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Applications and Prospects of Environmental DNA (eDNA) Technology for Coral Reef Fish Species Identification in Hainan Island

Yanlin Wang [1], Haimei Wang [2]

1 Tropical Animal Resources Research Center, Hainan Institute of Tropical Agricultural Resources, Sanya, 572025, Hainan, China

2 Hainan Institute of Biotechnology, Haikou, 570206, Hainan, China

Corresponding author: haimei.wang@hitar.org

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Abstract Environmental DNA (eDNA) technology has made its mark in biodiversity monitoring in recent years, providing a new means for traditional coral reef fish surveys. This study takes the identification of coral reef fish species in Hainan Island as the core, and systematically reviews the principles, methods of eDNA technology and its application progress in tropical waters. This paper introduces the current status of coral reef ecosystems in Hainan Island and the limitations of traditional fish monitoring methods, explains the background and advantages of the rise of eDNA technology; analyzes the source, characteristics and stability of eDNA, summarizes the collection and preservation methods of water samples and the standard procedures for DNA extraction, amplification and sequence analysis. On this basis, the development and main achievements of eDNA technology in aquatic ecology research are summarized, including fish diversity monitoring cases in typical coral reefs such as Hawaii and Okinawa, Japan, and the adaptability of applications in different sea areas and differences with traditional methods are compared. By reviewing the current research status of fish diversity in coral reefs in Hainan Island, we pointed out the distribution of key protected species and endemic species and the shortcomings in current monitoring. This study believes that eDNA technology has the advantages of high sensitivity and non-invasiveness, which can effectively make up for the shortcomings of traditional methods and has important application value in the protection and management of fish diversity in Hainan Island coral reefs.

Keywords Environmental DNA; Coral reef fish; Species identification; Hainan Island; Biodiversity monitoring; Marine protection

1 Introduction

Hainan Island is located in the northern part of the South China Sea and is one of the most concentrated distribution areas of tropical coral reefs in China. Coral reef ecosystems are known as "a tropical rainforest in the ocean" because of their high biodiversity and abundant fishery resources. According to statistics, China's offshore coral reef fish species are extremely abundant, with a total of about 2 855 species, of which more than 400 coral reef fish are recorded around Hainan Island (Shi et al., 2022). As one of the centers of global coral reef biodiversity, the South China Sea has more than 1 120 types of coral reef fish, accounting for about one-third of the world's coral reef fish. These fish species include butterflyfish, damselfish, groupers, and the humphead wrasse (*Cheilinus undulatus*), many of which are key functional species and economically important species. However, affected by climate change and human activities, Hainan Island coral reefs have experienced coral bleaching and habitat degradation in recent years, and the structure of fish communities has also changed (Huang et al., 2024).

Traditional fish diversity monitoring mainly relies on methods such as underwater visual census and net sampling, but there are obvious shortcomings in complex coral reef environments. Visual investigations are often limited by visibility, underwater terrain, and can only observe species with a certain size and daytime activity, and insufficient detection of hidden cave fish and nocturnal species (Dugal et al., 2023). For example, traditional surveys are difficult to spot small fish hiding in the cracks of reefs or night-infested species, and these "hidden biota" may account for a considerable proportion of the diversity of coral reef fish. Secondly, artificial diving surveys are time-consuming and labor-intensive, and are subject to manpower and diving time, making it difficult to achieve large-scale and high-frequency long-term monitoring. In addition, the method of obtaining specimens by using destructive means such as fried fish and poisoned fish has long been banned, and collection methods such as trawling are not applicable

in reef areas. Therefore, in the context of the growing demand for ecological environment monitoring, it is urgent to introduce new technical means to obtain more efficient and comprehensive information on the diversity of coral reef fish.

In recent years, the rise of environmental DNA (eDNA) technology has brought revolutionary changes to ecological monitoring. eDNA refers to trace DNA fragments left by organisms in the environment. By collecting water samples and analyzing the eDNA in it, information about the existence of species in the environment can be obtained indirectly. Compared with traditional methods, eDNA technology has significant advantages such as non-invasive, high sensitivity and high throughput (Gold et al., 2021). Based on these characteristics, eDNA technology has developed rapidly since the mid-2010s and has been widely used in species monitoring in various ecosystems such as rivers, lakes and oceans. A large number of cases have proven that eDNA can compensate for the shortcomings of traditional methods for inadequate detection of rare, occult and small species. Therefore, introducing eDNA technology into the survey on fish diversity in coral reefs in Hainan Island is expected to break through the limitations of existing monitoring and provide more comprehensive and scientific data support for coral reef protection.

2 Basic Principles and Technical Processes of eDNA Technology

2.1 Source, characteristics and stability of environmental DNA

Environmental DNA is usually derived from metabolites or tissue fragments of organisms in the environment. These gene fragments exist in water as free DNA or cellular forms and can be collected as "genetic traces" of the presence of the target species. However, the stability of eDNA in the environment is affected by a variety of physicochemical factors, and its concentration and fragment length rapidly decline over time (Mauvisseau et al., 2022). The eDNA in water can be in various forms (such as dissolving state, adhering to particles, ingesting by microorganisms, etc.), and the degradation rates of DNA in different forms vary. Environmental conditions such as temperature, pH, UV radiation, microbial activity, etc. will significantly affect the degradation process of eDNA (Sahu et al., 2025). Generally speaking, in tropical nearshore waters, high temperatures and strong light will accelerate DNA degradation, making the eDNA signal relatively short, and the detection of species information that has existed in recent days. Some studies have reviewed the retention time of eDNA in water bodies and found that in most cases, environmental DNA is completely degraded within a few days to weeks. On the other hand, the spatial distribution of eDNA is also localized and localized. In open seas, water flow will cause DNA to spread, but the complex structure of coral reefs can form a small locally closed environment, so that eDNA still mainly represents the composition of local species within the scale of tens of meters. Jaquier et al. (2024) study in the Western Indian Ocean scattered archipelago shows that the composition of eDNA in water bodies near the reef disk is significantly different from the offshore 250 meters away: samples close to the reef are mainly settled benthic fish DNA, while more oceanic fish are detected (Jaquier et al., 2024). This discovery supports the spatial limitations of eDNA signals, that is, environmental DNA mainly reflects biological communities within a certain range around the sampling point, rather than information at infinite distances. Therefore, in practical applications, it is necessary to reasonably design sampling and distribution points based on the ecological characteristics and current conditions of the target species to ensure that representative eDNA signals are obtained.

