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REVIEW, CONCEPT, AND SYNTHESIS

An innovative approach for marine macro-organism monitoring: methodology and future perspectives of environmental DNA (eDNA) technology

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Abstract

Monitoring macro-organisms in the marine ecosystems has become a subject of significant interest in recent years. The utilization of environmental DNA (eDNA) technology presents a promising avenue for marine biomonitoring, offering an efficient and convenient approach. However, there is a scarcity of comprehensive studies that provide an overview of the development and application of marine eDNA technology. The present study involves a comprehensive investigation and compilation of all publications from January 2012 to November 2024 pertaining to the application of eDNA technology for monitoring marine macro-organisms. It presents a comprehensive overview of the current status of eDNA technology application in marine macro-organism monitoring, focusing on the historical development, technical processes, and practical applications. And the limitations of eDNA technology have also been proposed. A review of current research hotspots and innovation trends suggests that the application of eDNA technology will provide a novel approach to biodiversity conservation, macro-organisms monitoring in marine ecosystems.

Keyword eDNA · Macro-organism · Marine ecosystems · Biomonitoring

Introduction

Marine ecosystems are extensive and diverse, serving not only as critical zones for geochemical cycles but also as invaluable resources for human societies (such as providing essential services such as food and raw materials, contributing to cultural and spiritual enrichment) (Sala et al. 2021; James et al. 2024). However, human activities, global climate change, and other factors are destroying the biodiversity and balance of marine ecosystem (Wernberg et al. 2024). In this context, biodiversity monitoring is essential for marine ecosystems management and conservation. The traditional approaches, e.g., nets, traps, to biome surveying pose challenges with harm to organisms or ecosystems. In addition to being restricted by the water's depth and turbidity, traditional biological monitoring techniques can cause stress and injury to the captured species (English et al. 2024). Thus, the emergence and evolution of environmental DNA (eDNA) technology for biological monitoring has provided a solution to the limitations and drawbacks associated with traditional investigations.

eDNA refers to DNA that can be obtained from environmental samples without isolating the target organism. The presence of DNA can be traced to the organism itself or its remnants, such as skin, mucosa, and feces (Taberlet et al. 2018b). Community DNA is defined as DNA isolated from a large mixture of organisms found in environmental samples

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(Deiner et al. 2017). The eDNA technology is comprised of two primary analytical methodologies: species-specific detection and metabarcoding. Species-specific detection is a method of amplifying the DNA of a target organism species present in the environment using primers specific to the target species, which enables specific yet accurate monitoring of known species through quantitative PCR techniques (Capo et al. 2021). eDNA metabarcoding is a monitoring technique used to identify multiple species in environmental samples, which can be used in wider biodiversity surveys (Lamb et al. 2019). It is imperative to note that all references to eDNA technology in this paper pertain to monitoring technology that incorporates both species-specific detection and metabarcoding methods. The process of eDNA technology frequently encompasses the collection of environmental samples, eDNA extraction, quantitative or non-quantitative PCR amplification, library construction, sequencing, and data analysis (Thompson and Thielen 2023). It provides a new, convenient, and cost-effective method for macro-organism monitoring and study in marine ecosystem.

Recently, eDNA technology is increasingly applied to monitoring the macro-organism taxa in aquatic ecosystems. In 2008, it was first used to monitor rare and invasive macro-species (e.g., American bullfrogs) in waters, and the steps for macro-organism monitoring and subsequent bioinformatics analysis have established (Ficetola et al. 2008). In 2012, a thorough advancement in eDNA technology has emerged, and gradually applied to monitoring macro-organisms in marine ecosystems (Thomsen et al. 2012). eDNA technology has advanced over the next dozen years, undergoing enhancements in instrumentation, experimental methods, and other aspects of the methodological process. The most representative advance is the emergence of eDNA metabarcoding technology, which represents a momentous revolution based on the advancements in molecular biology (Miya 2022). The main experiences and improvements in the process of eDNA monitoring marine macro-organisms from 2012 to November 2024 have been summarized in our review as follows: I) water sampling (water volume and number of replicates), II) eDNA collection (i.e., filtration, including filter types and pore size), III) eDNA extraction, IV) quantitative and qualitative analysis, V) PCR primers, VI) application. Finally, the limitations, challenges, and prospects of eDNA technology in marine ecosystems have been succinctly outlined, with the aim of providing insights for future advancements in this field.

Overview of publications in Web of Science

Here, we conducted a comprehensive review of the literature on the utilization of eDNA technology for macro-organism monitoring of marine ecosystems. The Web of Science database (www.webofscience.com), encompassing publications across a wide spectrum of life science disciplines, was selected. Literatures were screened according to three dimensions: Was eDNA technology used? Were samples collected from marine ecosystems? Were macro-organisms studied? To accomplish our specific objectives, we excluded subject areas such as microbiology, pharmacology, prophylactic immunology, and medicine, along with conference papers, book chapters, and notes. Four hundred and sixteen publications were finally screened out. The following information was systematically recorded for each publication: study country / region, publication time, taxa studied, volume of water per sample, number of sample replicates, filter type and pore size used, DNA extraction methods employed, analytical methods utilized, and PCR primers employed.

The "Full record and cited reference" option was used to export 416 publications from the Web of Science as plain text. Subsequently, bibliometric network analysis was conducted by coupling keywords using “VOS viewer” (version 1.6.20). The method of binary counting for keyword co-occurrence was selected, wherein synonyms were replaced to ensure that only one keyword expressing that meaning is retained, and the minimum word frequency value was set to "5." The node size represented the number of citations, while links and link strengths indicated the relevance between keywords. Additionally, different colors were used to represent distinct clusters. The network successfully identified 88 out of 1812 keywords that met the predefined threshold, which were subsequently automatically classified into five distinct clusters based on their significance (Fig. 1). As a result, a total of 1570 links were generated, exhibiting an impressive aggregate link strength of 5167. The most frequently occurring keywords were "eDNA," "biodiversity," and "eDNA metabarcoding," with an average publication volume of 304, 194, and 116, respectively. Similarly, the terms "fish," "community," and "conservation" were also frequently mentioned, with an average occurrence of 99, 84, and 70 volumes, respectively. The keywords "sea" and "tool" exhibited a high average publication volume (51 and 47) as well. The results of occurrence frequency, links, and link strengths suggested that eDNA metabarcoding emerged as a popular tool for monitoring marine macro-organisms, particularly in assessing fish communities diversity.

The publications from countries affiliated with the first author exhibited distinct regional characteristics in terms of statistics (Fig. 2a). The United States had the largest

