Journal of *Marine Science and Engineering*

MDPI

Review

Artificial Intelligence-Assisted Environmental DNA Metabarcoding and High-Resolution Underwater Optical Imaging for Noninvasive and Innovative Marine Environmental Monitoring

Jing Yang [1], Chao Li [2,3], Linus Shing Him Lo [1], Xu Zhang [4], Zhikui Chen [4], Jing Gao [4], Clara U [4,5], Zhijun Dai [6], Masahiro Nakaoka [7], Huayong Yang [2,*] and Jinping Cheng [1,*]

1 Department of Science and Environmental Studies, The Education University of Hong Kong, New Territories, Hong Kong SAR, China; s1152459@s.eduhk.hk (J.Y.); linuslo@s.eduhk.hk (L.S.H.L.)
2 Southern Marine Science and Engineering Guangdong Laboratory (Guangzhou), Guangzhou 511458, China; lichao@gmlab.ac.cn
3 Ocean College, Zhejiang University, Zhoushan 316021, China
4 DUT School of Software Technology, DUT-RU International School of Information Science and Engineering, the Key Laboratory for Ubiquitous Network and Service Software of Liaoning Province, Dalian University of Technology, Dalian 116620, China; xza@dlut.edu.cn (X.Z.); zkchen@dlut.edu.cn (Z.C.); gaojing@dlut.edu.cn (J.G.); accord@epd.gov.hk (C.U.)
5 Environmental Assessment Division, Environmental Protection Department, Government of the Hong Kong SAR, Hong Kong SAR, China
6 State Key Laboratory of Estuarine and Coastal Research, East China Normal University, Shanghai 200241, China; zjdai@sklec.ecnu.edu.cn
7 Field Science Center for Northern Biosphere, Hokkaido University, 1 Aikappu, Akkeshi, Hokkaido 088-1113, Japan; nakaoka@fsc.hokudai.ac.jp
* Correspondence: yanghy@gmlab.ac.cn (H.Y.); jincheng@eduhk.hk (J.C.)

check for updates

Citation: Yang, J.; Li, C.; Lo, L.S.H.; Zhang, X.; Chen, Z.; Gao, J.; U, C.; Dai, Z.; Nakaoka, M.; Yang, H.; et al. Artificial Intelligence-Assisted Environmental DNA Metabarcoding and High-Resolution Underwater Optical Imaging for Noninvasive and Innovative Marine Environmental Monitoring. *J. Mar. Sci. Eng.* **2024**, *12*, 1729. https://doi.org/10.3390/jmse12101729

Academic Editors: Gianluca Quarta and Milva Pepi

Received: 7 July 2024
Revised: 27 August 2024
Accepted: 18 September 2024
Published: 1 October 2024

Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

Abstract: To effectively protect the marine environment, it is crucial to establish effective environ mental monitoring platforms. Traditional marine environmental monitoring methods heavily rely on morphological identification and field expertise, with the sampling process being disruptive and potentially destructive to vulnerable marine environments. In light of emerging biomonitoring needs and biodiversity declines, we reviewed the urgently needed, ongoing advances in developing effective, noninvasive, and innovative monitoring methods and systems to examine the complex marine environment for better strategic conservation and protection, using the coral ecosystem as one of the representative forefront examples in marine protection. This review summarizes current trends and efforts in transitioning into more standardizable and automatable utilizations of environmental DNA metabarcoding-based monitoring strategies and high-resolution underwater optical imaging monitoring systems as two of the promising pillars for the next generation of noninvasive biomonitoring and associated applications. The assistance of artificial intelligence for environmental DNA metabarcoding and high-resolution underwater optical imaging into an empowered, all-rounded monitoring platform for enhanced monitoring capacity is discussed as a highly potent direction for future research exploration. This review will be a cornerstone reference for the future development of artificial intelligence-assisted, noninvasive, and innovative marine environmental monitoring systems.

Keywords: biodiversity monitoring; machine learning; environmental DNA; metabarcoding; underwater optical imaging-based monitoring

1. Introduction

Oceans cover approximately 71% of the Earth's surface area. As one of the major repositories of the Earth's biodiversity, oceans have significant ecological, economic, and social value for humankind [1]. Each species in the marine food web and the diverse

marine communities play a crucial role in maintaining the balance and health of the marine ecosystem, providing important ecological services to the Earth, such as oxygen production, carbon conservation, and nutrient cycling [2]. Therefore, marine biodiversity is critical to maintaining global biodiversity and environmental health. However, the oceans are facing enormous threats and challenges from climate change and overexploitation, making marine environmental conservation an urgent worldwide concern [3,4]. To address biodiversity decline and to protect and restore marine ecosystems and biodiversity [5], the United Nations has implemented the Strategic Plan for Biodiversity 2011–2020 [6] and followed with the next Decade of Marine Science for Sustainable Development (2021–2030). Real-time, accurate, comprehensive, and large-scale monitoring of the oceans' physicochemical, biodiversity, and health status is fundamental and a critical foundation for the enhanced protection and management of the marine environment [7].

Traditional biomonitoring methods such as electrofishing, netting, trapping, bottom trawling, etc., have been serving as crucial and useful tools for understanding species composition and abundance in the marine environment. However, these traditional biomonitoring methods are mostly capture-oriented in their means of obtaining samples for analysis, which are invasive and potentially destructive when applied to reflect the status of the environment [8]. Furthermore, morphological identification-based biomonitoring methods can be limited in their ability to identify and quantify rare species at low densities and differentiate morphologically similar or identical species (i.e., cryptic species) [8]. For important but vulnerable marine environments, such as the coral reef ecosystems and other marine protected areas, capture-based methods will cause significant damage and destabilize the inhabiting community, which increases the environmental burden and offsets the protection initiatives. Consequently, it is crucial to develop noninvasive marine monitoring technologies for enhanced marine protection and management strategies.

In recent years, various environmentally friendly and high-resolution monitoring platforms have emerged, such as optical (sensing) and acoustic methods, biosensors, and molecular biology-based monitoring methods (metagenomics, environmental DNA (eDNA) metabarcoding) [8,9]. Automated vehicles/equipment that reduce human labor in front-end operations have also been incorporated into such monitoring platforms to improve monitoring outcomes [10]. For example, the integration of high-resolution image capture equipment on unmanned platforms has profoundly transformed the field of underwater optical imaging-based monitoring to achieve high-resolution imaging with labor-saving operations [11]. No matter how cryptic a species may be, it is bound to leave its DNA in the environment; it is theoretically possible to detect these species by sampling and extracting environmental DNA. Moreover, by using multiple primers to amplify target DNA fragments after collecting eDNA from environmental samples, it is also possible to simultaneously obtain compositional or structural information of multiple taxa, such as the "tree of life metabarcoding" [12,13]. Hence, eDNA metabarcoding-based monitoring has also emerged as a promising tool for enhanced marine monitoring because of its noninvasive nature in sampling. Furthermore, integrating eDNA metabarcoding-based monitoring with morphology-based optical monitoring can complement each other further to empower the monitoring outcomes with enhanced monitoring efficiency.

