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eDNA Metabarcoding, a Promising Tool for Monitoring Aquatic Biodiversity in the Estuaries of Reunion Island (South-West Indian Ocean)

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ABSTRACT

Reunion Island is in the South-West Indian Ocean (SWIO), where all freshwater fish species are diadromous. The ecological status assessments of freshwater in watersheds have revealed a continuing deterioration in these fish populations due to anthropic pressures. In this context, monitoring the fish's biological sustainability is crucial to ensure the health of these estuarine ecosystems. The aim of this study was to compare the efficacy of conventional electrofishing monitoring (EF) with the environmental DNA metabarcoding tool to evaluate fish biodiversity in the estuaries. We measured the diversity and structure of the fish community in three estuaries with various geographical, hydrological, and anthropogenic conditions over different seasons. To this end, fish were captured by EF, and we then isolated DNA from the water samples to perform bioinformatic analyses derived from eDNA, using the 12S marker. Statistical analyses were carried out to compare the results of these two methods. For all watersheds combined, a comparison of the results for measuring fish richness showed that eDNA performed significantly better than EF. Indeed, the eDNA detected 31 species, whereas the EF detected only 12 species. For both methods, we observed significant differences in community structure between watersheds, with a significant nestedness phenomenon where the fish assemblage obtained from EF captures is a sub-assemblage of that obtained from eDNA. Moreover, compared to EF, eDNA enabled the detection of endemic to the Mascarene region species (e.g., *Cotylopus acutipinnis*), introduced exotic species (e.g., *Oreochromis niloticus*), and species difficult to capture and identify due to their juvenile life stage through EF (e.g., *Anguilla* sp.). Our data confirm the effectiveness of eDNA to detect fish species, both taxonomically and in terms of species richness and proves to be an effective tool for monitoring fish diversity of the islands of the SWIO.

1 | Introduction

Estuaries are important transition zones between marine and freshwater ecosystems (Levin et al. 2001; Borja et al. 2011). By promoting the exchange of nutrients along the longitudinal axis of watershed, these connectivity zones ensure the survival and migration of many aquatic organisms, including fish (Shao et al. 2019). A large proportion of these fish have a diadromous life cycle, migrating between rivers and coastal areas during their lifetime (March et al. 2003). Anthropogenic pressures from watershed, such as agricultural pollution, hydraulic infrastructure, and urbanization, are major and growing threats to this aquatic biodiversity (Dudgeon et al. 2006; O'Brien et al. 2019). Fish communities, because of their

sensitivity to environmental disturbances, are useful bioindicators of the health of ecosystems (Borja et al. 2011). Fish respond to the cumulative effects of physical and chemical disturbances in the water in which they live, providing an integrated view of the state of their environment over long periods of time (Badu Borteley Eugenia, Armah, and Dankwa 2019). The monitoring of freshwater fish communities is traditionally based on tracking, either capturing individuals by electrofishing (EF) or gillnetting, or via underwater visual observation (Bonar et al. 2009). Although these approaches provide a variety of information about fishes' life history (sex, life stage, and size), they can have a negative impact on organisms such as inducing physiological stress and behavioral changes, including reduced feeding and aggression rates, or inactivity rendering them more approachable (Mesa and Schreck 1989; Snyder 2003). Further, they are generally conducted over a restricted range of areas (Fischer and Quist 2014), require substantial human and logistical resources, as well as skills in morphological identification (Minamoto et al. 2020).

To overcome these difficulties, new approaches based on the analysis of environmental DNA (eDNA) are emerging. eDNA is the genetic material isolated directly from environmental samples such as soil or water. As DNA can spread rapidly and persist in the environment, it is possible to determine the recent or past presence of an organism in the environment, beyond the point of sampling (Deiner et al. 2016; Thomsen and Willerslev 2015). eDNA can be used to detect mobile species or species present in low abundance because they are cryptic or in the early stages of invasion (Rishan, Kline, and Rahman 2023). eDNA is a more integrative tool than electrofishing, as it can detect a wide range of species from simple water samples, covering vast geographical areas and providing a more comprehensive view of biodiversity over longer periods, whereas electrofishing remains limited to the species and sites sampled at a specific time (Fischer and Quist 2014; Sahu et al. 2023). The use of environmental DNA is more appropriate in this case as it is, a noninvasive and rapid method that can detect and identify species present in an environment without the negative sampling impacts of the populations studied (Bohmann et al. 2014). The ease of sampling makes it possible to analyze the dynamics of species, populations, and communities, mapping their geographic distribution over long periods and large spatial scales (Beng and Corlett 2020; Nakagawa et al. 2018). Although eDNA approaches are becoming increasingly popular and democratized for conservation and environmental management applications, it is pertinent to compare these approaches with more traditional means of monitoring to determine the limitations and benefits of each (Shen et al. 2022). Previous studies have already shown that eDNA can provide assessments of fish diversity that are comparable to, or even better than, traditional ecological approaches (Czeglédi et al. 2021; Fujii et al. 2019; Goutte et al. 2020). However, there remains a lack of comparative studies in tropical island environments, where unique ecological conditions and distinct community compositions may impact the applicability and accuracy of eDNA. This gap in the literature highlights the importance of conducting focused research in these ecosystems to better understand how eDNA might complement or even replace more invasive traditional methods like electrofishing.

Due to their isolation, tropical volcanic islands are naturally variable ecosystems, with a high species turnover rate and significant richness of endemic freshwater species (Jaisankar 2018; Kinch et al. 2010). High rates of endemism, low alpha diversity, small population sizes and genetic bottlenecks make this biodiversity sensitive to natural and anthropogenic disturbances (Jaisankar 2018; Keppel et al. 2014). Given the growing pressures of habitat degradation, introduction and spread of invasive species, overexploitation, pollution and disease, the vulnerability of tropical river ecosystems is increasing. There is a need to establish unified, robust, and easy-to-use methodologies for their management and conservation (Barlow et al. 2018; Jaisankar 2018; Smith, Covich, and Brasher 2003).

This study was carried out on the tropical volcanic island of Reunion, which is an isolated tropical island of 2500 km^2 located in the south-west Indian Ocean (SWIO), known as a biodiversity hotspot (Myers et al. 2000; Roberts 2002) and facing a decline in these fish populations rivers (Keith 2002).

The aim of the present study was to show that, eDNA metabarcoding can offer real contributions and new applications for monitoring these tropical fish populations compared with traditional monitoring. In Reunion, the most used method for monitoring fish populations is electrofishing, due to its effectiveness in capturing freshwater species (Lagarde et al. 2021). But electrofishing may have limitations, such as the inability to detect rare or cryptic species, or to sample large areas. eDNA metabarcoding can provide an alternative assessment of this unique biodiversity, helping to better understand and conserve these ecosystems (Beng and Corlett 2020). In addition, as Reunion Island faces challenges related to invasive species, which can threaten native biodiversity and ecosystem functioning, eDNA metabarcoding can be used to monitor and detect invasive species in estuarine ecosystems, enabling early detection and rapid response measures to mitigate their impacts. Especially as invasive exotic species, such as Tilapia (*Oreochromis* sp.), are on the increase in Reunion's waters and impacting native fauna by predation (Cassemiro et al. 2018; DEAL Réunion et al. 2019). In this way, fish data generated by eDNA metabarcoding can contribute to conservation planning and management strategies on Reunion Island. By identifying biodiversity hotspots, priority species and areas of high ecological importance, decision-makers can better allocate resources and implement targeted conservation actions to protect and restore these ecosystems. As a result, fish eDNA data is helping to monitor the health of these estuarine ecosystems by providing information on the impacts of human activities, climate crisis, and other stressors on biodiversity and ecosystem functioning. This has already been demonstrated in different stressful contexts such as land use change (Li et al. 2023a), pollution (Xu et al. 2023), bottom trawling (Good et al. 2022), oil and gas drilling (Laroche et al. 2018), and invasive species (Everts et al. 2024).

Here, we (i) examined the similarities and differences in the detection of taxa using electrofishing (hereafter, EF) and the DNA-based sampling method, (ii) determined the patterns of fish community structure between the two methods, (iii) revealed the role of eDNA metabarcoding for the detection of species of high ecological interest and for the detection of invasive species.

Finally, we discussed the possibility of using eDNA metabarcoding for river monitoring.

2 | Methods

2.1 | Reunion Island Freshwater System Description

Reunion's rivers are home to 26 species of fish (Keith 2002). Of these species, 18% have been introduced, while 16% are endemic to the Mascarene region. Here, the freshwater fauna is characterized by the absence of primary fish (i.e., fish strictly confined to fresh water due to their perceived physiological intolerance to salinity; Sparks and Smith 2005), and the island's rivers are mainly colonized by diadromous amphidromous fish species that complete their life cycle by migrating between the island's rivers (or bodies of water) and the Indian Ocean (Keith 2002). The 2019 study report for the protection of Reunion's freshwater fish and crustacean species highlighted the International Union for Conservation of Nature (IUCN) status of these species (DEAL Réunion et al. 2019). These evaluations indicate that on a regional scale, that is, Reunion Island, four diadromous species are considered to be critically endangered (*Anguilla bicolor bicolor*, *Anguilla marmorata*, *Anguilla mossambica*, and *Cotylopus acutipinnis*), four fish species are considered vulnerable (*Awaous commersoni*, *Eleotris acanthopoma*, *Kuhlia rupestris*, and *Kuhlia sauvagii*), one species has been classified as near-threatened (*Sicyopterus lagocephalus*), and another as of minor concern (*Eleotris klunzingerii*). Another assessment of the biological status of fish populations in freshwater was carried out in 2019 and showed a deterioration in these fish populations in Reunion's rivers (Office de l'Eau Réunion and DEAL 2019). This observation shows the necessity of implementing a real strategy to protect these populations, on the scale of the Reunion basin (DEAL Réunion et al. 2019).