2.2 Methods for collecting and preserving water samples

The first step in environmental DNA analysis is water sample collection. A reasonable sampling plan should consider factors such as time, space and frequency to cover the heterogeneity of the target area as much as possible. In the coral reef environment of Hainan Island, multiple sampling points can be set according to the distribution of reefs and current conditions, including typical habitats such as lagoons, reef slopes and open waters in the reef. Each point is repeatedly sampled in different seasons or day-night cycles to capture information on dynamic changes in communities. Contamination should be avoided during the sampling process: the water collector and bottle utensils should be sterilized in advance, the sampling personnel should avoid contacting the bottle mouth, and collect blank control samples on site to monitor background DNA. Usually a certain volume of water is collected per sample point and eDNA is enriched by on-site filtration (Kristin et al., 2020). Filtration is a key step in capturing eDNA.

Commonly used filter membrane materials include cellulose acetate, nylon, etc., with a pore size of generally 0.45 μm or 0.22 μm, which can intercept free DNA and cell debris. The material and pore size of the filter membrane will affect DNA recovery. Hinlo et al. (2017) compared different filtration and precipitation methods and found that filtration method can more effectively enrich eDNA in water, while filter membrane types (such as nitrofibrous filter vs. polycarbonate filter membrane) also have an impact on DNA yield (Hinlo et al., 2017). Generally speaking, 0.45 μm fiber filter membranes are often used in seawater samples because they take into account the faster filtration speed and higher DNA capture efficiency. After filtration is completed, the filter membrane needs to be stored immediately. Common methods include placing the filter membrane in anhydrous ethanol, or storing it at low temperature after adding DNA protection reagents, or quick-freezing of the filter membrane on site. These measures are designed to inhibit the DNA enzyme activity of microorganisms on the filter membrane and prevent eDNA degradation. During transportation and laboratory preservation, the filter membrane should be kept in a freezing environment of -20 °C or even -80 °C until DNA is extracted (Troth et al., 2020). Studies have shown that timely cryopreservation can significantly improve the success rate and yield of subsequent DNA extraction.

2.3 DNA extraction, amplification and sequence analysis process

Extraction of DNA from filter membranes is the second step in eDNA analysis. Currently commonly used DNA extraction kits (such as Qiagen DNeasy, etc.) have been proven to be effective in recovering environmental DNA from filter membranes. During the extraction process, the filter membrane is usually cut into pieces and placed in the lysate, and total DNA is obtained by digestion and elution of protease K. Because the total DNA concentration in the water sample is very low and the target DNA fragment length is short (usually 100~300 bp), specific gene fragments need to be amplified by polymerase chain reaction (PCR) to improve the detectability of the DNA of the target species. The MiFish primers developed by Miya et al. are a set of primers that are widely used in fish eDNA metabarcodes and can cover the vast majority of bone and cartilage fish species (Miya et al., 2015). These primers amplify 12S gene fragments about 170 bp long, suitable for high-throughput sequencing platforms. The amplification reaction requires strict prevention of contamination, and usually a negative control (no DNA template) and a positive control (DNA of known sequences) are set up to monitor PCR quality. After successful amplification, the product was purified and a sequencing library was constructed, and sent to a high-throughput sequencer to obtain massive sequence data. Next is bioinformatics analysis: the output sequences are quality-controlled and deredundantly processed, clustered into operational taxonos (OTUs) or resolved into amplicon sequence variants (ASVs), and then aligned with the reference database for species annotation. After data comparison, information such as species composition and sequence read abundance in the sample can be obtained. Finally, the species list and readings are combined with statistical analysis to compare community differences at different points or different time periods, and the species diversity index can be evaluated.

3 Development of eDNA Technology in Aquatic Ecology Research

3.1 Main research results and typical cases

3.1.1 EDNA monitoring of fish diversity in offshore coral reefs in Hawaii

The coral reef ecosystems around the Hawaiian Islands have abundant species and are highly endemic, and have always been one of the hot spots for marine biodiversity research. Although there are relatively limited research on eDNA published in Hawaiian coral reef fish, relevant attempts have shown that the technology is feasible in the region. Marine biologists conducted pilot studies in a protected area near Oahu Island in Hawaii. Through eDNA analysis, a large number of coral reef fish DNA signals, including butterfly fish, cannonball fish, etc., were detected, and were highly consistent with the list of species surveyed by traditional underwater visual surveys. Another team study of NOAA plans to conduct a comprehensive application of eDNA on the west coast of Hawaii's Great Island in 2022. The program combines eDNA with three-dimensional reef imaging and artificial visual census to compare the pros and cons of different methods. These efforts herald the hope of eDNA to achieve integrated monitoring of the entire coral reef ecosystem “from microorganisms to large fish” in waters such as Hawaii (Friedlander et al., 2022). Although there are not many specific eDNA research results in Hawaii at present, it can be foreseen that as the methods become more mature, more eDNA monitoring cases will appear in the future for fish diversity in Hawaii coral reefs.