Artificial intelligence (AI) systems leverage data and algorithms to enable capabilities such as perception, learning, reasoning, and decision-making, allowing them to emulate and surpass human performance in tackling complex problems and tasks [14]. Machine learning, a fundamental branch of AI, aims to develop models and optimize algorithms through training on large datasets. By learning and improving predictions autonomously using neural networks and deep learning techniques, these optimized algorithms and models can perform direct calculations on new input data to obtain predicted results [15]. In the ecological domain, AI has been used to estimate ecosystem status [16,17] and predict ecosystem responses to management changes [18]. The growing availability of large datasets from optical imaging platforms and environmental DNA (eDNA) metabarcoding has further highlighted the crucial role of AI in assisting with the processing and analysis

of these rich data sources. For instance, integrating imaging and sequencing data with AI-powered techniques, such as using machine learning to cross-validate eDNA-based taxonomic classification and identification against extensive image libraries, can significantly improve the efficiency and reliability of these advanced biomonitoring platforms [19].

In this review, a literature search for marine environment monitoring methods was conducted on the Web of Science (https://www.webofscience.com) using the keywords "Environmental DNA metabarcoding" and "environment monitoring" as the search topic on 27 August 2024. A total of 187 publications from 2012 to 2024 were summarized. Considering the corresponding literature, this review focused on high-resolution underwater optical imaging-based monitoring technology and eDNA metabarcoding and (1) their advantages in being noninvasive and applicability for noninvasive marine monitoring; (2) their key components or methodologies in current applications as well as their current limitations; (3) how AI can assist these two monitoring methods respectively; (4) their application in marine environment monitoring and protection with AI technology or novel concepts for biodiversity conservation such as citizen sciences; and finally the advantages of combining the usage of the two monitoring methods and their application in marine environmental monitoring were discussed.

2. High-Resolution Underwater Optical Imaging-Based Monitoring Technology as a Powerful Monitoring Tool for Marine Ecosystem

High-resolution underwater optical imaging-based monitoring technology utilizes advanced optical instruments and techniques to capture detailed and high-quality images or videos of underwater environments. This technology provides observational data that are high resolution and noninvasive in nature and have been proven to have advantages in capturing details that traditional methods tend to overlook. These methods are also much less labor-intensive as they can be operated remotely; specialized devices are highly efficient, capable of storing and effectively processing large amounts of data and transmitting data in real time. This technology has been helpful in studying underwater ecosystems, biodiversity, and various physical and biological processes with precision and accuracy [20].

2.1. High-Resolution Underwater Optical Imaging-Based Monitoring Platform for Marine Environmental Monitoring

The key components of high-resolution underwater optical imaging-based monitoring include specialized underwater cameras, remote-operated vehicles (ROVs), and autonomous underwater vehicles (AUVs) equipped with cameras, lights, illumination systems, image and video processing systems, data transmission and storage systems. Underwater cameras are designed with high-resolution sensors, lenses, and durable housings to withstand the underwater environment and capture fine details. ROVs and AUVs, on the other hand, can navigate underwater, collect data, and transmit them in real time for analysis. The advantages of using underwater ROVs for monitoring are to reduce labor-intensive work, human safety risks, and high costs associated with field investigations. Underwater ROVs also enable the analysis of collected videos or images to extract the spatial distribution characteristics, which is crucial for image signal analysis. Additionally, the proposed deep learning-based scheme using underwater ROVs can accurately reveal the biological diversity and distribution, making it a practical alternative to traditional inspection methods [21,22]. However, elevated pressure, poor visibility, streamflow regime, brightness, turbidity, and the importance of structural tightness are some factors that must be considered during the design and later in the implementation phase [23]. Underwater target detection via ROVs has been widely used for purposes such as fish cage inspection [24].

To ensure high-resolution imaging, additional lighting systems are used to compensate for the lack of natural light at depth. Advanced image and video processing techniques can further enhance the quality of obtained images and video through techniques such as removing visual noise and enabling accurate and relevant information to be extracted from the captured data. Real-time data transmission from the underwater environment

to the surface is achieved using underwater communication systems, while efficient storage and management systems handle the large volumes of data generated. For example, researchers have adopted the iterative sparse reconstruction theory to study orthogonal frequency-division multiplexing communication and estimated the channel of the system [25]. Then, a new FFT technology was introduced and implemented to reduce the inter-carrier interference of time-varying underwater acoustic channels [26] and achieve high-efficiency transmission.

Marine ecology research benefits from high-resolution imaging, enabling investigations into the behavior of marine organisms, species interactions, and ecosystem dynamics. Biodiversity monitoring aims to study and document underwater species and habitats, contributing valuable information for biodiversity conservation [27]. High-resolution underwater optical imaging-based monitoring technology is vital in advancing our understanding of underwater ecosystems and their dynamics. It empowers scientists to gather detailed data, visualize underwater environments, and make informed decisions for marine resource conservation and sustainable management.

2.2. Artificial Intelligence (AI)-Based Data Processing Strategies to Overcome Limitations of Captured Image or Video Data

Traditional methods require a significant amount of manual effort to examine features and patterns in images that are relevant to ecological reality. Such manual approaches cannot efficiently handle the ever-increasing volume of raw data generated by modern sensing technologies and are not suitable for large-scale monitoring of complex ecosystems [28]. To address this challenge, ecological studies increasingly rely on advanced computational methods to automate data processing and extract knowledge from ecological records [29]. Thus, obtaining image and video data and its processing technologies are essential in vision-based underwater monitoring and biodiversity assessment.

With the introduction of machine learning and artificial intelligence techniques, the identification task can be intuitively modeled as a target detection and recognition task in the field of image signal processing. The statistics of biological species and quantity can be completed during image processing by providing a clear and high-quality image basis for underwater organism identification and statistical work after it. For example, with the help of target detection technology, AI-driven deep-sea creature sampling missions [30] and underwater biodiversity data collection [31] are already possible. Advanced object detection frameworks, such as You Only Look Once (YOLO), are also being applied to underwater bio-detection [32], while applications such as fish size monitoring [33], penguins and their prey detection [34], and marine animals tracking [35] also demonstrated the important role of image processing techniques in vision-based underwater monitoring and biodiversity assessment. However, due to the harsh, unexplored, and unpredictable nature of the marine environment, impact due to waves, absence of light, and photos taken at close range, reliable and long-term monitoring remains a challenge for smart underwater monitoring systems. The use of computer vision and machine learning algorithms, which heavily rely on the reliability and quality of image data, also imposes pressure on data processing considerations such as correcting color distortion and handling image clarity in turbid water areas.

Camera performance limitations, such as resolution and color distortion, have constrained long-term monitoring of optical image diversity in the marine environment. In other words, the outcome of monitoring tasks depends on the quality of the image. In recent years, advancements in technology and equipment have significantly enhanced optical imaging-based marine monitoring [36]. The design, optimization, transmission, enhancement, and renovation of the optical image system, along with intensity retrieval and allotment, encompass a series of critical steps that are refined through image quality assessment (IQA) and underwater-image-quality-measurement (UIQM) methods. Incomplete and low-quality data in underwater images and videos are primarily due to insufficient information. To address this deficiency, single-modal and multi-modal approaches are commonly employed, as depicted in Figure 1.

