater depths (Bessey et al. 2025), while active sampling of surface waters can provide a solid baseline for detection on which passive approaches can build. Future studies could further investigate the role of soaking time in the performance of GenoCartridges, with the aim of optimizing passive eDNA collection over shorter deployment periods.

Interestingly, the PCoA revealed that the temporal and vertical organization of the eDNA signal around the FAD outweighed differences associated with sampling method. Both PERMANOVA and linear models consistently showed that sampling day and depth account for a larger fraction of the variance among samples, whereas the effect of sampling method, although statistically significant, remained comparatively modest.

Short term eDNA studies in coastal marine systems have shown marked changes in community composition across hours to days at a fixed station, consistent with the idea that fish behavior and/or hydrodynamics can generate strong temporal structure in eDNA signals (Ely et al. 2021; Jensen et al. 2022; Dowell et al. 2024). Likewise, a study in small lakes has demonstrated that spatial and temporal replicates can differ considerably in MOTU composition, implying that sampling date and structure of replication (i.e., number and distribution of replicates) influence the communities inferred from metabarcoding data (Beentjes et al. 2019). Notably, during our field study, the first day was characterized by rougher sea conditions than the other two days. Despite its qualitative character, these observations may help explain the higher number of OTUs detected by active sampling—likely reflecting contributions from species originating over a wider area—and the lower number detected by passive sampling, potentially due to reduced retention of eDNA in the GenoCartridges under stronger current regimes (Figure 7B). This may also explain why *Hemiramphus far*, a coastal species that is not generally found at FADs, is driving the PCoA results along the day gradients (Figure 8E). Furthermore, despite the limited temporal scope of this study, our results may also indicate that significant variations in species composition and abundance around the experimental FAD occurred over consecutive days. Few studies have monitored species occurrence at a single FAD over such short timescales as those examined in this study. Acoustic tagging studies could reveal the associative behavior of multiple pelagic species over several days, highlighting species-specific diel patterns at FADs, with different species exhibiting stronger associations during daytime or night-time depending on their ecology (Forget et al. 2015). However, such diel patterns are unlikely to explain our results, as all samples were collected consistently at the same time of day. Similarly, these studies allowed quantifying the amount of time spent in proximity of FADs by tagged individuals, unveiling species-specific characteristics (Rodriguez-Tress et al. 2017; Tolotti et al. 2020). However, these studies cannot inform about the presence of untagged individuals/species and thus offer only a partial view of the aggregation. Finally, data from commercial echosounder buoys allow for accurate assessment of the presence or absence of tuna in FAD aggregations (Baidai et al. 2021) and reveal that FADs alternate between periods of several days or weeks being occupied or unoccupied by tuna. A similar phenomenon may have occurred during this field study, with a departure of a school of *Decapterus* sp. after Day 1, as highlighted by the importance of this species in the PCoA (Figure 8E). However, echosounder buoys do not provide species-level information, and no data is available for nontuna species. Finally, underwater visual surveys, which provided information on species presence at depths and distances from FADs similar to those in this study (Gaertner et al. 2008; Taquet, Sancho, et al. 2007; Forget et al. 2020), highlighted high inter-FAD variability in species occurrence—even among nearby FADs—but did not sample over consecutive days. Further studies comparing eDNA with simultaneous underwater visual censuses may help determine whether the observed daily variability reflects actual changes in species composition or is due to other factors affecting eDNA detectability and availability. Finally, the *Abudefduf* sp. is the main species driving the PCoA results along the depth gradient. This well-known *intranatant* species lives in close association with the FAD structure (<0.5 m) and occupies shallow waters (Parin and Fedoryako 1999; Fréon and Dagorn 2000), which is consistent with our results, as the *Abudefduf* sp. vector in the PCoA points toward most data collected at 1 m depth.

