文章编号：1674-5566(2025)05-0978-17 DOI：10. 12024/jsou. 20250304816

eDNA-based detection of non-indigenous species in marine environments

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Abstract: As climate change, international trade, and human activities increasingly disrupt traditional geographic barriers in the oceans, non-indigenous species (NIS) have successfully established themselves outside their native ranges. Outbreaks of NIS can pose significant threats to local ecosystems and economies, making them a critical issue for marine biodiversity and biosecurity. Biological invasions in marine habitats differ significantly from those on land or in freshwater. Detection and identification of NIS in marine habitats is particularly challenging due to difficulties in sampling, morphological identification, and visualization in the early stages of outbreaks. Environmental DNA (eDNA) approaches have emerged as reliable and cost-effective methods for both qualitative and quantitative detection of marine NIS, particularly in the introductory phase. In this review, we summarize recent applications and advances in eDNA-based detection of marine NIS. We emphasize that innovations in eDNA sampling equipment, improvements in detection methods, and further refinement of the reference genomic database for marine species are crucial for the future development of this field.

Key words: eDNA; non-indigenous species; marine ecosystems; marine biodiversity and biosecurity; detection and identification

中图分类号：Q 178. 1；S 944. 4 **文献标志码**：A

Biological invasions in marine ecosystems, driven by climate change, global trade, and anthropogenic activities, are escalating worldwide. Human-induced breakdown of natural barriers heightens nonindigenous species (NIS) introduction risks. Established NIS disrupt food webs, displace native species, degrade habitats, and impair ecosystem services, triggering biodiversity loss and functional ecosystem alterations[1-2]. Transoceanic shipping represents one of the most significant vectors for marine NIS transfer, primarily through organism transfer via ballast water discharge, hull fouling, and port activities[3]. Additionally, marine NIS spread through pathways such as commercial trade (e.g., live seafood imports) and natural dispersal mechanisms[4]. Intraregional or leisure boating may also enhance the spread of NIS[3]. Moreover, the costs associated with controlling NIS and mitigating their impacts are significant[5-6], highlighting the urgent need for effective monitoring and management strategies. Marine ecosystems, characterized by high salinity and stratified horizontal/vertical life zones, host species uniquely adapted to these conditions. Given vast unexplored oceanic regions, this review

收稿日期：2025-03-31 **修回日期**：2025-05-30

基金项目：国家重点研发计划(2022YFC2601300)

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prioritizes biomes integral to maritime economies and human impacts (e. g., neritic and photic zones). Although NIS encompass diverse taxa, current marine environmental DNA (eDNA) detection predominantly targets invasive animals.

Detecting and identifying NIS in marine environments presents several challenges. First, marine biomes cover vast areas of water, and sampling sites are often located miles offshore, making them difficult to access and reliant on water surface vehicles. Second, traditional methods for detecting and identifying NIS in marine ecosystems, such as visual surveys[7-8], specimen collection (using tools such as nets, dredges, or traps)[9-10], settlement plate monitoring[11-12], hydroacoustic surveys[13] and ballast water and hull fouling surveys[14], require specialized training in morphological identification. Additionally, these methods have a high likelihood of missing detections, thus face significant limitations in terms of scalability, accuracy, and efficiency.

Molecular methods, particularly eDNA technologies, effectively augment traditional approaches by analyzing organisms' environmental genetic material for species detection and quantification. These sensitive, cost-efficient tools prove particularly valuable for early invasion monitoring of marine NIS. This review examines eDNA's advancements in marine NIS detection, emphasizing innovations in sampling equipment, detection methodologies, and genomic reference database development. Integrated with conventional techniques, eDNA has become a critical strategy for marine biosecurity and biodiversity conservation.

1 Using eDNA approaches to detect marine NIS

Organisms residing in marine habitats continuously release cellular materials, such as feces, mucus, gametes, shed skin, and carcasses into the surrounding water. These materials contain nuclear or mitochondrial DNA, which can be extracted from water samples and analyzed using molecular techniques. The concept of using eDNA to detect organisms was first proposed by Olsen et al. in 1986, who utilized 5S and 16S ribosomal RNA to study the microbial population in environmental samples[15]. However, it was not until the early 2000s that researchers began actively using eDNA to assess species in freshwater[16]. Since then, eDNA has been increasingly recognized as an effective molecular tool that enables scientists to detect the presence of specific species within an ecosystem. Nevertheless, the application of eDNA for marine detection commenced later than its utilization in terrestrial and freshwater ecosystems, with initial prioritization given to coastal monitoring, as scientists from the University of Copenhagen published two research papers using eDNA to detect marine fish biodiversity[17] and harbor porpoises[18]. Their work representatively utilized two primary strategies: eDNA metabarcoding and barcoding, which allowed for the detection of a broader range of biological communities as well as species-specific individuals.