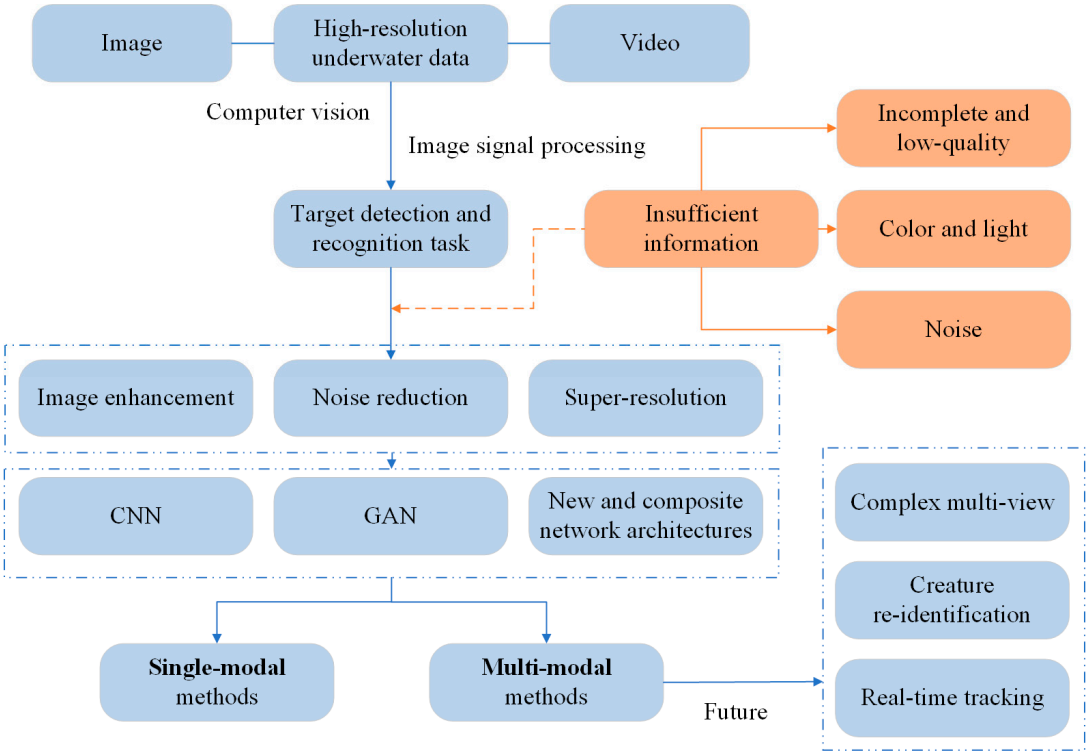

Figure 1. Flow chart summaring the components (blue boxes) and existing problems (orange boxes) involved in high-resolution underwater optical imaging-based monitoring technology. The collected high-resolution underwater data, images, and video data are generally incomplete, low quality, high noise, and other problems. Through the use of deep learning methods, such as Convolutional Neural Networks (CNN), Generative Adversarial Networks (GAN), and new network architectures, construction, single-modal/multi-modal methods, purposes such as real-time tracking, creature reidentification, and more can be achieved.

2.2.1. Single-Modal Methods

Single-modal strategies focus on mining deep into the information from the visual modality itself, thereby focusing more attention on improving the quality of the raw data. One of the most representative techniques is image enhancement, covering dehazing [37], color restoration [38], and feature enhancement [37] under the empowerment of artificial intelligence, which has been applied in underwater animal detection and classification at cabled observatories [39]. Noise reduction methods also behave as image enhancement strategies [40]. Super-resolution (SR) enhances high-resolution images from low-resolution counterparts and has been increasingly exploited for perceptual image quality improvement, but underwater images with SR are relatively under-explored as most existing methods are unable to reduce the adverse effects of being underwater, and corresponding techniques are yet to be developed [41]. Within these methods, techniques such as transfer learning, multiscale, and residual learning are also introduced for better information mining [42,43].

2.2.2. Multi-Modal Methods

Multi-modal strategies are the current trend for method development, making better use of the multidimensional information carried by the sample and fusing its multi-view and context knowledge. Most existing methods find both models from the visual perspective [44] or decompose existing images [45]. The image fusion of infrared and visible video can guide the complementary advantages of different modal image information, which significantly improve image quality and feature engineering efficiency, and extending the idea to the underwater environment will be a very meaningful exploration [46]. In terms of

noise reduction, multi-modal methods need to be able to process complex noise in different models simultaneously instead of separating different views for separate processing in traditional methods [47]. It is necessary to design novel end-to-end processing network models to combat different frequencies of ocean noise through attention mechanisms, adaptive algorithms, ADMM algorithms, etc. [48].

Regarding the architecture of deep methods, the framework derived from CNN and its extended architecture are still the main ones [49,50]. Although GAN is helpful in supplementing information, correcting color and contrast, and reconstructing occluded objects, the generated fake samples limit the authenticity of underwater exploration tasks [51,52]. In response to the multiple challenges of underwater tasks, researchers have focused on new network architecture based on various technologies. A cascade of neural networks has been designed for automated marking of underwater animals [53]. Meanwhile, FA^{+}Net [54], SFGNet [55], PA-UIENet [56], are advanced architectures emerging in recent years.

2.3. The Promising Use of AI in Underwater Optical Imaging-Based Monitoring Application

Relevant ecological information has been recorded through noninvasive and nondestructive underwater optical imaging-based methods as a meaningful new way to monitor and evaluate biodiversity. At the same time, the trend of underwater optical imaging-based monitoring applications is leading to multi-modal methods, which obtain and utilize multi-dimensional information, even beyond pure visual modal analysis. The empowering role of artificial intelligence has been given research attention to improve the overall intelligence of models and processes, such as creature reidentification, real-time tracking, etc. [57]. Although it is desirable to select multiple models with as wide a gap as possible, such as among text, visual, acoustic, and even eDNA signals, to complement multi-dimensional information from a broader perspective, it is currently difficult to integrate multi-modal heterogeneous feature information.

From the perspective of underwater animal detection and recognition application, a multimodal-based, scene-aware, framework for aquatic animal segmentation has been proposed [58]. However, there are many challenges and limitations in the generalization of methods in the underwater environment. For example, the morphological characteristics of underwater organisms can be significantly different, and the aforementioned limitations of camera hardware conditions will also be obstacles. To sum up, developing multi-modal underwater optical imaging-based monitoring and biodiversity assessment in complex underwater environments requires introducing and combining more advanced technologies [59,60]. With the help of artificial intelligence, multi-modal methods have advantages in the ability to examine the same sample from various perspectives, extract and fuse crucial feature information from each modal, and leverage intermodal and intramodal knowledge to enhance underwater object detection.