2.2 | Study Sites

The island experiences a humid tropical climate influenced by the ocean, with two distinct seasons shaping its hydrology and temperature fluctuations. The austral winter, spanning from May to October, brings cooler and drier weather, while the austral summer, from November to April, is characterized by hot and humid conditions (Réchou et al. 2019). Geographically, the island exhibits variations: the eastern, "windward" coast receives heavy rainfall due to southeast trade winds, resulting in substantial precipitation throughout the year, whereas the western, "leeward" coast experiences lighter rainfall and a drier climate (Réchou et al. 2019). Additionally, the leeward coast faces increased anthropogenic pressures like agriculture and urbanization (Lagabrielle et al. 2009).

Two biological inventory methodologies were compared for three estuaries on Reunion Island, with different geographical

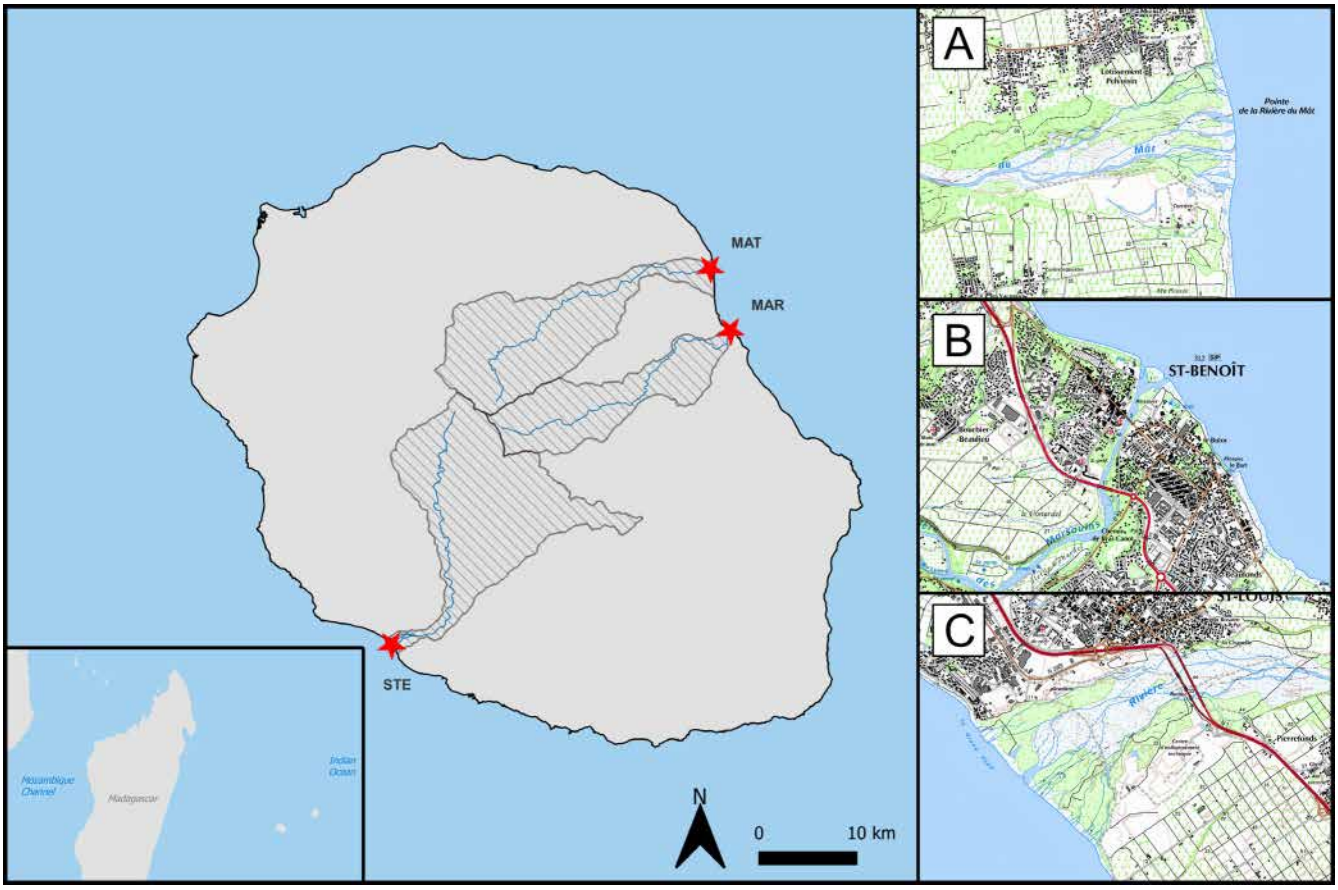

FIGURE 1 | Sampling site locations in Reunion Island (southwestern Indian Ocean). To the east, or the "windward" coast, are the stations of (A) Rivière du Mât: MAT and (B) Rivière des Marsouins: MAR. To the west, or the "leeward" coast, (C) Rivière Saint-Etienne station: STE.

characteristics: the Rivière du Mât (MAT) and the Rivière des Marsouins (MAR) on the eastern side of the island, and the Rivière Saint-Etienne (STE) on the western side of the island (Figure 1). Four sampling campaigns were conducted on these rivers to cover the two seasons of winter and summer, and the transition periods between the two seasons, referred to here as spring and fall. The first took place in November 2021 (spring: C1), the second in January 2022 (summer: C2), the third in March 2022 (fall: C3), and the last in July 2022 (winter: C4).

2.3 | Sampling Methodology

The water samples for eDNA analysis and the electrofishing samples were collected on the same day. However, to avoid contamination, the water was sampled before the electrofishing took place.

2.3.1 | Electrofishing Survey

Electrofishing sampling was conducted using the "eel abundance index" method, derived from the Point Abundance Sampling method (PAS; Germis 2016; Laffaille et al. 2004). We used a 24 V Smith-Root LR-24 back-pack electrofishing unit with a standard 400-mm anode ring and braided cable rat tail for a cathode (Smith-Root Inc., USA). The river was surveyed in a zig-zag pattern between each bank passing through open water, with 30 sampling points in sectors where the water level did not exceed 60 cm. Due to the dynamic nature of Reunion's rivers, the width of the estuaries varied according to the period sampled. On average, over the period from November 2021 to July 2022, the widths were 24.3 m for MAT, 45.1 m for MAR, and 15.6 m for STE. Each point was fished for 30 s (Germis 2016). The fish were captured near the anode. The individuals collected were identified, sexed, measured, weighed, assigned a developmental stage, and counted by species (Keith, Vigneux, and Bosc 1999). Some juvenile fish were difficult to identify to species level, so they were identified to genus level only. After identification according to the Fish Atlas of Keith, Vigneux, and Bosc (1999), the fish captured were returned to the waterbody alive. The abundance of each taxon (genus or species level) at each site was recorded.

2.3.2 | Environmental DNA Sampling and Extraction

The water sampling protocol was modified from Majaneva et al. (2018), and according to the recommendations of Pawlowski et al. (2020). Briefly, the steps are as follows. At each sampling site, three independent replicates of water samples (4000 mL/sample) were collected using cans previously decontaminated with a 50% bleach solution and single-use gloves. The samples were then placed in independent decontaminated coolers with ice during transport from the study site to the laboratory. Filtration was carried out within 6 h of collection. All procedures were carried out on a bench cleaned with a 50% bleach solution, and the equipment was decontaminated between each filtration. Using a vacuum pump, the water sample replicates were filtered through magnetic funnels onto independent filters made from a mixture of cellulose esters (MCE: nitrate and acetate; Merck Millipore; 47 mm diameter; 0.45 μm pores). Like Peixoto et al. (2021), the water was filtered until the filter membrane clogged (1250–4000 mL). A filtration control was performed on the same day, that is, 2 L of cleaning water (i.e., laboratory water). Following Allison et al. (2020), the filters were stored in 2-mL tubes containing silica beads, which dry out the filters and prevent DNA degradation. The sample filters were then stored at −20°C until extraction. The DNA was extracted from the filters using a protocol modified from the DNeasy Blood and Tissue DNA extraction kit (Qiagen, Hilden, Germany) in order to include a bead-beating pretreatment step (Closek et al. 2019). Laboratory control was carried out by performing a filter less extraction. The samples were stored at −20°C prior to sequencing. Amplification and high-throughput sequencing were conducted according to the protocols of the ADNid-Qualtech group laboratory (ADNid, Monteferrier-sur-Lez, France). The 12S rRNA region, selected to target vertebrates, is mainly used for fish studies (Miya et al. 2015). Polymerase chain reaction (PCR) amplification was performed using MiFish primers which amplify 171 bp of the 12S rRNA gene (Miya et al. 2015). Quality control was performed on an agarose gel. A second PCR was performed for sample indexing and library preparation, followed by pooling of libraries in equimolar quantities. Qualitative and quantitative controls were performed at Fragment Analyzer (Agilent Technologies Inc., Santa Clara, United States). Libraries were deposited on Miseq v2 flow cells and sequenced on the Illumina Miseq platform in a 2 × 250 bp format.