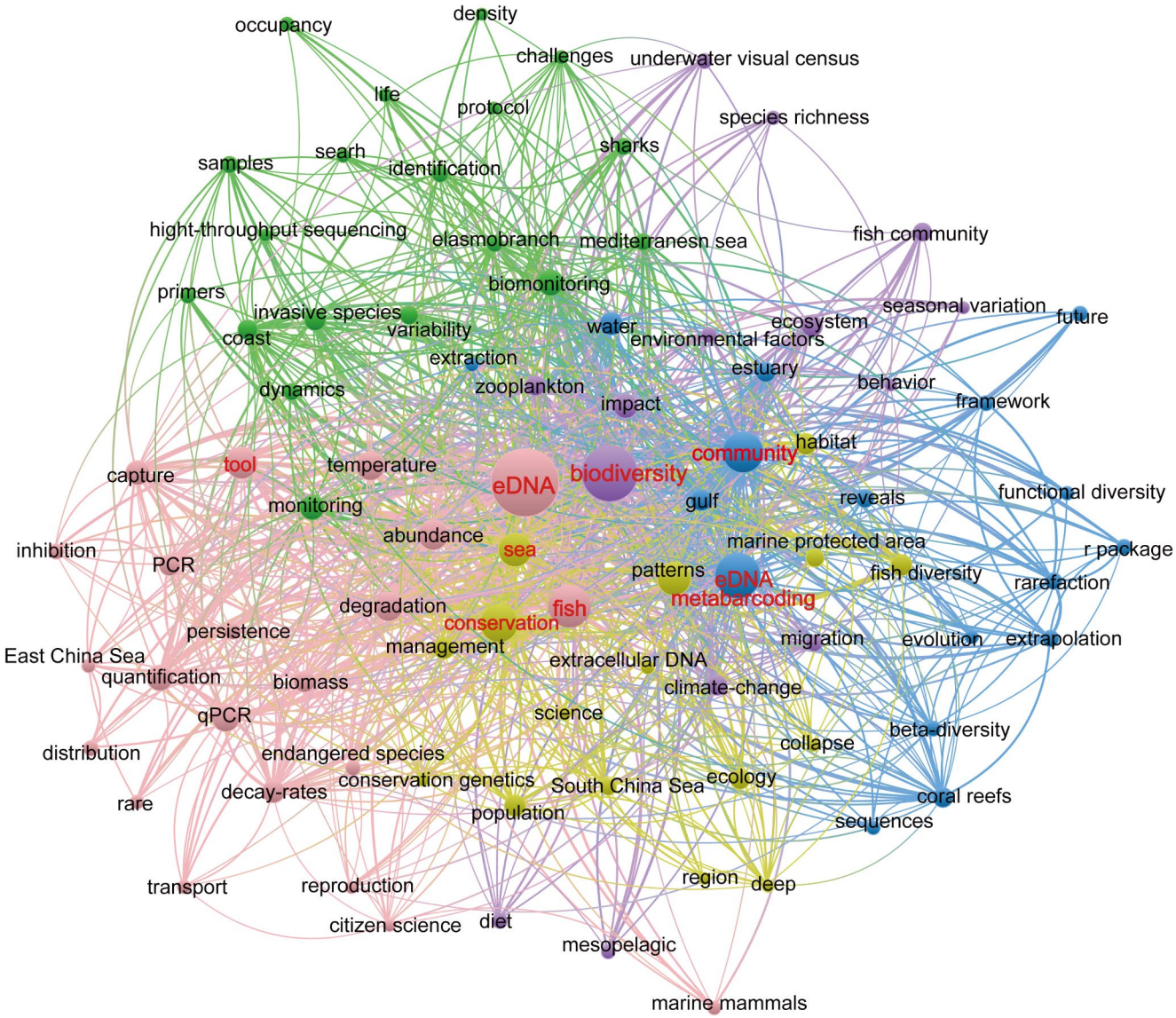

Fig. 1 Network diagram illustrates the co-occurrence pattern of keywords in relation to eDNA studies on marine macro-organisms using the VOS Viewer software. Different colors represent distinct clusters, while the nodes correspond to different keywords. The size of each node represents the frequency of keyword occurrences

proportions (20.4%), closely followed by China (14.6%), Japan (12.3%), and Australia (7.7%). The aforementioned countries possess superior technological and industrial capabilities, coupled with abundant marine resources. Additionally, eDNA technology was also employed for marine biomonitoring in France (6.5%), Italy (4.6%), Spain (4.3%), Denmark (3.8%), Canada (3.8%), and the United Kingdom (3.4%). Generally, these countries/regions were predominantly situated in coastal areas.

The year 2012 is widely recognized as a pivotal milestone in the progressive advancement of macro-eDNA technology, coinciding with the inaugural publication on macro-organism monitoring within marine ecosystems (Thomsen et al. 2012). The studies on macro-organism monitoring in marine ecosystems have been steadily increasing since 2015, with the top three years being 2024 (93, up to November, not the whole year), followed by 2023 (80), and then 2022 (63) (Fig. 2b). The innovations in technology and methods have led to increasing research on eDNA techniques for monitoring macro-organisms.

Fish play a pivotal role in marine ecosystems, serving as essential nutrient recyclers, which are not only a vital source of sustenance and nutrients for both humans and animals but also serve as reliable indicators of ecosystem health (Pinna et al. 2023). As a result, the taxon that was most frequently studied (56.3%) and exhibited an increasing trend was fish (Fig. 3a). The second most frequently studied taxa were multiple target taxa (17.1%), as research objectives

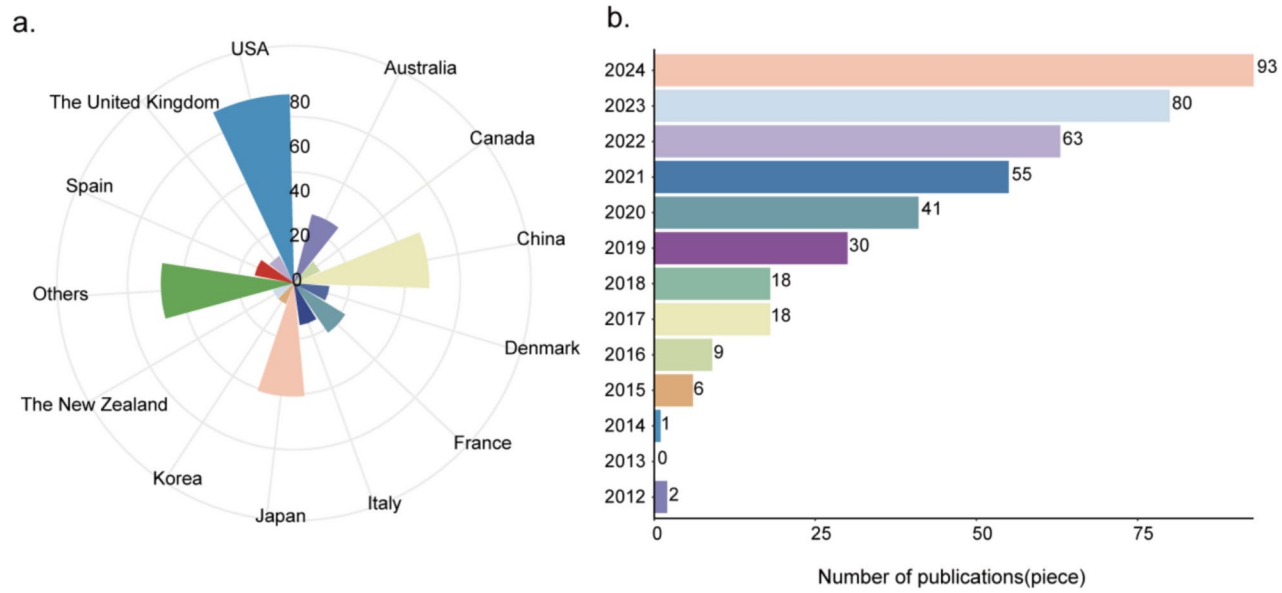

Fig. 2 Publication status of eDNA-related studies on marine macro-organisms retrieved from Web of Science between January 2012 and November 2024. **a**. Rose diagram showing the country or region of the first author; **b**. Number of publications on eDNA-related studies in each year

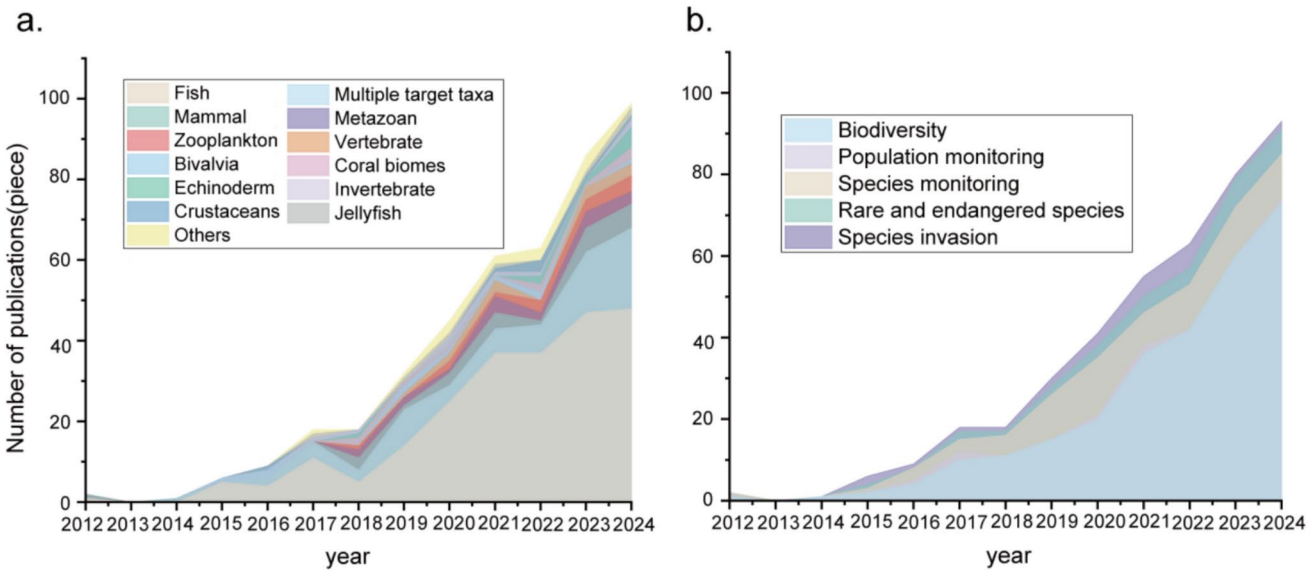

Fig. 3 Fluvial maps depicting the primary taxa studied in marine ecosystems using eDNA technology and their respective applications. **a**. Targeted taxa; **b**. Applications. The term "vertebrates" and "metazoans" in the article refer to studies that monitor all species in these taxa. The term "multiple target taxa" refers to studies that monitor two or more taxa at the same time, e.g., fish and crustaceans or fish and bivalves at the same time, etc

often focused on investigating the composition and diversity of various biocenoses (e.g., simultaneous monitoring of multiple taxa such as fish, shellfish and crustaceans) in a given region (Yang et al. 2024). The subsequent taxa examined were marine mammals, metazoans, and zooplankton, with respective proportions of 6.0%, 4.3%, and 3.4%. The endangerment of numerous marine mammals, as widely acknowledged, has garnered significant attention from both scientists and the public (Boldrocchi et al. 2024). The role of zooplankton extends beyond carbon and nutrient cycling, climate regulation, and fisheries; it also contributes significantly to human productive activities (Johnston et al. 2022). Generally, the marine macro-eDNA studies have predominantly focused on fishes, multiple targeted taxa, and bivalves, while the exploration of biocenosis diversity has witnessed a surge since 2017.