Because of their low cost, high efficiency, and high sensitivity, eDNA approaches have greatly expanded their applications in marine NIS detection. Zaiko et al. first demonstrated the use of eDNA metabarcoding to assess NIS in ballast water and marine coastal waters[19], which was then rapidly applied to various marine NIS detection scenarios[20-21]. By year 2024, literature indicated over 140 marine NIS species have been monitored by metabarcoding or barcoding in multiple research programs, according to the literature (Tab.1). The main advantage of environmental eDNA in detecting NIS in marine environments is its ability to identify species even when they are present in low numbers shortly after their introduction. This early detection is crucial because it often occurs when the population is still localized and sparse, increasing the likelihood of successful eradication or effective local management[22-25]. Additionally,

eDNA approaches can be utilized in diverse marine environments, including coastal, estuarine, and pelagic areas. This method enables effective monitoring without extensive physical sampling or direct observation. It can simultaneously detect multiple species from a single water sample and often does not require prior knowledge of taxonomy based on morphological characteristics. The integration of next-generation sequencing techniques or quantitative PCR (qPCR) has enhanced the monitoring of marine NIS, making it more comprehensive, standardized in laboratory practice, and scalable for large-scale applications[17, 26].

Tab. 1 Summary of studies on detection for key marine NIS using environmental DNA

Class	Species	Gene	Reference
Actinopterygii	*Cynoglossus* sp.	CO Ⅰ	KARAHAN et al.[27]
Ascidiacea	*Ascidiella aspersa/Styela plicata/Ciona robusta/ Didemnum vexillum/ Pyura mirabilis/Didemnum molle/Ciona intestinalis/Styela clava/ Botrylloides violaceus/Botryllus schlosseri*	CO Ⅰ	GARGAN et al.[28] ; BAE et al.[29] ; BAE et al.[30] ; ARDURA et al.[31] ; MATEJUSOVA et al.[32]
Aulopiformes	*Saurida macrolepis*	CO Ⅰ	KARAHAN et al.[27]
Beloniformes	*Parexocoetus mento*	CO Ⅰ	KARAHAN et al.[27]
Beryciformes	*Sargocentron rubrum*	CO Ⅰ	KARAHAN et al.[27]
Bivalvia	*Magallana gigas/Xenostrobus secures/Rangia cuneata/Dreissena polymorpha/ Corbicula fluminea/Xenostrobus securis/Talonostrea/Mytella strigata*	CO Ⅰ /16S rRNA/D-LoopD/ Cytb/12S rRNA/ ND2/ND4/ND5	ARDURA, et al.[33] ; MIRALLES et al.[34] ; ARDURA[35]; YIP et al.[36] ; EGETER et al.[37] ; WELLS et al.[38]
Clupeiformes	*Etrumeus golanii/Herklotsichthys punctatus*	CO Ⅰ	KARAHAN et al.[27]
Hydrozoa	*Ectopleura crocea*	CO Ⅰ	KIM et al.[39]
Maxillopoda	*Amphibalanus eburneus*	18S rRNA/CO Ⅰ	CASTRO-CUBILLOS et al.[40]
Perciformes	*Micropterus dolomieu/Alepes djedaba/Apogon queketti/Apogon smithi/Callionymus filamentosus/ Champsodon nudivittis/Cheilodipterus novemstriatus/Decapterus russelli/Equulites klunzingeri/Nemipterus randalli/Oxyurichthys petersii/Petroscirtes ancylodon Pomadasys stridens/Scomberomorus commerson/Siganus luridus/Siganus rivulatus/Sillago sihama/ Sphyraena chrysotaenia/Terapon puta/Trypauchen vagina/Upeneus moluccensis/Upeneus pori/ Neogobius melanostomus/Sciaenops ocellatus*	CO Ⅰ	KARAHAN et al.[27] ; STOECKLE et al.[41] ; FRANKLIN et al.[42] ; O'SULLIVAN et al.[43] ; WANG et al.[44]
Pleuronectiformes	*Cynoglossus sinusarabici*	CO Ⅰ	KARAHAN et al.[27]
Polychaeta	*Ficopomatus enigmaticus/Hydroides elegans/ Polydora cornuta/Polydora triglanda/Sabella spallanzanii/Hediste diversicolor/Capitella capitata* *Sabella spallanzanii/Hediste diversicolor/ Capitella capitata*	CO Ⅰ /18S rDNA/16S rDNA	BORRELL et al.[31] ; MUÑOZ-COLMENERO et al.[45] ; VON AMMON et al.[46] ; WOOD et al.[47] ; SUAREZ-MENENDEZ et al.[48] ; BRAND et al.[49] ; ZIRNGIBL et al.[50] ; ARDURA et al.[51] ; SCRIVER et al.[52]
Salmoniformes	*Oncorhynchus gorbuscha/Salmo salar*	12S rRNA/12S rRNA/CO Ⅰ	O'SULLIVAN et al.[43] ; BOYSE et al.[53]
Scorpaeniformes	*Pterois miles*	CO Ⅰ	HARTLE-MOUGIOU et al.[54]
Scyphozoa	*Aurelia coerulea*	CO Ⅰ	WANG et al.[55]
Siluriformes	*Plotosus lineatus*	CO Ⅰ	KARAHAN et al.[27]
Syngnathiformes	*Fistularia commersonii*	CO Ⅰ	KARAHAN et al.[27]
Tentaculata	*Mnemiopsis leidyi*	CO Ⅰ	CRÉACH et al.[56]
Tetraodontiformes	*Lagocephalus sceleratus/Lagocephalus guentheri/ Lagocephalus suezensis/Stephanolepis diaspros/ Torquigener flavimaculosus*	CO Ⅰ	KARAHAN et al.[27]