3.1.2 Monitoring of fish community structure in coral reefs in Okinawa, Japan

Okinawa is located in the Ryukyu Islands in the Western Pacific and has a subtropical coral reef ecology similar to Hainan Island. As early as the late 2010s, Japanese scholars had conducted eDNA research on fish in coral reef lagoons in Okinawa. Oka et al. (2021) conducted eDNA monitoring in a small coral reef lagoon in the northern part of Okinawa's main island and detected 291 species of fish (including bony and cartilage fish), far exceeding the 217 species recorded in traditional fishing surveys, adding dozens of species that had not been recorded before. This study is the world's first case of using eDNA for fish biodiversity surveys in tropical coral reef lagoons, demonstrating that eDNA metabarcoding technology can achieve good results in highly species-rich coral reef environments. The study also found significant differences in the composition of eDNA species at different sampling points, indicating that there may be microhabitat differentiation within the lagoon, such as higher proportion of reef fish detected near coral-rich areas, while the proportion of oceanic migratory fish appearing near the outsea channel increases (Oka et al., 2021). Another Japanese team used eDNA to monitor reef-making corals on the slopes of Okinawa's coral reefs. Yoshida et al. (2022) reported the identification of the presence of multiple coral genus using eDNA around the main island of Okinawa, partly a hidden species not recorded in traditional investigations, indicating that eDNA also has potential in the monitoring of the diversity of corals itself (Yoshida et al., 2022).

3.2 Analysis of the adaptability of technology in different sea areas

Environmental DNA technology has been applied in various waters from tropical to temperate zones, and the environmental characteristics of different sea areas have an impact on the effect of eDNA detection. In areas with gentle water flow and strong enclosure (such as lagoons and lagoons), eDNA can often retain local information better, and species detection is highly corresponding to on-site communities. On the contrary, in the open sea, currents and tides will rapidly dilute and spread DNA, reducing the sensitivity of local detection. Therefore, the application of eDNA in different sea areas requires consideration of the influence of hydrodynamic factors. Gelis et al. (2021)'s study in the coral triangular area of Indonesia showed that in coral reef bays with relatively stable water flow, the fish community structure detected by eDNA is highly consistent with the survey results of traditional fish fried fish, and can reflect the diversity and dominant species composition of the reef area; while on the outer slopes of the reef with high waves and rapid flow, the number of sampling times is needed to capture instantaneous species signals (Gelis et al., 2021). For Hainan Island, strong water flow disturbances caused by summer typhoons and monsoons may temporarily reduce the eDNA detection rate, while calm seasons are conducive to DNA accumulation and detection. In terms of cross-sea comparison, Mathon et al. (2022) analyzed eDNA monitoring of multiple coral reefs in the Atlantic and Indo-Pacific Oceans and found that although the species composition varies greatly in different regions, the biogeographic pattern revealed by eDNA is consistent with the understanding of traditional diving surveys. This shows that eDNA technology has consistent applicability across regions and can reveal large-scale biodiversity patterns. It is worth mentioning that in some special sea areas such as the deep sea and the polar regions, the application of eDNA is also being explored. For shallow sea ecosystems such as coral reefs, there is sufficient evidence that eDNA can adapt to conditions of different water temperatures, salinity and habitat complexity (Malik et al., 2025).

3.3 Comparison between traditional survey methods and eDNA technology

As application cases increase, researchers began to systematically compare the similarities and differences between eDNA and traditional surveys to evaluate the advantages and limitations of new technologies. Overall, most studies have found that the number of species detected by eDNA methods is no less than that of traditional methods, and the detection rate for certain occult and rare species is higher. Gold et al. (2021) compared eDNA with diving surveys in the protected areas of the Channel Islands in California, USA, and found that the eDNA method captured about 76% of the species in visual surveys, and also detected 23 additional fish that were not observed in the naked eye but existed in historical records (Gold et al., 2021). This suggests that eDNA can complement or even expand the list of species surveyed in traditional surveys. On the other hand, traditional methods still have certain advantages in quantitative terms. At present, eDNA readings can only roughly reflect the relative abundance of species, and it is difficult to directly correspond to the number of individuals or biomass in the community. Visual census can estimate information such as population density and body length structure. Therefore, some scholars advocate

combining the two methods and complementing each other's strengths (Muenzel et al., 2024). For example, Muenzel et al. (2024) proposed to use eDNA to quickly screen species lists, supplemented by fine visual surveys of key species to obtain quantitative parameters, so as to take into account breadth and ensure accuracy in protected areas planning. Of course, there are also a few cases where eDNA fails to detect visually discovered species. In Gold and other studies, a few quasi-perids fish cannot be distinguished because they share the same 12S sequence, and a very small number of cave-born fish that only appears visually (maybe due to extremely low DNA release). This suggests that the species resolution and specificity of eDNA primers, as well as the species DNA generation and diffusion mechanism, will affect the detection results.