The application of marine eDNA technology has become increasingly diverse due to advancements and breakthroughs

in various fields. It has successfully applied in monitoring marine macro-biocenoses, encompassing various categories such as biodiversity assessment (66.1%), individual species monitoring (32.2%: economic/important species (19.2%), rare and endangered species (7.2%), invasive species (5.8%)), and population monitoring (1.7%) (Fig. 3b). The overall trend shows a steady increase in applications for biodiversity monitoring over the years, although the majority of population monitoring applications still remain in the exploratory phase.

The procedures of eDNA technology

Sampling

The quality of sample collection is determined by two factors, namely the volume of water sample and the number of replicates, directly impacting the outcomes of marine macro-organism monitoring (Fig. 4). Theoretically, the volume of water samples collected is directly proportional to the detectable proportion of local biodiversity. However, larger volumes of seawater consequently require increased expenditures on labor, logistics, and machinery consumption. Before 2015, the majority of eDNA studies utilized a diverse range of water sample volumes, encompassing both smaller and larger sample volumes (Thomsen et al. 2012). The advancement of technology has facilitated the extensive collection of seawater samples, enabling the acquisition of 30 or 50 L water samples in a single instance (Macé et al. 2024). Whether sampling for aquarium experiments or field experiments, larger volume of water samples, more different biocenonses can be detected (Mirimin et al. 2020). From 2016 to 2024, over one-third of the studies opted for collecting water samples ranging from 500–1,000 mL (40.1%), with a majority of them being 1,000 mL (38.0%) (Fig. 5a). The collection of 1 L water samples is more cost-effective and convenient for transportation compared to larger volumes, while still being sufficient for detecting representative taxa in the ocean (Takahashi et al. 2023).

The sampling strategies employed during biomonitoring processes should be customized to minimize the risk of

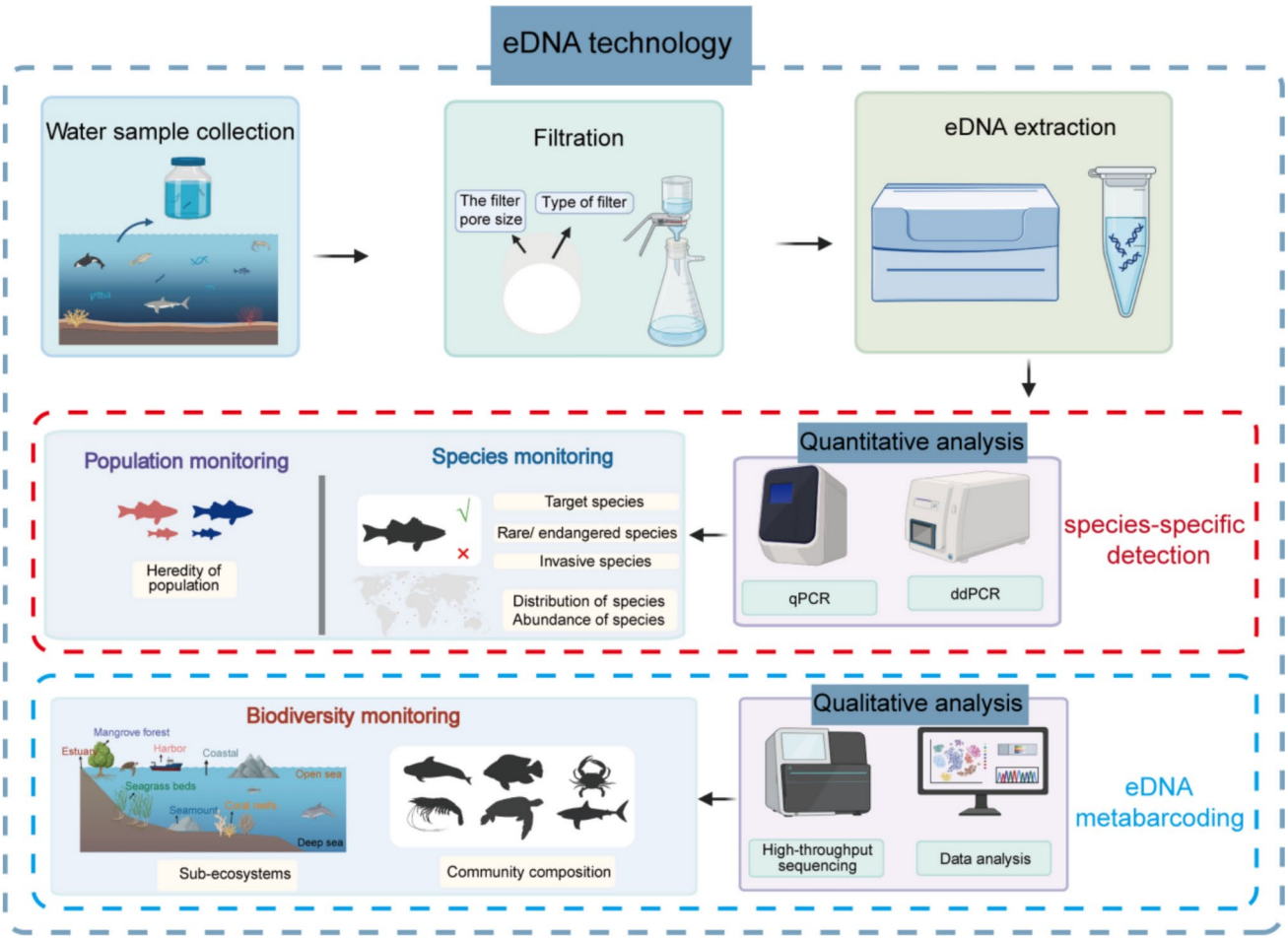

Fig. 4 Flow chart of eDNA technology. eDNA technology often includes sample collection, eDNA capture (filter membrane material and pore size), eDNA extraction, PCR amplification (quantitative and non-quantitative), sequencing, and data analysis

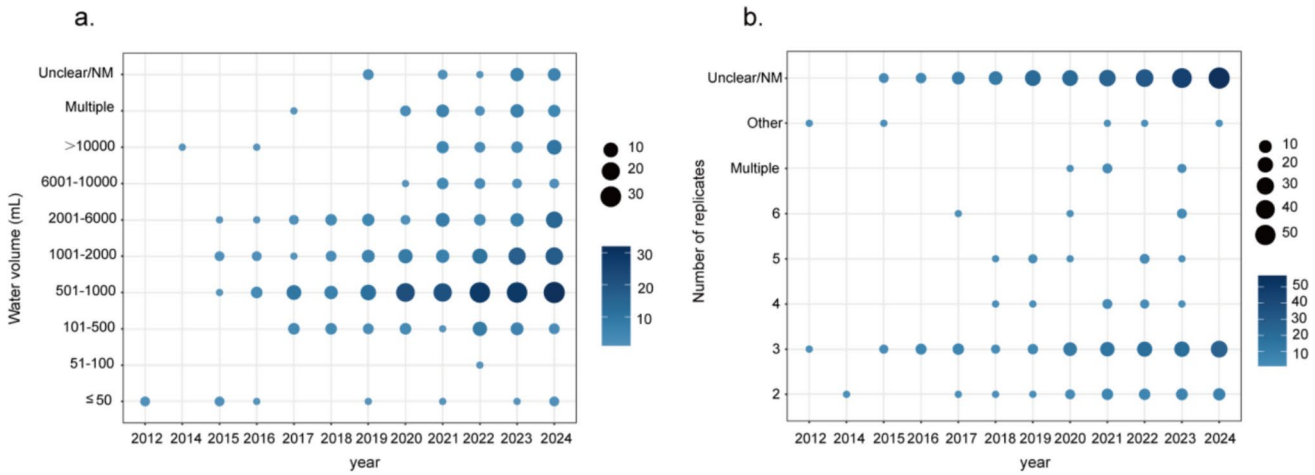

Fig. 5 **a**. The volume of sampling water in publications; **b**. The number of sampling replicates. The size and color of the bubbles represent the number of publications