2.4 | Bioinformatic Analyses

Bioinformatic analyses were performed using the FROGS pipeline according to Escudié et al. (2018) using default parameters. FROGS is a set of independent tools that process amplicon reads coming from Illumina sequencing technologies. Each replicate was treated individually. A first quality/filtration step was performed to remove primers, short sequences, ambiguous bases. Sequences were clustered at 97% similarity using Swarm (Mahé et al. 2014) to form OTUs (Operational taxonomic units). Chimera detection and elimination were based on VSEARCH with the de novo UCHIME method (Edgar et al. 2011; Rognes et al. 2016). FROGS guidelines recommend applying an abundance filter prior to the taxonomic affiliation process. OTUs with low abundances were removed from the samples, specifically those with a relative read abundance (RRA) below 0.02% within a sample or with fewer than 10 reads. Subsequently, the OTUs were compared with reference databases, such as NCBI nt (GenBank) and MiFish, and assigned to taxa with a similarity threshold of 97% or greater. To eliminate false positives or sequencing errors, we removed from all samples the total number of reads detected in the controls for each OTU. Sequences associated with contamination, such as humans, birds, or bacteria, were removed.

2.5 | Statistical Analyses

Statistical analyses were carried out using R 4.3.1 (R Core Team 2023) and the Vegan package for diversity analysis (Oksanen et al. 2022). Replicates were pooled (water = three samples per site) before the following statistical analyses. To do this, we pooled the number of reads corresponding to each OTU

per site (MAR, MAT, and STE) for each sampling period (C1, C2, C3, and C4). For these analyses, and to ensure comparable information from both methodologies (eDNA vs. RF), species identification for *Anguilla* (*Anguilla bicolor bicolor*, *A. marmorata*, and *A. mossambica*) and *Eleotris* (*Eleotris acanthopoma*, *E. fusca*, and *E. klunzingerii*) was grouped as *Anguilla* sp. and *Eleotris* sp. for both eDNA and EF, due to uncertainties in individual identification during electrofishing.

To test the relationship between the number of individuals captured by electrofishing and the number of reads per species obtained through eDNA, we used a linear model. The number of individuals captured by electrofishing at each station for each sampling campaign was compared to the corresponding eDNA reads.

2.5.1 | Alpha Diversity

We measured α-diversity by calculating the observed fish richness of each sample. We used linear models (ANOVA) to test differences in species richness between watersheds (MAR, MAT, and STE), sampling campaigns (C1-4), and fish detection methodologies (EF, eDNA) and to investigate potential interactions between the effect of detection methodology and both sampling campaigns and watersheds. We evaluated the relative importance of each effect by comparing models containing all combinations of effects in a selection framework (Burnham and Anderson 2002). The difference between the small-sample corrected Akaike's information criterion (AICc, Burnham and Anderson 2002) of each model, and the lowest AICc of all models, ΔAICc, as well as the Akaike weights derived from the AICc (AICc-w) (Burnham and Anderson 2002), were used to identify the best combination(s) of effects to explain the variation in the dependent variable (species richness). All models with a ΔAICc value <2 were considered as having equivalent levels of support in the data and were retained in a set of "best models." We used Akaike weights (AICc-w) to estimate the relative importance of each effect by summing the AICc-w across the "best models" in which they were included. If several models were part of the "best models" set, Akaike weights were used to weight an average of single models estimates to produce multimodel estimates of effects strengths (Burnham and Anderson 2002). We used the R package MuMIn (Bárton and MK 2023) to estimate information-theoretic criteria and infer multimodel estimates.

2.5.2 | Beta Diversity

We investigated the differences in species composition between samples (β diversity) by calculating the Jaccard pairwise dissimilarity index (βjac) between all pairs of samples after reducing abundance data (reads for eDNA and individuals for EF) to presence/absence data. Because beta diversity can result from both species replacement and/or from species gains and losses between assemblages, we also quantified the independent relative contribution of the two components of beta diversity to the overall Jaccard dissimilarity: turnover (βjtu), depicting species replacement between samples and nestedness (βjne), depicting the dissimilarity between samples due to differences in species richness using the "Betapart" R package (Baselga et al. 2021; Baselga and Orme 2012). We then summarized the relative contribution of the two components (βjne and βjtu) to the overall dissimilarity (βjac) by calculating the ratio between the species turnover component (βjtu) and βjac: $\beta\text{ratio} = \beta\text{jtu}/\beta\text{jac}$ (Albouy et al. 2012). Values of βratio greater than 0.5 indicate that species turnover is the main driver of βjac, whereas values lower than 0.5 indicate that βjac is mostly caused by nestedness. For a βratio value equal to 1, turnover component is the sole driver of βjac. When βratio equals 0, this indicates that the species nestedness is the sole driver of βjac.

We first investigated how the 12 assemblages differed between detection methods (using βjac, βjtu, and βjne) and if these differences in composition varied across watersheds and sampling campaigns. We then investigated if patterns of spatial dissimilarity (between watershed dissimilarity for each sampling campaign) and temporal dissimilarity (intra watershed, between sampling campaign dissimilarity) differed between detection methods separately for βjac and βratio. We used the same model selection procedure as for species richness to investigate for effects on the difference in βjac and βratio estimations between detection methods: respectively $\Delta(\beta\text{jac}) = \beta\text{jac}_{\text{eDNA}} - \beta\text{jac}_{\text{EF}}$ and $\Delta(\beta\text{ratio}) = \beta\text{ratio}_{\text{eDNA}} - \beta\text{ratio}_{\text{EF}}$. We investigated for the effects of watershed (MAR, MAT, and STE) and the particular sampling campaign comparison (C1_C2, C1_C3, ..., C3_C4) on the temporal $\Delta(\beta\text{jac})$ and $\Delta(\beta\text{ratio})$ and effects of sampling campaign (C1—C4) and the particular watershed comparison (MAR_MAT, STE_MAR, and STE_MAT) on the spatial $\Delta(\beta\text{jac})$ and $\Delta(\beta\text{ratio})$.

To explore how dissimilarities between all assemblages are structured and test the effects of sampling methods, campaigns, and watershed on this structure, we used a nonmetric multidimensional scaling (NMDS) on the pairwise Jaccard dissimilarities and quantified the relative importance of effects using permutational multivariate analyses of variance (PERMANOVA, adonis2 function in the R package Vegan, Oksanen et al. 2022).

3 | Results

3.1 | Electrofishing Detection

For all sites combined and all periods, 665 individuals were captured, of which 51 and 150 were identified to the species and genus levels, respectively. We found 12 species belonging to four orders (Anguilliformes, Centrarchiformes, Gobiiformes, and Syngnathiformes), five families (Anguillidae, Eleotridae, Gobiidae, Kuhliidae, and Syngnathidae), and seven genera (Figure 2a, Table S1). The most frequently caught order is Gobiiformes (93.7% of captured individuals), for which the species *Sicyopterus lagocephalus* was the most abundant (53%), followed by unidentified species of the genus *Eleotris* (21.6%), then *Cotylopus acutipinnis* (12.5%), *Awaous commersoni* (3.2%), and finally, two *Eleotris* species, *E. acanthopoma* (2%) and *E. klunzingerii* (1.5%) (Figure 2a, Table S1). The second most abundant order was the Anguilliformes (4.5%), with *Anguilla marmorata* (3.5%), *Anguilla bicolor bicolor* (0.5%), and unidentified individuals of the genus *Anguilla* (0.6%) (Figure 2a, Table S1). The order Centrarchiformes accounted for only 1.5% of total

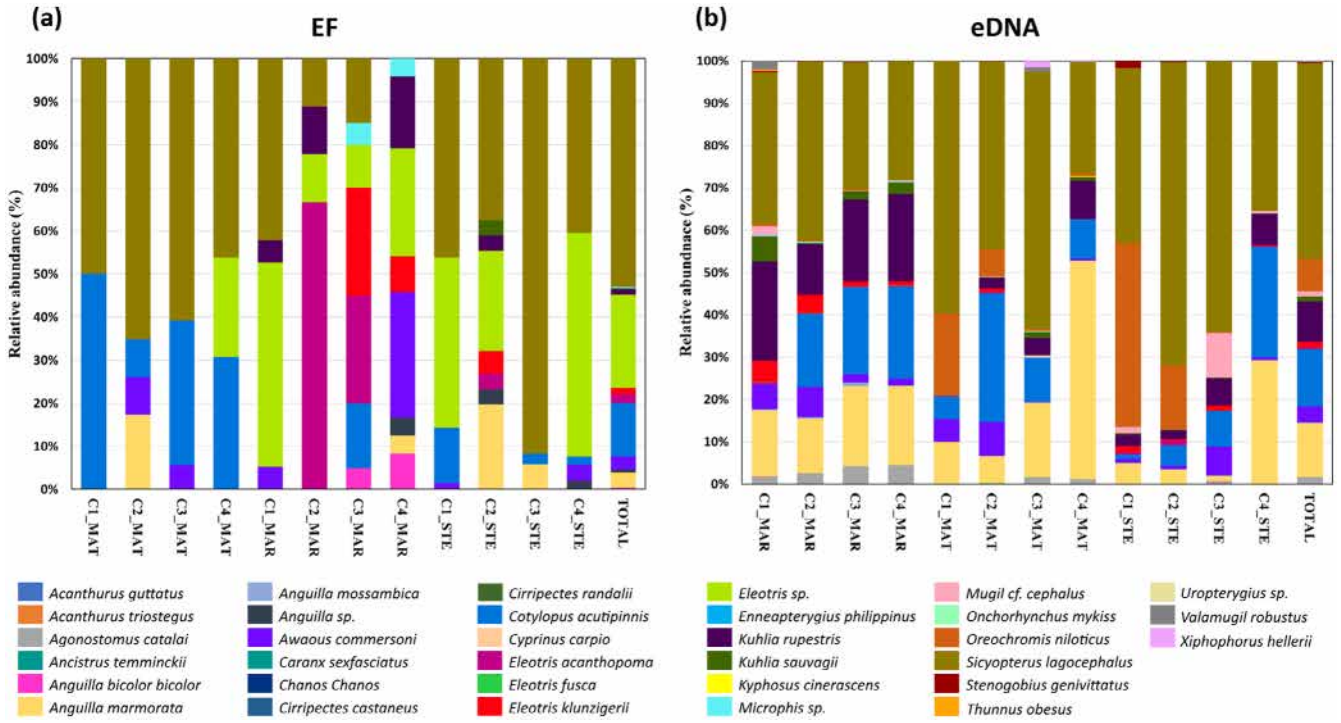

FIGURE 2 | Relative abundance of fish community in different sites for each period by (a) electrofishing (EF; % number of species) and (b) eDNA metabarcoding (% reads). Samples are named according to the sampling sites and period, of which "C1," "C2," "C3," and "C4" represents period, "MAR," "MAT," and "STE" represents sites (respectively MAR for the Rivière des Marsouins, MAT for the Rivière du Mât, and STE for the Rivière Saint-Etienne). TOTAL represents data from all periods and all sites combined for each method.

