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METHODS

Use of environmental DNA in assessment of fish functional and phylogenetic diversity

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Abstract

Assessing the impact of global changes and protection effectiveness is a key step in monitoring marine fishes. Most traditional census methods are demanding or destructive. Nondisturbing and nonlethal approaches based on video and environmental DNA are alternatives to underwater visual census or fishing. However, their ability to detect multiple biodiversity factors beyond traditional taxonomic diversity is still unknown. For bony fishes and elasmobranchs, we compared the performance of eDNA metabarcoding and long-term remote video to assess species' phylogenetic and functional diversity. We used 10 eDNA samples from 30 L of water each and 25 hr of underwater videos over 4 days on Malpelo Island (pacific coast of Colombia), a remote marine protected area. Metabarcoding of eDNA detected 66% more molecular operational taxonomic units (MOTUs) than species on video. We found 66 and 43 functional entities with a single eDNA marker and videos, respectively, and higher functional richness for eDNA than videos. Despite gaps in genetic reference databases, eDNA also detected a higher fish phylogenetic diversity than videos; accumulation curves showed how 1 eDNA transect detected as much phylogenetic diversity as 25 hr of video. Environmental DNA metabarcoding can be used to affordably, efficiently, and accurately census biodiversity factors in marine systems. Although taxonomic assignments are still limited by species coverage in genetic reference databases, use of MOTUs highlights the potential of eDNA metabarcoding once reference databases have expanded.

KEYWORDS

accumulation curves, biodiversity, eDNA metabarcoding, functional traits, Malpelo, marine protected area, tropical reefs, video

Uso de ADN Ambiental en la Evaluación de la Diversidad Funcional y Filogenética de los Peces

Resumen: La evaluación del impacto de los cambios globales y la efectividad de la protección es un paso fundamental para el monitoreo de peces marinos. La mayoría de los métodos tradicionales de censos son demandantes o destructivos, por lo que las estrategias no letales y no intrusivas basadas en videograbaciones y en el ADN ambiental (ADNa) son alternativas a los censos visuales submarinos y a la pesca. Sin embargo, todavía no se conoce la habilidad que tienen estos métodos para detectar diferentes factores de la biodiversidad más allá de la diversidad taxonómica. Para los peces óseos y los elasmobranquios, comparamos el desempeño de la caracterización genética con ADNa y del video remoto de larga duración para evaluar la diversidad funcional y filogenética de las especies. Usamos diez muestras de ADNa tomadas de 30 litros de agua cada una y 25 horas de vídeos submarinos grabados durante cuatro días en la Isla Malpelo (costa del Pacífico de Colombia), un área marina protegida remota. La caracterización genética con el ADNa detectó 66% más unidades taxonómicas moleculares operacionales (UTMOs) que el video. Encontramos 66 y 43 entidades funcionales con un solo marcador de ADNa y con el video, respectivamente, y una riqueza funcional más alta para el ADNa que el video. A pesar de los vacíos en las bases de datos genéticos usadas como referencia, el ADNa también detectó una diversidad filogenética más alta que aquella en los videos; las curvas de acumulación mostraron cómo un solo transecto de ADNa detectó tanta diversidad filogenética como 25 horas de video. La caracterización genética con ADN ambiental puede usarse para censar los factores de biodiversidad de manera asequible, eficiente y certera en los sistemas marinos. Aunque las atribuciones taxonómicas todavía están limitadas por la cobertura de especies en las bases de datos genéticos de referencia, el uso de los UTMOs resalta el potencial que tiene la caracterización genética con ADNa una vez que las bases de datos de referencia sean expandidas.

PALABRAS CLAVE:
área marina protegida, arrecifes tropicales, biodiversidad, características funcionales, caracterización genética con ADNa, curvas de acumulación, Malpelo, video

摘要
评估全球变化影响及保护有效性是海洋鱼类监测的关键步骤。然而, 大多数传统种群调查方法都有较高的要求或具有破坏性。基于视频和环境 DNA 的非干扰性、非致死性方法则是水下视觉调查或鱼类捕捞的替代方法。然而, 这些方法检测传统分类学多样性之外其它生物多样性因子的能力尚不清楚。本研究关注硬骨鱼类和软骨鱼类, 比较了环境 DNA宏条形码和长期远程视频监测在评估物种的系统发育多样性和功能多样性中的表现情况。我们在一个偏远的海洋保护区——马尔佩洛岛 (哥伦比亚太平洋海岸) , 收集了10个分别取自 30 升水体的环境 DNA 样品, 以及4天共25小时的水下视频。结果发现, 环境 DNA 宏条形码比水下视频多检测出66%的分子操作分类单元 (MOTUs) 。我们利用单一环境DNA标记或视频分别发现了66个和 43 个功能实体, 利用环境 DNA 检测出的功能丰富度高于水下视频。尽管遗传参考数据库仍存在一些空缺, 但环境DNA 还是比视频检测出更高的鱼类系统发育多样性;累积曲线展示了1个环境 DNA 样带检测到的系统发育多样性相当于25小时的水下视频。环境 DNA 宏条形码可以经济、有效且准确地用于调查海洋系统中的生物多样性因子。尽管分类学鉴定仍受到遗传参考数据库中物种覆盖范围的限制, 但 MOTUs 的使用显现了在参考数据库得到扩充后环境 DNA 宏条形码所具备的潜力。**【翻译:胡怡思;审校:聂永刚】**

关键词: 视频, 环境DNA宏条形码, 生物多样性, 功能性状, 累积曲线, 马尔佩洛岛, 海洋保护区, 热带珊瑚礁

INTRODUCTION

In a context of global changes, monitoring species communities is essential for biodiversity assessment and the evaluation of management strategies (Cinner et al., 2020). In most biodiversity inventories each species is considered independently of its evolutionary history or functional traits (Cardoso et al., 2014). Yet, species diversity alone does not provide sufficient information on ecosystem states and processes because not all species are equivalent (Brun et al., 2019; Craven et al., 2018). A multifaceted approach to biodiversity assessment is often required to better understand community changes and conservation outcomes (Mbaru et al., 2020; Monnet et al., 2014; Trindade-Santos et al., 2020). So far, few researchers have compared the ability of inventory methods to measure multiple facets of biodiversity.