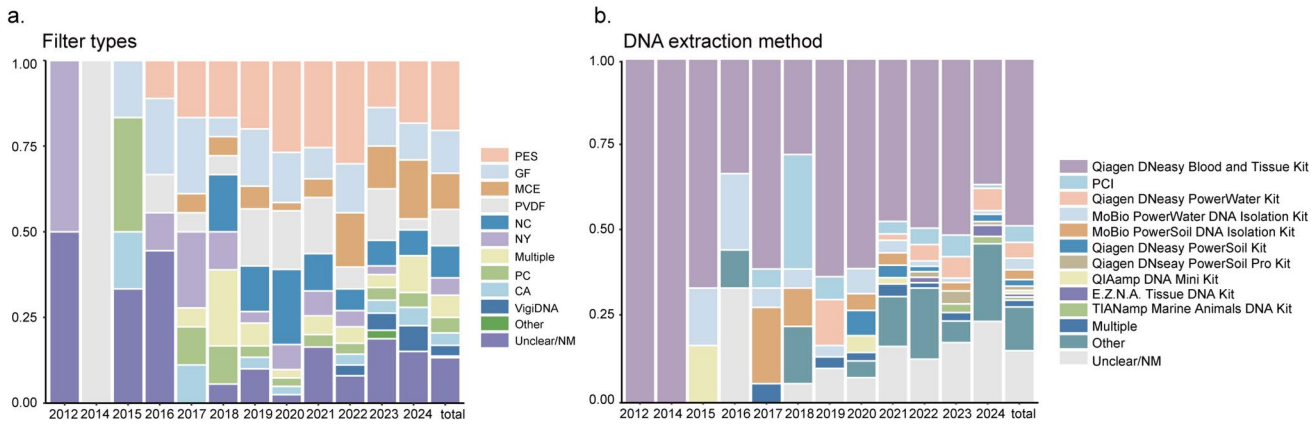

Fig. 6 **a**. Types of filter membrane; **b**. The methods and kits for eDNA extraction. Abbreviations for each filter material and eDNA extraction methods are as follows: nitrocellulose (NC), mixed cellulose ester (MCE), polyethersulfone (PES), polyvinylidene fluoride (PVDF), nylon (NY), polycarbonate (PC), cellulose acetate (CA), glass fiber (GF), phenol–chloroform isoamyl alcohol (PCI).

false-negative detections, either by increasing the number of replicates or the volume of water sampled in eDNA collections (Kawakami et al. 2023; Takahashi et al. 2023). The number of sampling replicates varies depending on the specific research objectives and geographical regions, typically ranging from 2 to 10 or more. The most preferred sampling strategy during the eDNA technology was to conduct three repetitions (27.6%), followed by two repetitions (8.2%). Occasionally, four (1.9%) or five (1.9%) repetitions were taken into consideration (Fig. 5b). Combining with the water volumes and replicates, the sampling strategy of 1 L water collection with three replicates (accounted for 13.7%) is an appropriate and universally applicable alternative when utilizing macro-eDNA technology in marine ecosystems.

eDNA capture

Filter types

The collection and preservation of eDNA is an essential task in eDNA technology, which involves the applications of vacuum peristaltic pumps, filter cartridges, and filter membranes. The marine macro-biomonitoring utilized a range of filter membranes, including polyethersulfone (PES, 20.4%), glass fibre (GF, 12.5%), polyvinylidene fluoride (PVDF, 10.6%), mixed cellulose (MCE, 10.6%), nitrocellulose (NC, 9.4%), nylon (NY, 5.0%), and other types (Fig. 6a). The utilization of GF, MCE, and PES filter membranes has experienced continuous growth and diversification since 2017. Synthetic thermoplastic filter membranes such as PES, polyvinylidene fluoride (PVDF), and NY possess advantages in thermal stability, chemical resistance, and mechanical strength (toughness) (Takahashi et al. 2023).

Studies have revealed that different filters possess distinct advantages and disadvantages, with eDNA obtained from PES being highly portable, facilitating on-site filtration and sample preservation even in remote and challenging field conditions (DeHart et al. 2023; Holmes et al. 2024).

Filter pore size

In marine ecosystems, the filtration rate is primarily influenced by the pore size of filter membranes (Wang et al. 2021a). The current range of available filter membrane pore sizes widely spans from 0.1 µm to 20 µm, with the preferred aperture sizes for fish community eDNA monitoring being 0.22 µm and 0.45 µm (Fig. 7). The pore size of 0.45 µm is additionally employed for the capture of taxa such as crustaceans and fishes, while a pore size of 0.22 µm is commonly utilized for vertebrates and coral reef communities (Sun et al. 2023; Loughlin et al. 2024). The utilization of filtering membranes with pore sizes of 0.2 µm (11.1%), 0.7 µm (6.0%), 0.8 µm (1.7%), and 1.2 µm (3.1%) demonstrated effective eDNA capture capabilities. The filtration rate of a 0.22 µm membrane in certain environments exhibits a positive correlation with the filtration volume; however, it is highly susceptible to saturation after processing 2–3 L and does not yield improved eDNA quantity/quality. The 0.45 µm membrane, on the contrary, exhibits exceptional water sample filtration efficiency and effectively retains even the tiniest biological particles (Valsecchi et al. 2021). Therefore, the adoption of multiple diverse pore sizes (8.9%) in a progressive manner was preferred over relying solely on a single pore-size filter membrane. For instance, one could initially employ a membrane with 10 µm pore size followed by progressively reducing the pore size to 0.8 µm and eventually to 0.2 µm (Suarez-Menendez et al. 2020). The limitations associated with filtration speed and suboptimal eDNA capture resulting from using a single pore size can be overcome by employing a combination of membranes with varying pore sizes, thereby enhancing the recovery efficiency.

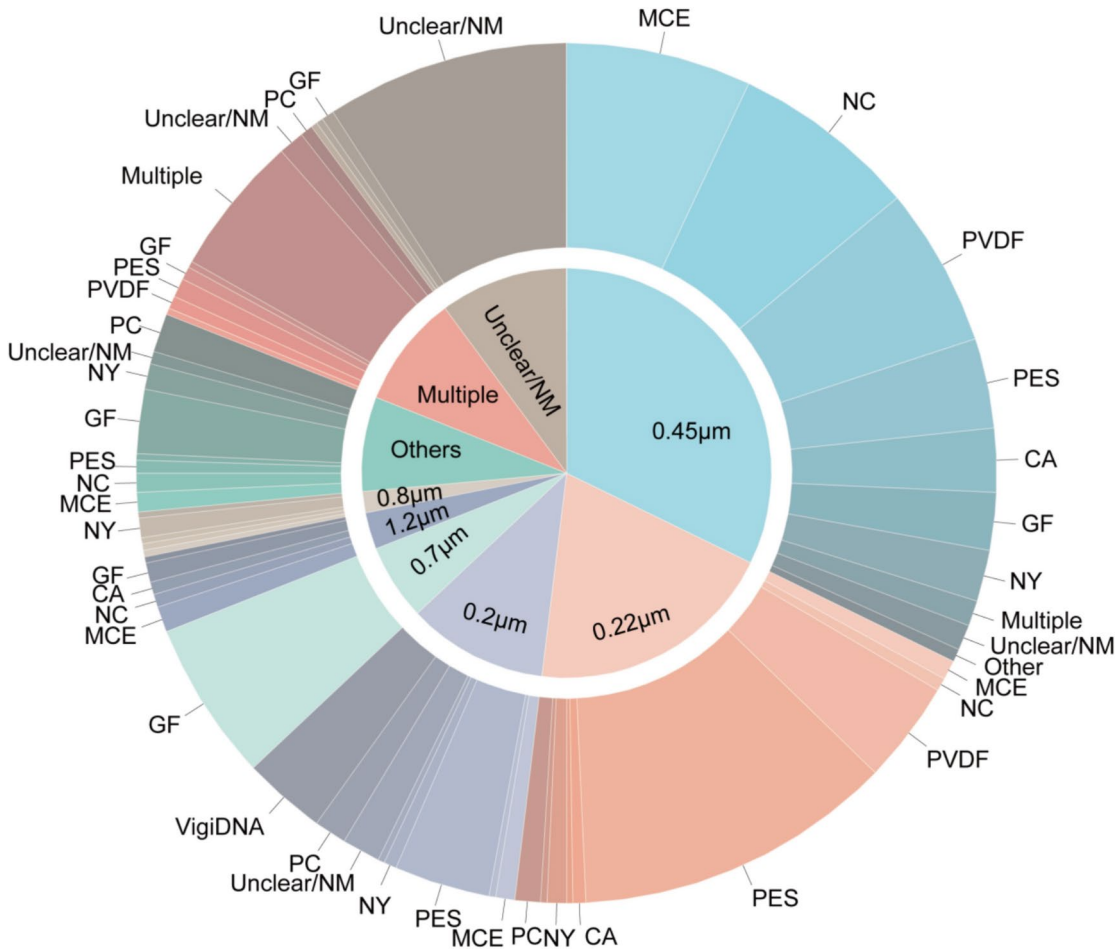

Fig. 7 The proportion of filter pore size and the types of filter membrane for eDNA capturing. The inner circle of the sunburst chart represents the percentage of filter pore sizes, while the outer circle depicts the percentage of filter types corresponding to different pore sizes. For the abbreviations of filter materials are listed in Fig. 6