abundance, with two *Kuhlia* species, *K. rupestris* (1.2%) and *K. sauvagii* (0.3%). The least abundant order was Syngathiformes (0.3%), represented by only one species of *Microphis* (Figure 2a, Table S1). For the Rivière des Marsouins site (MAR), 11 species out of the 12 identified were caught, compared with five for the Rivière du Mât (MAT) and 10 for the Rivière de Saint-Etienne (STE) (Table S1). The most common species caught in the Rivière des Marsouins (MAR) was *Eleotris* sp. (25%), followed by *Sicyopterus lagocephalus* (17%); only *Kuhlia rupestris* was not caught (Figure 2a). In contrast, in the Rivière du Mât (MAT) and the Rivière Saint-Etienne (STE), *S. lagocephalus* was the most frequently caught species (59% and 57%, respectively). Some species were caught only once. *Kuhlia sauvagii* was only caught at the Rivière Saint-Etienne, and *Microphis* sp.1 was only caught at the Rivière des Marsouins (Figure 2a, Table S1).

3.2 | eDNA Detection

In total, 730,093 reads were obtained before filtering and 705,645 reads were retained after, which corresponds on average to 58,803 reads per sample (ranging from 9763 to 80,134; Table S2). A total of 46 OTUs were recovered from the dataset: Three OTUs were assigned at genus level and the remaining 43 OTUs were successfully assigned at the species level (Table S2). The total volume filtered during each sampling was not significantly different between watersheds (*t*-test, $p = 0.37$) or between sampling campaigns (*t*-test, $p = 0.23$). The total number of reads obtained during each sampling was independent of the water volume filtered ($t = -0.129$, $p = 0.90$; Table S3) and also differed across samples ($H\ (11) = 11.00$, $p = 0.44$). The number of reads per species was also independent ($t = 0.042$, $p = 0.970$; Table S3) as was the number of species detected during each sampling ($t = 0.34$, $p = 0.74$; Table S3).

Across all sites and time periods, eDNA metabarcoding of the 12 samples identified 31 taxa, including 29 species spanning 25 genera, 20 families, and 16 orders (Table S4). Gobiiformes was the most common order (65.4%), represented by seven species, *Sicyopterus lagocephalus* (46.2%), *Cotylopus acutipinnis* (13.6%), *Awaous commersoni* (3.7%), three species of *Eleotris* genus (*E. klunzingerii* [1.4%], *E. acanthopoma* [0.3%], *E. fusca* [0.02%]), and *Stenogobius genivittatus* (0.2%) (Figure 2b, Table S4). The two other most represented orders, sites and stations combined, are the Anguilliformes, representing 13% of total relative abundance, with the species *Anguilla marmorata* being the most detected (13%), and the order Centrarchiformes (10.6%) with the species *Kuhlia rupestris* being the most detected (9.5%) (Figure 2b, Table S4).

The species *Sicyopterus lagocephalus* is widely represented at all stations, whatever the sampling period: At the MAR site, it represents 33.6%, compared to 50.2% at the MAT site and 59.6% at the STE estuary (Figure 2b). Similarly, two other species of Gobiiformes, *Awaous commersoni* and *Cotylopus acutipinnis*, were found in each of the estuaries studied at almost similar relative abundance. At each site, the relative abundance of *A. commersoni* was around 4%, while the relative abundance of *C. acutinipinnis* was around 16.5% for MAR and MAT, and 6% for STE (Figure 2b, Table S4). The same pattern was found for *Anguilla marmorata*, which has a higher relative abundance in MAR and MAT (around 16%) than in STE (4.4%) (Figure 2b, Table S4).

We found eight strict marine species in our samples: *Acanthurus guttatus*, *Acanthurus triostegus*, *Cirripectes castaneus*, *Cirripectes randalli*, *Enneapterygius philippinus*, *Kyphosus cinerascens*, *Thunnus obesus*, and *Uropterygius* sp. These species were only detected at one or two stations in a sampling campaign. *A. gutattus* and *A. triostegus* were only detected on MAT during sampling campaign 3 (C3). *Cirripectes* species were only detected at the MAR station in sampling campaign 2 (C2). *E. philippinus* was detected twice (C1_MAT and C3_STE), as was *T. obesus* (C1_MAR and C2_MAR). *Kyphosus cinerascens* and *Uropterygius* sp. were detected only on C4_MAT and C1_MAT, respectively (Figure 2b, Table S4). These species represent a small percentage of total reads (< 0.01%; Table S4).

Sporadic species were also detected, of the order Mugiliformes, *Agonostomus telfairii*, *Mugil* cf. *cephalus*, and *Valamugil robustus*; and exotic species of Clichiformes order, with *Oreochromis niloticus*, accounting for 7.6% and 3.1% of total number of reads, respectively (Figure 2b, Table S4). An invasive exotic species in this region, *Ancistrus* cf. *temminckii* was detected only in the Rivière des Marsouins (MAR) in July 2022 (Figure 2b, Table S4).

3.3 | Relationship Between the Number of Reads From the eDNA Method and the Number of Individuals Caught by Electrofishing

The results of the model correlating the number of fish caught and the number of reads from eDNA showed no significant relationship for MAR and MAT ($t = 0.29$, $p = 0.78$; $t = 0.95$, $p = 0.37$; Table S5), whereas they were significant for STE ($t = 2.90$, $p = 0.01$; Table S5). For STE, however, the variation in the number of reads explained only a moderate proportion of the variation in the number of individuals ($R^2 = 0.392$; Table S5).

3.4 | Alpha Diversity

Models' selection indicated a support in the data for the effects of detection method, watershed, and an interaction between these two variables but never for sampling campaign (ΔAICc < 2; Table S6). The relative importance of these effects is as follows: method > watershed > interaction between method and watershed.

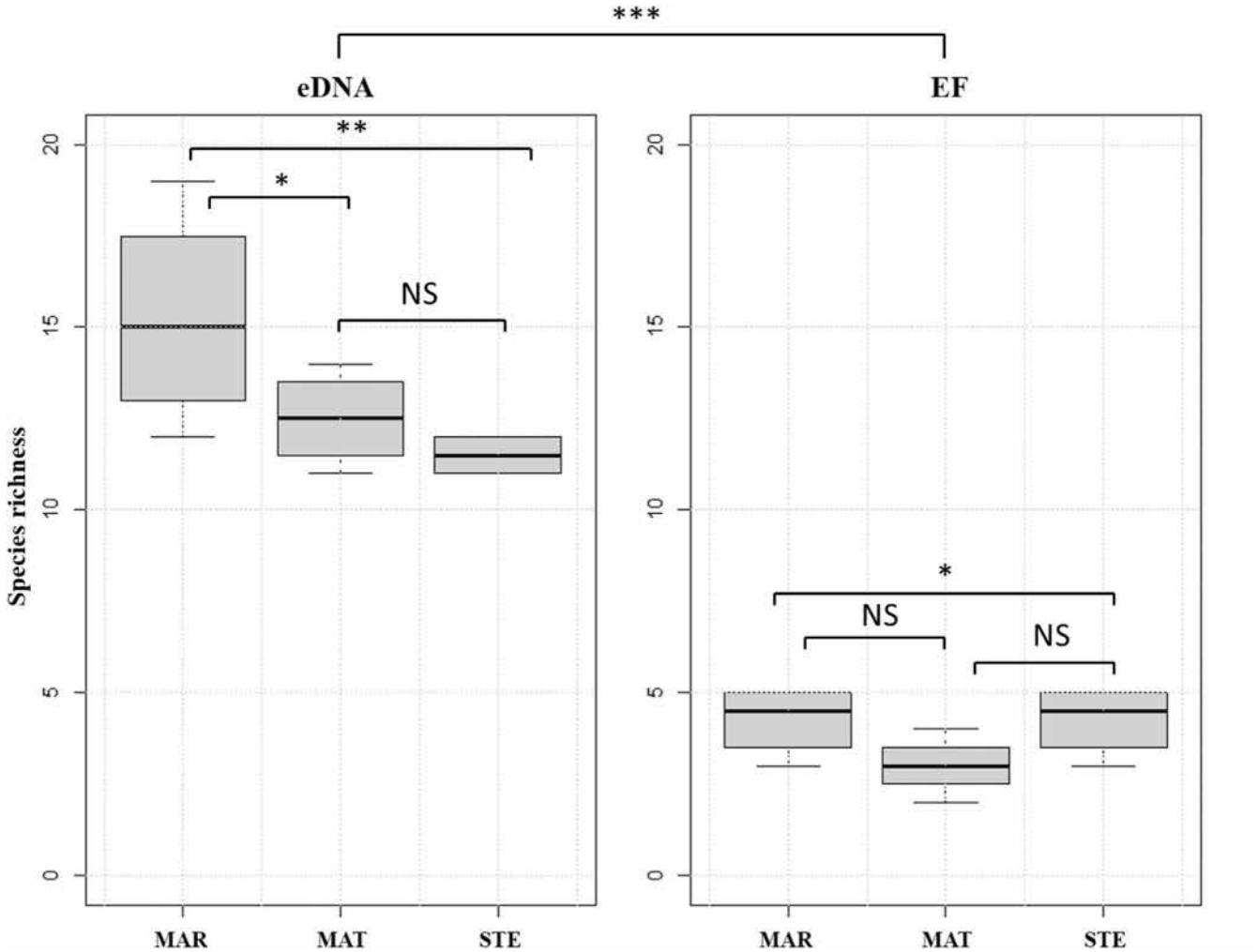

FIGURE 3 | Species richness (number of species) detected at each site using eDNA metabarcoding or electrofishing method (EF). The difference between the two methods or between the sites were determined using *t*-tests (number of species followed a normal distribution, Table S7). MAR for the Rivière des Marsouins, MAT for the Rivière du Mât, and STE for the Rivière Saint-Etienne. Asterisk (*) indicates significant effect (NS not significant; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