Taxonomic diversity (TD) represents the sum of species present in a given community and is the most widely used measure of biodiversity (Cardoso et al., 2014). Yet, TD ignores ecological differences among species (Jarzyna & Jetz, 2016). Two prominent approaches have been proposed to complement taxonomic information by accounting for species' ecological features and evolutionary divergence (McGill et al., 2006; Webb et al., 2002). Phylogenetic diversity (PD) quantifies the extent of evolutionary history in a given community, a key facet in biogeography, conservation, and ecosystem functioning (Forest et al., 2007; Tucker et al., 2019). Functional diversity (FD), the extent of species' trait values, sheds light on community assembly rules and ecosystem functioning (Mouillot, Graham, et al., 2013). Although PD has been considered a surrogate for FD, recent studies challenge this assumption (Mazel et al., 2018) or reveal an asynchrony in responses of both facets to disturbances (Devictor et al., 2010; Monnet et al., 2014). Thus, TD, FD, and PD are complementary and should be considered in parallel as part of a comprehensive assessment of biodiversity.

In marine coastal ecosystems, monitoring is traditionally performed using underwater visual censuses (UVCs) (Cinner et al., 2020), remote underwater video systems (RUVs), or environmental DNA (eDNA) metabarcoding, a molecular method that recovers DNA traces from the environment (water, sediments, etc.) (Deiner et al., 2017). Although UVCs have known biases (e.g., limited sampling time and space or diver avoidance [MacNeil et al., 2008]), video-based assessments can provide many hours of sampling without diver presence (Dickens et al., 2011). Remote videos recover about the same TD as most historical UVC methods because small benthic and low-range species are missed, but large predators more detected (Bosch et al., 2017; Colton & Swearer, 2010; Langlois et al., 2010). Environmental DNA can recover more or about the same TD than traditional methods such as netting, UVC, or RUVs (Boussarie et al., 2018; Nguyen et al., 2019), and most often provide a complementary inventory (Stat et al., 2019). In the Mediterranean Sea, Aglieri et al. (2021) showed how eDNA detects a larger functional breath than UVC, BRUVs, and small-scale fisheries methods, despite the latter detecting more TD. Yet, few researchers have focused on tropical systems, and, to our knowledge, no one has compared the ability of eDNA versus video surveys to measure all 3 biodiversity facets together.

We used eDNA metabarcoding and long-duration videos to survey marine fishes and sharks off Malpelo Island, a marine protected area and World Heritage Site. We compared TD, FD, and PD results derived from both methods. Underwater life there is highly diverse, with around 300 bony fish, shark, and ray species, including 5 endemic species (Chasqui Velasco et al., 2016).

METHODS

Study site and sampling

We sampled around the Sanctuary of Fauna and Flora in Malpelo, a remote oceanic island 490 km off the Colombia in the eastern tropical Pacific (Figure 1), for 4 days (25–28 March 2018) at 1 site (El Arrecife). Malpelo is surrounded by deep water and fishing activities are prohibited in the surrounding 8757 km^2 (Edgar et al., 2011). The reef ecosystem around the island is influenced by major oceanic currents (Rodríguez-Rubio et al., 2003) and local upwelling, and the benthos is bare rock with low coral cover (Quimbayo et al., 2017). Malpelo Island is one of the most pristine and vulnerable reef ecosystems in the tropical eastern Pacific. Fish biomass and biodiversity are high (>250 vertebrates species) and provide a baseline for undisturbed assemblages in this marine province (Quimbayo et al., 2017).

We deployed 1 long-duration RUV (Extrem-Vision, Rivesaltes, France) that films up to 12 hr (screenshots in Appendix S1). The camera was 40 cm above the seafloor (13 m deep, 04.00600°, −81.60433°) and had a 90° field of view in which benthic and pelagic areas were recorded over 10 m^2. Resolution was 1920 × 1080 pixels, and 30 frames/s were shot. Recording occurred on 25 (day and night) and 28 March (day) (Figure 1c). Cameras filmed 24 hr and 50 min of video. At night, 2 dive lights illuminated the camera's view. A Hero 5 (GoPro, San Mateo, California) was mounted on top of the RUVs to film in the opposite direction for the first 2 hr of deployment of each daylight recording. Three hours and 30 min were recorded with the GoPros. During video recordings, we sampled eDNA above the camera in round surface transects. We did 5 identical transects at different times, corresponding to 10 samples (i.e., eDNA filters) (Figure 1) because we collected 2 samples/transect. Transects sampled from a boat, and we pumped 30 L of water/sample. We used an Athena peristaltic pump (Proactive Environmental Products, Bradenton, Florida) (nominal flow 1.0 L/min) on each side of the boat to filter water through a VigiDNA 0.20 μm cross-flow filtration capsule (SPYGEN, le Bourget du Lac, France). To avoid contamination, we used only disposable sterile tubing and gloves for each filtration capsule. Immediately after filtration, the filter units were filled with CL1 Conservation buffer (SPYGEN) and stored at room temperature (20–25 °C) for 5.5 months until DNA extraction.

Video processing

Two frames per second were extracted from all videos. Fishes were identified at the lowest taxonomic level possible, following Fishbase taxonomy (Froese & Pauly, 2000), by trained personnel, who recorded the first occurrence of each species in each of the videos (i.e., number of individuals per species was not recorded).

Environmental DNA processing

The DNA extraction was performed in a dedicated laboratory for eDNA extraction equipped with positive air pressure, UV treatment, and frequent air renewal and decontamination procedures conducted before and after all manipulation. For DNA extraction, we followed the protocol in Fernández et al. (2021).