Despite its promising potential, there are several challenges associated with using eDNA for detecting NIS in marine environments. One major challenge is the low concentration and degradation of eDNA in seawater. Environmental factors can influence the persistence of eDNA in aquatic environments[57]. Effective eDNA detection faces constrained temporal-spatial windows in high-energy marine systems like the open ocean. Moreover, eDNA metabarcoding accuracy hinges critically on optimized genetic marker selection, efficient primer-PCR compatibility, and access to robust marine genomic reference databases—prerequisites for multispecies detection via eDNA approaches.

2 Persistence of eDNA in marine systems

Marine genetic material originates from both biological processes (e. g., metabolism, tissue decomposition, predation-parasitism) and anthropogenic activities (e. g., fishing, shipping, mariculture, pollution). Natural sources include organismal secretions (mucus, saliva) and waste, while human actions amplify its dispersal. Compared to RNA, eDNA is relatively stable and can persist in the water column for hours to days, depending on environmental conditions[58]. Researchers have assessed eDNA compositions using filters with different pore sizes, and the results indicate that eDNA in water is primarily found in mitochondria or within cells[59], additionally, the donor organism (e. g., fish, invertebrates, microbes) and the tissues the DNA is shed from (e.g., skin cells, mucus, feces or the blood and tissue fragment from injured or decaying organisms) all have an intricate influence on persistence[60].

In marine systems, the persistence of eDNA is influenced by a complex interplay of physical, chemical, and biological factors (Tab. 2). Key physical factors mainly include water temperature, water movement, and ultraviolet (UV) radiation. Research has shown that eDNA can remain detectable for extended periods—up to several days—in cold, deep waters where it is shielded from UV radiation[61]. Specifically, studies have demonstrated that eDNA degradation rates are significantly minimized under conditions of low temperature (5 ℃) and reduced UV-B exposure. For instance, eDNA has been found to remain detectable for up to 81 days when stored in the absence of light and under refrigeration[61]. Currents and waves can disperse and dilute eDNA, reducing its concentration and making detection more challenging. Study also revealed that the degradation rate of eDNA in nearshore environments is 1.6 times faster than that in offshore environments[62]. Additionally, the persistence and concentration of eDNA in sediment are significantly higher than in flowing water, highlighting the role of sediment as a reservoir for eDNA[63]. The high salinity of seawater can help stabilize eDNA to some extent, it can also complicate the extraction and purification processes. While eDNA is generally stable in the neutral to slightly alkaline pH range (approximately pH 7-8), extreme pH conditions (whether acidic or alkaline) can accelerate degradation through processes such as depurination, denaturation or hydrolysis of DNA molecules. The pH of seawater typically ranges from 7.5 to 8.4 but can be out of range due to pollution or in specific locations such as hydrothermal vents. Ocean acidification driven by rising atmospheric CO_2 levels, lowers the pH of seawater, may also potentially reduce eDNA persistence. High levels of organic matter can bind to eDNA, potentially protecting it from degradation or making it less accessible for detection[64-66]. Biological factors, such as nucleases released by microorganisms (including bacteria and fungi), can further degrade DNA into smaller fragments. Microbial and enzymatic activities typically interact with other factors like temperature and nutrient availability. For instance, warmer and more nutrient-rich aquatic

environments tend to accelerate eDNA degradation and diminish its persistence. The persistence of eDNA is also influenced by microbial activity. For instance, filter-feeding behaviors can significantly reduce the concentration of eDNA in water bodies and shorten its residence time. Additionally, certain microorganisms actively uptake eDNA from the environment to facilitate their metabolic processes[61, 67-68]. These findings highlight the importance of considering environmental conditions when designing eDNA-based studies and interpreting results.