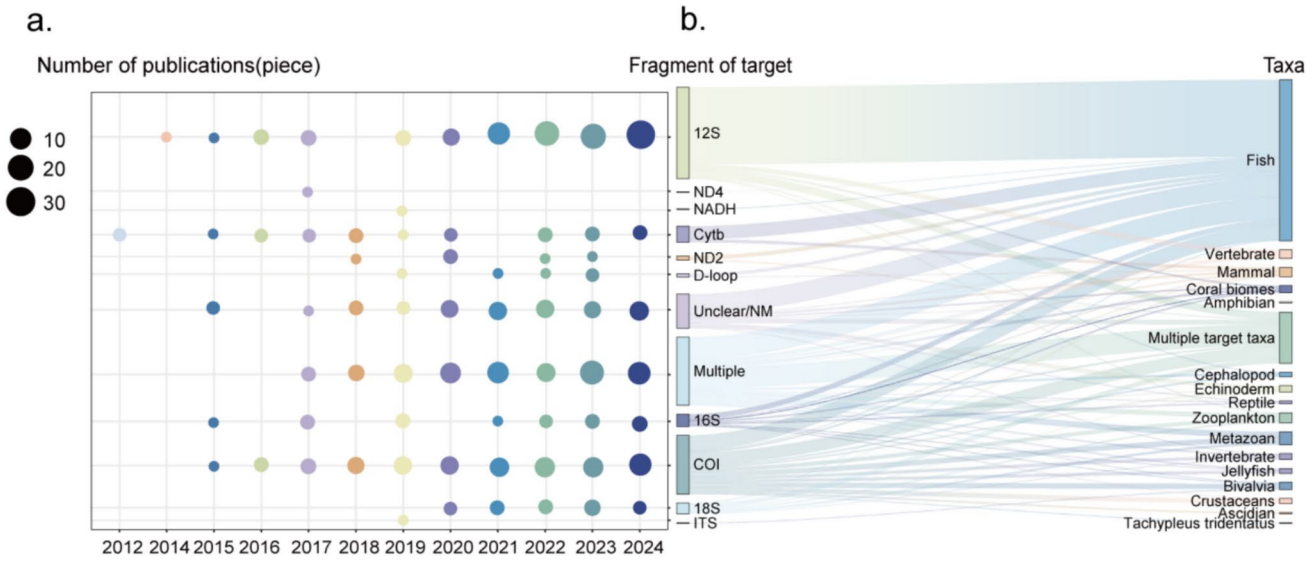

Fig. 8 The application of target fragments. **a**. Bubble diagrams depicting the temporal allocation of targeted fragments. **b**. The sankey diagram illustrates the application of targeted fragments and its corresponding taxa

The filter membrane and pore size demonstrate a highly robust correlation, resulting in variations in the efficiency and quality of eDNA collection across different combinations of filter membrane-pore sizes (Fig. 7). The utilization of different combinations of filter membranes with identical pore sizes leads to a wide range of outcomes (Clark et al. 2024). The five most frequently utilized combinations include 0.22 μm PES (12%), 0.45 μm NC (6.9%), 0.45 μm MCE (6.9%), 0.45 μm PVDF (6.0%), and 0.7 μm GF (6.0%). Similarly, the performance of filter membranes with different pore sizes for eDNA collection also exhibits variations (Kawakami et al. 2023). The selection of a 0.22 μm PES membrane is recommended due to its extensive usage and well-documented effectiveness in capturing eDNA from various taxonomic groups. The use of alternative membrane types is recommended to select the pore size of 0.45 μm.

eDNA extraction

The effectiveness of DNA extraction methods depends on the specific organism and sample substrate involved. Therefore, it is crucial to carefully select an appropriate extraction protocol when extracting eDNA from filter membranes. The utilization of traditional eDNA extraction methods, such as cetyltrimethylammonium bromide (CTAB) (only three publications) and phenol–chloroform isoamyl alcohol (PCI) (4.8%), has been relatively limited in the context of macro-organism marine ecosystem. The PCI method offers extraction efficiencies comparable to those provided by commercial kits, while increasing the likelihood of eDNA detection at a relatively low cost (Leurs et al. 2023). Commercial kits offer a more straightforward and efficient approach than traditional extraction methods, while containing fewer harmful reagents (Goldberg et al. 2016). Consequently, commercial eDNA extraction kits have consistently been the predominant choice for extraction protocols, with an application rate surpassing 80% over the past decade (Fig. 6b). The most commonly employed kits for extracting eDNA from marine ecosystems include the Qiagen DNeasy Blood and Tissue Kit (48.6%), the Qiagen DNeasy PowerWater Kit (4.6%), the MoBio PowerWater DNA Isolation Kit (3.4%), the MoBio PowerSoil DNA Isolation Kit (2.9%), as well as several other similar kits (Fig. 6b). As the field of kit production continues to advance, an increasingly diverse range of kits is being utilized for eDNA extraction and collection.

The consideration of several factors is crucial in effectively capturing or collecting eDNA samples, encompassing aspects such as efficiency, reliability, comparability of methodologies, cost-effectiveness, and ease of utilization (Wang et al. 2021a). The reliability of different extraction protocols varies, so it is vital to conduct a comparative analysis of the various extraction methods to identify the optimal method. A comparative analysis of the PCI, Qiagen DNeasy Blood and Tissue Kit, Mo Bio PowerWater DNA Isolation Kit, and the other extraction methods has revealed that the Qiagen DNeasy Blood and Tissue Kit demonstrates superior efficacy compared to the aforementioned extraction techniques. The observed outcome can be ascribed to the implementation of proteinase K within the Qiagen DNeasy Blood and Tissue Kit, in conjunction with an extended digestion period, thus ensuring sufficient eDNA capture (Jeunen et al. 2019).

Table 1 The ten most frequently utilized primer pairs in all marine macro-organism researches. The names of the primers, the target taxa, the sequence, the original reference, the targeted region, the amplicon size and the number of applications are presented

Target taxa	Primer names	Sequence (5′–3′)	Original reference	Targeted region	Amplicon size, bp	Publications
Fish	MiFish-U-F	GTCGGTAAAACTCGTGCCAGC	(Miya et al. 2015)	12S	163–185	89
	MiFish-U-R	CATAGTGGGGTATCTAATCCCAGTTTG				
	MiFish-E–F	GTTGGTAAATCTCGTGCCAGC		12S	163–185	
	MiFish-E-R	CATAGTAGGGTATCTAATCCTAGTTTG				
Metazoan	mlCOIintF	GGWACWGGWTGAACWGTWTAYCCYCC	(Geller et al. 2013; Leray et al. 2013)	COI	313	47
	jgHCO2198	TAIACYTCIGGRTGICCRAARAAYCA				
Fish	Teleo_F	ACACCGCCCGTCACTCT	(Valentini et al. 2016)	12S	Under 100	27
	Teleo_R	CTTCCGGTACACTTACCATG				
Fish	Tele02 (F)	AAACTCGTGCCAGCCACC	(Taberlet et al. 2018a)	12S	129–167	14
	Tele02 (R)	GGGTATCTAATCCCAGTTTG				
Fish	16SF/D	GACCCTATGGAGCTTTAGAC	(Deagle et al. 2007; Berry et al. 2017)	16S	Approx.200	13
	16S2R (degenerate)	CGCTGTTATCCCTADRGTAACT				
Eukaryotes	18S_uni_1F	GCCAGTAGTCATATGCTTGTCT	(Pochon et al. 2013)	18S	400	8
	18S_uni_400R	GCCTGCTGCCTTCCTT				
Vertebrate	12S-V5 (F)	ACTGGGATTAGATACCCC	(Riaz et al. 2011)	12S	85–117	5
	12S-V5 (R)	TAGAACAGGCTCCTCTAG				
Fish	FishF1	TCAACCAACCACAAAGACATTGGCAC	(Ward et al. 2005; Fields et al. 2015)	COI	127	6
	FishF2	TCGACTAATCATAAAGATATCGGCAC				
	SharKCOI-MINIR	AAGATTACAAAAGCGTGGGC				
Metazoan	mlCOIintF	GGWACWGGWTGAACWGTWTAYCCYCC	(MEYER CP 2003; Leray et al. 2013)	COI	313	4
	dgHCO2198	TAAACTTCAGGGTGACCAAARAAYCA				
Eukaryotes	380F	TCCCTGCCHTTTGTACACAC	(Amaral-Zettler et al. 2009)	18S	87–186	3
	1510R	CCTTCYGCAGGTTCACCTAC				