For PCR amplification, we used 3 different primer pairs targeting distinct taxonomic groups: teleo, targeting teleost fishes and elasmobranchs (Valentini et al., 2016); Chon01, targeting elasmobranchs; and Vert01 (Taberlet et al., 2018), targeting vertebrates in general (primer sequences in Appendix S2). The PCR mixture was denatured at 95 °C for 10 min, followed by 50 cycles of 30 s at 95 °C, 30 s at 55 °C for teleo and Vert01 and 58 °C for Chon01 and 1 min at 72 °C, and a final elongation step at 72 °C for 7 min. Twelve replicates of PCRs were run

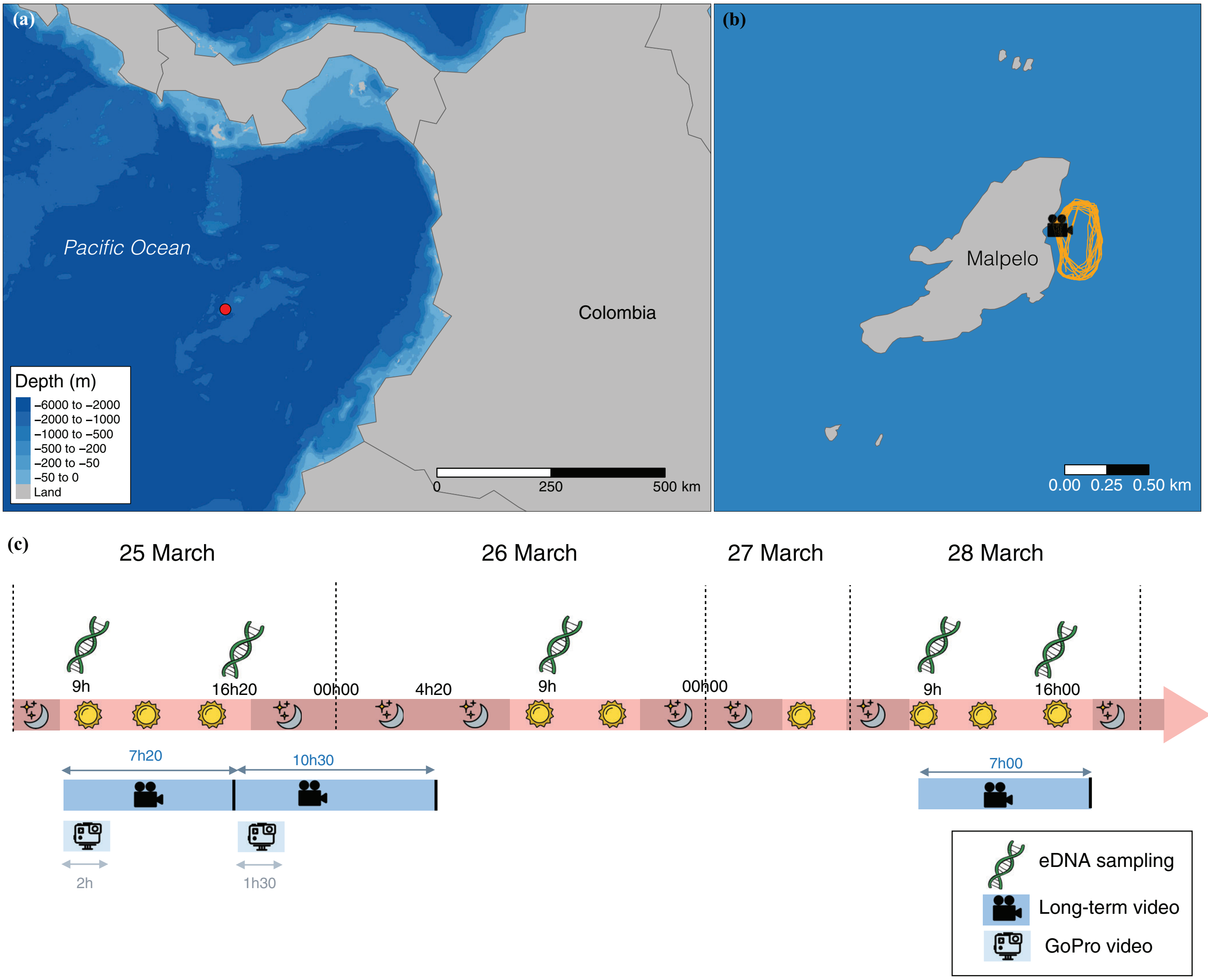

FIGURE 1 (a and b) Location of environmental DNA (eDNA) transects (tracks) and video cameras (camera symbol) near Malpelo Island, Colombia, and (c) timing of video recording and eDNA sampling

per sample (i.e., 24/transect because we had 2 field duplicates/transect). The primers were 5′-labeled with an 8-nucleotide tag; there were at least 3 differences between any pair of tags. The tag combinations were unique to each sample for Chon01 and Vert01 primers and unique to each PCR replicate for teleo primer. The tagging system allows assignment of each sequence to the corresponding sample during sequence analysis. After amplification, samples were titrated using capillary electrophoresis (QIAxcel [Qiagen GmbH, Hilden, Germany]) and purified using a MinElute PCR purification kit (Qiagen GmbH, Hilden, Germany). The purified PCR products were pooled in equal volumes to achieve a theoretical sequencing depth of 1,000,000 reads/sample/marker. Library preparation and sequencing were performed at Fasteris via a ligation protocol (Geneva). Three libraries were prepared using the MetaFast protocol (Fasteris, Plan-les-Ouates, Switzerland). For all libraries, a paired-end sequencing (2 × 125 bp) was carried out with an Illumina HiSeq 2500 sequencer on 2 HiSeq Rapid Flow Cells (version 2) with the HiSeq Rapid SBS Kit (version 2) (Illumina, San Diego, CA, USA). Library preparation and sequencing were performed at Fasteris (Geneva). Three negative extraction controls and 1 negative PCR control (ultrapure water, 12 replicates) were amplified per primer pair and sequenced in parallel with the samples to monitor possible contaminants.

Bioinformatics

Following sequencing, reads were processed using clustering and postclustering cleaning to remove potential errors and estimate the number of species based on molecular operational taxonomic units (MOTUs) (Juhel et al., 2020; Marques et al., 2020). Design of amplicon sequence variants through denoising is also used to analyze eDNA (Callahan et al., 2016). Marques et al. (2020) and Sales et al. (2021) demonstrated the accuracy of MOTU clustering in estimating the number of species in the absence of complete genetic reference databases. An approach focused solely on denoising would be insufficient to remove all errors and thus would overestimate the number of species (Brandt et al., 2021). Estimation of species diversity with MOTU richness was only performed using the teleo marker because other markers have not been tested extensively and their performance for estimating species richness remains unassessed.