Tab. 2 Key Factors Influencing the Persistence of eDNA in Marine Environments

	Key Factors	Reference
Physical factors	Water temperature: Warmer temperatures accelerate DNA degradation, while colder temperatures can prolong eDNA persistence.	MOYER et al.[69]
	UV radiation: Sunlight, particularly UV-B and UV-A, can cause DNA damage and fragmentation, reducing its detectability.	ANDRUSZKIEWICZ et al.[70]
	Water movement: Currents, waves, and turbulence can disperse eDNA, diluting its concentration and making it harder to detect。	CARIM et al.[63]
Chemical factors	Salinity: High salinity can stabilize DNA to some extent, but it may also influence degradation rates.	HARRISON et al.[71]
	pH: Extreme pH levels (either acidic or alkaline) can accelerate DNA degradation.	ZULKEFLI et al.[72]
	Organic matter: High levels of organic matter can bind to eDNA, potentially protecting it from degradation or making it less accessible for detection.	POURMOGHADAM et al.[66]
Biological factors	Source of eDNA: The type of organism (e.g., fish, invertebrates, microbes) and the amount of DNA shed (e.g., skin cells, mucus, feces) influence persistence.	BARNES et al.[60]
	Predation and filter feeding: Organisms that consume or filter eDNA from the water column can reduce its persistence.	SEYMOUR et al.[68]
	Microbial activity: Microbes in the water column and sediments can rapidly degrade eDNA, reducing its persistence.	YIN et al.[73]
	Enzymatic degradation: Nucleases released by organisms or present in the environment break down DNA.	VAN BOCHOVE et al.[74]

3 Obtaining eDNA for downstream applications

In the marine environment, the process of obtaining eDNA for further analysis involves several key steps: seawater collection, eDNA enrichment, eDNA extraction and purification, and DNA preservation[75]. Collecting seawater samples containing eDNA is essential, as it provides the foundation for all subsequent molecular detection methods. The sampling strategy, site selection, and adherence to standardized protocols are crucial for ensuring the quality and reliability of the collected data. The marine environment is highly dynamic and complex, influenced by various factors such as wave action, UV radiation, and fluctuations in water temperature, etc., which have been discussed above. These variables can create significant challenges for effective eDNA collection, making careful planning and execution vital for successful outcomes[70, 76]. Therefore, researchers are strongly encouraged to design appropriate field sampling plans and conduct pilot detection experiments tailored to the specific marine species of interest before proceeding with sampling. When eDNA is released by marine organisms, it disperses horizontally and vertically throughout the marine environment. Typically, eDNA can be found within a range of several tens of meters from the organisms' activity depths[77]. Comparative studies on eDNA abundance of *Acanthaster cf. solaris* between surface and benthic seawater samples revealed significantly higher concentrations in benthic samples. This disparity was hypothesized to correlate with the proximity of

benthic sampling sites to *A. cf. solaris* habitats. Furthermore, the study recommended that site selection for eDNA sampling should account for hydrodynamic factors (e. g., tidal cycles and current patterns) and species-specific biological parameters (e. g., habitat preferences of *A. cf. solaris*). Additionally, optimal sampling timing was proposed to align with the species' summer breeding season, when eDNA signals are likely amplified due to increased biological activity[78]. As a result, multiple sampling points should be strategically planned to encompass the depths and areas where the target species is active, necessitating a basic understanding of the species' life habits[79-80]. In addition, optimizing sampling times by considering the reproductive and active seasons of marine organisms can further increase the likelihood of detecting the target species[81].

In most cases, researchers need to venture out to sea in a boat or use sampling devices to collect water samples from the shore. Among the common water sampling devices, the Niskin bottle is widely used. It can be modified and attached to various instruments to collect seawater[82]. In recent years, advancements in drone technology have provided new conveniences for water sample collection in offshore eDNA studies. One study proposed the use of a drone for water sampling and verified its reliability through field experiments, finding no significant differences between samples collected by drones and those obtained using traditional sampling methods[83]. Additionally, to address the complexity and variability of marine environments, various automated water collection devices have been developed to meet different sampling needs[84-86]. We anticipate that in the future, water sampling equipment for eDNA will become more diversified and widely used to meet the needs of different operating environments.

After collecting water samples, it is crucial to promptly concentrate eDNA to prevent its rapid degradation. Currently, three primary methods are used for eDNA concentration: sedimentation, centrifugation, and filtration[87]. The sedimentation method involves adding organic reagents, such as ethanol, to promote eDNA precipitation at the bottom of the container. However, this method is limited by the volume of water that can be processed and often yields low eDNA concentrations[88]. Similarly, the centrifugation method uses centrifugal force to concentrate eDNA but also faces constraints in processing large water volumes[89]. In contrast, filtration is the most widely used and effective method for eDNA concentration. This technique involves passing large volumes of water through filter membranes, which capture eDNA for subsequent analysis. The efficiency of filtration depends heavily on the filter membrane material and pore size. Filter membranes made from materials such as nitrocellulose, mixed cellulose esters, and polysulfone have been shown to provide satisfying eDNA concentration efficiency[90]. However, due to variations in seawater composition and eDNA characteristics across marine environments, there is no universal standard for filter membrane material so far[91]. Pore size is another critical factor influencing filtration efficiency. Smaller pore sizes can capture more eDNA, including both intracellular and extracellular DNA, but may clog more easily, reducing sampling efficiency. Therefore, selecting an appropriate pore size should be based on the water quality of the sampling area[92]. When in situ eDNA concentration is impractical, samples can be preserved chemically (e. g., sodium acetate) or cryogenically. Beyond conventional protocols, integrated water sampling-concentration systems have emerged, exemplified by marine-optimized high-throughput eDNA samplers with in situ filtration that boost processing capacity. These systems demonstrate enhanced benthic invertebrate detection efficacy relative to conventional methods[93].