3. eDNA Metabarcoding as the Next-Generation Biomonitoring and Biodiversity Conservation Tools: Its Noninvasive Nature and Advantages

eDNA metabarcoding-based monitoring methods involve collecting and extracting DNA from environmental samples such as air, water, sediment, soil, etc., and then utilizing molecular techniques such as polymerase chain reaction (PCR) and high-throughput sequencing (HTS) to obtain biological information from the environmental samples. eDNA-based methods only require collecting environmental samples, which is advantageous in being noninvasive and minimizes the destruction or invasion of the environment compared to traditional capture-based methods. Environmental samples typically contain multiple types and sources of DNA, such as those from captured microbes, living cell DNA released into the environment through urine, feces, mucus, etc., and extracellular DNA released due to cell fragmentation after the death of organisms [61]. These eDNA contain biological information of their hosts, which when coupled with bioinformatics, can serve as evidence for the presence of various species in the environment and further provide information on the distribution and functional characteristics of a community without the need to directly

observe or capture any organisms [61,62]. Furthermore, eDNA metabarcoding enables simultaneous monitoring of diverse taxa by concurrently amplifying multiple specific DNA fragments using single or multiple universal primers. Combined with HTS technology, eDNA metabarcoding thus can detect a wide range of taxa rapidly and at lower costs than traditional methods [63,64]. In addition, the use of eDNA metabarcoding also promotes the transition of species identification from morphological recognition, which can be inherently deceptive, to molecular biology, which can be more reliable and reproducible. The new concepts and approaches for noninvasive biomonitoring [65,66], which eDNA provides over visual and morphology-based monitoring, are appealing for detecting many marine species that are naturally rare and cryptic, endangered, or at juvenile stages, which are essential to be protected from invasive and destructive activities [67]. Given the characteristics and advantages described above, eDNA metabarcoding has been widely used in ecological environment monitoring and biodiversity conservation, with the potential to further integrate with new concepts and technologies such as citizen science and machine learning (ML).

4. Progress in Key eDNA Metabarcoding Methodologies

eDNA metabarcoding is conducted based on the assumption that all organisms leave behind DNA and the DNA present in the environmental samples could realistically reflect and represent the biodiversity in the environment [62]. The use of eDNA metabarcoding in marine ecosystems is considered more challenging than in freshwater ecosystems, because eDNA dilution and mixing are higher in the bulk water environment of the ocean due to ocean currents, salinity, tides, and other abiotic factors that can affect the migration and degradation of genetic materials [68,69]. In the methodology of eDNA metabarcoding, there are various factors, in addition to the sampling environment, that could affect how informative the samples represent the biodiversity in the environment. Thus, ongoing efforts have been dedicated to optimizing the sampling process, the efficiency of DNA collection and extraction, the selection and design of PCR primers, and the completeness of the reference database. For instance, the sampling process and sample size, according to the study's purpose, the study site's environmental factors, and target species, must be thoroughly considered before the experiment to optimize the result outcomes. Over the past decade, eDNA metabarcoding-associated procedures have advanced significantly. For example, methods for collecting environmental samples have evolved from manual sampling to a wide variety of automated sampling to facilitate standardization. Therefore, this section summarizes the progress made in sampling methods, reference databases, and primers development, which are the key processes and foundations for eDNA metabarcoding-based monitoring (Figure 2).

4.1. The Nondestructive Sampling in eDNA Metabarcoding-Based Monitoring

In the marine environment, eDNA metabarcoding is most frequently carried out by collecting water and sediment samples [70], though new forms of sampling such as biofilm sampling have emerged [71]. Water and sediment samples cover different environmental compartments and behave differently in reflecting target types of organisms or communities [72–74]. For example, the DNA of aquatic insects, mollusks, and oligochaetes is preferentially derived from sediments compared to amphibians, fish, mammals, etc. [75]. Tagliabue et al. [74] collected both sediment and benthic water samples to detect benthic communities, and the structure of benthic communities reflected differed significantly between samples, with more pelagic and nektonic species in the water samples. Thus, the appropriate selection of water or sediment samples, or the simultaneous collection of both samples, is critical for obtaining eDNA representative of scientific objectives.

For water sampling, surface seawater is often directly collected from sampling sites using simple tools such as buckets [76–78], wide-mouth containers [79], or Niskin bottles [80], while larger volume samplers tend to be used for deep seawater sampling, such as Nansen metal water sampler [81] or Niskin-style rosette sampler [82]. Similarly, depending on

water depth and substrate type, different types of corers or grabs for sediment sampling can be employed, such as Kajak corer, piston corer, gravity corer, or a Van Veen grab, which are operated by hand or by line [83,84]. Though these samplers have been widely used to collect water or sediment samples, they still need manual effort for operation and transport. In recent years, various semi- or full-automatic and passive samplers that can be deployed in marine environments have been used for sampling in eDNA metabarcoding, which reduces labor and allows for the standardization potential of the sampling procedure.

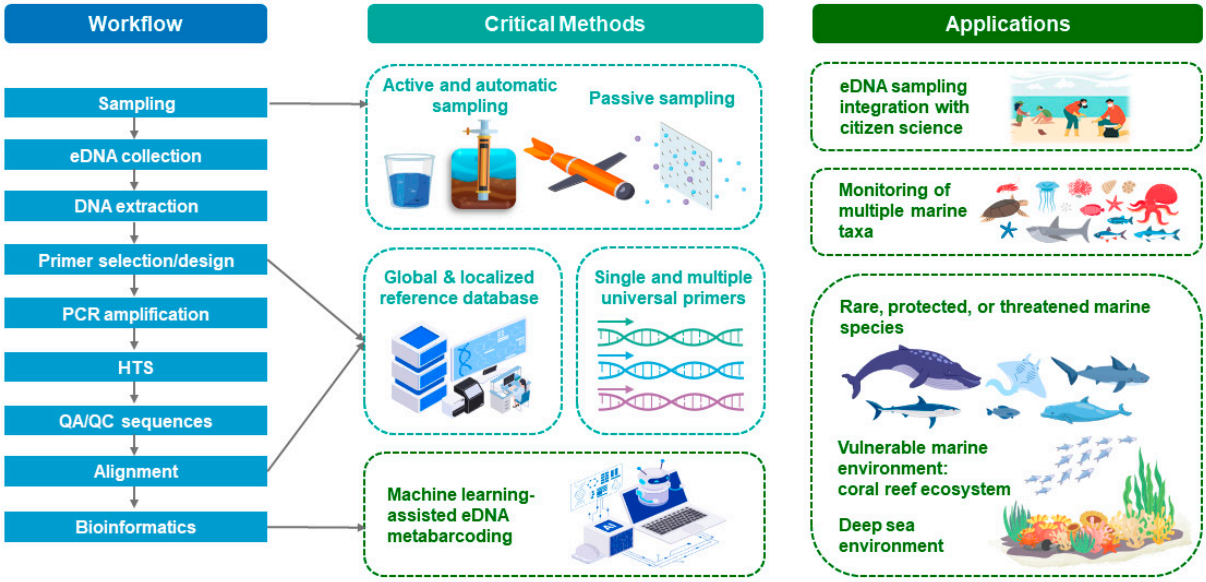

Figure 2. Thematic diagram of environmental DNA (eDNA) metabarcoding-based monitoring in marine protection and management. PCR: polymerase chain reaction; HTS: High-throughput sequencing.