For all markers, reads were assembled using Vsearch (Rognes et al., 2016) and then cut using Cutadapt (Martin, 2011). We used Swarm (Mahé et al., 2015) for clustering; the minimum distance of 1 mismatch between clusters followed Marques et al. (2020). We discarded all observations with fewer than 10 reads, corresponding to untargeted taxa or present in only 1 PCR in the dataset, to avoid spurious MOTUs originating from a PCR error because it is unlikely for the same error to be generated several times in distinct PCRs. Chimeric sequences were discarded using UCHIME. All sequences with a frequency of occurrence <0.0006/plate position in the same sequencing batch and <0.001/library were discarded to avoid index cross talk (MacConaill et al., 2018) and tag jumps (Schnell et al., 2015). These thresholds were empirically determined per sequencing batch with experimental blanks (combinations of tags not present in the libraries). For the teleo marker only, we applied the postclustering algorithm LULU to further refine diversity estimations based on MOTUs proxy (Frøslev et al., 2017; Marques et al., 2020). For all markers, taxonomic assignments of MOTUs sequences were carried out with the ecotag program from the OBITOOLS toolkit (Boyer et al., 2016); the European Nucleotide Archive (Leinonen et al., 2011) was used as a reference database (release 141). Taxonomic assignments were corrected to avoid overconfidence in assignments: species assignments were validated only for a 100% sequence match, genus for a 90–99% match, and family for an 85–90% match (Juhel et al., 2020). Fish names were verified using the rfishbase R package (Boettiger et al., 2012). All taxa assigned to deep water or mesophotic species or lineages were flagged and not analyzed due to a lack of trait values for the functional analysis.

Trait-based analyses

Six traits acted as proxies for species' contributions to ecosystem functions: body size, mobility, period of activity, schooling behavior, vertical position in the water column, and diet (Villéger et al., 2017), coded as categories (Mouillot et al., 2014). When a recorded species was absent from the database, trait values came from the literature or the dominant trait value within its genera was used. Species with the same trait values were grouped into functional entities (FE) (Mouillot et al., 2014). The functional distances between all pairs of species were computed using Gower's distance, which accounts for several types of variables (Legendre, & Legendre, 1998). To construct a multidimensional functional space, we performed a principal coordinate analysis (PCoA) on this distance matrix and kept the 4 first axes, which provided a faithful representation of the initial trait-based distance between species according to the mSD quality index (Maire et al., 2015). For the MOTUs not assigned at the species level due to gaps in the genetic reference database, trait values were assigned based on trait values from clades at higher taxonomic levels. More precisely, when a MOTU was assigned only to a genus, we randomly sampled 1 species among all species from the same genus occurring in the tropical eastern Pacific. If no species among the region had trait data, we randomly sampled 1 species among all species from the genus. When an MOTU was assigned to a family, the same method was applied among species from the same family. To evaluate the effect of assigning trait values to MOTUs based on 1 species from the same genus or family, we conducted the same analyses based only on MOTUs assigned at the species level.

Comparing fish biodiversity estimates

We compared taxonomic, phylogenetic, and FD computed from video and eDNA data. Beyond the comparison of family identities and considering the limited number of species identified with eDNA, we compared the methods based on MOTUs generated with eDNA as a proxy for species. Because species identity is not accessible for most MOTUs, we used them as proxies for species for MOTUs generated with the teleo marker to make comparisons at a higher taxonomic level (i.e., family). For each family, we estimated the number of species detected by each method without having to assign a species. Functional richness was the proportion of the functional space occupied by a species assemblage (Villeger et al., 2008). We generated accumulation curves over recording time. All recordings were combined in chronological order to create 1 long, continuous video.

For eDNA, we considered each of 10 filters individually and arranged them in chronological order to create the accumulation curves. Because field duplicates are taken at the same time, they were randomly placed first or second. Taxonomic richness and FE richness were computed to generate accumulation curves through time. We used R package vegan to generate a randomized MOTU richness accumulation curve for richness and FEs and R package PDcalc to generate a rarefaction curve for PD. We computed functional dissimilarity between the 2 census methods as the proportion of nonoverlap in the functional space between the convex hulls shaping the taxa recorded by each method and as the contribution of turnover to this dissimilarity (Villéger et al., 2013). These indices were calculated with R package betapart (Baselga & Orme, 2012). We computed Faith's PD for identified taxa with picante R package applied to 100 supertrees (Rabosky et al., 2018) pruned at the genus level for teleosts (bony fish). Faith's PD represents the sum of the length of branches linking all taxa present in an assemblage. All genus and species MOTUs were considered for PD analysis. Elasmobranchs were not included in the PD analysis.

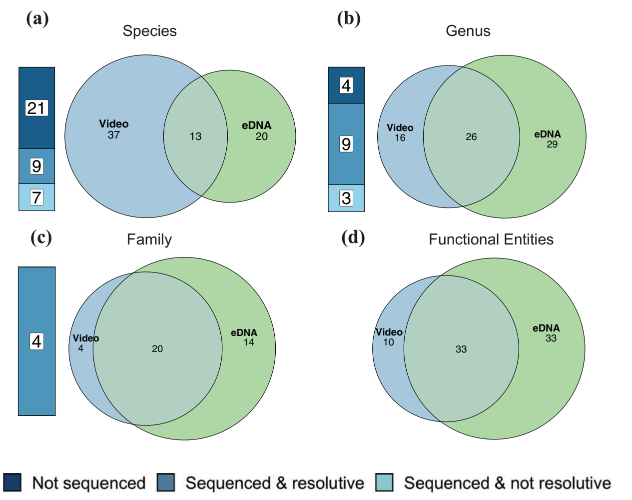

FIGURE 2 Comparison of the number of (a) species, (b) genera, and (c) families detected by eDNA and video and (d) the number of functional entities for the teleo marker (numbers in the bars: species was sequenced, species was sequenced and resolutive at the species level, and species was sequenced and not resolutive, respectively, from dark blue to light blue)

Results

Biodiversity estimates

We recovered 3.3 million DNA sequences after bioinformatic quality filtration, corresponding to 130 distinct MOTUs (Appendix S3). Among these, 23 MOTUs were assigned to a taxonomic level higher than family (percent similarity <85%) and not included in our analyses. Twenty-two MOTUs were assigned to deep-water fishes (e.g., *Diplophos taenia* or *Triphoturus mexicanus*) and were removed from analyses. Overall, among eDNA sequences belonging to a shallow-water taxon identified at least to family, 3 million sequences from 85 MOTUs were retained (Appendix S4). Thirty-three MOTUs could be assigned to species, so we considered the lowest taxonomic assignment for each MOTU and thus performed some analyses at the family level to allow a more representative comparison between methods.