The heatmaps of the most abundant OTUs indicate that several of the dominant eDNA signals correspond to epipelagic fishes that are widely reported at anchored and drifting FADs, though it should be recalled that the biogeography-aware filtering applied to the reference database in our study restricted detections to marine fish species known to occur in Indonesian waters. *Selar crumenophthalmus*, for instance, is commonly found in

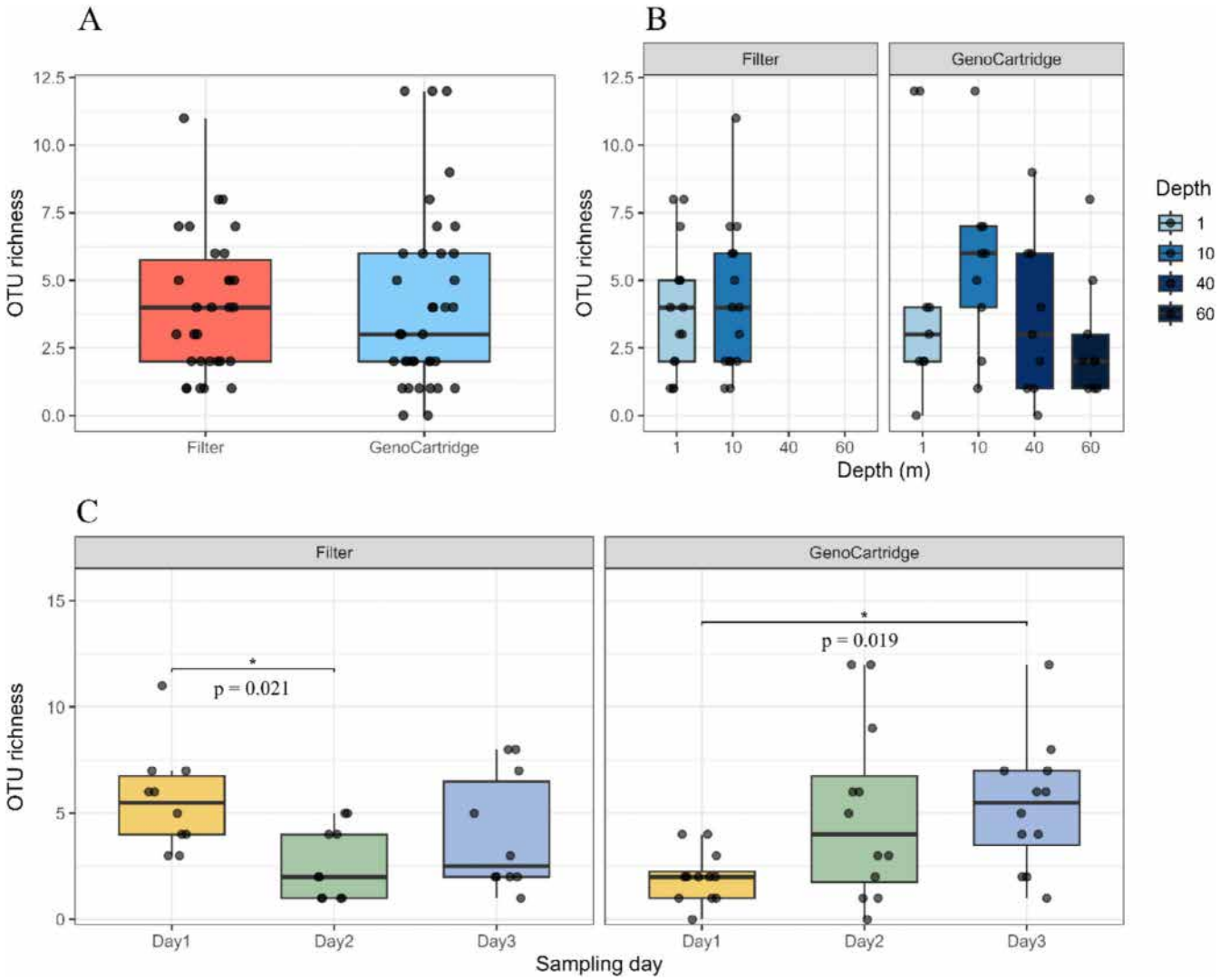

FIGURE 6 | Patterns of OTU richness detected by filtration and GenoCartridge passive sampling. (A) Comparison of overall OTU richness between Filter and GenoCartridge samples. (B) OTU richness by depth (1, 10, 40, and 60 m) for each sampling method. (C) OTU richness across the three sampling days for filtration and GenoCartridge methods. Dots represent individual samples, and boxplots summarize the distribution and median of OTU richness values within each group. Significance brackets indicate the results of Kruskal-Wallis tests, with asterisks denoting statistically significant differences ($p < 0.05$).