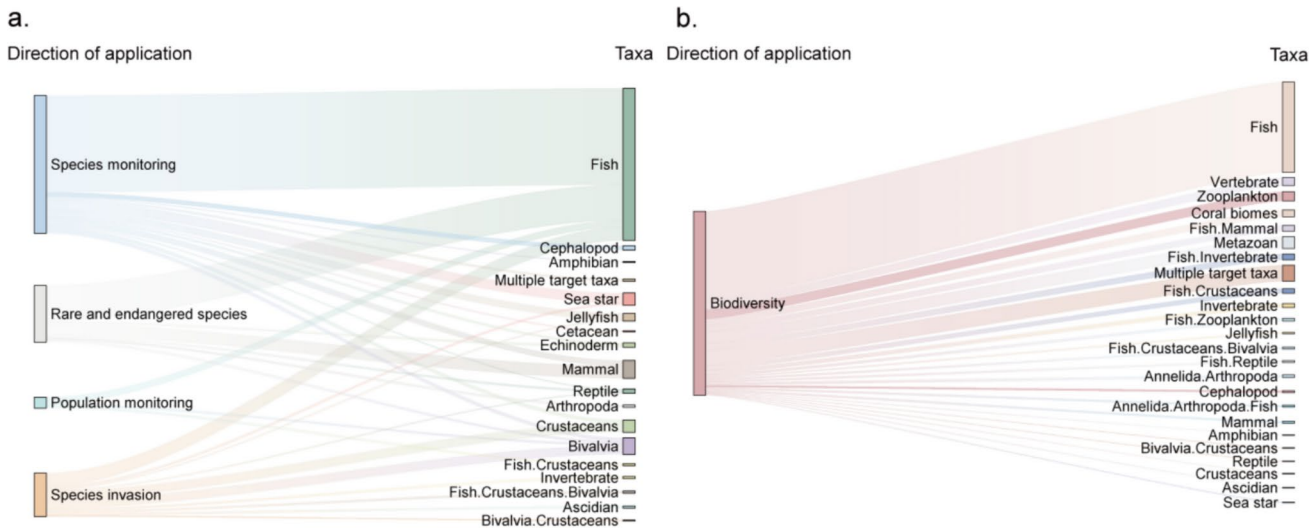

Fig. 9 Sankey diagram depicts the application domains of eDNA technology and their corresponding taxa. **a**. Application of eDNA technology in species and population monitoring; **b**. eDNA technology in biodiversity monitoring. The relative size of the nodes represents the proportion of publications in each domain and the frequency of studies on the corresponding taxa

Primers

The selection of appropriate PCR primers is undeniably a pivotal step in the effective implementation of eDNA technology, as it fundamentally influences the nature of the data being analyzed. The use of mitochondrial DNA for PCR primer design in eDNA technology is more advantageous for

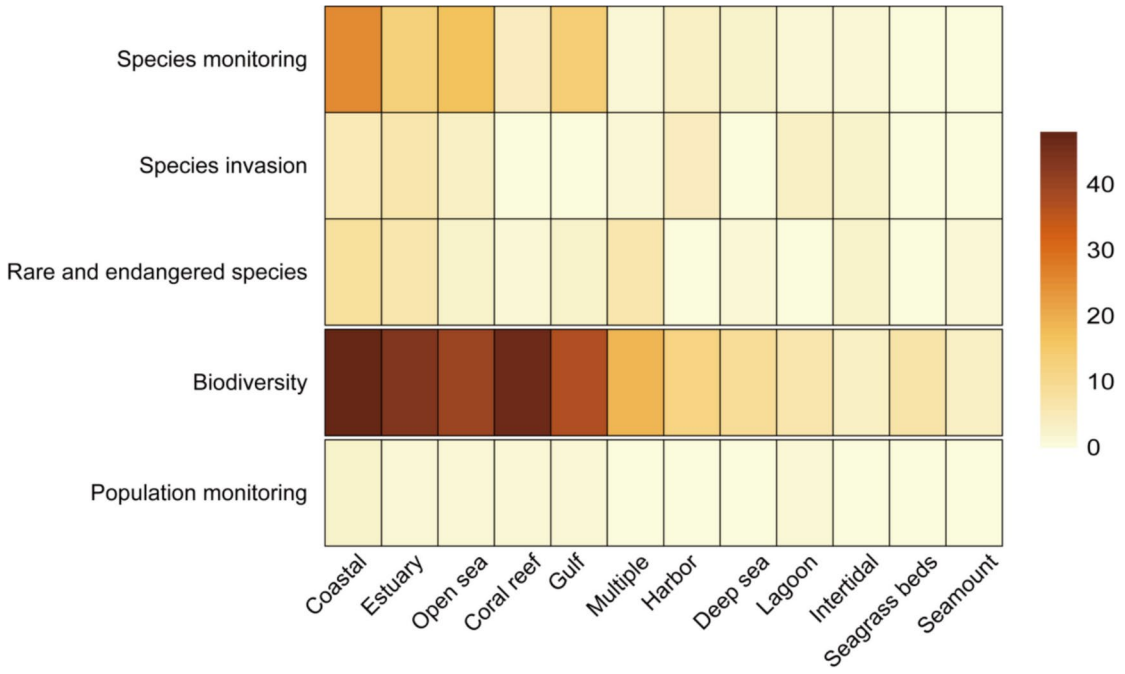

Fig. 10 The application of eDNA technology in various sub-ecosystems. The color of the heatmap corresponds to the quantity of publications

species identification compared to nuclear genes, due to its larger size, greater stability, and higher conservation across regions and copy numbers (Schroeter et al. 2020). The most frequently utilized targeted fragments in eDNA technology include 12S rDNA (30.3%), cytochrome c oxidase I (COI, 19.5%), cytochrome b (Cytb, 5.3%), 16S rDNA (4.1%), and 18S rDNA (3.6%). Additionally, the applications of ND2 (6 publications), D-loop (5 publications), ITS (1 publication), NADH (1 publication), and ND4 (1 publication) have been observed in multiple instances (Fig. 8a). Among these, the mitochondrial gene COI is a widely utilized genetic marker and is regarded as the standard 'taxonomic unit barcode' for most biocenoses (Leray et al. 2013). For fish monitoring, primers derived from mitochondrial DNA, including those targeting 12S rDNA, COI, 16S rDNA, Cytb, ND2, and D-loop are employed predominantly in marine ecosystems.

The ten most commonly utilized pairs of PCR primers in macro-organisms monitoring studies of marine ecosystems are presented in Table 1. MiFish-U and Tele02 have been proven to be highly specificity, discriminatory, and reproducible for fish (Jaquier et al. 2024; Wang et al. 2024). The MiFish-U/E primer (most used) shows significantly superior amplification capacity compared to other competitive primers when targeting short segments of fish DNA from a variety of environmental samples (Miya et al. 2015). And the other commonly employed primers in fish community monitoring include Teleo_F/R (N = 27), Tele02 F/R (N = 14), and 16SF/D-16S2R (N = 13). After the MiFish-U/E primers, the primer mlCOIintF and jgHCO2198 (N = 47) have demonstrated superior efficacy in monitoring macro-organism diversity, including metazoan and zooplanktonic community (Zhang et al. 2024).

With the advancement of eDNA technology, there has been a steady increase in the proportion of studies utilizing multiple pairs of amplification primers derived from different targeted fragments (22.6%), with the aim of enhancing assay precision (Fig. 8b). The simultaneous use of multiple pairs of distinct primers improves the resolution of low-level classifications, mitigates primer bias and false negatives, and ultimately optimizes community-level analyses, thereby yielding more precise and thorough outcomes in species detection (He et al. 2023; Chiquillo et al. 2024). The concurrent utilization of existing primers can be considered in conjunction with the novel designed primers for targeted monitoring macro-organisms in marine ecosystems, aiming to improve accuracy in future studies.

eDNA analysis

Qualitative analysis

The qualitative analysis in eDNA technology primarily involves the assessment of biocenosis composition and biodiversity, encompassing the identification and examination of exotic, rare, and endangered species. Among the 416 publications, a majority studies (N = 260, 62.5%) exclusively employed a qualitative approach, while an additional proportion (N = 41, 9.9%) utilized a combination of qualitative and quantitative methods. Notably, 72.4% of the studies referenced qualitative analysis specifically focused on

biodiversity assessments, while 6.25% related to species monitoring, and the remaining studies addressed population monitoring.