4 Current Status of Research on Fish Diversity in Coral Reefs in Hainan Island

4.1 Overview of fish community structure and diversity

The composition of coral reef fish in Hainan Island is affected by geographical location and environmental conditions, showing the characteristics of tropical Western Pacific fish species, and is similar to other coral reefs in the South China Sea. According to previous surveys, coral reef fishes along the coast of Hainan mainly include Dambescaridae, Snake family, Grouper family, Parrotaceae, Floyceae, and Floyceae. They include small-scale coral reef settlements, as well as migratory economic fish occasionally entering the reef area (Zhang et al., 2021). The overall species richness is quite high. Shi et al. (2022) used eDNA technology to detect 41 coral reef fish at one time in the waters near the West Island of Sanya. These species cover all major vegetative layers of tropical coral reef ecosystems, including algae-eating fish, plankton-eating fish, benthic invertebrate eaters, and top predators. Traditional field surveys also confirm the high diversity of coral reef fish in Hainan Island: underwater visual census in the reef areas of Sanya and Lingshui recorded about 200 species of fish, including damselfish, mullets and snapperidaceae as dominant groups. However, species composition varies across regions and habitats. Coral reefs around islands (such as West Island and Fenjiezhou Island) are significantly higher in fish species and numbers than those on the mainland shore reefs (Li, 2024). Research in the West Island waters provides the first list of Hainan coral reef fish based on eDNA, indicating that there are common species widely distributed in the Indo-Western Pacific, such as the large blacktip shark (*Carcharhinus melanopterus*) and the humphead wrasse (*Cheilinus undulatus*) have been observed, along with some species that are endemic to or rare in the South China Sea, such as the Hainan surgeonfish and the Chinese golden trevally..

4.2 Key protection and distribution of endemic fish

Hainan Island coral reefs are home to a variety of rare and endangered fish and endemic species in China, which have important conservation value. Among them, humphead wrasse (also known as Napoleon fish, scientific name *Cheilinus undulatus*) is one of the largest coral reef fish in the world. It has been listed as an endangered species by IUCN due to overfishing and is a national second-class protected animal in China. Humphead wrasse has historically been distributed in the sea areas of Sanya, Xisha, Hainan, and other waters. At present, the density of wild populations is extremely low. Its juvenile fish often live in coral clusters in lagoons and lagoons. Traditional diving surveys occasionally record adult fish individuals, and eDNA technology is expected to detect trace DNA, thereby confirming the existence of these occult species. In addition to the humphead wrasse (*Cheilinus undulatus*), large groupers, such as the brown-marbled grouper (*Epinephelus fuscoguttatus*) and the camouflage grouper (*Epinephelus polyphekadion*), also serve as important apex predators within the coral reef ecosystems of Hainan., but due to the fishing of egg-spawning groups, the population has dropped significantly (Zhang et al., 2021). Most of these groupers are national second-class protected aquatic animals or are included in the China Aquatic Wildlife Protection List, and they need to focus on monitoring their population dynamics. There are also some small fish unique to the South China Sea around Hainan Island. Because these endemic species are small in size and difficult to detect, there was a lack of information in the past.

4.3 Deficiencies and demands of current monitoring research

Although there has been a certain accumulation of research on the diversity of coral reefs in Hainan Island, traditional monitoring is still insufficient, mainly reflected in the following aspects: the monitoring coverage is limited, and previous investigations have mostly focused on protected areas or tourist dive sites with less man-made

interference (such as West Island, Fenjiezhou Island, etc.), while there is less attention to some remote waters (such as the Qiongxi Coast, near Linggao) and severely damaged shore reefs. This leads to a lack of comprehensive understanding of the spatial distribution pattern of fish diversity in coral reefs in Hainan Island. The monitoring frequency is not high enough, and coral reef fish communities may change significantly due to seasons, years and even disaster events (such as typhoons, red tides), but most studies are one-time or short-term surveys, lacking long-term continuous monitoring data and cannot reveal community succession and species dynamics. There is insufficient monitoring of key species, lack of systematic population assessment and on-site monitoring, and only sporadic records, so the effectiveness of protection measures cannot be evaluated. The methods and methods are relatively single, mainly relying on divers' visual or catch statistics, and the data accuracy is limited and it is difficult to detect early juvenile fish (Chen et al., 2024). There is room for improvement in the current research on the diversity of coral reefs in Hainan in terms of breadth and depth. Management departments and scientific researchers urgently need to introduce new technical means to achieve more comprehensive, long-term and fine monitoring of coral reef fish. This is an opportunity for eDNA technology to show its strengths - by deploying conventional water eDNA sampling programs in typical coral reefs in Hainan, we are expected to obtain high-resolution data on fish diversity across regions and seasons to fill the gap in traditional monitoring.

5 The Impact and Response Strategies of Environmental Characteristics of Hainan Island Coral Reefs on eDNA Application

5.1 Effect of tropical high-temperature environment on eDNA degradation rate

The coral reefs in Hainan Island are tropical marine environments, with high water temperatures per year (usually in the range of 20 ℃~30 ℃), and high temperatures will significantly accelerate the degradation of eDNA. Studies have shown that the higher the water temperature, the shorter the eDNA retention time in the water body, and the degradation rate increases exponentially. For example, McCartin et al. (2022) experiments found that marine eDNA can maintain detectable concentrations for more than two weeks under low temperature (≤10 ℃) conditions, and can be completely degraded at high temperatures (water temperature ≥20 ℃). This strong control effect of temperature on eDNA stability is consistent with the temperature dependence of the biochemical reaction rate: high temperature will accelerate the activity of degraded enzymes such as DNA enzymes and the chemical degradation of DNA, making eDNA molecules fragmented and degraded faster (McCartin et al., 2022). In tropical coral reef areas, strong solar radiation and ultraviolet rays may also accelerate the photodegradation of eDNA in surface water bodies, further shortening the half-life of eDNA. Therefore, compared with temperate or cold water environments, eDNA signals in the high-temperature sea areas of Hainan Island are more transient and fleeting. If not collected in time, the target DNA fragments may have been degraded and difficult to detect.