The extraction and purification of eDNA from seawater pose significant challenges due to the typically low concentrations of eDNA and the

complex chemical composition of seawater. Currently, the primary methods for extracting marine eDNA involve the use of chemical organic reagents and commercial DNA extraction kits[94]. Traditional chemical methods (e. g., phenol-chloroform, isoamyl alcohol) offer cost-efficient eDNA extraction but involve toxic reagents requiring cautious handling. Residual solvents/salts from these protocols may inhibit downstream enzymatic reactions (PCR, ligation, and fragmentation). Commercial kits like Qiagen DNeasy Blood & Tissue and PowerWater are widely adopted despite limited seawater-specific options, providing standardized alternatives to conventional approaches[95]. While these commercial kits tend to be more expensive, they offer several advantages, including simplified extraction procedures and the effective removal of PCR inhibitors. Furthermore, magnetic beads, which can be modified for various purposes, have been increasingly applied in eDNA extraction, and have proven to significantly improve both extraction efficiency and DNA yield[96].

eDNA undergoes critical quality control (QC) before downstream analysis to assess its integrity, purity, and concentration, ensuring reliable results[97]. To assess eDNA integrity, agarose gel electrophoresis and microfluidic capillary electrophoresis are commonly employed to determine whether eDNA has fragmented due to degradation[98]. Purity is evaluated spectrophotometrica (e. g., using Nanodrop) via the A_{260}/A_{280} ratio (optimal range 1.7-2.1); deviations from this range indicate contamination requiring further purification[99]. When quantifying eDNA concentration, both spectrophotometric and fluorescence-based methods are utilized[100]. These QC protocols collectively optimize eDNA sample integrity, ensuring analytical reliability in downstream workflows.

4 eDNA barcoding and metabarcoding

The premise of managing NIS in marine ecosystems begins with assessing the species composition of target organisms. As eDNA is shed and released into surrounding water bodies, these DNA fragments are collected for PCR amplification and sequencing workflows, enable indirect inference of organism distribution and diversity through residual genetic traces, circumventing direct specimen collection. This non-invasive biomonitoring approach increasingly supports invasive species tracking, conservation efforts, and ecosystem health evaluations[16].

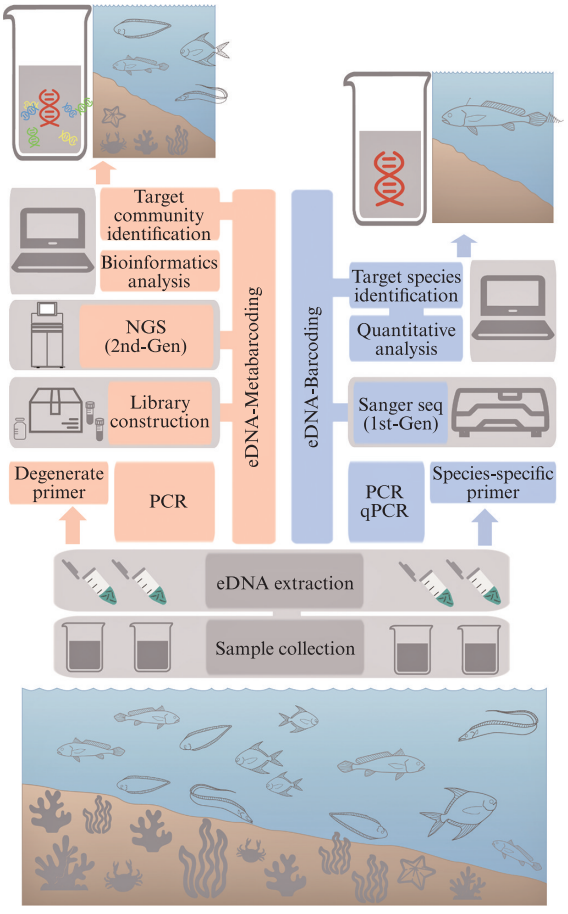
PCR based eDNA detection methods are primarily categorized into two main approaches: eDNA barcoding and eDNA metabarcoding. eDNA barcoding utilizes species-specific primers and probes designed to target particular organisms, coupled with PCR to selectively amplify and detect the DNA of the target species. In contrast, the most notable feature of eDNA metabarcoding technology lies in its utilization of degenerate primers coupled with next-generation sequencing (NGS), which enables simultaneous identification of diverse taxa within environmental samples without requiring prior knowledge of their species composition (Fig. 1). Both methods offer powerful tools for biodiversity monitoring and ecological research[101]. The integration of qPCR into eDNA barcoding significantly enhances its capabilities, enabling not only the detection of target species but also the quantification of their DNA in water bodies. This quantification is, to some extent, correlated with species abundance. For example, researchers developed species-specific primers and probes for the invasive fish species *Sciaenops ocellatus*. By employing qPCR, their study revealed a distinct regional distribution pattern of this species in the East China Sea, demonstrating the effectiveness of eDNA methods in ecological and conservation research[44]. Digital PCR (dPCR) represents a groundbreaking advancement in nucleic acid detection and quantification, offering exceptional sensitivity and precision. By partitioning a sample into thousands of individual reactions, dPCR