The qualitative investigation of eDNA technology encompasses several crucial procedures: eDNA extraction, PCR amplification, library construction, high-throughput sequencing, and bioinformatics data processing. The data processing represents a crucial stage in the eDNA biomonitoring, and the Cutadapt and DADA2 plug-ins are commonly utilized for initial data screening, filtering, and removal of duplicates, followed by sequence classification and annotation in this stage (Suzzi et al. 2023). The predominant algorithms applied in data processing encompass operational taxonomic unit (OTU)-based clustering (N = 90) in conjunction with amplified sequence variation (ASV)-based noise reduction techniques (N = 81). The other two commonly employed clustering methods are molecular operator taxonomic unit (MOTU) (N = 40) and zero-radius operational taxonomic unit (ZOTU) (N = 15). Furthermore, a small proportion of studies have also conducted concurrent data analysis using multiple algorithms (N = 10).

The definition of OTU typically involves clustering sequences that exhibit sequence homology above a predetermined threshold, commonly set at 97%, for the purpose of monitoring taxonomic units. The empirical evidence suggests that this threshold provides a reliable method for accurately classifying species into their respective family, genus, and species categories (Lyu et al. 2023). Besides, the ASV method is considered equivalent to the OTU method for clustering sequences with 100% similarity, and suggesting as a viable alternative for future studies (Diao et al. 2022). The ASV methodology has the capability to infer biological sequences from samples prior to amplification and sequencing errors, effectively distinguishing sequence variants with as little as a single nucleotide difference (Callahan et al. 2017). The application of ASV clustering to the data enhances the accuracy of species identification and has been successfully employed for monitoring marine macro-organisms (Dugal et al. 2023; Zhang et al. 2023).

The database is a crucial determinant that impacts the accuracy of data processing results following OTU/ASV clustering, serving as the fundamental dataset for conducting diversity analysis on macro-organisms. The pre-processed data is commonly subjected to BLAST analysis for querying and comparing sequences within the database, enabling the selection of the most closely related sequences for annotation (Cananzi et al. 2022). Globally, several widely used databases have been established, such as NCBI (www.NCBI.nlm.nih.gov/Genbank), FishBase (http://www.fishbase.org), the Barcode of Life Database (BOLD) (http://www.boldsystems.org), MitoFish (http://mitofish.aori.u-tokyo.ac.jp/), World Register of Marine Species (WoRMS. http://www.marinespecies.org/), and others. The establishment of globally recognized databases housing extensive sequence data for the COI, 12S, 16S, and Cytb "DNA barcode" regions has facilitated the exploration of valuable new eDNA markers specific to species or biocenosis. In order to enhance the study of organisms within their respective regions, certain nations or regions have established databases of indigenous species (Zhang et al. 2023). However, the current reference databases are inadequate to meet the research requirements for monitoring macro-organisms in marine ecosystems, necessitating several enhancements, including the continual updating of existing databases and the establishment of localized species lists (Zhu et al. 2023).

Quantitative analysis

The term "quantitative analysis of eDNA" typically refers to the systematic monitoring of a single species' distribution and abundance in order to accurately assess its biomass. The most commonly employed quantitative analysis method in the macro-organic studies of marine ecosystems was qPCR (with 131 publications). The qPCR technology enables the quantitative assessment of eDNA concentration by constructing a standard curve based on known eDNA concentrations and determining the DNA copy number through calculation (Yates et al. 2021). It not only enables the quantification of eDNA concentration but also exhibits a remarkable level of specificity and sensitivity, rendering it extensively utilized in marine macro-organism monitoring studies (Wang et al. 2021b; Valsecchi et al. 2022).

In turbid, high-dissolved-organic-matter, or eutrophic aquatic environments, the PCR reaction is often hindered, resulting in reduced efficiency of DNA polymerase (Brys et al. 2021). Recently, the advancement of PCR technology and equipment has resulted in an increased utilization of ddPCR in marine macro-organism monitoring. ddPCR is a technique that randomly splits the sample DNA into separated droplets so that most droplets contain at most one template, ultimately quantifying the target nucleic acid sequence (template) accurately (Hou et al. 2023). ddPCR can effectively overcome PCR inhibitors due to its independent of amplification efficiency, with superior precision and reproducibility in distinguishing samples with small concentration differences (Sidstedt et al. 2020). The ddPCR (14 publications) become the second most commonly used technology in eDNA quantitative analysis, and the utilization of both qPCR and ddPCR procedures concurrently has been observed in a limited number of studies (six publications). However, it should be noted that ddPCR entails substantial investment in equipment and incurs higher operational costs compared to real-time qPCR (Tsuji et al. 2019). Therefore, further advancements and cost reduction efforts are

necessary before ddPCR can be deemed a feasible alternative to conventional methods for quantifying eDNA concentrations in eDNA studies.

Application of eDNA technology to macro-organisms

Species, rare and endangered species, and invasive species monitoring

The monitoring of species, encompassing the assessment of biomass, abundance, and distribution, constitutes a crucial field where eDNA technology finds significant applications. Approximately 19.2% of these studies utilized eDNA technology at the species level, and the target species primarily focusing on economical fish (N = 56), seastars (N = 5), crustaceans (N = 2), and bivalvia (N = 2) (Fig. 3b and 9a). Certain macro-organisms exhibit migratory behavior during overwintering, reproduction, and foraging. This strategy of life history not only determines the fate of individuals but also exerts profound influences on the structure and dynamics of populations and communities in marine ecosystems (McLean et al. 2023). The utilization of eDNA technology provides numerous advantages in elucidating and delineating the migratory patterns of macro-organisms (Yatsuyanagi and Araki 2020; Harris et al. 2022). Furthermore, eDNA technology demonstrated a notable correlation between the frequency of spawning events and the concentration of eDNA (Holmes et al. 2022).

The term "invasive alien species" refers to a specific category of alien species that possess a strong ability to disperse widely in non-native habitats, resulting in significant negative impacts on the surrounding environment and human livelihoods. The absence of local natural enemies allows invasive species to proliferate, thereby impacting various ecosystem attributes. Ultimately, it can lead to a disturbance in the equilibrium of indigenous ecosystems (Pyšek et al. 2020). A total of 24 studies (5.8%) were conducted on exotic species using eDNA technology, including fish (N = 7), bivalvia (N = 5), crustaceans (N = 4), and others (Fig. 9a). The application of eDNA technology has demonstrated its efficacy in accurately detecting invasive / non-native species across various marine regions (Danziger and Frederich 2022; Hartle-Mougiou et al. 2024).

The endangerment and potential extinction of numerous species in marine ecosystems are attributed to a combination of human activities, pollution, natural disasters, and other contributing factors. A total of 30 studies focused on the monitoring of endangered species, primarily encompassing elasmobranchs (N = 13), osteichthyans (N = 7), and marine mammals (N = 6). The eDNA technique also holds the potential to effectively monitor the activities and spawning events (i.e., reproduction) of endangered species, thereby contributing significantly to their conservation efforts (Koyama et al. 2022).

Biodiversity monitoring

Biodiversity serves as a crucial indicator of the vitality and resilience of ecosystems, encompassing the variety of biological communities that comprise a wide array of species within an ecosystem. The application of eDNA technology in marine biodiversity monitoring was extensive, with 275 publications (66.1%) being published. Studies on marine eDNA technology to monitor macro-organisms were first initiated in 2012, specifically focusing on the assessment of fish community diversity and the identification of species that are infrequently or never detected through conventional methods. The primary taxa comprised fish (49.1%), metazoan (6.5%), zooplankton (5.1%), vertebrates (4.4%), coral biomes (3.6%), and others (Fig. 9b). Furthermore, eDNA metabarcoding represents a highly effective method for investigating and monitoring the diversity of pelagic zooplankton and their vertical assemblages (Feng et al. 2022). The eDNA technology facilitates the simultaneous monitoring of biodiversity across various trophic levels and a wide range of marine taxa within a specific area (24.4%) (Dugal et al. 2023).