Model selection showed that the fish richness at each site detected by eDNA metabarcoding was always higher than that captured by electrofishing (Figure 3; Table S7). eDNA metabarcoding detected all species inventoried (Table 1). On average, 9.87 species were not detected with EF in each sample (Table S7). The fish richness identified using eDNA metabarcoding in the Rivière des Marsouins (MAR, $n = 21$), the Rivière du Mât (MAT, $n = 21$), and the Rivière de Saint-Etienne (STE, $n = 15$) was higher than that captured by electrofishing (Figure 3; Table S8). Using eDNA for fish detection revealed a gradient in species richness across the watersheds studied (MAR > MAT > STE) that was not detected using EF: as exemplified by averaged model coefficients (Table S7), the marked difference in species richness between MAR and STE when sampled using eDNA was not detected using EF (Figure 3).

This heightened species richness is further supported by species composition comparisons. Fish species obtained by the two methods comprised 31 species in 23 genera, 18 families and 15 orders: the eDNA method identified 31 species, compared with 12 species using the EF method (Tables S1–S4). Ten species were detected using both methods, with *Sicyopterus lagocephalus* and *Eleotris* sp. being the most prevalent species across all sites (Tables S1–S4). eDNA was able to detect all three *Eleotris* species (*E. acanthopoma*, *E. klunzingerii*, and *E. fusca*), compared with only two with electrofishing (Table 1). Similarly, for species of the *Anguilla* genus, eDNA metabarcoding distinguished the three species (*Anguilla bicolor bicolor*, *Anguilla marmorata*, and *Anguilla mossambica*), whereas identification after electrofishing was essentially possible at the genus level, leading to zero identification of *Anguilla mossambica* (Table 1). In addition, eDNA detected several marine species (such as *Acanthurus guttatus* or *A. triostegus*), exotic species (such as *Oreochromis niloticus* or *Cyprinus carpio*), and an invasive species (*Ancistrus Temminckii*) that were never catched with electrofishing (Figure 2b, Table S4).

3.5 | Structure of Fish Community

Beta diversity analyses revealed a higher beta diversity for eDNA data, and demonstrated that the compositional variations between the two methodologies were essentially a nestedness phenomenon (βjtu = 0; Table 2). Null turnover values indicated that the assemblages obtained by EF were sub-assemblages of those obtained by eDNA (Table 2).

The PERMANOVA, testing the effect of method and sampling site on community structure, indicated that there was indeed a significant effect of method ($F = 15.55$, $R^2 = 0.38$, $p < 0.05$, Table S9), and sampling site ($F = 2.46$, $R^2 = 0.12$, $p < 0.05$, Table S9). Beta diversity with NMDS showed a distinct separation of fish communities between the two methods (Figure 4a). For the eDNA method, a significantly different community structuring was observed between the three sites ($F = 1.66$, $R^2 = 0.27$, $p < 0.05$), particularly between the MAR site and those of MAT and STE (Figure 4b). The fish community composition of STE was relatively similar to that of MAT (Figure 4b). The same was observed for the electrofishing method, with the community composition differing significantly between the different sites ($F = 2.79$, $R^2 = 0.38$, $p < 0.05$), with nevertheless some overlap of certain species (Figure 4c).

3.5.1 | Temporal Beta Diversity

Based on these results, we decomposed the temporal beta diversity, that is, the intra-site beta diversity. We compared βjac and βratio between the methods and investigated for the effects of watershed and sampling campaign comparison on the differences between methods estimations of βjac and βratio. The βjac values were generally greater for EF than for eDNA except for three campaign comparisons at MAT and one at STE (Figure 5a, Table S10). Model selection analyses indicated that there was a watershed effect on the difference in βjac estimation (Table S10). The temporal Jaccard index, was on average, overestimated for STE and MAR but not for MAT (Figure 5c). We observed no effect of campaign pairs on dissimilarity patterns between methods (Figure 5e, Table S10). Similar to βjac, we detected only an effect of watershed on the differences in βratio estimations between methods (Table S10); however, the overestimations of βjac at MAR and STE (Figure 5c) corresponded respectively to overestimates and underestimates of βratio (Table S10, Figure 5d). Differences in βratio further revealed that the differences in βjac estimation between the detection methods could be due to both over- or underestimates of the turnover component (Figure 5a,b). For example, in the case where the βjac estimations were equivalent for the two methods, as was the case for the C1-C2 campaign pair for MAR (blue square in Figure 5a), βratio estimates diverged greatly: βjac was composed of 50% turnover and 50% nestedness for eDNA, whereas the two campaigns were completely nested for EF (Figure 5b). When βjac was underestimated (STE C1_C4, MAT C1_C3, MAT C1_C4, and MAT C2_C3), this was always due to a null estimation of the βjtu component with EF data, whereas this component contributed substantially to the βjac using eDNA data (Figure 5b,f). When βjac was overestimated with EF data, very different situations were encountered. Strong overestimates in βjac can be due to complete nestedness underestimation (e.g., MAR C1_C3 and MAR C2_C3, respectively, the yellow and purple squares in Figure 5) or alternatively to a substantial overestimation of nestedness (e.g., STE C2_C3). In the same vein, equivalent estimates of βratio obtained with EF (e.g., 0.75 for MAR C2_C3, STE C2_C3, and MAR C2_C4; Figure 5b) can correspond to very different estimates using eDNA data (e.g., 0, 1 and 0.3, respectively; Figure 5b).

3.5.2 | Spatial Beta Diversity

We compared βjac and βratio between the methods and investigated for the effects of watershed comparison and sampling campaign on the differences between methods estimations of βjac and βratio. The βjac indices were higher for EF than eDNA and this was modulated according to the spatial comparison, that is, the watershed pair studied (Figure 6a). Model selection analyses indicated that there was

TABLE 1 | Species determined by electrofishing (○), eDNA metabarcoding (▲) and both methods (▧) for the four sampling periods and three estuaries. "MAR," "MAT," and "STE" represent sites (MAR for the Rivière des Marsouins, MAT for the Rivière du Mât, and STE for the Rivière Saint-Etienne, respectively).

		Spring			Summer			Fall			Winter		
Type	**Species**	**C1_ MAR**	**C1_ MAT**	**C1_ STE**	**C2_ MAR**	**C2_ MAT**	**C2_ STE**	**C3_ MAR**	**C3_ MAT**	**C3_ STE**	**C4_ MAR**	**C4_ MAT**	**C4_STE**
Marine	*Acanthurus guttatus*								▲				
	Acanthurus triostegus								▲				
	Caranx sexfasciatus	▲											
	Cirripectes castaneus				▲								
	Cirripectes randalli				▲								
	Enneapterygius philippinus		▲							▲			
	Kyphosus cinerascens											▲	
	Thunnus obesus	▲			▲								
	Uropterygius sp.		▲										
Sporadic marine	*Agonostomus telfairii*	▲			▲	▲	▲	▲	▲	▲	▲	▲	▲
	Chanos chanos				▲								
	Mugil cf. *cephalus*	▲	▲	▲	▲	▲	▲	▲		▲	▲		▲
	Valamugil robustus	▲			▲		▲	▲	▲	▲			
Diadromous	*Anguilla bicolor bicolor*	▲		▲				▧		▲	○		
	Anguilla marmorata	▲	▲	▲	▲	▧	▧	▲	▲	▧	▧	▲	▲
	Anguilla mossambica	▲		▲	▲	▲	▲	▲		▲	▲		
	Anguilla sp.[a]					○	○	○		○	○		○
	Awaous commersoni	▧	▲	▧	▲	▧	▲	▲	▧	▲	▧	▲	▧
	Cotylopus acutipinnis	▲	▧	▧	▲	▧	▲	▧	▧	▧	▲	▧	▧
	Eleotris acanthopoma	▲		▲	▧	▲	▧	▧			▲		▲
	Eleotris fusca	▲	▲		▲			▲			▲		
	Eleotris klunzingerii	▲	▲	▲	▲	▲	▧	▧		▲	▧	▲	▲
	Eleotris sp.[a]	○		○	○		○	○			○	○	○
	Kuhlia rupestris	▧	▲	▲	▧	▲	▧	▲	▲	▲	▧	▲	▲

(Continues)

TABLE 1 | (Continued)

Type	Species	Spring			Summer			Fall			Winter		
		C1_MAR	C1_MAT	C1_STE	C2_MAR	C2_MAT	C2_STE	C3_MAR	C3_MAT	C3_STE	C4_MAR	C4_MAT	C4_STE
	Kuhlia sauvagii	◀		◀	◀	◀	▨	◀	◀	◀	◀	◀	◀
	Microphis sp. 1	◀		◀	◀	◀		◀			▨		
	Sicyopterus lagocephalus	▨	▨	▨	▨	▨	▨	▨	▨	▨	◀	▨	▨
	Stenogobius genivittatus	◀	◀	◀	◀	◀	◀	◀					◀
Exotic	*Ancistrus* cf. *temminckii*										◀		
	Cyprinus carpio								◀				
	Oncorhynchus mykiss				◀				◀		◀		◀
	Oreochromis niloticus	◀	◀	◀	◀	◀	◀	◀	◀	◀		◀	
	Xiphophorus hellerii	◀	◀		◀	◀		◀	◀			◀	

[a]The detection of *Anguilla* sp. and *Eleotris* sp. are only to be considered for the EF.