5.2 Characteristics of spatial distribution of eDNA under complex hydrodynamic conditions

The hydrodynamic processes of coral reef environments are complex and changeable, including tidal fluctuations, waves and coastal currents, which significantly affect the spatial distribution and migration of eDNA. The review of Jo et al. (2019) pointed out that the horizontal diffusion distances of eDNA in different water bodies vary greatly: in still water waters, eDNA can usually only diffuse horizontally to several several meters to dozens of meters, while in open ocean waters, it can reach hundreds of meters or even kilometers, depending on the local water flow condition. In coastal environments like coral reefs, eDNA may be carried by water flow away from the original release site. There was field research to collect water samples from different distances along the coast of tropical islands and reefs, and found that the composition of eDNA species near the reef has been significantly different from that of hundreds of meters away from the reef, showing that the eDNA signal has spatial limitations: the eDNA of benthic reef fish is mainly distributed in waters close to coral reefs, while more species widely distributed in the ocean are detected far away from the reefs (Figure 1) (Jaquier et al., 2024). In this study, there was obvious species replacement and turnover between fish community DNA from 0 meters offshore of the coral reef and 250 m ~ 750 m offshore samples, and the abundance of reef groups detected in reef water samples dropped sharply in distant samples (Jaquier et al., 2024). This phenomenon shows that in coral reef environments with relatively mild hydrodynamics and complex terrain, eDNA signals mainly reflect local biomes and have less long-distance transmission. In addition, the seasonal flow and vortex of water masses may also cause eDNA enrichment or dilution

in certain regions. Layering and mixing of water columns can also lead to differences in vertical distribution: the concentration and composition of the eDNA of the surface and bottom water bodies may differ depending on the intensity of the turbulent mixing.

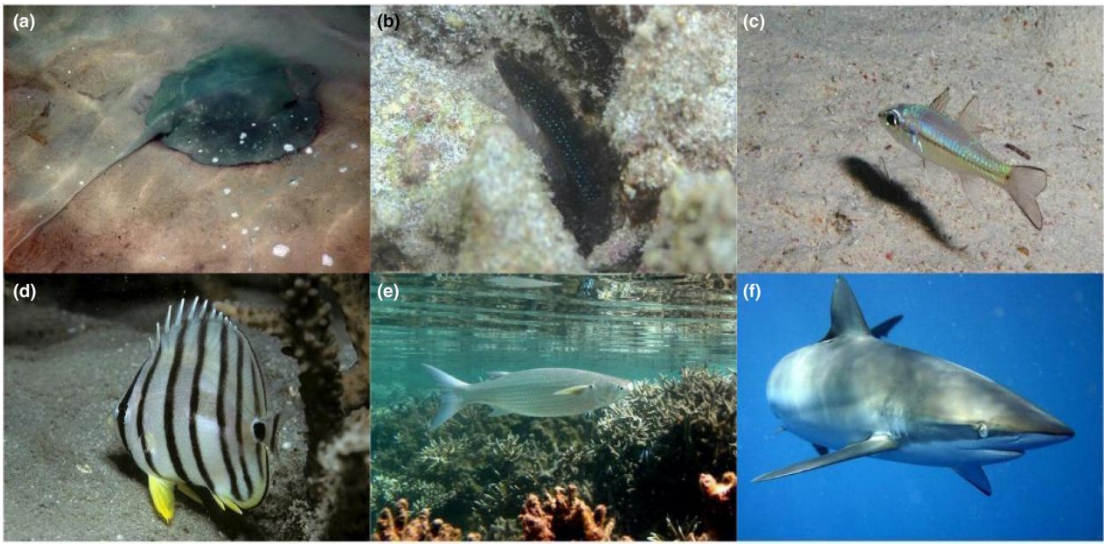

Figure 1 Species detected using environmental DNA (Adopted from Jaquier et al., 2024)

5.3 The mechanism of action of coral reef sediments on eDNA preservation and release

The seabed of coral reefs is usually covered with a large amount of calcium carbonate sand particles, coral debris and other sediments. Studies have shown that a considerable portion of the eDNA in water bodies settles into benthic sediments in the form of particles, thereby reducing the DNA concentration in columnar water samples. Stoeckle et al. (2020) compared the eDNA attenuation under sediment conditions in the experimental environment, and found that the DNA concentration in the water sample was significantly reduced in the presence of sediment, while delayed release of DNA was detected in the sediment: even after the source organism was removed, the sediment still slowly released previously adsorbed DNA, resulting in the continued appearance of target DNA signals in the water. This phenomenon may be manifested in the wild as the target species has actually disappeared or distanced, but its DNA is lagging due to sediment storage and resuspended effects (Wu et al., 2024). Further quantitative comparisons found that the degradation rate of eDNA in sediments is more than one order of magnitude lower than that in water, and the concentration of DNA accumulated in unit mass sediments can be dozens or even thousands of times higher than that of water samples, indicating that coral reef substrates can serve as an important reservoir of eDNA. At the same time, the species composition reflected by the sediment and water bodies is slightly different in quality and quantity: water body eDNA is more representative of the current active species, while sediment eDNA is superimposed on species information over the past period (Sakata et al., 2020). Therefore, in the coral reef environment of Hainan Island, seabed sediments will prolong the tail of eDNA signals in time, which is not only conducive to detecting the historical existence of rare species, but also may bring the risk of "false positive".