4.1.1. Active and Automatic Sampling

Currently, the major challenge of employing eDNA metabarcoding remains in the standardization of sampling and processing methods. Several methods and guidelines for eDNA monitoring in aquatic environments have been published by the United States Geological Survey (USGS) [85], the Federal Office for the Environment (FOEN) of Switzerland [75], European Cooperation in Science & Technology (COST) [86] etc., providing a reference for standardized operation for eDNA applications. However, applying these guidance methods rigorously in practice is often not feasible. For example, the volume of water required in sampling may conflict with the need for timely transportation of samples back to the laboratory to reduce risks of DNA degradation [87]. Methods to preserve eDNA in environmental samples, such as the addition of storage reagents to samples and field-based filtration systems to reduce the need for laboratory-dependent work, are constantly revised and explored for improved sampling effectiveness.

To improve sampling efficiency and reduce labor, a portable eDNA sampling system (https://store.smith-root.com/products/edna-sampler-backpack-lith-combo, accessed on 27 August 2024) was designed to integrate a pole for water collection and a portable backpack that includes a pump with sensors that can be remotely controlled, as well as a device for filtering and preserving water samples. This integrated eDNA sampler reduces the need for human contact and the risk of DNA degradation, as water samples can be sequentially filtered by the portable pump and preserved in the system. Meanwhile, its programmable sampling rate and other parameters allow for standardizing sampling methods. The flow and pressure experiment for this portable sampler carried out by Thomas et al. [88] showed that these systems could capture enough DNA when applying 5 µm filters in specific parameter settings such as a flow rate threshold of 1.0 L/m.

Representative eDNA sampling coverage can be challenging in the field, especially in relatively inaccessible environments. In recent years, ROVs or Unmanned Aerial Vehicle

(UAV; drone) coupled with sampling containers and filtration instruments have been used to collect water samples for eDNA studies. For example, 1 L water bottles attached to the flying drones submerging into the surface water have been used to collect water samples [89]. Further, a drone combining a filter capsule that can take one sample consisting of 10 to 20 sub-samples of 50 or 100 mL water was developed (https://sylphium.com/eng/edna-sampling-drone/, accessed on 27 August 2024). To minimize destruction while collecting samples closest to corals, an underwater mini ROV and a seawater sampler have also been applied to collect seawater 1–2 m above the coral reef top to survey the scleractinia corals. This method proved to have sufficient detection efficiency to identify targets at the genus level by collecting about 0.5 L water [90]. Nevertheless, complete and accurate sampling requires practiced skills to maneuver a UAV or ROV proficiently, and the effectiveness of these sampling methods still needs further verification.

Meanwhile, a series of fully automated eDNA samplers have been designed to sample and preserve eDNA samples without researchers on-site. The environmental sampling processor (ESP) generation 3 is one of the most advanced automated samplers that can be coupled with an autonomous underwater vehicle (AUV) and contains 60 filter cartridges that filter water samples under a set program which can be kept stable for 21 days [91]. The ESP sampler provides high-frequency and continuous sampling and, because of its capability for unmanned operation, can travel to remote and inaccessible field sites, greatly reducing the cost of time, labor, and other resources. A test of eDNA quantification for ESP has confirmed its comparable capability and feasibility with manual sampling methods [91]. ESP has also been employed on an uncrewed surface vessel (USV) surveyor SD 1200 to collect water samples for regular intervals over a 4200-km, 29-day transit, which would otherwise require enormous human and material resources using traditional manual sampling methods [92]. However, it is undeniable that ESP samplers are expensive, especially for small research groups, and are still in research and development phases without widescale implementation potential yet. Thus, a range of automated eDNA samplers were also recommended for a lower cost. A representative, open-sourced, single-filter system, subsurface automated sampler for eDNA (SASe) with a relatively low cost (~280 USD) was designed by the National Oceanic and Atmospheric Administration (NOAA, USA), which is submersible to 55 m and can filter and preserve eDNA samples *in situ* [93]. There are also relatively low-cost samplers with a multi-filter system for eDNA collection, such as the Large Volume eDNA Samples [94], as well as a compact and automated eDNA sampler that have both sample preservation and self-cleaning capabilities designed by Hendricks et al. [95]. The development of these automated samplers can be highly beneficial to reduce logistic challenges in eDNA monitoring.

4.1.2. Passive Sampling

In active sampling, water and sediment samples are commonly collected manually or via automated samplers, paired with filtration systems to concentrate DNA. While labor efficiency compared with conventional capture-based monitoring methods have considerably improved, some of the limitations of active sampling, such as the need for pump equipment, could be time-consuming or costly. Thus, passive sampling, a low-cost and easily deployable alternative method, was developed using natural (e.g., sponge) or artificial materials. For example, two types of passive samplers, including positively charged nylon and non-charged cellulose ester membranes fixed in a pearl oyster aquaculture frame, were submerged in seawater ~1 m below the water surface for 24 h, and presented a 97% detection rate of fish taxa compared with active eDNA sampling [96]. Membranes submerged underwater for 24 h to collect eDNA also proved able to detect comparable species richness with eDNA metabarcoding conducted by active sampling [97]. Furthermore, the sorbent material used to capture eDNA is a key factor in passive sampling. Chen et al. [98] reported that the best-performing material was glass fiber filters after comparing the ability to capture the eDNA of the Chinese giant salamander (*Andrias davidianus*) among 12 artificial materials. These studies demonstrated

the usefulness and potential of passive eDNA sampling. However, limitations remain, such as the fixation of sorbent materials mentioned above in inaccessible water or remote deep seawater and the potential of biofilm development and fouling process.

In summary, both widely accepted manual sampling methods and emerging automated samplers or passive sampling strategies have their advantages and limitations. Choosing and optimizing appropriate eDNA sampling methods and maximizing and rationalizing the sampling process according to experimental needs to avoid errors and risk are crucial steps for high-efficiency monitoring.

4.2. The Reference Database for Marine Species and the Practical Standards for eDNA Metabarcoding-Based Marine Monitoring

Following amplification of the target DNA fragments, the sequence information obtained by sequencing needs to be aligned with known species genomic sequence information, i.e., reference databases. Moreover, the selection of appropriate marker genes and the design of appropriate primers, as well as the subsequent taxonomic assignment of sequenced marker genes, are all tightly bound to the coverage and quality of the reference databases. Thus, the integrity and breadth of the reference sequence database are crucial for exact species identification [99].