Among taxa detected with eDNA, 66 had distinct FEs, 52 of which represented 1 taxon, 10 represented 2 taxa, 3 represented 3 taxa, and 1 represented 4 taxa (Figure 2). On videos we identified 51 taxa, 50 species, and 1 genus (*Mobula* sp.). The 51 taxa encompassed 43 FEs: 37 FEs were composed of 1 species, 5 FEs of 2 species, and 1 FE of 4 species. Combining the methods generated 77 FEs, among which around half (33) were shared. Ten were unique to videos, and 33 were unique to eDNA (Figure 2). For species-assigned MOTUs, 25 FEs were detected only on videos, 11 only by eDNA, and 18 by both methods (Appendix S5).

Taxonomic congruence between methods

The difference in taxonomic-level assignment between methods prevented a straightforward comparison of detected taxa; 98% of taxa were identified at the species level on videos, as opposed to 40% for eDNA. Video detected more species than eDNA: 50 versus 33, respectively (13 shared). However, only 24% (9 of 37) of species detected exclusively with video were sequenced and detectable at the species level with eDNA (Figure 2). Environmental DNA with a single marker detected more genera (55) than video (42) and more families (34 vs. 24, respectively) (Figure 2). For all 20 families detected with eDNA and video, eDNA detected more or the same amount of MOTUs compared with species from video (Figure 3). For 13 families, the number of video-detected species was the same as the number of eDNA-detected MOTUs. For the 7 remaining families, eDNA detected more MOTUs compared with the number of species detected with video. Among the 14 families detected exclusively with eDNA, we detected 5 MOTUs of Scombridae and 2 MOTUs of Gobiidae; videos showed no species of these families. Combined, the teleo marker and Chon01 and Vert01 markers (Appendix S6) revealed 34 extra taxa (Appendices S7 & S8), including 15 species. The combination of all 3 markers changed the number of shared species with videos from 13 to 17, genera from 26 to 35, and families from 20 to 22 (Appendix S9). Multimarker eDNA detected 46 families (24 not detected on videos), whereas 2 families were detected with videos exclusively (Scaridae and Aulostomidae). The Vert01 primer detected 3 taxa of marine mammals (Delphinidae, *Grampus griseus*, and *Kogia sima*) and 1 marine bird (*Sula* sp.), which were not included in our analyses.

Functional and phylogenetic congruence between methods

Combined, eDNA with the teleo marker and videos revealed 71 fish genera, 67 teleosts representing a Faith's PD of 4603. The 37 genera detected with videos revealed a PD of 2729 (59% of total), whereas the 49 genera detected with eDNA revealed a PD of 3767 (82% of total). Four genera detected with videos only were not detectable with eDNA due to gaps in genetic reference database (Figure 2). Extending the eDNA analysis to multimarkers revealed 14 extra genera, extending the assemblage PD to 5322, with a PD of 4971 for multimarker eDNA alone (Appendix S10). Thus, 93% of total PD was detected with multimarker eDNA versus 51% with video.

Video-recorded species filled a smaller functional space (i.e., convex hull delimited by the most extreme combination of traits values) than eDNA-recorded taxa (Figure 4 & Appendix S11). The dissimilarity (β diversity) between those convex hulls was 0.37, and turnover contributed to 16% of this dissimilarity, highlighting that taxa recorded on video filled mostly a subset of the space of eDNA-recorded taxa. The portion of the functional space filled by eDNA only was driven by a few taxa (e.g., *Psenes cyanophrys*, *Mobula tarapacana*, or *Canthigaster jactator*) that were strictly pelagic, planktivorous, or small omnivorous species. The small functional space filled only by video-detected taxa was due to the small invertivorous cryptobenthic blenny (*Hypsoblennius maculipinna*), which was not detected with eDNA. Including eDNA from all 3 markers showed that