TABLE 2 | Beta diversity metrics, which measure dissimilarity in assemblages between eDNA and EF, including Jaccard dissimilarity (βjac), turnover (βjtu; species replacement), and nestedness (βjne: Species loss) in three estuaries of Reunion Island (MAR for the Rivière des Marsouins, MAT for the Rivière du Mât, and STE for the Rivière Saint-Etienne, respectively).

Watershed	Sampling	βjac	βjtu	βjne
MAT	C1	0.833	0	0.833
MAT	C2	0.692	0	0.692
MAT	C3	0.786	0	0.786
MAT	C4	0.727	0	0.727
MAR	C1	0.75	0	0.75
MAR	C2	0.842	0	0.842
MAR	C3	0.643	0	0.643
MAR	C4	0.583	0	0.583
STE	C1	0.636	0	0.636
STE	C2	0.583	0	0.583
STE	C3	0.75	0	0.75
STE	C4	0.545	0	0.545

a watershed comparison effect on the difference in βjac estimation (Table S11). We observed no effect of campaign on dissimilarity patterns between methods (Figure 6e). The highest difference in βjac, that is, the highest dissimilarity, was observed for the MAR_MAT (Figure 6b,c). As with temporal beta diversity, the differences in βjac observed between the methods could be due to over- or underestimates of nestedness or turnover (Figure 6a–b). In the case of an overestimation of βjac, the composition of dissimilarity could be extremely different from one method to another. This was the case for C1 STE_MAT where a turnover of 100% was observed for the EF (βjac = 1), while for the eDNA, the βjac was zero, indicating 100% nestedness (Figure 6b,d,f). In the case of an equivalent βjac value, as for C4 STE_MAR, the decomposition indicated a turnover rate of 0% in EF, compared with nearly 100% in eDNA (Figure 6b,f). However, in some situations, the decomposition was the same whatever the method. For example, for C2 STE_MAT, the βjac decomposition indicated 0% turnover in EF and eDNA (βratio = 0, Figure 6b,d,f).

4 | Discussion

This study is the first on Reunion Island to inventory fish species in estuaries using eDNA and to compare eDNA metabarcoding to electrofishing methods. In comparing both methods, our study confirmed that eDNA metabarcoding detected a similar or higher diversity, particularly in detecting transient or less abundant species. This matches with other published findings. Indeed, recent studies like the ones carried out in the China or in Switzerland, have also shown eDNA's effectiveness compared with the classical methods such as trawling or electrofishing for analyzing fish community structure, further supporting its value

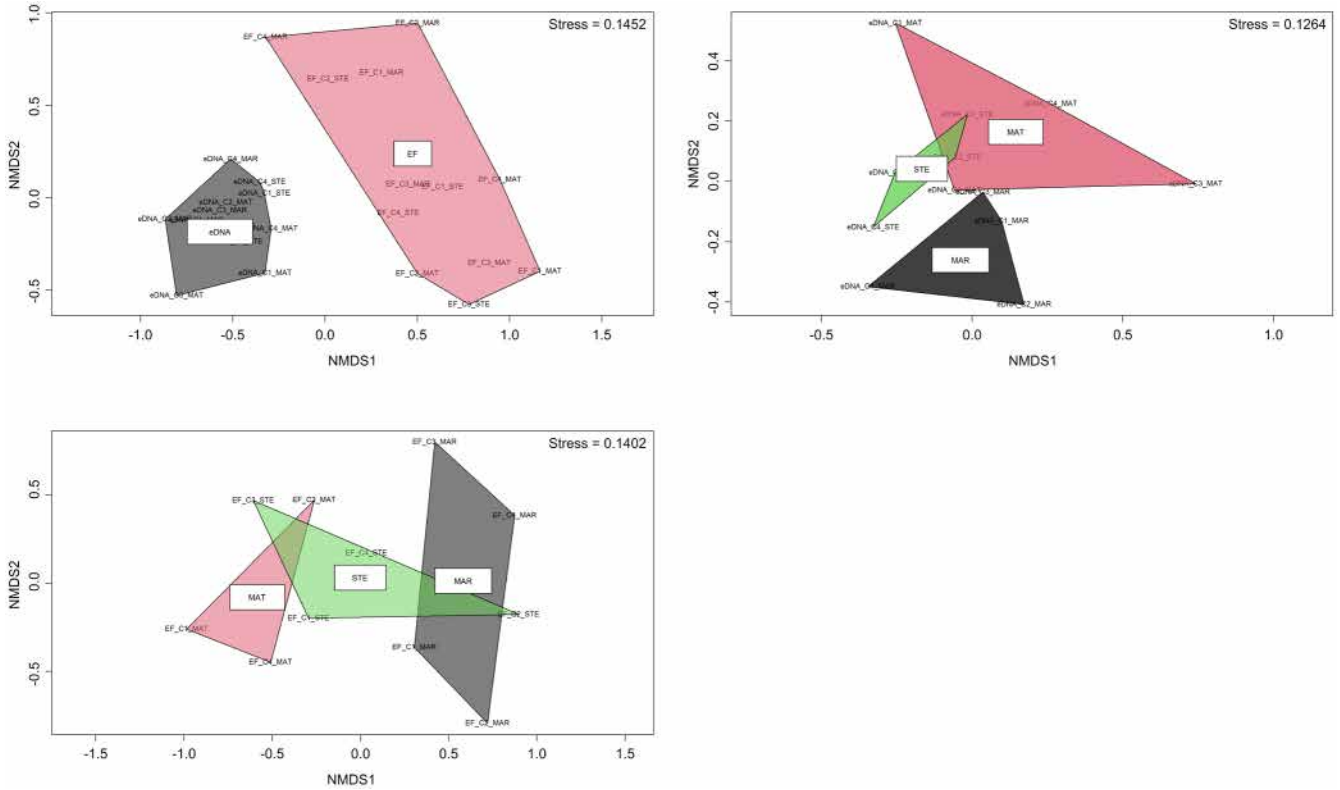

FIGURE 4 | Beta diversity visualized using nonmetric multidimensional scaling (NMDS) with Jaccard dissimilarity distances of community compositions for: (a) all data and both methods; (b) eDNA method and sites; (c) Electrofishing method (EF) and sites. MAR for the Rivière des Marsouins, MAT for the Rivière du Mât, and STE for the Rivière Saint-Etienne.

as a complementary tool to traditional methods (Brantschen and Altermatt 2024; Jiang et al. 2023; Li et al. 2023b).

4.1 | Evaluation of the Diversity and Composition of Species Obtained by eDNA

Employing traditional methods like electrofishing or gillnets for monitoring species diversity can be challenging due to the rarity of certain species, fluctuating detection probabilities, and the substantial field effort required to ensure comprehensive coverage (Olds et al. 2016; Senapati et al. 2019). Recently, the adoption of eDNA metabarcoding has emerged as a noninvasive approach for characterizing aquatic environments due to the more promising detection of taxa in diverse water bodies (Golpour et al. 2022). Its efficacy in monitoring fish communities has been demonstrated for both large and small water bodies (Shen et al. 2022). In our study, we showed that by using 4 L of water, we achieve a high level of taxonomic detection, identifying 31 species, including all the freshwater species previously documented in Reunion's freshwater environments (Keith et al. 2006). Our eDNA results revealed a higher species richness than that obtained through electrofishing across all study sites (MAR, MAT, and STE), highlighting the effectiveness of eDNA in providing a comprehensive view of fish communities. Additionally, our findings align with those of Lagarde et al. (2021) regarding the number of species, as well as their distribution and diversity across watersheds in Reunion's freshwaters. However, our study offers additional insights enabled by eDNA analysis, specifically through the detection of rare (*Anguilla mossambica*), cryptic (*Eleotris fusca*), and invasive species (*Ancistrus* cf. *temminckii*) that were not identified using EF methods alone. This underscores eDNA's capability to detect species that may otherwise go unnoticed, providing a more complete understanding of biodiversity in these environments.

However, similarity analyses revealed significant differences in species composition between these three sites, notably with the presence of sporadic or marine species. We detected marine species such as *Acanthurus* sp., *Cirripectes* sp., *Thunnus obesus* and *Chanos chanos* at the east coast stations (MAT and MAR). The detection of these species may be explained, on one hand, by their actual presence at some point within the estuary. Sporadic or marine species tend to enter estuaries in search of food or exploration, and remain there as juveniles (Keith et al. 2006). On the other hand, the position and hydrology of these watersheds may be responsible for their presence.