4.2.1. Globally Accessible Reference Databases

A collection of publicly available reference databases that can be used for eDNA metabarcoding has been established and is constantly updated. For example, Barcode of Life Database (BOLD) (https://www.boldsystems.org/, accessed on 27 August 2024) is a web platform dedicated to collecting and organizing DNA barcodes of organisms worldwide. Researchers and citizen scientists from worldwide can upload the DNA barcodes of their collected biological samples to the BOLD database. Currently, BOLD provides a collection of 15,976,000 barcodes for 352,000 species of fish, mammals, plants, fungi, and other species (as of 10 March 2024) and is expected to expand its coverage to 1.5 million species by 2025. In addition to databases specializing in collecting DNA barcodes, the GenBank nucleotide database affiliated with NCBI is one of the most comprehensive and commonly used databases, containing sequences from more than 165,000 organisms. Further, MitoFish is a specialized mitochondrial genome database with precise taxonomical annotation of fish, including complete mtDNA sequences for 4605 fish species (as of 8 February 2024). For ribosomal DNA, the commonly used marker gene in addition to mitochondrial DNA, SILVA (https://www.arb-silva.de/, accessed on 27 August 2024) is one of the most commonly used databases alongside the Ribosomal Database Project (https://www.glbrc.org/data-and-tools/glbrc-data-sets/ribosomal-database-project, accessed on 27 August 2024) providing bacterial, archaeal, fungal, and eukaryotic rRNA sequences and corresponding analysis tools. More specific information on current reference databases is summarized in Table 1.

4.2.2. Localized References Databases

Although many reference databases have been established and are widely used, current reference databases are still insufficient considering the great diversity of species [100]. Such databases currently suffer from a huge redundancy of data volume, frequent comparison errors due to low upload thresholds, and an imbalance in both geospatial and species coverage [101]. A recently realized need is for excessive data to be summarized into more effectively accessible and processible information such as class-specific or pathogen-specific databases. For example, Kasmi et al. [102] targeting mitochondrial genes in fish, have extracted all 12S rRNA and COI fish sequences in databases such as NCBI, European Nucleotide Archive (ENA), BOLD, etc. and revised corresponding taxonomic information according to the Integrated Taxonomic Information System (ITIS) and FishBase, create the Mare-MAGE database. Gold et al. [103] reported that regional databases using sequences of local fishes provide higher alignment accuracy than comprehensive global databases

such as GenBank. Therefore, establishing regional, indigenous DNA databases and updating DNA barcodes globally are crucial for species monitoring and conservation. Several regional databases have been established, such as the Canadian Centre for DNA Barcoding (CCDB) and the European Molecular Biology Laboratory database (EMBL). Recently, the Wuhan Institute of Aquatic Biology, Chinese Academy of Sciences, also constructed China's first comprehensive aquatic organisms eDNA database, AeDNA (http://159.226.163.221/, accessed on 27 August 2024) (Table 1), by integrating DNA sequence, metadata, and taxonomic information from public databases, as well as DNA sequences of local aquatic organisms, which lays a much-needed foundation to benefit future environmental monitoring efforts. Concerning more localized regions worldwide, such as Hainan Island [104] and Pearl River Estuary [105], the eDNA metabarcoding reference database of fish also has been preliminarily constructed. These regional and localized databases can further broaden the species coverage of public databases, and they are expected to increase the rate of species identification from eDNA metabarcoding data.

Table 1. Summary of commonly used public genome reference database.

Database	Main Content	Source	Website *
GenBank	Nucleotide sequences for over 478,000 officially classified species	National Center for Biotechnology Information (NCBI)	https://www.ncbi.nlm.nih.gov/genbank/
European Molecular Biology Laboratory database (EMBL)	Data resources and analysis tools to support life science research	29 member states	https://www.embl.org/
Barcode of Life Database (BOLD)	An assembly of DNA barcode data with primers, electropherograms, images and sequences.; sub-database for fish (FISH-BOL), mammals, bird species, plant species, and more.	International Barcode of Life (iBOL) Project; Canada	https://www.boldsystems.org/
Greengenes 2—16S rRNA database	A reference tree that unifies the genome and 16S rRNA databases in a consistent, integrated resource by inserting sequences into a genome-wide phylogenetic tree	Lawrence Berkeley National Laboratory, Berkeley, CA, USA	https://greengenes2.ucsd.edu/
SILVA rRNA database	Aligned small (16S/18S, SSU) and large subunit (23S/28S, LSU) ribosomal RNA (rRNA) sequences for all three domains of life (Bacteria, Archaea and Eukarya).	Free online resource from Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures GmbH, Braunschweig, Germany.	https://www.arb-silva.de/
Ribosomal Database Project (RDP)	Quality-controlled, aligned and annotated Bacterial and Archaeal 16S rRNA sequences, and Fungal 28S rRNA sequences; A series of analysis tools	United States	http://rdp.cme.msu.edu/
PR2-metaPR2	Eukaryotic 18S rRNA metabarcodes that have been reprocessed and assigned using PR2	Vaulot et al., 2022 [106]	https://app.metapr2.org/metapr2/
UNITE	Eukaryotic nuclear ribosomal ITS region	Northern European initiative	https://unite.ut.ee/
MIDORI Reference 2	DNA and amino acid sequences used for taxonomic assignments of Eukaryota mitochondrial DNA sequences	Biodiversity Research Center, Academia Sinica, Taiwan	https://www.reference-midori.info/index.html
MitoFish	Standardized fish mitochondrial genome	Atmosphere and Ocean Research Institute, the University of Tokyo, Japan.	https://mitofish.aori.u-tokyo.ac.jp/
AeDNA	Aquatic DNA barcodes and genomes; Habitat types cover rivers, lakes, seas, glaciers and hot springs.	Institute of Aquatic Biology, Chinese Academy of Sciences, Wuhan, China	http://159.226.163.221/

* All URLs were accessed on 27 August 2024.

4.3. eDNA Metabarcoding-Based Monitoring from the Use of Single to Multiple Primers

eDNA is naturally degrading and can be minuscule in quantity in aquatic environments. A recent study showed that fish-derived eDNA fragments extracted from natural seawater samples accounted for only 0.004% of total sequences when using shotgun

sequencing [12]. Thus, following the DNA collection from environmental samples, specific/targeted DNA fragments known as the "barcode", which involves highly conserved gene regions across species and highly variable regions capable of distinguishing species, need to be amplified for subsequent high-throughput sequencing and analysis. Appropriate PCR primers for PCR amplification are critical in eDNA metabarcoding-based methods as they determine the taxonomic coverage of amplified DNA, which can ultimately determine monitoring outcomes [107]. Significantly different diversity was detected when different primers were used for samples obtained in the same study area and with the same eDNA metabarcoding workflow [63]. A well-designed primer must consider the variable and conserved regions of the gene for its intended target coverage and specificity, in addition to other practical considerations such as amplicon size, structure, and primer compatibility. Sources of gene sequences that can be referenced for primer design include verified genome sequences in established reference databases or genome sequences of target species obtained by sequencing. The performance of designed primers can then be tested by in silico evaluation [108], in vitro experimental tests [109], and/or surveys in laboratory aquariums or natural environments [110].