enables the absolute quantification of target DNA, even at ultra-low concentrations, such as a single molecule of eDNA within complex samples. This methodology eliminates standard curve dependencies inherent to conventional PCR, minimizing procedural complexity and error propagation. Such technical merits—exceptional accuracy, reproducibility, and throughput—position dPCR as a transformative tool for advancing environmental biomonitoring and biodiversity analytics[102]. eDNA metabarcoding extends the principle of eDNA barcoding by using universal or group-specific genetic markers (barcodes) to target organisms of interest from a wider range of biological communities. This is accomplished primarily by using degenerate primers designed to target specific taxonomic groups to amplify DNA fragments of the target taxa, followed by high-throughput amplicon sequencing. The resulting DNA sequences are subsequently analyzed by comparing them against comprehensive reference databases, enabling the identification of species present within complex environmental samples (Fig. 1). Its advantages of low cost, high efficiency, and high sensitivity have significantly expanded its applications in the study of aquatic invasive species[103] (Tab.1).

Successful eDNA barcoding and metabarcoding depend on several critical elements to achieve accurate and reliable results. While proper sampling techniques and DNA template preparation have been addressed in earlier sections, this part will primarily focus on the molecular pipelines essential for generating high-quality sequencing data.

5　Reference genomes of marine species

The ocean, which covers the majority of the Earth's surface, serves as the cradle of life, harboring an estimated 95% of the planet's biomass and 38 out of the 39 known animal phyla. Despite its vast biodiversity, a significant portion of marine organisms remains undiscovered and poorly



In eDNA metabarcoding, degenerate primers are typically used to amplify eDNA samples, coupled with next-generation sequencing(NGS) to detect target biological communities. In contrast, eDNA barcoding generally employs species-specific primers or combines species-specific probes with qPCR for target species identification.

Fig. 1　Schematic diagram comparing eDNA metabarcoding and eDNA barcoding workflows

understood[104]. The genomes of marine species provide an invaluable resource with immense potential for applications across medicine, food production, and environmental sustainability[105]. In the realm of environmental eDNA detection, the genomes of marine organisms play a pivotal role, directly impacting the efficacy of marine species detection and identification. To study the distribution of marine organisms at sampling sites, researchers typically depend on publicly available reference genomes to design primers and probes for amplicon generation or direct quantitative PCR analysis. With the integration of eDNA detection and next-generation sequencing, both amplicon

sequencing and shotgun sequencing have become widely adopted. These strategies rely heavily on the availability of reference genomes for marine species to enable effective bioinformatics analysis following library sequencing.

Amplicon sequencing, a targeted sequencing approach, involves the PCR amplification of organism-specific DNA fragments. The sensitivity and accuracy of the sequencing results are highly dependent on the design of PCR primers used to generate the amplicons[106]. Most studies have successfully employed mitochondrial genes, such as COI, Cytb, 12S, and 16S, as detection targets for marine organisms, including fish, amphibians, and mammals, demonstrating robust outcomes[107]. In bioinformatics analyses, sequence alignment is a critical step for identifying taxonomic matches, underscoring the necessity of accurate and comprehensive reference databases[108]. Shotgun sequencing, a non-targeted sequencing technique, indiscriminately sequences all marine eDNA without the need for specific taxon enrichment. This method is widely employed in marine eDNA studies for detecting microorganisms[109] and can also be effectively applied to other taxonomic groups without limitations. Unlike amplicon metabarcoding, shotgun sequencing targets both mitochondrial and nuclear genomes in an unbiased manner, demanding higher sequencing depth and broader reference genome coverage. However, the scarcity of marine-specific reference databases-often fragmented-compromises detection sensitivity, underscoring the imperative for enhanced genomic repositories.