The monitoring of marine biodiversity is primarily focused on five representative marine ecosystems, namely coral reefs (17.1% of biodiversity monitoring studies), coasts (17.5%), estuary (16%), open ocean (14.5%), and gulf (13.5%) (Fig. 10). Some studies concurrently monitor biodiversity in multiple habitats (N = 19), such as conducting comparative surveys of coral reefs, estuaries, seagrass beds, and intertidal zones simultaneously (West et al. 2021). And the focus of several other studies is directed towards specific marine habitats, encompassing harbors (N = 12), deep sea (N = 9), seagrass beds (N = 7), lagoons (N = 6), intertidal zones (N = 3), and submarine mountains (N = 3). The estuaries, intertidal zones, coasts, bays, and other similar habitats, characterized by their distinct environmental conditions, diverse biological resources, and susceptibility to human activities, constitute crucial areas of focus for biodiversity monitoring (Fieseler et al. 2023).

The coral reef ecosystems are distinguished by their remarkable biodiversity, serving as crucial habitats for more than half of the marine species. The decrease in biodiversity within the coral reefs presents a significant menace to the integrity of coastal habitats and the overall well-being of marine ecosystems (Mora et al. 2011). Monitoring biodiversity in coral reefs is crucial for the assessment of anthropogenic impacts, thereby generating scientific data that can

boost the restoration and conservation efforts for these ecosystems. Studies on macro-biodiversity in coral reef ecosystems through eDNA technology have facilitated an in-depth exploration of coral reefs in diverse geographic regions, thereby paving the way for the comprehensive monitoring and conservation (Dugal et al. 2023; Gómez-Buckley et al. 2023).

Population monitoring

Population genetic data can offer valuable insights into the demographic characteristics of species, and the utilization of eDNA in the investigation of it is still at an early stage. The field of population monitoring through eDNA technology has been characterized by a scarcity of research, with only seven articles acknowledging its existence. The relative frequencies of haplotypes in whale shark populations captured by eDNA were comparable to those obtained from tissue samples, but the use of eDNA allowed for a greater retrieval of haplotypes compared to traditional tissue sample survey methods (Dugal et al. 2022). In addition, the seasonal utilization dynamics of inland habitats in lagoon carp were investigated through eDNA technology, elucidating the differences between two genotypes based on the ratio of native and non-native single nucleotide polymorphism alleles at mitochondrial loci. (Uchii et al. 2017). The eDNA technology has undoubtedly advanced to the extent where it can facilitate population monitoring and provide more comprehensive and accurate information on population genetics. The applications of this technique in marine macro-organisms, however, remain limited and require further comprehensive investigation.

Limitations, challenges and future prospect

Degradation of eDNA

The degradation of eDNA is a significant influencing factor in the extraction process of eDNA from macro-organisms, potentially leading to inadequate capture of the target species and subsequent inaccuracies in the final monitoring data (Ely et al. 2021). During transportation, eDNA can undergo degradation, and the extent of degradation tends to increase with longer durations (Shogren et al. 2018). Both abiotic factors (e.g., salinity, temperature, hydrological conditions, UV radiation, and pH) and biotic factors (e.g., microbial activity and extracellular enzymes) impact the degradation processes of DNA (Collins et al. 2018; McCartin et al. 2022). The recovery and degradation of eDNA can be affected during the sampling process, such as the presence of obstructed filters, improper storage conditions, and the utilization of inappropriate extraction methods (Ramón-Laca et al. 2021). Accordingly, it is imperative to carefully select appropriate sampling and extraction techniques based on the specific site conditions to optimize the eDNA collection and minimize its degradation. Experiments have demonstrated that frozen transport or the addition of preservation solutions (e.g., ethanol, sodium acetate solution, and cationic surfactants) can effectively reduce the degradation of eDNA during filter storage and transport (Thamke et al. 2024).

Biased amplification and reproducibility

The application of PCR for amplifying target fragments of eDNA is often prone to bias, leading to the generation of inaccurate assay results, thereby compromising their accuracy and authenticity (Nichols et al. 2018). Amplification bias can be attributed to various factors, including degradation of eDNA, primer selection, and other biotic and abiotic influences. The degradation of eDNA in the oceans, advection of ocean currents, and presence of other substances have been found to impede DNA amplification, leading to a bias in targeted sequence binding observed in both qPCR and eDNA metabarcoding (Andruszkiewicz et al. 2019). It is crucial to emphasize that the prudent selection of species- or taxon-specific primers is indispensable for ensuring the precision and dependability of PCR amplification. The lack of such primers may give rise to amplification bias, potentially leading to errors in species identification (Wilcox et al. 2018). The primary source of errors and bias in PCR amplification lies in the gene and taxon-specific primers required for amplifying the barcode region, as they selectively target specific sequences while disregarding others (Yang et al. 2021). The presence of these biases can lead to the unjustified omission of information during periods of targeted population and species expansion. Besides, the amplification bias will lead to the preferential amplification of DNA that is relatively abundant, thereby obscuring species with lower amounts of DNA in a sample (Yu et al. 2022). The amplification bias can be effectively addressed through modifications in PCR protocols, such as increasing the number of PCR replicates, optimizing denaturation temperatures, extending the time, and pre-determining the optimal primer set (Bylemans et al. 2018).

The attainment of high accuracy and reproducibility will enhance the efficacy of eDNA detection and quantification, which is contingent upon the filtration of water samples, the employed extraction method, and the utilized PCR reaction system during the process of eDNA technology (Stoeckle et al. 2022). The reproducibility and consistency of the assays obtained through different filtration and extraction methodologies exhibit discrepancies, and the accuracy and

reproducibility of experiments can be improved by conducting multiple PCR reactions on a single sample (Ramón-Laca et al. 2021).

Incomplete and insufficient database

The current limitations, including incomplete databases and inadequate data abundance, pose significant obstacles to the progress of eDNA technology. The accuracy of species identification in marine macro-organism monitoring studies is contingent upon the extent of sequence data available in the reference database (Wang et al. 2021a). The data abundance can serve as an indicator of the presence and relative abundance of diverse organisms in the eDNA samples, thereby providing a benchmark for subsequent species identification and community analysis (Jiang et al. 2023). It is reasonable to assert that existing public databases can facilitate data analysis to a certain extent; however, it has to acknowledge the inherent limitations of these public databases. Firstly, the sequence data in public database still possess rudimentary quality, contain errors, and be susceptible to misclassification (Abidin et al. 2022). Secondly, The current eDNA databases lack sufficient coverage and documentation of numerous species in marine environments due to the instable nature and distinct geographical distribution of eDNA (He et al. 2023).

The lack of geographic specificity in public databases hampers effective data analysis and comparison (Gómez-Buckley et al. 2023). The presence of incomplete databases often leads to the exclusion of relevant sequence data and incorrect taxonomic matches, thereby hindering the precise identification of species (Dziedzic et al. 2023). The establishment of high-quality reference databases on the subject is one of the most direct and effective methods for enhancing the monitoring of local biodiversity, and it can be achieved by implementing standardized procedures for sample collection, data management, and data sharing (Goldberg et al. 2016). Besides, the investigation of macro-organisms in diverse habitats should be expanded to enhance the inclusiveness of eDNA databases, which will enhance the value of eDNA technology in various fields.

The future trajectory of development

The advancements in science and technology have led to significant innovation potential for marine eDNA technology, particularly in the field of macro-biomonitoring. Besides, advocating for the standardization of eDNA technology in marine ecosystems biomonitoring is crucial, because standardized protocols enables data comparisons across different studies at broader temporal and spatial scales. The use of standardized eDNA technology can also serve as a valuable reference tool for non-specialists (Takahashi et al. 2023). Previous studies have demonstrated the necessity of optimising and standardizing eDNA metabarcoding techniques through comparing different capture schemes, extraction methods, and the experimental yielding results (Zou et al. 2020; Li et al. 2024). Noteworthily, there are multiple sets of guidelines for aquatic organisms monitoring, but they have not yet been universally accepted and implemented (Shaw et al. 2016). Currently, standardized protocols and guidelines for marine macro-organism monitoring still necessitate continuous exploration.

Comprehensive investigations into species composition and biodiversity in certain marine ecosystems (e.g., the polar region, the abyssal zone) have been limited primarily due to technological constraints and extreme environmental conditions. eDNA technology presents a highly effective tool for investigating such regions, which have been successfully employed to assess various taxonic community composition and diversity in the polar regions (Zhang et al. 2024). Besides, conducting surveys and continuously monitoring biodiversity in deep-sea ecosystems is fraught with numerous challenges, primarily including logistical constraints, exorbitant costs, and limited sampling opportunities. eDNA technology can overcome most of the challenges and efficiently monitor biodiversity in marine ecosystems without the above drawbacks (Fujiwara et al. 2022; Boldrocchi et al. 2024). The continuous advancement of technology may render it feasible to further research on the biodiversity.

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Data availability This paper is a review article, and the data presented are bibliometric summary data, which are authentic and reliable.

Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

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