Mitochondrial genes are generally more widely used as marker genes than nuclear genes, as the copy number of mitochondrial DNA (mtDNA) is larger than that of nuclear DNA. Since the concentration of genetic materials in the environment is low, mtDNA copies are more likely to be detected [111,112]. Moreover, current genome databases contain many mitochondrial DNA sequence information, which eases species identification. The cytochrome b (Cytb) gene, the D-loop gene, and the mitochondrial 12S and 16S ribosomal DNA are now widely used as molecular markers for eukaryotic species identification, particularly in fish [113]. A universal primer that targets a specific DNA fragment across a wide range of taxonomic groups is often used in eDNA metabarcoding-based methods to facilitate broad-scale community-level study in the marine environment [76]. Targeting teleost fish, Zhang et al. [13] compared 22 sets of primers for eDNA metabarcoding analysis using in silico PCR and metabarcoding. In general, commonly used primers designed to target 12S rRNA genes, such as MiFish-U [114] and Teleo [115], compared to primers targeting 16S rRNA or COI genes, such as Ve16S [116], FishCBL/CBR [76], etc., were able to detect higher fish diversity [117]. As reported by Xing et al. [113], the Cytb gene is the most used marker for species-specific fish detection, followed by COI. For other marine invertebrates, some primers are commonly used, such as mlCOIintF/jgHCO2198 [118] targeting COI genes, 16S_Inv_For/16S_Inv_Rev [119] targeting 16S rRNA. New sets of primers are also constantly developed, such as MollCOI154/MollCOI255 for marine mollusks [120].

Simultaneous amplification of multiple target fragments using multiple universal primers enables monitoring of multiple taxa-groups and overall biodiversity in the ecosystem. This is the prominent advantage demonstrated by eDNA metabarcoding-based methods. For example, Liu and Zhang [121] recommended the simultaneous use of multiple markers for eDNA metabarcoding analyses after investigating biodiversity in deep-sea sediments using 18S rRNA (V1–2 and V9) and 28S rRNA primers in conjunction. In a systematic review, groupings of (a) COI and 18S rRNA, and (b) COI, 16S rDNA and 18S rRNA were the most commonly used strategy for marine eDNA metabarcoding [122]. In addition, one of the most recognized examples by Stat et al. [12] simultaneously used 10 pairs of primers with nuclear 18S rDNA, mitochondrial COI, mitochondrial 16S rDNA, and chloroplast 23S rDNA as marker genes targeting Eukaryotes, Metazoans, Fish, Mammals, Crustaceans, Cephalopods, *Symbiodinium*, Plants, and Prokaryotes, etc., to achieve "tree of life" metabarcoding at the ecosystem level.

5. eDNA Metabarcoding-Based Monitoring for Marine Conservation and Management

eDNA metabarcoding, which benefits from the development of molecular technologies and bioinformatics [123], has shown great potential to be used for population- and community-level or large-scale monitoring with implications for conservation strategies and policy-making through biodiversity monitoring. This section focuses on complex

marine environments such as coral reef ecosystems and deep sea to show the application of eDNA metabarcoding for marine conservation and management. Further, we introduced regional and/or global monitoring trends by integrating citizen science or machine learning with eDNA metabarcoding (Figure 2).

5.1. eDNA Metabarcoding for Marine Biodiversity Monitoring

eDNA metabarcoding significantly facilitates and improves the assessment of biodiversity in marine ecosystems. It effectively captures community dynamics and has emerged as a crucial tool for informing management decisions. In 2012, eDNA metabarcoding was first applied in marine environments for the purpose of fish biodiversity monitoring [76]. The detection coverage achieved with the eDNA metabarcoding method, utilizing only 0.5 L water samples from a temperate marine ecosystem, was reported to be equal to or superior to that of nine conventional methods, including bottom trawling. Currently, eDNA metabarcoding is widely employed for monitoring community composition and structure across spatiotemporal gradients, ecosystem-level assessments [13], ecological status evaluations [63,124], early warnings of harmful algal blooms [125], and the impacts of environmental changes and human activities. This method targets various marine taxa, including fish [126], invertebrates [127], elasmobranchs, cetaceans, and even entire eukaryotic communities [128]. The below section summarizes the applications of eDNA metabarcoding in the context of rare or threatened marine species of high conservation concern, as well as sensitive marine ecosystems and deep-sea environments. It highlights the advantages and applications of eDNA metabarcoding-based methodologies for marine environmental protection and management.

5.1.1. eDNA Metabarcoding-Based Monitoring for Rare, Protected, or Threatened Marine Species

Monitoring the status and population trends of rare, protected, or endangered marine species is essential for informing conservation efforts and guiding effective management strategies to prevent further declines and promote recovery. Many of these rare, protected, or threatened species occur at naturally low population densities or are challenging to observe, making traditional survey methods ineffective, time-consuming, and resource-intensive. Recent studies have demonstrated that eDNA metabarcoding-based monitoring serves as a rapid, safe, sensitive, and cost-effective method for detecting and studying rare marine species [129]. For instance, the impact of increased human fishing activities on elasmobranch diversity and richness was revealed through the use of two forward primers, FishF2 and VF2, along with one reverse primer, “Shark COI-MINIR”, in eDNA metabarcoding analyses [130]. For species such as sharks, which are challenging to capture for survey purposes, eDNA metabarcoding detected approximately 44% more shark species than underwater visual census (UVC) and baited video methods, with a sampling effort that was two orders of magnitude lower [131]. Notably, sharks were identified in human-impacted areas where no shark species had previously been recorded using these methods [131]. Similarly, for threatened cetaceans that exhibit low abundance and elusive behavior, such as dwarf sperm whales [132], humpback whales, and bottlenose dolphins [133], diversity can be effectively monitored using 12S rDNA primers, including Vert01 and Mamm01, within eDNA metabarcoding frameworks. In addition to diversity assessments, eDNA metabarcoding employing universal primer MiFish-U and modified primer Elas02 provided rapid and extensive insights into the abundance and temporal variation of sharks and rays around an Indian Ocean island, surpassing traditional visual and capture-based techniques [134]. Furthermore, leveraging the advantages of eDNA metabarcoding to monitor multiple taxa allows for the simultaneous assessment of the spatiotemporal distribution and abundance of marine mammals, such as bottlenose dolphins, minke whales, and harbor porpoises, along with their foraging fish. This is achieved using two sets of primers, MarVer1 and MarVer3, offering critical information for understanding and predicting their distribution and informing conservation strategies [135].

With the enhancement of methodological infrastructure, such as the establishment of local reference databases, eDNA metabarcoding methods can detect a broader range of species diversity, including those that have not been previously observed or recorded in certain areas. For instance, de la Hoz Schilling et al. [136] identified 27 elasmobranch species using the MiFish-E (12S) primer set in the Banc d'Arguin National Park, Mauritania, of which 12 species were newly documented. Notably, 67.9% of the detected species are listed in the IUCN Red List of Threatened Species. Additionally, the implementation of passive filter-collection eDNA samplers within nets used for routine fishing activities allows for the detection of bycatch, including elasmobranchs, while further reducing sampling effort [137]. These vulnerable marine organisms can act as valuable bioindicators, offering insights into the overall health and functioning of the broader marine ecosystem. Their presence, abundance, and behavioral patterns may indicate changes in environmental conditions. Thus, eDNA metabarcoding methods hold significant potential for providing enhanced and comprehensive information for the future monitoring and protection of these important marine species.