6 Challenges and Solutions For the Application of eDNA Technology

6.1 Technical sensitivity and environmental interference issues

Although the advantages of eDNA technology are obvious, it also faces challenges in sensitivity and reliability in practical applications. The eDNA test results may be affected by environmental conditions and there are false negatives or false positives. When the target species has extremely low density or only occasionally passes, its DNA concentration may be below the detection limit, thus missing detection. This is particularly prominent in large and rare fishes, requiring increased sensitivity by increasing water sample volume or enrichment means. Research by Bessey et al. (2020) shows that in tropical sea islands and reef areas, the volume of water sample has a significant impact on the number of species detected by eDNA: the number of fish OTUs detected by filtering 20 liters of seawater is about 30% higher than the 2 liter sample, and it is recommended to increase the sampling volume as

much as possible to capture more DNA (Bessey et al., 2020). Secondly, environmental interference factors such as ocean currents and waves will have a dilution effect on the spatial distribution of eDNA. If the sampling coincides with strong water flow erosion, it may cause the local DNA to disperse rapidly. In this case, multiple parallel samples can be selected or repeated sampling in different tidal periods to reduce the influence of instantaneous factors. The presence of PCR inhibitory substances (such as humic acid, heavy metals, etc.) in seawater may also affect sensitivity (Pritam Banerjee et al., 2022). For waters affected by river runoff in the offshore Hainan, additional purification steps are required after DNA extraction, or inhibitor-resistant enzymes are used during amplification to overcome potential PCR inhibition. Another problem is the fragmentation of environmental DNA: DNA degradation is faster in high temperature seawater, and the fragments may be too short to make it impossible to amplify barcode fragments of the expected length. Solutions include the use of shorter amplification fragments (such as very short barcode mini-barcode) or the addition of storage solution immediately after sample collection to reduce the degradation rate.

6.2 The impact of imperfect species identification database

A core link in environmental DNA analysis is the species annotation of sequences, which relies on a complete database of reference sequences. The current challenge is that species endemic to certain regions or not sequenced lack corresponding sequences in the database, resulting in the inability to accurately identify eDNA sequences. This is particularly prominent in biodiversity hotspots such as Hainan Island. Some endemic fish in the South China Sea have not yet determined the DNA barcode sequence, so eDNA can only identify the level of family or genus when detecting its DNA, and the species name cannot be confirmed. Jaquier et al. (2024) found through global analysis that there is a clear "species vacancy" phenomenon in the fish DNA database: about 20% to 30% of known fish species lack 12S barcode sequences. The imperfection of this database directly affects the accuracy of eDNA application in species identification. To solve this problem, it is necessary to carry out regional DNA reference library construction work: collect tissue samples of common and endemic fish in Hainan Island and the South China Sea, determine standard barcode sequences and upload them to the database. Continuing to improve the sequence data of local species will greatly improve the success rate and accuracy of eDNA species allocation (Read et al., 2022). Another strategy is to adopt multigene loci to improve identification resolution. For example, sequencing the mitochondrial *COI* gene (often used in DNA barcode) and the *12S* gene simultaneously will help distinguish relatives. However, this also requires the completeness of the COI database.

6.3 Construction of standardized processes and regional adaptability

To truly apply eDNA technology to routine monitoring, the problem of standardization and local adaptation needs to be solved. At present, different research teams may have differences in primer selection, filtration volume, data analysis threshold, etc., resulting in limited comparability of the results. In order to serve the management of Hainan's marine ecology, it is necessary to establish a set of eDNA operation specifications suitable for the region. This includes: formulating standard operating procedures (SOPs), which should be based on the optimization results of previous trials and are in line with international common practices to make the data have horizontal comparison significance. Secondly, establish quality control and data interpretation standards. For example, set the sequence reading threshold, and species records below a certain reading should be treated with caution; it is clear that no fish sequence was detected by negative controls as a qualified experiment, otherwise the entire batch of data will be invalid. Again, in terms of regional adaptability, adjustments can be made to the particularity of Hainan sea area (Figure 2). For example, if the water temperature along Hainan coast is high, you can consider shortening the interval between sampling and experiments, or refrigerating the water samples on site to protect DNA integrity. A local reference database update plan for Hainan should also be formulated, and new species sequences should be supplemented every year, and taxonomic changes in the database should be calibrated in a timely manner. Management departments can also participate in the formulation of relevant standards to include eDNA monitoring as part of the official monitoring specifications (Thomsen et al., 2024). With the interaction between scientific research and application, continuously improving the process to make it easier and more cost-controllable, which is also the key to improving promotion. At present, each water sample costs about a few hundred yuan from collection to analysis. If the cost can be reduced through local reagents or combined sampling, it will be conducive to large-

scale application.

Figure 2 eDNA detection operation experiment (Adapted from Douglas et al., 2020)

7 The Application Value of eDNA in Hainan's Marine Ecological Protection and Management

7.1 Assisted biological monitoring of marine protected areas

There are many marine reserves and coral reef nature reserves around Hainan Island, such as Hainan Coral Reef National Nature Reserve (West Island-Dianjiezhou Area). Effective protected area management relies on dynamic monitoring of biological resources within the area. Traditional protected areas monitoring often focuses on indicators such as coral coverage, economic fish resources, and lacks timely grasp of overall biodiversity. Applying eDNA technology to protected area monitoring can achieve broad-spectrum biological monitoring: a water sample can detect the existence of microfishes to large migratory fishes in one water sample, thereby comprehensively assessing the ecological health status of protected area. eDNA can also assist in monitoring and protecting effectiveness. Gold et al. (2021) found that eDNA can sensitively reflect the differences in fish schools inside and outside California reserves, proving that the establishment of reserves does maintain a high species richness. In Hainan, the eDNA results in waters adjacent to protected areas and non-protected areas can also be compared to evaluate the spillover effects and effectiveness of protected areas. If the species diversity and rare species frequency in the protected area is significantly higher than that outside, it means that the conservation measures are effective. On the contrary, if there is no obvious difference, the protection strength or scope needs to be reviewed. Because the eDNA method is fast and non-invasive, monitoring frequency can be higher, and the acquired time series data can help capture seasonal migration or abnormal events (such as fish school changes after coral bleaching). For groups that are difficult to evaluate in traditional methods (such as nocturnal fish and juvenile fish), eDNA also provides an observation window, making the reserve manager more clear. In the future, eDNA monitoring may be one of the standard projects for routine monitoring of Hainan Marine Protection Areas, providing a scientific basis for assessment and adjustment of protection effects.