coastal FAD aggregations and shows pronounced daytime association (Soria et al. 2009; Capello et al. 2012). The dolphinfish *Coryphaena hippurus* is likewise among the main species recorded at FADs and exhibits prolonged residence times and repeated visits to individual devices (Taquet, Dagorn, et al. 2007). Round scads *Decapterus* sp. and spotted sardinella *Amblygaster sirm* dominate the catches of anchored FAD fisheries in the Java Sea (Potier et al. 1997), while underwater visual surveys in the western Indian Ocean show that *Canthidermis maculata* can form dense schools immediately beneath drifting FADs (Taquet, Sancho, et al. 2007). The concentration of these taxa in our 1–10 m samples is therefore consistent with an epipelagic assemblage typically associated with FADs in conventional fisheries and visual surveys. The occurrence of *Kyphosus vaigiensis* around the FAD is consistent with the ecology of kyphosids, whose juveniles can associate with floating objects and occur well offshore (Knudsen and Clements 2016). By contrast, *K. cinerascens* is primarily an herbivorous, reef-associated species grazing on macroalgae over shallow hard substrates (Knudsen and Clements 2016), so its detection near a coastal FAD is less expected and may reflect eDNA advected from nearby reefs or contributions from pelagic early stages. Additionally, occasional detections involved taxa that typically inhabit deeper waters. In particular, *Nealotus tripes* and *Myctophum brachygnathum* were detected also in some of our surface samples (3 samples; < 5000 reads in total) in both filters and GenoCartridge replicates. Although predominantly mesopelagic (Scott and Scott 1988; Mundy 2005), there are explanations for shallow detections. *N. tripes* exhibits nyctoepipelagic behavior and can occur in the upper 100 m during night hours (Yatsu et al. 2005; Alt et al. 2018), while early life stages of *M. brachygnathum* are reported from epipelagic layers (Loeb 1979).

Two additional OTUs among the most abundant detections correspond to taxa that are not typically associated with FAD habitats. The pearlfish *Carapus mourlani* is a commensal species inhabiting benthic invertebrate hosts (Parmentier et al. 2016), and the stomiiform *Photonectes albipennis* is a deep pelagic

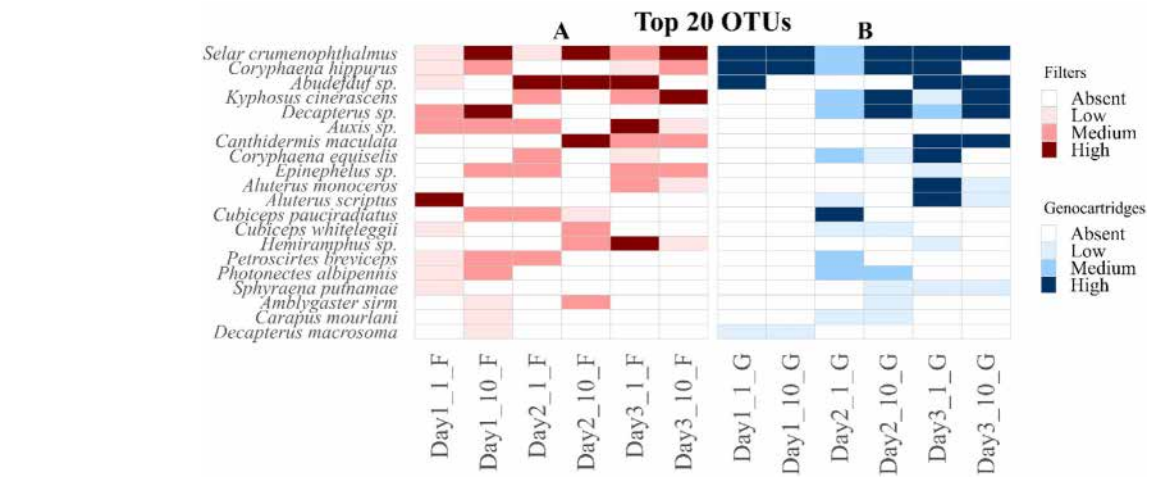

FIGURE 7 | Heatmap of the top 20 operational taxonomic units (OTUs) detected by (A) Filters and (B) GenoCartridges, considering only samples collected at 1 and 10 m. For each sampling day-depth combination on the *x*-axis, OTU read counts were summed across replicate samples. Nonzero read counts were log10 transformed across all groups from both methods combined, and the 33rd and 66th percentiles of this distribution were used as global thresholds (Absent = 0; Low = log10(count) ≤ 33rd; Medium = 33rd < log10(count) ≤ 66th; High = log10(count) > 66th).