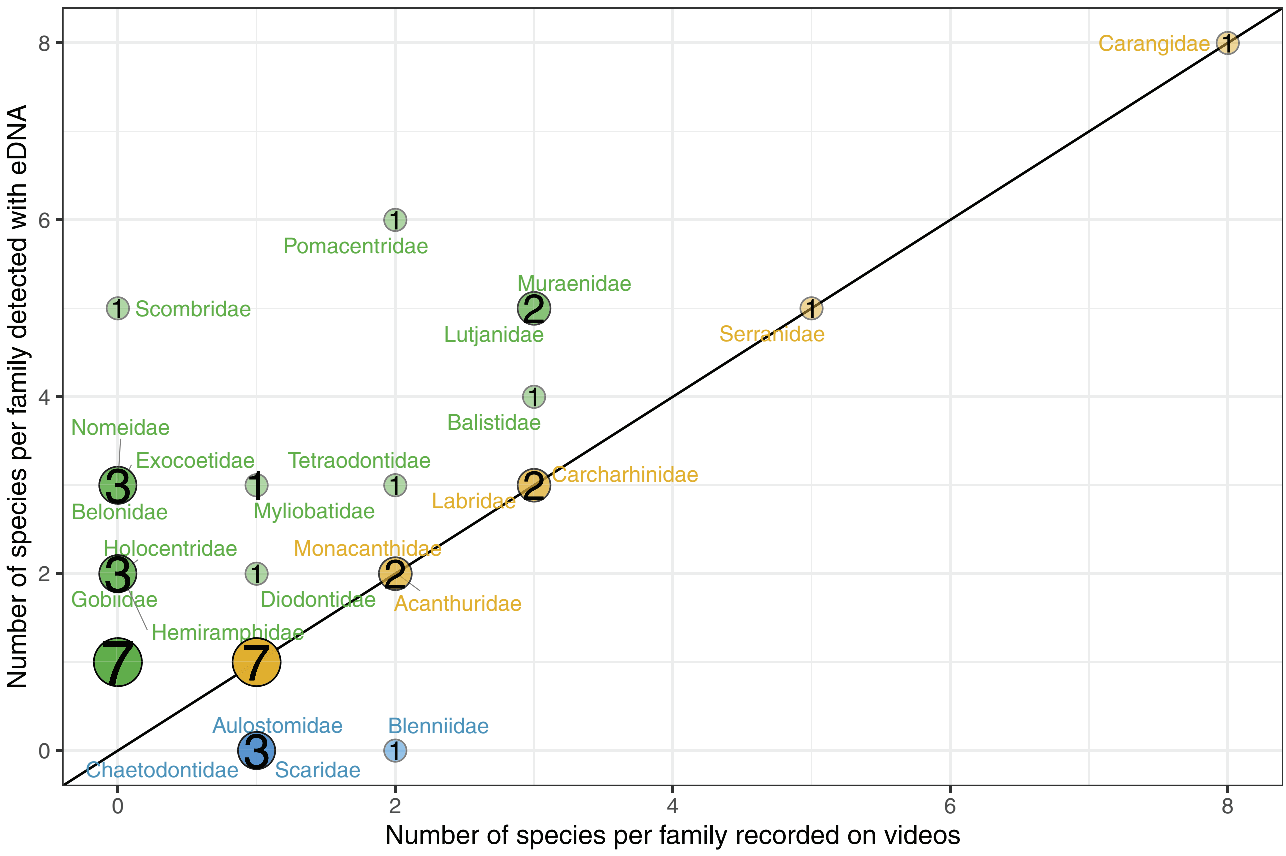

FIGURE 3 Comparison of the number of species per family, without deep-water families, based on environmental DNA (eDNA) teleo marker and video (numbers in circles, number of families at that point; green, eDNA performs better; blue, video performs better; purple, both methods similar performance). Family identities are indicated, except when more than 5 families co-occur at the same point

video-recorded taxa filled part of the functional space that contained all eDNA-detected taxa (Appendix S10).

Biodiversity accumulation curves and asymptotes

One hour of video resulted in the detection of 63% of species (32) and 70% of FEs (30) identified over 25 hr of video (Figure 5a, c). After 2 hours, 7 more species and 4 FEs were recorded; 76% (39) of species and 81% (34) of FEs. In 7 hours of video, 90% of all FEs were detected on videos (39 of 43). After 25 hr of video, 56% of all FEs detected with both eDNA and videos (43 of 77 total). In 6 hr of video, 90% of total PD was recorded. For eDNA, 67% of MOTUs (57) and 70% of FEs (46) were detected in 2 samples (1 transect) (Figure 5b, d). After 4 transects (8 samples), 93% of MOTUs (79) and 92% of FEs (61 of 66) were detected. Two eDNA samples detected as much Faith's PD as 25 hr of video (PD = 2735) (Figure 5e, f), but 10 eDNA samples with the teleo marker did not detect as much PD as 10 eDNA samples with the combination of all 3 markers (PD = 4971) or the combination of all methods (i.e., all eDNA primers and video combined [PD = 5322]) (Appendix S10).

DISCUSSION

We found that eDNA metabarcoding outperformed long-duration remote video recording in estimating several facets of fish biodiversity. More MOTUs (species proxy) and higher FE, FD, and PD richness were detected with eDNA than in 25 hr of video. Fast and reliable estimations of biodiversity with eDNA should help scale up the current spatiotemporal extent of sampling and bring a multifaceted perspective to reef fish biodiversity (Cinner et al., 2020).

RUVs and eDNA were complementary methods to survey species diversity in reef ecosystems, but eDNA detected more genera and families. Fourteen families were detected with eDNA only, whereas 4 were detected with video only. This advantage was more pronounced when combining multiple primer pairs: overlap between methods was higher and more taxa were detected with eDNA only. Twenty-four families were exclusively eDNA detected and 2 were exclusively video detected. Both methods detected mobile yet elusive predators well (e.g., jacks and sharks); more taxa were detected with eDNA. Unbaited cameras seem to perform well for shark detection, but this may be because Malpelo Island is a shark gathering place (Bessudo et al., 2011; Ketchum et al., 2014). Hence, the detection probability on video was likely higher around Malpelo