7.2 Evaluate the effects of ecological restoration and resource restoration

Hainan has taken a variety of measures in the ecological restoration of coral reefs in recent years, including artificial transplantation of corals and setting up fish reefs. The ultimate goal of these measures is to restore biodiversity and ecological function, and eDNA technology can serve as an evaluation tool to measure the effectiveness of repair. Traditionally, evaluating coral reef restoration often involves measuring coral survival rates or observing changes in fish populations, but these indicators may be one-sided. With eDNA, we can test whether repair has brought about

the expected ecological improvement from the perspective of the entire biome. If the eDNA results show that the number of species has gradually increased, especially the increase in DNA frequency of reef-based fish, it means that the reef has successfully attracted and supported more fish to stay, that is, the repair is effective. Similarly, whether the seedlings that are artificially proliferated and released can also be confirmed by detecting the DNA of specific species. Another application is post-disaster assessment. When coral reefs experience extreme events, whether fish communities decline or migrate accordingly, eDNA can provide a rapid diagnosis. Compared with traditional surveys, eDNA can be screened in a large range in a short time, which is very suitable for repeated use at different stages of the restoration project. Similar practices have been found internationally: after the installation of artificial structures of damaged coral reefs, fish diversity was monitored with eDNA. The results showed that the number of species in the recovery area was significantly higher than that in the unrepaired area, demonstrating the effectiveness of the intervention (Ushio et al., 2020). In Hainan, eDNA indicators (such as species richness and biodiversity index) can be included in the ecological restoration effect assessment system to quantify the ecological benefits of restoration.

7.3 Improve scientific management and policy decision-making support

The new data types provided by environmental DNA technology will greatly enrich the knowledge base of Hainan marine ecosystem managers and help achieve more scientific decision-making. Long-term eDNA monitoring can establish big data, and statistical modeling can identify the drivers of fish community changes. For example, by correlating environmental factors such as eDNA species abundance and water quality, water temperature, etc., it can be found which species are most sensitive to environmental changes, and then serve as indicators of environmental health (He et al., 2022). Secondly, eDNA can help optimize protection planning. For Hainan, we can draw a distribution map of fish diversity in coastal coral reefs based on eDNA surveys and find the species abundance center and the distribution points of endangered species. This provides a scientific basis for the construction or adjustment of the scope of marine protected areas. Again, eDNA technology can also be applied to law enforcement and supervision. When cracking down on illegal fish frying, electric fish and other activities, abnormally high concentrations of fish DNA fragments often remain in the water on site. Using portable rapid detection of eDNA can help law enforcement personnel determine whether illegal fishing has occurred in the near future. In terms of public education and community co-management, eDNA results intuitively reflect regional biodiversity and can be used to increase public awareness of environmental protection. Showing the rich list of species obtained by eDNA analysis in local waters to fishermen and communities can help them recognize the preciousness of the ocean around them and thus support the implementation of conservation policies.

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Conflict of Interest Disclosure

The authors confirm that the study was conducted without any commercial or financial relationships and could be interpreted as a potential conflict of interest.

References

Banerjee P., Stewart K.A., Dey G., Antognazza C.M., Sharma R.K., Maity J.P., Saha S., Doi H., Vere N., Michael, Chan W.Y., Lin P.Y., Chao H.C., and Chen C.Y., 2022, Environmental DNA analysis as an emerging non-destructive method for plant biodiversity monitoring: a review, AoB Plants, 14(4): plac031. https://doi.org/10.1093/aobpla/plac031

Bessey C., Jarman S.N., Berry O., Olsen Y.S., Bunce M., Simpson T., Power M., Mc Laughlin J., Edgar G.J., and Keesing J., 2020, Maximizing fish detection with eDNA metabarcoding, Environmental DNA, 2(4): 493-504. https://doi.org/10.1002/edn3.74

Chen X., Li S., Zhao J., and YaoM., 2024, Passive eDNA sampling facilitates biodiversity monitoring and rare species detection, Environment International, 187: 108706. https://doi.org/10.1016/j.envint.2024.108706.

Douglas K.E., Shea P., Porzecanski A.L., and Naro-Maciel E., 2020, What's in the water, Using environmental DNA for marine monitoring and planning, Lessons in Conservation, 10: 29-48. https://doi.org/10.5531/cbc.linc.10.1.3

Dugal L., Thomas L., Meenakshisundaram A., Simpson T., Lines R., Colquhoun J., Jarman S., and Meekan M., 2023, Distinct coral reef habitat communities

characterized by environmental DNA metabarcoding, Coral Reefs, 42(1): 17-30.
https://doi.org/10.1007/s00338-022-02301-3

Friedlander A., Aeby G., and Brainard R., 2022, The state of coral reef ecosystems of the main Hawaiian Islands, The State of Coral Reef Ecosystems of The United States and Pacific Freely Associated States, 17: 222-269.

Gold Z., Sprague J., Kushner D.J., Zerecero Marin E., Barber P.H., 2021, eDNA metabarcoding as a biomonitoring tool for marine protected areas, PLoS ONE, 16(2): e0238557.
https://doi.org/10.1371/journal.pone.0238557

Handley L.L., Blackwell T., Broadhurst H.A., Clark K., Davison P.I., England J., Mariani S., Mcdevitt A., Pillay K., Read D., Walsh K., Nisbet A., and Creer S., 2022, UK DNA working group eDNA week, Environmental DNA, 5(1): 18-24.
https://doi.org/10.1002/edn3.364.