The eastern coast is characterized by very high rainfall and is subject to prevailing winds blowing from the southeast (the trade winds), which generates a larger swell and facilitates the entry of certain species into the estuaries or their genetic material (Réchou et al. 2019). This is why the detection of these species

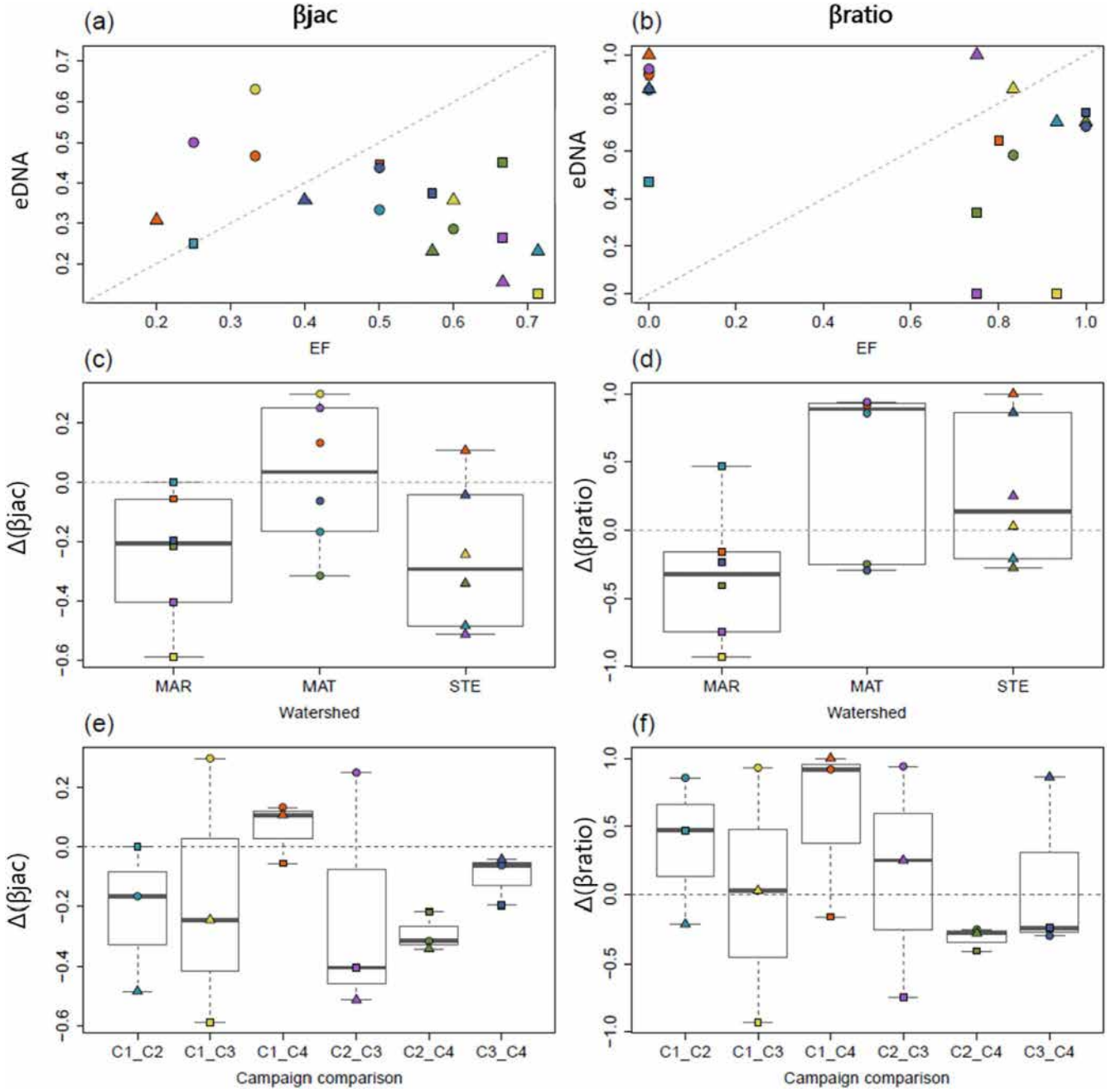

FIGURE 5 | Temporal beta diversity, or intra-site beta diversity, expressed as Jaccard dissimilarity (βjac) and its composition (βratio) obtained for the two sampling methods (eDNA metabarcoding and EF electrofishing) for each site (MAR for the Rivière des Marsouins, MAT for the Rivière du Mât, and STE for the Rivière Saint-Etienne) between each campaign pair ("C1," "C2," "C3," and "C4" represent each campaign). (a) βjac between the methods (eDNA, EF); (b) βratio between the methods (eDNA, EF); (c) Effects of watershed (MAR, MAT, and STE) on the temporal $\Delta(\beta$jac); (d) Effects of watershed (MAR, MAT, and STE) on the $\Delta(\beta$ratio); (e) Effects of comparison of sampling campaigns (C1_C2, C1_CE, ..., C3_C4) on the temporal $\Delta(\beta$jac); (f) Effects of comparison of sampling campaigns (C1_C2, C1_CE, ..., C3_C4) on the $\Delta(\beta$ratio). The shapes correspond to the watershed (□: MAR; ○: MAT; △: STE). The colors correspond to the campaign pair (Blue: C1-C2; yellow: C1-C3; orange: C1-C4; violet: C2-C3; green: C2-C4; dark blue: C3-C4).

does not necessarily mean that they are or were present in the environment, but that their DNA was transported to the estuaries by water currents or tidal flows, or carried downstream from areas further up the river (Deiner et al. 2016; Harrison, Sunday, and Rogers 2019).

Similarities in community composition are observed and are due to the presence of diadromous species (freshwater indigenous species) such as *Sicyopterus lagocephalus* and *Cotylopus acutipinnis*. These species are known to be widely distributed across the island, with a higher abundance of *S. lagocephalus* (Hoareau 2005). On Reunion Island, the detection of diadromous species is an important piece of information for managers. Indeed, their particular biological characteristics make them particularly sensitive to major anthropogenic pressures, such as the increasing development of rivers, or the increase

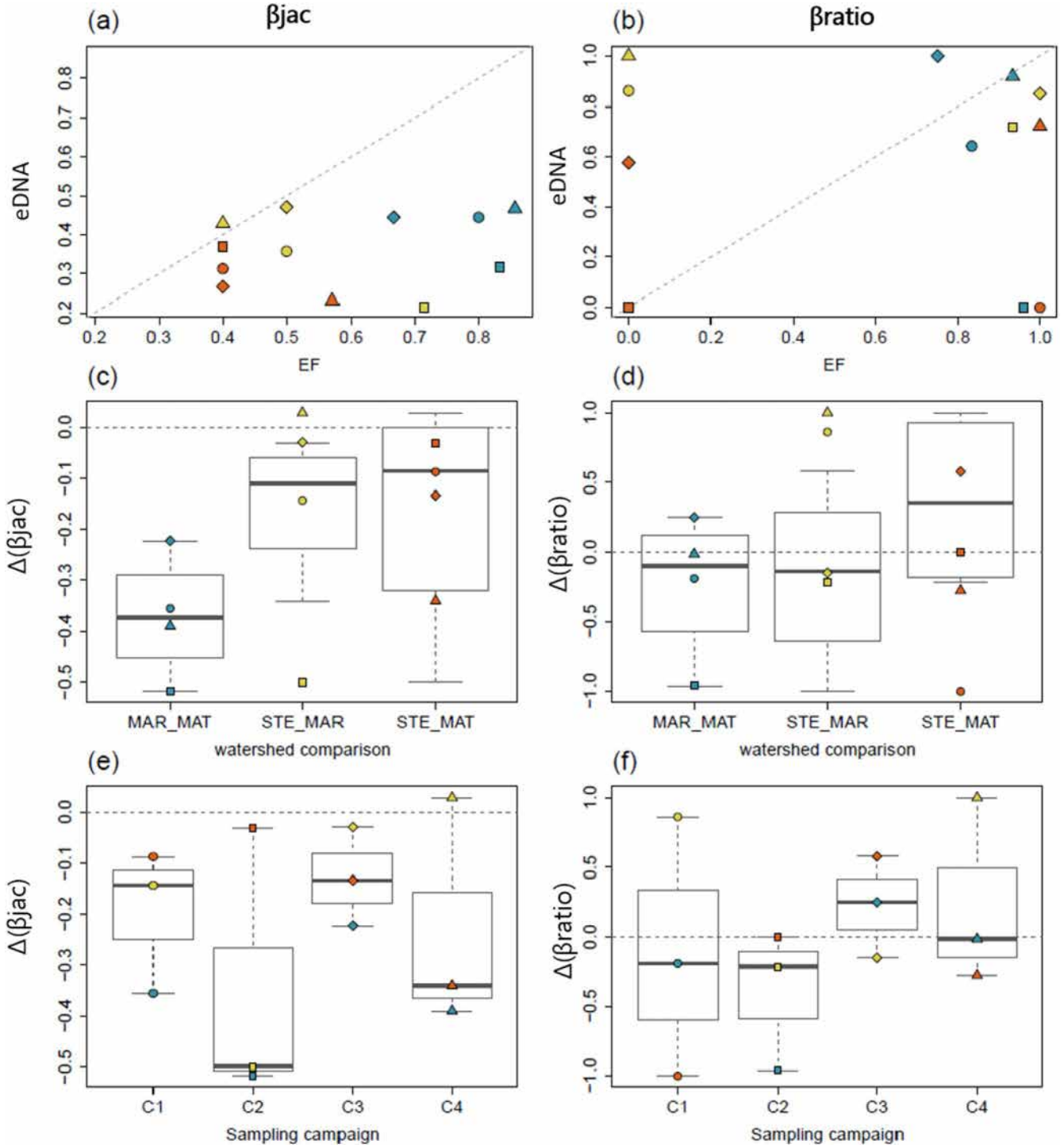

FIGURE 6 | Spatial beta diversity, or inter-site beta diversity, expressed as Jaccard dissimilarity (βjac) and its composition (βratio) obtained for the two sampling methods (eDNA metabarcoding and EF electrofishing) for each campaign ("C1," "C2," "C3," and "C4" represent the period) between each watershed pair (MAR for the Rivière des Marsouins, MAT for the Rivière du Mât, and STE for the Rivière Saint-Etienne). (a) βjac between the methods (eDNA, EF); (b) βratio between the methods (eDNA, EF); (c) Effects of watershed comparison (MAR_MAT, STE_MAR, and STE_MAT) on the spatial $\Delta(\beta$jac); (d) Effects of watershed comparison (MAR_MAT, STE_MAR, and STE_MAT) on the $\Delta(\beta$ratio); (e) Effects of sampling campaign (C1, C2, C3, and C4) on the spatial $\Delta(\beta$jac); (f) Effects of sampling campaign (C1, C2, C3, and C4) on the $\Delta(\beta$ratio). The shapes correspond to the sampling campaigns (○: C1; □: C2; ◇: C3; △: C4). The colors correspond to the pair of watersheds (blue: MAR_MAT; yellow: STE_MAR; orange: STE_MAT).

in pollutant discharges of agricultural, industrial, or domestic origin (Hoareau 2005). They are therefore considered to be indicators of the quality of aquatic environments (Hoareau 2005; Keith et al. 2006).

eDNA has made it possible to detect introduced exotic species in all three watersheds, confirming local declarations of the presence of species such as *Oreochromis niloticus* and *Xiphophorus hellerii*. These species, introduced in the 1960s

for aquaculture or biological mosquito control, were found in all low-lying areas: in rivers and coastal ponds, for *O. niloticus*, and the green swordtail (*X. hellerii*) in almost all rivers (Keith 2002).