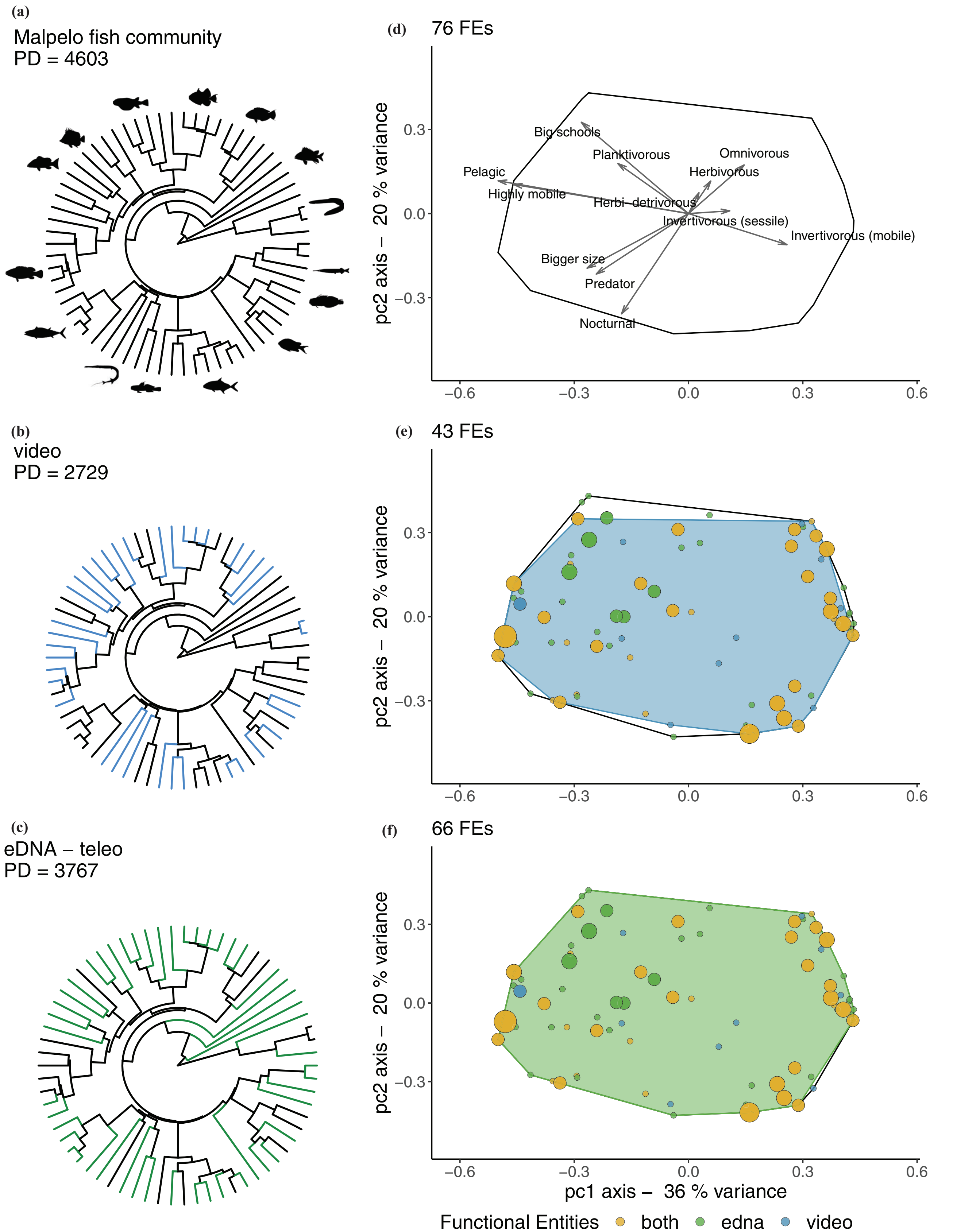

FIGURE 4 Comparison of phylogenetic diversity and functional diversity detected via eDNA and video: (a) phylogenetic tree of teleost fish at the genus level based on (a) a combination of eDNA with teleo and video, (b) video only, and (c) eDNA with only the teleo marker and the functional space filling in for the 2 principal component (PC) axes at the best taxonomic level for (d) the entire assemblage, (e) video only, and (f) eDNA with only the teleo marker (arrows, traits driving each PC axis scaled to the hull envelop extreme values; PD, Faith's phylogenetic diversity)

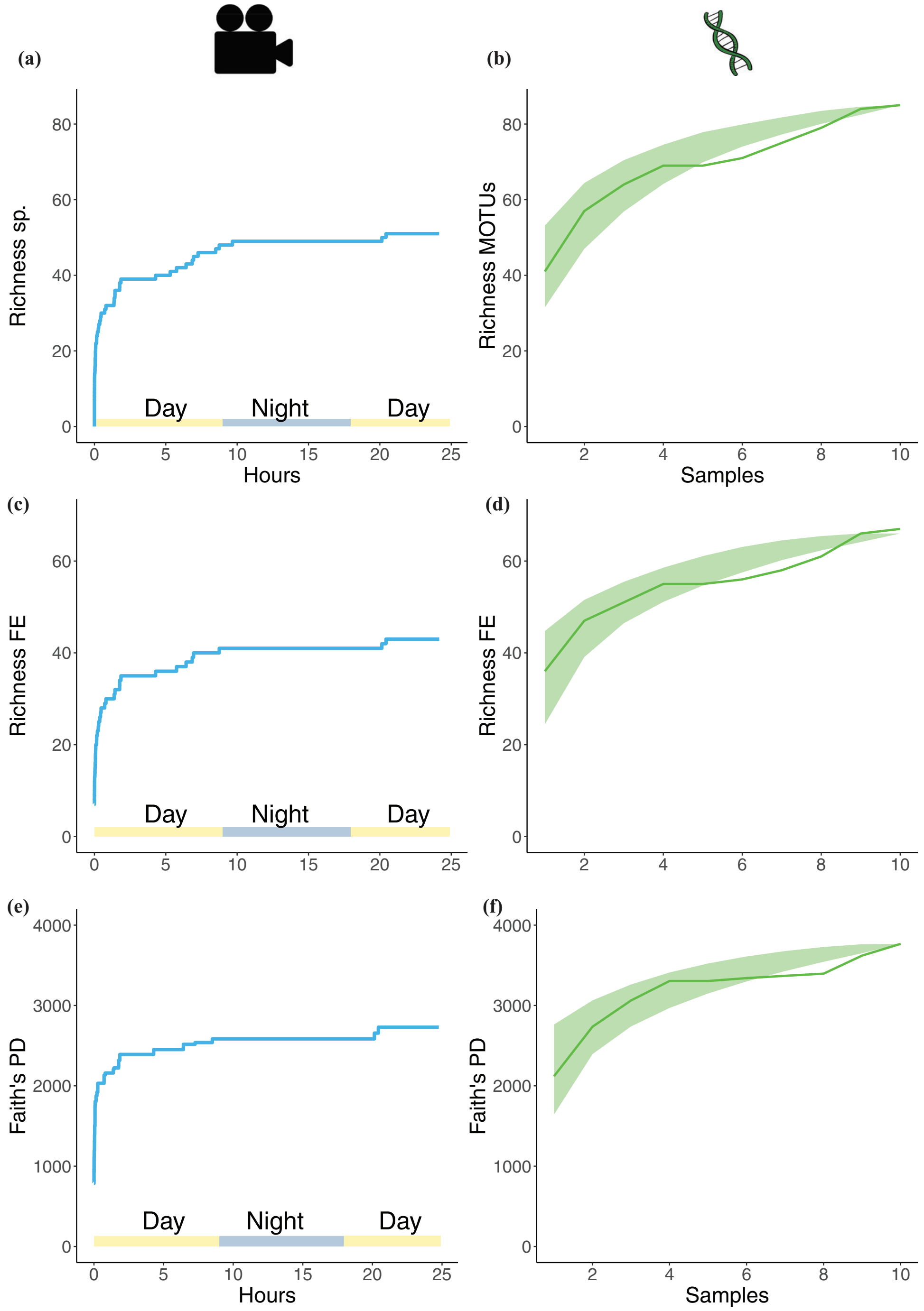

FIGURE 5 Accumulation curves for (a) species richness on videos, (b) molecular operational taxonomic unit (MOTU) richness for eDNA, and richness of functional entities (FEs) for (c) videos, (d) eDNA, and (e) Faith's phylogenetic diversity (PD) with video and (f) with eDNA (a single marker used for eDNA) (green ribbon, accumulation curve with standard deviation based on a randomized saturation approach for eDNA; green line, real values ordered by sampling date)

Island than around a typical reef, where sharks are scarcer and more cautious (Juhel et al., 2019) and thus where eDNA may perform better than other methods (Bakker et al., 2017; Boussarie et al., 2018).