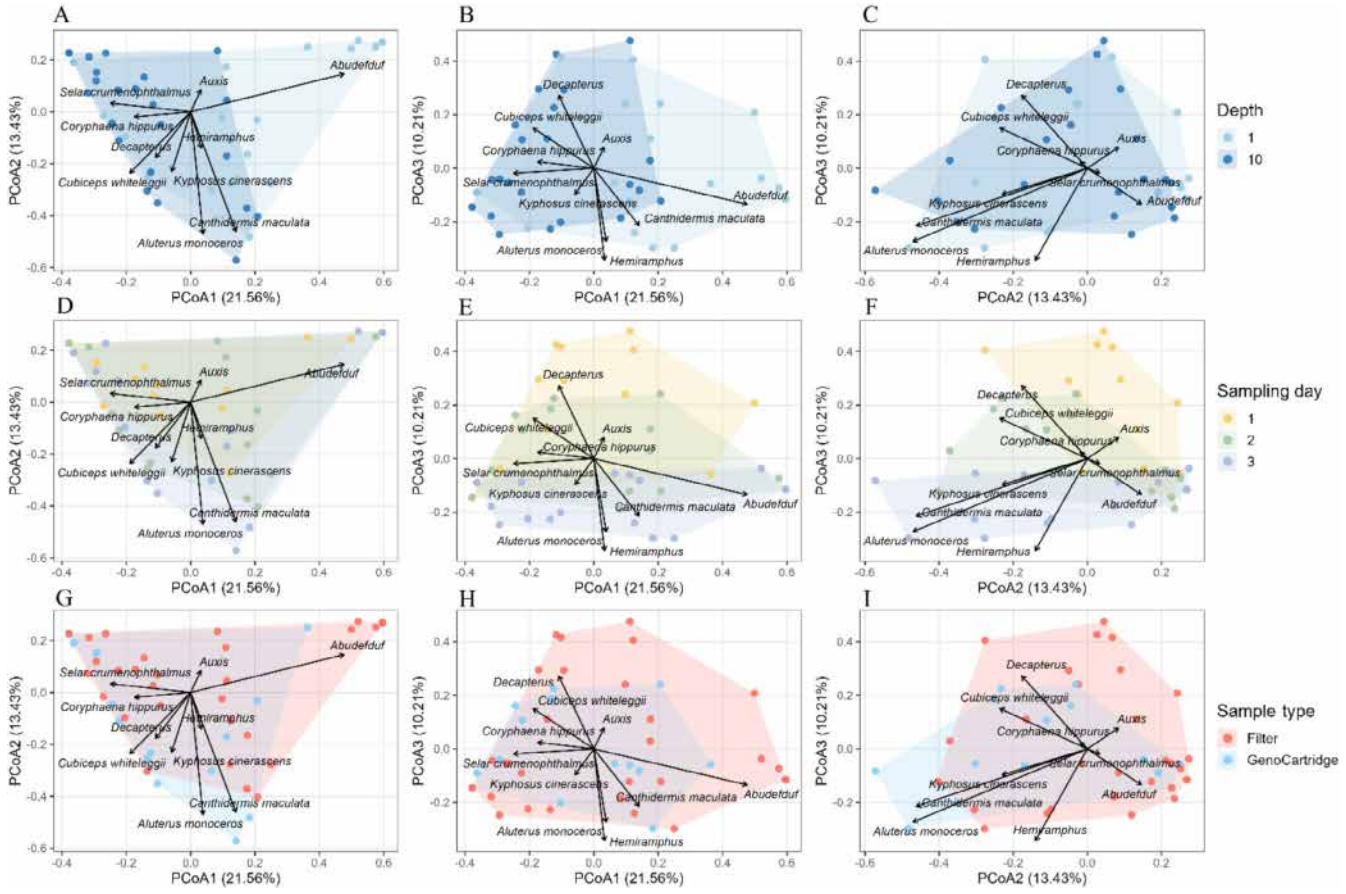

FIGURE 8 | Multi-panel PCoA based on Dice (Sørensen) dissimilarity from presence-absence data. Points represent samples, colored and grouped by depth (A–C), sampling day (D–F), or sample type (G–I); shaded convex hulls show group spread and arrows show the 10 most frequently detected taxa in the dataset (ranked by occurrence) projected onto the ordination space; arrow lengths were scaled by a constant multiplier (1.3) without affecting direction.

taxon generally occurring in mesopelagic to bathypelagic layers. Their detection in the upper 1–10 m is therefore unlikely to reflect a local aggregation beneath the FAD and is more plausibly attributed to eDNA advected from nearby benthic or deeper pelagic habitats. In this sense, these taxa represent ecological outliers within the otherwise epipelagic community identified around the device. On the other hand, skipjack tuna (*Katsuwonus pelamis*) is expected around FADs, but was not

TABLE 2 | Linear models testing the effects of depth, sample type and sampling day on sample scores along the first three PCoA axes, for the full dataset and for the 1–10 m subset. Reported are F values, p values and adjusted R^2; asterisks indicate significance levels (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

PCoA axis	Dataset	Predictor	F	p	Adj_R^2
PCoA1	Full	Depth	4.788	0.005**	0.208
16.25%		Sample_type	4.834	0.032*	
		Sampling_day	1.651	0.201	
PCoA2	Full	Depth	4.765	0.005**	0.115
11.76%		Sample_type	1.072	0.305	
		Sampling_day	5.858	0.005**	
PCoA3	Full	Depth	6.516	< 0.001***	0.386
11.59%		Sample_type	6.707	0.012*	
		Sampling_day	0.030	0.971	
PCoA1	Subset	Depth	15.699	< 0.001***	0.305
21.56%	1–10 m	Sample_type	4.465	0.041*	
		Sampling_day	1.999	0.148	
PCoA2	Subset	Depth	0.549	0.463	0.082
13.43%	1–10 m	Sample_type	2.552	0.118	
		Sampling_day	2.497	0.095	
PCoA3	Subset	Depth	0.142	0.708	0.335
10.21%	1–10 m	Sample_type	0.369	0.547	
		Sampling_day	13.354	< 0.001***	

detected in this study. However, its absence in the metabarcoding output may be methodological since the amplified 12S region is very conserved in Katsuwonus and Auxis (specifically *A. rochei* and *A. thazard*). ASV and technical reason can drive assignment by VSEARCH into *Auxis* sp.