Current public amplicon databases such as RDP, Greengenes, and SILVA contain sequences related to marine organisms and are widely used for taxonomic annotation in metagenomic sequencing[110-112]. Similarly, sequence alignment in eDNA sequencing also requires comprehensive taxonomic annotated reference databases, with commonly used resources including NCBI, GTDB, and others[113]. This paper reviews public marine eDNA databases (Tab. 3), highlighting their variable data volumes. While NCBI hosts the most extensive gene repository, uncurated redundancies and inaccuracies hinder precise taxonomic annotation. Establishing a curated, high-coverage marine genomic database remains a critical yet formidable undertaking.

With the rapid advancement of sequencing technologies and the significant reduction in sequencing costs, marine organism genome sequencing is increasingly benefited from long-read assembly methods, which has significantly improved the quality of genome assembly[114]. Despite vast sequencing efforts, marine biodiversity remains inadequately represented, with inconsistent genome quality and coverage. Research indicates that the majority of marine vertebrate genomes have yet to be sequenced, and the currently available genomes are predominantly derived from short-read sequencing, with a significant bias toward resource-rich regions[115]. Globally, the number of marine genome sequencing projects is growing, yielding encouraging outcomes. Notably, the Earth BioGenome Project (EBP) has set an ambitious goal to sequence and characterize the genomes of all eukaryotic life on Earth, encompassing plants, animals, fungi, and microorganisms[116]. The Qingdao BGI Research Institute in China has made significant contributions in marine genomics, releasing four specialized marine organism genome databases focused on aquatic vertebrates, algae, marine invertebrates, and marine microorganisms[117-120]. Meanwhile, numerous ongoing sequencing initiatives are expected to produce more comprehensive and high-quality marine genome databases in the near future, advancing our understanding of marine biodiversity and its genetic underpinnings.

Tab. 3 Publicly accessible databases containing marine genome and amplicon information

Database name	Description	Key features	Link/Reference
SILVA	a database of Bacteria, Archaea and Eukaryota domains gene sequences.	Focuses on microbial identification using rRNA Sequences; includes marine microorganism.	https://www.arb-silva.de/
RDP	a database of Archaea,Bacteria and Fungi gene sequences.	Focuses on microbial identification using aligned and annotated rRNA Sequences; includes marine microorganism.	http://rdp.cme.msu.edu/
UNITE	a database of Fungi sequences.	Focuses on Fungi identification using ITS Sequences; includes marine Fungi.	https://unite.ut.ee/
GreenGenes	a database of Archaea and Bacteria gene sequences.	Focuses on microbial identification using 16S rRNA Sequences; includes marine microorganism.	http://greengenes.lbl.gov/
PR2	a database of unicellular eukaryotes gene sequences.	Focuses on protist identification using Small SubUnit rRNA and rDNA sequences; includes marine species.	https://pr2-database.org/
PFR2	a resource of planktonic Foraminifera gene sequences.	Focuses on planktonic Foraminifera identification using 18S ribosomal sequences; includes marine Foraminifer.	http://pfr2.sb-roscoff.fr/
MIDORI2	a database of eukaryotes gene sequences.	Focuses on eukaryotes identification using mitochondrial gene sequences; includes marine species.	https://www.reference-midori.info/
BOLD (Barcode of life data system)	A repository for DNA barcode sequences.	Focuses on species identification using barcodes; includes marine species.	http://www.boldsystems.org/
MitoFish Database	A curated database of fish-specific eDNA sequences.	Focuses on fish species identification using mitochondrial 12S rRNA gene.	http://mitofish.aori.u-tokyo.ac.jp/
GTDB	A comprehensive database of Archaea and Bacteria gene sequences.	Focuses on microbial species identification using Microbial genomes; includes marine microorganism.	https://gtdb.ecogenomic.org/
EDomics	An animal evolution and development multi omics comprehensive database platform.	Focuses on developmental and evolutionary issues using multiple omics datas; includes marine species.	http://edomics.qnlm.ac/
MolluscDB 2.0	A comprehensive functional genomics database specifically for molluscs.	Focuses on omics data mining using functional genomic resources; includes marine mollusks.	http://mgbase.qnlm.ac/home/
NCBI	A comprehensive public database of nucleotide sequences.	Includes marine genome sequences; supports BLAST searches and genome analysis.	https://www.ncbi.nlm.nih.gov/

With the rapid advancement of environmental eDNA projects, researchers have begun to develop a variety of eDNA-related databases, primarily aimed at organizing eDNA metabarcoding data and enabling effective data visualization. For instance, Chen et al. created the Aquatic eDNA Database (AeDNA), which consolidates over 600 000 reference sequences encompassing a wide range of aquatic organisms and environments[121]. Another notable resource is the MitoFish database, which specializes in mitochondrial genes of fish, housing data from thousands of fish species. Building on this foundation, researchers have also introduced two innovative tools: MitoAnnotator, designed for annotating fish mitochondrial genomes, and MiFish Pipeline, tailored for bioinformatics analysis of fish metabarcoding data[122]. Additionally, in the field of eDNA and metagenomics, the largest marine microbiome database has been established, hosting tens of thousands of marine microbial genomes and hundreds of millions of gene sequences. This extensive repository allows researchers to explore valuable gene resources, such as plastic-degrading enzymes and antimicrobial peptides, offering significant potential for biotechnological applications, and benefiting eDNA research as well[123].