Our results contrast with those of the only other study comparing eDNA and camera-based fish surveys (Stat et al., 2019), where the authors found complementarity in detection between methods at the genus level. Such differences may derive from use of short-duration filming (1 h) of baited cameras instead of long-duration filming of unbaited cameras (25 h) or from a different eDNA protocol. They used 500-ml water samples with a 16S marker, whereas we used a 12S marker and 30-L samples over a surface transect, which is expected to yield more detections due to eDNA particle dilution in marine environments (Thomsen et al., 2012). In highly diverse systems, traditional methods generally perform better than eDNA for TD, but this disadvantage seems mostly due to reference database gaps (McElroy et al., 2020). As expected, the reference database completeness impaired our use of eDNA, but our clustering approach allowed derivation of some TD metrics and revealed a strong potential for a fast TD census in marine ecosystems once reference databases are more populated.

Assessment of FD assessment with eDNA was better than with video; more FEs were detected and functional richness was higher. Despite disparities in taxonomic inventory, both methods revealed a close set of FEs: 77% of FEs (33/43) detected on video were also detected with eDNA and 50% (33/66) of FEs detected with eDNA were also detected on videos. Using only species-assigned MOTUs revealed 29 FEs, 11 exclusive to eDNA despite a low number of species-level assignments. This suggests the higher number of FEs detected with eDNA with random assignments of traits at higher taxonomic levels is probably not an artifact and shows the potential of eDNA-based inventory as genetic reference databases become more populated. Long-duration filming was necessary to capture most FEs. One hour of video recovered 70% of all FEs detected with videos, and 7 hr sampled 90% of FEs. One eDNA transect with 2 filters detected as much FE as 25 hr of videos, highlighting its ability to quickly inventory FD. Environmental DNA also detected a higher functional richness, meaning FEs detected exclusively with eDNA exhibited more extreme and distinct trait combinations than those detected with video only. The larger breadth of functional composition detected with eDNA was due to large pelagic piscivorous and planktivorous species that are vertices of the convex hull of the entire fish assemblage in the study area. The recording of large pelagic taxa with cameras was probably due to Malpelo being a remote oceanic island and the long-duration video, which can capture rare events or mobile species with low abundance. Other studies on marine systems suggest eDNA integrates a wider spatial signal (hundreds of meters), enabling detection of pelagic species (Boussarie et al., 2018; Aglieri et al., 2021; Valdivia-Carrillo et al., 2021), but can still delineate distinct habitats (Nguyen et al., 2019; West et al., 2020). Our eDNA inventory went beyond TD and measured FD without creating bias among FEs; thus, eDNA sampling can provide information on ecosystem functioning with little sampling effort.

We found that even considering a single taxonomic group with a single marker, eDNA outperformed video in PD detection. Some lineages were detected by eDNA and missed completely with video. Additional markers targeting teleosts expanded the PD detected with eDNA from 3767 to 4971 (almost 2 times the PD recovered on video). Limited sampling effort was required to identify much of the PD from the community; 1 transect detected as much diversity as 25 hr of video. Most video-based inventories do not film continuously for such long periods due to battery limitations and processing time (Mallet & Pelletier, 2014). Short-duration filming is likely to miss rare and mobile gregarious, large species from underrepresented lineages. Rare species are more distinct functionally and phylogenetically compared with their more common counterparts (Mi et al., 2012; Mouillot, Bellwood, et al., 2013). Under unprecedented global changes, phylogenetically diverse communities could have stronger evolutionary potential (Lavergne et al., 2010; Winter et al., 2013). It is crucial for monitoring methods to measure accurately the full evolutionary diversity of a community so that conservation can be implemented rapidly (Pollock et al., 2017) and global change effects can be tracked (Monnet et al., 2014).

Reference database coverage and marker resolution are among the main limitations to large-scale deployment of eDNA metabarcoding (Juhel et al., 2020; Jackman et al., 2021). Only about 13% of all fish species are currently sequenced using our teleo 12S marker (Marques et al., 2021), and alternative marker locations with larger reference sequences (e.g., on Cytochrome c oxidase subunit I - COI) are not appropriate for fish inventory because small fish-specific markers without amplification bias cannot currently be designed (Deagle et al., 2014; Collins et al., 2019; Zhang et al., 2020). The teleo marker has been sequenced for 107 species out of the 255 fish and shark species that occur or travel through the Malpelo ecosystem (Robertson & Allen, 2015). This sequence is unique (i.e., not shared with another species) to 36% (80/255) of them. To overcome reference databases limitations, it is useful to generate MOTUs to estimate the potential number of species present. Although MOTUs can accurately assess the level of biodiversity at all spatial scales (Marques et al., 2020; Sales et al., 2021), a MOTU may not translate into the presence of a species because it can represent several species within 1 cluster or several MOTUs belonging to 1 species if there is strong intraspecific variability or unfiltered PCR or sequencing errors. Lack of taxonomic resolution happens when distinct species share the same sequence, which can result in misidentification and underestimation of biodiversity. Our eDNA detection of *Carcharhinus obscurus* was probably a misidentification of the species *Carcharhinus galapagensis* because they are phylogenetically close and *C. galapagensis* was seen on videos but its barcode sequence is still unavailable. Our TD overlap between methods increased as taxonomic level increased due to gaps in genetic reference database, in accordance with previous eDNA studies (Valdivia-Carrillo et al., 2021). If a sequence does not match a referenced species, its genus or family can still be identified, which explains why we found a clear advantage for eDNA at higher taxonomic levels. Other biodiversity measures are also affected by this limitation; FD and PD could be better estimated if more sequences were identified to species level. Additional markers targeting the same taxonomic groups further expanded all measures of biodiversity, likely due to complementary reference database, although one can expect this advantage to fade in the medium term as reference databases expand. This finding reflects the potential of single-marker eDNA metabarcoding with larger genetic reference databases, although multiple-marker eDNA could still overcome the limitation of marker resolution.

Ecological functions provided by organisms and PD should be considered when measuring biodiversity (Cadotte et al., 2012; Diniz-Filho et al., 2013) because ecosystem functioning can be greatly altered without there being a strong impact on TD (D'Agata et al., 2014). Our results suggest that a multifaceted approach is feasible with eDNA metabarcoding, which delivered a faster and more exhaustive inventory than long-duration video. Video-based and eDNA methods can be complementary, mostly due to current limitations of genetic databases. Furthermore, fish size and behavior can also be monitored with video (Puk et al., 2020). Because eDNA analyses better estimate multiple facets of biodiversity, it has great potential for conservation, in which fast and accurate measures of diversity are required. Earlier detection of erosion of biodiversity facets would inform protection measures and improve understanding of the structure and functioning of communities (Benkwitt et al., 2020).

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