By detecting species described as present in freshwater rivers in Reunion Island, whether diadromous (e.g., *Anguilla* sp., *Sicyopterus lagocephalus*), sporadic (e.g., *Mugil* cf. *cephalus*, *Kuhlia* sp., and *Agonostomus telfairii*), or introduced exotics (e.g., *Oreochromis niloticus* or *Xiphophorus hellerii*), the eDNA metabarcoding tool proves its relevance for ichthyological monitoring, and more specifically for monitoring the ichthyological richness, of Reunion's estuaries.

4.2 | eDNA Versus Electrofishing

Together, eDNA metabarcoding and electrofishing (EF) methods identified 31 species, across all stations and sampling periods. eDNA sampling detected all species caught by electrofishing (12 species identified). Indeed, our results demonstrate a higher species richness with the eDNA metabarcoding method than with EF, confirming the result of other similar studies such as those of Czeglédi et al. (2021) or Olds et al. (2016). Here, this higher richness is mostly due to the detection of particular groups, such as marine or sporadic species. Furthermore, the structuring of fish communities between the two methodologies proved to be significantly different, and is essentially due to a phenomenon of nestedness between methods. This phenomenon corresponds to the loss (or gain) of species that involves the elimination (or addition) of species in one of the sites, and where the poorer assemblage corresponds to a strict subset of the richer assemblage (Baselga and Orme 2012). We demonstrate here that the fish species composition obtained by eDNA is richer and that the fish community composition obtained by electrofishing, is a sub-assembly of it. eDNA is more sensitive in detecting patterns of dissimilarity, such as nestedness and turnover, due to its finer taxonomic resolution and broader species richness. The dissimilarity between assemblage is generally lower with eDNA than with EF, as the additional species detected by eDNA (primarily marine species in this study) tend to homogenize assemblages, thereby reducing dissimilarity. In contrast, the randomness of EF captures increases dissimilarity each time new species are encountered. These differences have implications for temporal monitoring of assemblages, as EF tends to overestimate temporal dissimilarity, while eDNA allows for a more accurate assessment of the dynamics and geographical structuring of assemblages. eDNA reveals geographical differences in dissimilarity that are imperceptible with EF, which, by systematically under- or overestimating these dissimilarities, would provide a biased view of assemblage dynamics These results confirm that eDNA provides a more accurate picture of the temporal and spatial dynamics of populations and can detect species that are present intermittently or not captured by electrofishing (absent from the site or very poorly represented), as previously demonstrated (Milhau et al. 2019). This method mitigates against the substantial and unpredictable randomness bias of EF, where catch efficiency depends on many factors such as habitat characteristics (e.g., cross-sectional area, water velocity, granulometry of the substrate, conductivity; Pottier 2017; Pottier et al. 2020; Price and Peterson 2010).

4.3 | An Effective Tool for Identifying Aquatic Species

This study shows that eDNA can be used to detect species that are difficult to capture or identify without sacrificing the individual by EF, such as eel species, and to detect species that are rare or difficult to observe (Pfleger et al. 2016). The electric field generated by EF modifies the behavior of these species, which flee or dive toward the substrate, thus limiting their capture in a single fishing pass, especially if the individuals are small (Lambert, Feunteun, and Rigaud 1994; Pottier et al. 2022). Here, we were able to detect the three eel species, *Anguilla bicolor bicolor*, *A. marmorata*, and *A. mossambica*, which are generally difficult to catch and morphologically identify in their juvenile state, and whose presence is important information for managers due to their status on the IUCN red list (critically endangered for *A. bicolor bicolor* and *A. mossambica*, and near-threatened for *A. marmorata*).

Furthermore, in contrast to EF, we demonstrated the sensitivity of the eDNA tool for the detection of invasive species, as other authors had previously shown (King et al. 2022). We detected the presence of a species considered invasive in the region, *Ancistrus* cf. *temminckii*, during the last sampling campaign at the Rivière des Marsouins station. This taxon, native to South America, is present in aquaria (Chaumeton 2004), and was reported in 2022 in another area of the island at the Borbonica site (DEAL Réunion and Le Parc National de La Réunion 2023, Bras Long, Entre-Deux). The detection and monitoring of these species are of interest in the surveillance and management of biological invasions, and more particularly for early prevention of the introduction of a species (Sales et al. 2021). eDNA metabarcoding could also be used to study the ecological impacts of invasive alien species, as demonstrated by Everts et al. (2022), reinforcing the importance of these techniques in the management of biological invasions.

Finally, our study confirms that eDNA offers a better representation of community structure than traditional methods in terms of taxonomic level. For the *Eleotris* and *Anguilla* genera, eDNA enabled us to go as far as the species for each of the detections, whereas for individuals captured by EF, morphological identification did not enable us to go as far as the species at most of the sites studied. For *Eleotris*, these identification difficulties have already been recognized due to the lack of distinctive characters (absence of meristic characters) and the fact that the species have a similar brown appearance (Mennesson, Maeda, and Keith 2019). This taxonomic precision using eDNA has already been demonstrated by Penaluna et al. 2023, who were able to discriminate between two trout species (*Oncorhynchus mykiss* and *O. clarkii clarkiis*) that were difficult to identify morphologically.

4.4 | Limits and Benefits of eDNA

eDNA cannot provide the biological information that EF can, such as the abundance, size, sex, or health status of individuals.

In this study, we found no significant correlation between the number of reads obtained by eDNA metabarcoding and the number of individuals caught by electrofishing. However, in specific systems and for certain taxonomic groups, eDNA can approximate abundance. For example, Everts et al. (2022) observed a strong correlation between eDNA concentrations and the abundance of introduced bullfrog tadpoles in natural systems. Additionally, eDNA enhances species diversity estimates and taxonomic resolution, allowing for the identification of more species with minimal effort, reduced observer bias, and at a lower cost than EF.

Although eDNA is prone to potential false positives (Darling, Jerde, and Sepulveda 2021), these can be managed through careful detection expectations and error clarification, enhancing its reliability in ecological monitoring (Jerde 2019). The cost of EF sampling per station is 1200 euros (14,400 euros for 12 stations), requiring at least three people on site per day. In contrast, eDNA metabarcoding (three analyses per station) costs 400 euros (4800 euros for 12 stations) and can be completed by a single person within a day, including transport and processing. Thus, expected costs for eDNA sampling are lower than for EF (Evans et al. 2017).

Nonetheless, eDNA also incurs additional laboratory and sequencing costs and requires bioinformatics and ecological expertise for data analysis. The risk of false positives is another consideration, as species detected through eDNA may not be currently present but rather upstream (Darling, Jerde, and Sepulveda 2021; Jerde 2019). Therefore, identifying potential DNA sources is crucial for accurate interpretation. Our findings highlight the value of integrating eDNA metabarcoding with traditional methods to effectively monitor fish communities in estuarine ecosystems, facilitating a more comprehensive understanding of their composition, diversity, structure, and seasonal dynamics.

As reviewed by Wang et al. (2021), the methodology of fish eDNA is now well established, providing statistical models, effective tools for biomonitoring, and the innovative concept of in situ monitoring to address ecological and environmental challenges. However, depending on the environmental context in which fish communities are assessed, traditional methods, such as active and passive gear, along with newer approaches like video and acoustic techniques, remain relevant (Hammerl, Möllmann, and Oesterwind 2024).

5 | Conclusion

This study demonstrates the effectiveness of the eDNA metabarcoding tool for detecting fish communities in the estuaries of Reunion Island. We detected 31 species, including the 12 captured and identified by electrofishing, and species difficult to capture and identify by EF. eDNA metabarcoding can more accurately reflect community composition and reveal insights into the processes shaping these communities, providing a better understanding of the functioning of a transitional ecosystem. It is important to note that caution should be exercised regarding DNA detections that may not necessarily correspond to the actual presence of species but rather indicate the presence of their DNA. However, this tool offers opportunities for managers, both in terms of the ease of implementation and manpower required. These are lower than those required for electrofishing, as eDNA simply involves water sampling, whereas traditional fishing methods require more human resources and involve higher costs. As a reproducible tool, easy to set up and a non-exhaustive source of data, eDNA can be extended to other Indian Ocean Island territories for the monitoring and management of aquatic ecosystems.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

NGS raw data 12SrDNA sequences are deposited in zenodo data bank: https://doi.org/10.5281/zenodo.10495787.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.