6 Discussion and future directions

The introduction of NIS poses significant threats to marine biodiversity, ecosystem

functioning, and local economies as climate change, international trade, and human activities continue to disrupt natural geographic barriers, requiring effective and scalable detection methods. eDNA approaches have emerged as powerful tools to address these challenges, offering high sensitivity, cost-effectiveness, and the capability to detect species at low abundances during the early stages of invasion. This review highlights the advancements and applications of eDNA technologies in detecting marine NIS, emphasizing the importance of innovations in sampling methods, molecular techniques, and reference genomic databases.

The application of eDNA for marine NIS detection has advanced considerably since its inception. Initial studies showed the effectiveness of eDNA metabarcoding and barcoding in identifying marine biodiversity, including species like fish and harbor porpoises. Over the years, these methods have been refined and expanded to various marine environments, from coastal waters to the open ocean. The integration of NGS and qPCR has further improved the sensitivity and scalability of eDNA detection, enabling researchers to monitor NIS with greater accuracy and efficiency, and providing valuable insights into NIS distribution and ecological impact. Despite its potential, using eDNA for marine NIS detection faces several challenges. The persistence of eDNA in marine environments is influenced by the complex interplay of physical, chemical, and biological factors, such as water temperature, salinity, UV radiation, and microbial activity. These factors can lead to the rapid degradation of eDNA, which limits the window of detection and complicates sampling efforts. Additionally, the accuracy of eDNA metabarcoding relies heavily on the availability of comprehensive and well-curated genomic reference databases. While significant progress has been made in sequencing marine genomes, many species are still underrepresented, making it more difficult to identify NIS in complex environmental samples.

To overcome these challenges, researchers have developed innovative sampling and molecular techniques. Advances in sampling equipment, such as autonomous water samplers and drones, have improved the efficiency and scalability of eDNA collection in remote and dynamic marine environments. Additionally, the use of new filtration materials for eDNA concentration and magnetic bead-based extraction techniques has enhanced the yield and purity of DNA for downstream applications. These innovations have made it possible to detect NIS in a variety of marine habitats. The success of eDNA-based detection largely depends on the availability of high-quality reference genomic databases. Initiatives like EBP and specialized marine genome databases developed by institutions such as the Qingdao BGI Research Institute are working to fill these gaps by sequencing and annotating a wider range of marine species. These efforts are crucial for improving the accuracy, detection rate and reliability of NIS detection. In the context of quantitative analysis using eDNA technology, recent studies have demonstrated that leveraging the number of variable sites within environmental DNA released by target species enables more precise quantification of *Acanthogobius hast* and *Tridentiger bifasciatus*, thereby opening new avenues for methodological development in eDNA-based quantitative assessments[124].

The future of eDNA-based NIS detection relies on the continued integration of new technologies, such as CRISPR-Cas systems and collaborative efforts to expand genomic resources. CRISPR-Cas systems, particularly Cas12 and Cas13, offer unparalleled specificity in detecting target eDNA or RNA sequences at low concentrations[125-127]. Long-read sequencing technologies, such as those offered by PacBio and Oxford Nanopore, are expected to improve the quality of genome assemblies, enabling more accurate taxonomic identification[128]. Additionally,

the development of eDNA-specific databases, such as the Aquatic eDNA Database (AeDNA) and MitoFish, will facilitate the organization and visualization of eDNA data, making it more accessible to researchers and policymakers[121, 129]. As eDNA technologies continue to evolve, they will play an increasingly important role in marine biosecurity and biodiversity conservation, helping to mitigate the impacts of invasive species on marine ecosystems worldwide.

All authors declare that they have no conflicts of interest.

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海洋外来物种的eDNA检测技术研究进展

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摘 要: 随着气候变化、国际贸易和人类活动日益打破海洋中的传统地理阻隔,外来物种(NIS)已成功地在其原生地外建立种群。外来物种的暴发可能对当地生态系统和经济构成重大威胁,使其成为海洋生物多样性和生物安全的关键问题。海洋栖息地的生物入侵与陆地或淡水中的入侵存在显著差异。由于在暴发初期存在采样困难、形态学鉴定不易以及难以目击发现等问题,在海洋环境检测和识别外来物种尤其具有挑战性。环境DNA(eDNA)方法在海洋外来物种引入阶段已成为可靠且经济高效的定性与定量检测方法。本综述总结了基于eDNA的海洋外来物种检测的最新应用与进展。海洋物种eDNA采样设备的创新开发、检测方法的提升以及参考基因组数据库的进一步完善,对于该领域未来的发展至关重要。

关键词: eDNA; 外来物种; 海洋生物系统; 海洋生物多样性与生物安全; 检测与鉴定