Only three OTUs were detected exclusively in GenoCartridge samples collected below 10 m: *Rastrelliger kanagurta*, *Caranx sexfasciatus* and the deep-sea anglerfish *Cryptopsaras couesii*. The first two taxa have been reported in association with FAD fisheries or drifting FAD communities in the Indo-Pacific region (Taquet, Sancho, et al. 2007; Noranarttragoon et al. 2013) and their depth range includes the sampled depths, whereas *C. couesii* is a deep-water taxon and its detection is more plausibly attributed to transport of eDNA from deeper pelagic layers.

To our knowledge, eDNA studies explicitly targeting teleost diversity at pelagic tropical FADs have not yet been reported, which makes it difficult to compare the 39 teleost OTUs detected here with similar molecular surveys. In contrast, underwater visual census (UVC) surveys at drifting FADs reported 32 species across 33 FADs, with maxima of 20 species per FAD in equatorial waters and 13 in tropical waters (Taquet, Sancho, et al. 2007), while a standardized visual dataset reported 27 species across 22 censuses, with 7–18 species per census (Gaertner et al. 2008). Long-term monitoring at pelagic FADs further reported richness values in the low tens and highlighted differences between diver and video observations (Schneider 2023). Catch-based information from small purse seiners operating around coastal FADs in North Bali reported 20 species across 26 fishing trips (Purwanto et al. 2023). Future work could improve taxonomic coverage by adding an elasmobranch-targeted 12S assay (Elas02; Taberlet et al. 2018; Mariani et al. 2021) alongside the teleost marker (Liu et al. 2022), and increase species-level resolution in challenging groups such as scombrids by targeting longer fish amplicons, which generally provide higher discriminatory power among closely related taxa (Polanco et al. 2021; Yates et al. 2025). In the same perspective, the trade-off between reducing false positive assignments and potentially excluding genuine biological signals, as implemented in this study through biogeography-aware database filtering, should be carefully evaluated in relation to the specific aims of each eDNA study.