He W., Xu D., Liang Y., Ren L., and Fang D., 2022, Using eDNA to assess the fish diversity and spatial characteristics in the Changjiang River-Shijiu Lake connected system, Ecological Indicators, 139: 108968.
https://doi.org/10.1016/j.ecolind.2022.108968

Hinlo R., Gleeson D., Lintermans M., and Furlan E., 2017, Methods to maximise recovery of environmental DNA from water samples, PloS ONE, 12(6): e0179251.
https://doi.org/10.1371/journal.pone.0179251

Huang L., McWilliam M., Liu C., Yu X., Jiang L., Zhang C., Luo Y., Yang J., Yuan X., Lian J., Huang H., 2024, Loss of coral trait diversity and impacts on reef fish assemblages on recovering reefs, Ecol Evol, 14(11): e70510.
https://doi.org/10.1002/ece3.70510

Jaquier M., Albouy C., Bach W., Waldock C., Marques V., Maire E., Juhel J.B., Andrello M., Valentini A., Manel S., Dejean T., Mouillot D., and Pellissier L., 2024, Environmental DNA recovers fish composition turnover of the coral reefs of West Indian Ocean Islands, Ecol Evol, 14(5): e11337.
https://doi.org/10.1002/ece3.11337

Jo H., Kim D.K., Park K., and Kwak I.S., 2019, Discrimination of spatial distribution of aquatic organisms in a coastal ecosystem using eDNA, Applied Sciences, 9(17): 3450.
https://doi.org/10.3390/APP9173450.

Li M.M., 2024, Comparative genomics of fish: insights into evolutionary processes, International Journal of Molecular Zoology, 14(4): 222-232.
https://doi.org/10.5376/ijmz.2024.14.0020

Malik M.D.A., Ambariyanto A., Hartati R., Nursalim N., Kholilah N., Kurniasih E.M., Anggoro A.W., Prasetia R., Syamsyuni Y., Muh F., Cahyani N.K.D., 2025, eDNA uncovers hidden fish diversity in the coral reef ecosystems of Karimunjawa National Park, Indonesia, Regional Studies in Marine Science, 81: 103945.
https://doi.org/10.1016/j.rsma.2024.103945

Mauvisseau Q., Harper L.R., Sander M., Hanner R.H., Kleyer H., and Deiner K., 2022, The multiple states of environmental DNA and what is known about their persistence in aquatic environments, Environ Sci Technol, 56(9): 5322-5333.
https://doi.org/10.1021/acs.est.1c07638

McCartin L.J., Vohsen S.A., Ambrose S.W., Layden M., McFadden C.S., Cordes E.E., McDermott J.M., and Herrera S., 2022, Temperature controls eDNA persistence across physicochemical conditions in seawater, Environ Sci Technol, 56(12):8629-8639.
https://doi.org/10.1021/acs.est.2c01672

Miya M., Sato Y., Fukunaga T., Sado T., Poulsen J.Y., Sato K., Minamoto T., Yamamoto S., Yamanaka H., Araki H., Kondoh M., and Iwasaki W., 2015, MiFish., a set of universal PCR primers for metabarcoding environmental DNA from fishes: detection of more than 230 subtropical marine species, Royal Society open science, 2(7): 150088.
https://doi.org/10.1098/rsos.150088

Muenzel D., Bani A., De Brauwer M., and Beger M., 2024, Combining environmental DNA and visual surveys can inform conservation planning for coral reefs, Proceedings of the National Academy of Sciences, 121(17): e2307214121.
https://doi.org/10.1073/pnas.2307214121

Oka S.I., Doi H., Miyamoto K., Hanahara N., Sado T., and Miya M., 2021, Environmental DNA metabarcoding for biodiversity monitoring of a highly diverse tropical fish community in a coral reef lagoon: estimation of species richness and detection of habitat segregation, Environmental DNA, 3: 55-69.
https://doi.org/10.1002/edn3.132

Sahu A., Singh M., Amin A., Malik M.M., Qadri S.N., Abubakr A., Teja S.S., Ahmad Dar S., and Ahmad I., 2025, A systematic review on environmental DNA (eDNA) science: an eco-friendly survey method for conservation and restoration of fragile ecosystems, Ecological Indicators, 173: 113441.
https://doi.org/10.1016/j.ecolind.2025.113441

Shi J., Li C.H., Wang T., Zhao J.F., Liu Y., and Xiao Y.Y., 2022, Distribution pattern of coral reef fishes in China, Sustainability, 14(22): 15107.
https://doi.org/10.3390/su142215107

Stoeckle B.C., Jarman S.N., Berry O., Olsen Y.S., Bunce M., Simpson T., Power M., McLaughlin J., Edgar G.J., Keesing J., 2020, Maximizing fish detection with eDNA metabarcoding, Environmental DNA, 2(4): 493-504.
https://doi.org/10.1002/edn3.74

Thomsen P., Jensen M., and Sigsgaard E., 2024, A vision for global eDNA-based monitoring in a changing world, Cell, 187: 4444-4448.
https://doi.org/10.1016/j.cell.2024.04.019.

Troth C.R., Burian A., Mauvisseau Q., Bulling M., Nightingale J., Mauvisseau C., and Michael J., 2020, Sweet a development and application of eDNA-based tools for the conservation of white-clawed crayfish, Science of the Total Environment, 748: 141394.

https://doi.org/10.1016/j.scitotenv.2020.141394

Wu J.N., Xu X.Y., and Wu L.M., 2024., The physiological and ecological effects between ocean acidification and coral reefs, International Journal of Marine Science, 14(1): 14-20.
https://10.5376/ijms.2024.14.0003

Zhang K., Zhang J., Shi D., and Chen Z.Z., 2021, Assessment of coral reef fish stocks from the Nansha Islands, South China Sea, using length-based Bayesian biomass estimation, Frontiers in Marine Science, 7: 610707.
https://doi.org/10.3389/fmars.2020.610707