In conclusion, the application of eDNA provided good results and allowed an assessment of fish diversity near these structures. In future work, increasing the number of sampling days would help reduce the influence of temporal variability and better characterize local diversity, thereby improving the overall performance of the method.

Author Contributions

F.M., L.C., L.Z., M.C., and H.H. conceived and designed the experiments. H.H., M.C., J.H., and M.So. performed the field sampling. F.M. and G.P. carried out laboratory work. F.M. performed the bioinformatics and statistical analysis. F.M., L.Z., V.C., and M.Sp. tested and

contributed to the optimization of the GenoCartridge use. L.Z., L.C., W.W., and M.C. contributed sampling logistics, reagents, and analytical tools. F.M. wrote the paper with the contribution of H.H., M.C., L.C., L.Z., and T.L., and all authors revised and approved the final version.

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Ethics Statement

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The raw data underlying this study is available at Sequence Read Archive (SRA) at NCBI. BioProject ID: PRJNA1454365. Metadata and barcoding information are provided as "Supporting Information" of this article.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** Connections for tubing on the vacuum trap bottle cap: (1) air inlet, (2) liquid outlet, and (3) pressure release port. **Figure S2:** Components of the Smith-Root self-preserving eDNA filter (0.45 μm PES): (1) filter outlet, (2) filter inlet, and (3) the disposable rigid pipe. **Figure S3:** Attaching the extension tube to the Smith-Root eDNA filter while avoiding direct contact with the filter inlet to minimize contamination risk. **Figure S4:** GenoCartridge assembly sequence. **Figure S5:** Figures showing the GenoPod (1–2) and a close-up of the GenoCartridge (3). **Figure S6:** Loading of the GenoPod with the GenoCartridge (4–5). **Figure S7:** Safe removal of the GenoCartridge from the GenoPod. (6) Positioning the GenoPod for cartridge release. (7) Extracting the GenoCartridge while minimizing direct contact. **Figure S8:** Placement of the GenoCartridge into a 50 mL Falcon tube using the nylon thread (8), followed by its removal either by pulling or cutting the thread (9). **Figure S9:** Sealing the 50 mL Falcon tube containing the GenoCartridge with Parafilm to ensure proper sample conservation during transport to the laboratory. **Table S1:** Sequencing summary for samples (blank controls excluded) before QC (pre) and after blank removal (post). Total reads are the reads retained at each step. Assigned reads are sequences with a taxonomic assignment (assigned to at least one OTU). Total OTUs are the distinct OTUs detected in the dataset (present in at least one sample). **Table S2:** Sample-level sequencing summary and metadata for all libraries, including samples and negative controls. *SampleID* is the unique identifier of each library. *Day* indicates the sampling day. *Sample_type* specifies the material processed. *Control_type* classifies each entry as a biological sample or a negative control. *Depth* is the sampling depth in meters for samples (set to "na" for controls). *Total_reads* is the total number of reads obtained for that sample, including unassgned reads. *Reads_assigned* is the number of reads that received a taxonomic assignment (assigned to at least one OTU). **Table S3:** Demultiplexing information for all the sequenced samples and controls. SampleID indicates the unique sample identifier; barcode_FW and barcode_RV report the forward and reverse barcode sequences associated with each sample and used for demultiplexing the raw sequencing data. **Figure S10:** Rarefaction curves showing the relationship between sequencing depth (read count) and OTU richness for all samples (preQC). Sample completeness calculated with *iNEXT* (Hsieh et al. 2016; version 3.0.2) was 1 for all the samples. **Figure S11:** Multi-panel PCoA based on Dice (Sørensen) dissimilarity from presence-absence data on the entire dataset.