5.1.2. eDNA Metabarcoding-Based Monitoring for the Vulnerable Marine Ecosystem: Coral Reef Ecosystem

eDNA metabarcoding methods have proven effective in monitoring community composition and structure across diverse habitats [74,126,138]. Marine ecosystems, such as coral reefs and seagrass beds, are recognized as biodiversity hotspots and are particularly sensitive to disturbances. The noninvasive nature of eDNA metabarcoding maximizes its potential for conservation-oriented monitoring. In vulnerable marine environments like coral ecosystems, which are among the most hyper-diverse and sensitive, traditional underwater visual census (UVC) methods conducted via scuba diving may be inadequate for effectively monitoring cryptic organisms residing within coral structures. Visual obstructions and limitations in taxonomic identification further compound these challenges. For example, Brandl et al. [139] reported that half of all reef fish are cryptic species. In contrast, eDNA-based methods, which rely on the DNA released by organisms into the environment, mitigate biases associated with visual obstacles. A study collecting 226 seawater samples from five tropical regions (the Caribbean, Central and Southwest Pacific, Coral Triangle, and Western Indian Ocean) demonstrated that eDNA metabarcoding could monitor a greater diversity of reef-associated and cryptobenthic species compared to UVC methods [140]. Furthermore, coral spawning and sexual reproduction are critical events for maintaining coral reef ecosystems, typically occurring at night. Visual observations during these events are limited by nighttime light conditions, inaccessibility of certain locations, and the labor-intensive nature of such surveys, which are also prone to bias and may disrupt coral activities. By comparing eDNA abundance before and after coral spawning events, eDNA metabarcoding methods successfully monitored the spawning activities of 37 coral species and an associated 133 fish species that serve as indicators of spawning, all with reduced physical disturbance to coral reef organisms [141].

Recent advancements in eDNA-based monitoring methods have led to the development of coral-specific primers aimed at assessing species diversity (at least to the genus level) and relative abundance of corals. The efficacy of eDNA metabarcoding for coral monitoring has been evaluated in comparison to underwater visual census (UVC) methods. Nichols and Marko et al. [77] were among the first to design primers for amplifying the COI and 16S genes of coral genera in Hawaii specifically for eDNA metabarcoding. They created two corresponding primers for relatively short (~120 bp) and long (~400 bp) amplified fragments for each gene, as detailed in Table 2. When these primers were employed to estimate coral relative abundance, the eDNA read counts exhibited a high correlation with coral coverage measured via diving techniques, yielding consistent information regarding dominant species. Similarly, Shinzato et al. [142] designed two pairs of primers (Scle_12S and Scle_COI) based on the mitochondrial genomes of 71 scleractinian coral species (representing 36 genera and 15 families) sourced from the NCBI database. Their

findings revealed that 93% and 72% of the PCR-amplified fragments matched 12S and COI sequences in the database, respectively, demonstrating greater specificity than previous studies. Currently, the most commonly utilized primers are those developed by Alexander et al. [143], which include one reverse primer capable of detecting corals, excluding the genus Acropora, and another primer specifically designed for Acropora detection, based on ITS gene reference sequences of scleractinian corals in the NCBI database. eDNA metabarcoding employing these two primer pairs detected coral genera comparably to results obtained from scuba diving surveys. West et al. [144] further applied these primer pairs in eDNA metabarcoding monitoring of Lalanggarram marine parks. Although these methods were able to detect corals at the species level, they required the collection of benthic tissue samples, which conflicts with the original aim of being environmentally friendly. To enhance species-level monitoring, further enrichment of the ITS reference database is essential. Dugal et al. [145] established a reference database for 70 local coral species based on the ITS sequences of 94 coral species, demonstrating that utilizing local reference databases significantly improved the accuracy of coral species identification. There remains an urgent need for additional eDNA metabarcoding research focused on coral monitoring, with objectives to refine methodological processes, including sample optimization and the enrichment of primers and reference databases, to achieve high efficiency and resolution in coral monitoring.

Table 2. Reported primers targeting scleractinian corals monitoring using eDNA metabarcoding.

Markers	Primer Name	Primer Sequence (5′-3′)	Target Length (bp)	References
16S rRNA	HICOR16S_F1	CCGGTATGAATGGTRTCMCGA		Nichols & Marko [77]
	HICOR16S_R1	TMCAGTAAAGYTCCATGGGG	120	
	HICOR16S_R2	GTAACTTTTATTTGYTTATC	400	
COI	HICORCOX_F1	GAACAAGGRGCKGGBAC		
	HICORCOX_R1	CCVGGRGCYCKCATRTTAAA	120	
	HICORCOX_R2	GCAACAAAAGTYGGKATTAT	400	
Nuclear ITS	SCLER5.8SFor	GARTCTTTGAACGCAAATGGC		Alexander et al. [143]; West et al. [70]; Dugal et al. [145]
	SCLER28SRev	GCTTATTAATATGCTTAAATTCAGCG		
	Coralacro_874Rev	TCGCCGTTACTGAGGGAATC		
12S rRNA	Scle_12S_Fw	CCAGCMGACGCGGTRANACTTA	~366–465	Shinzato et al. [142]
	Scle_12S_Rv	AAWTTGACGACGGCCATGC		
COI	Scle_CO1_Fw	ATTGTNTGRGCNCAYCATATGTTTA	~296–302	
	Scle_CO1_Rv	CCCATAGARAGNACATARTGAAA		

Monitoring associated and symbiotic organisms in coral reef ecosystems is equally crucial, as these organisms are integral to the food web that supports trophic transfer. Thus, a multi-primer approach is necessary to target various taxa, including both micro- and macro-organism groups. For instance, Dugal et al. [146] using a pair of universal primers (18S_uni_1F and 18S_uni_400R) targeting the V1–3 hypervariable region of the 18S rRNA genes have successfully detected 14 metazoan phyla and 57 species within coral reef environments. By employing multiple primers that target mitochondrial 16S rRNA, COI genes, and the nuclear 18S rRNA gene, a diverse array of taxa—including bony fish, elasmobranchs, crustaceans, mollusks, and echinoderms—has been simultaneously identified in coral ecosystems [144,146]. Stat et al. [12] utilized ten pairs of primers to detect numerous target marine taxa in a single tropical coral reef in Western Australia. These multi-primer strategies yield more comprehensive outcomes and are increasingly adopted to meet marine biomonitoring needs. Furthermore, several studies have indicated that eDNA metabarcoding demonstrates comparable or superior detection performance while requiring less effort than traditional underwater visual census methods for monitoring corals [147] and reef fishes [148]. Utilizing eDNA metabarcoding allows for the assessment of coral community structure [77,142], species diversity, and β/γ biodiversity of reef

fishes [73,140], as well as coral symbionts [149], echinoderms [150], and their larvae [151]. This approach provides detailed information that can enhance the conservation of coral reef ecosystems.

5.1.3. eDNA Metabarcoding-Based Monitoring for Deep-Sea Environment

The deep-sea environment, defined as ocean depths below 200 m, encompasses nearly two-thirds of the Earth's surface and represents one of the planet's largest and most biodiverse yet least explored ecosystems [121,152]. The significant depths and immense pressure of the deep sea pose challenges for the deployment of widely available monitoring tools. Remote deep-sea areas are often unfeasible for transportation of monitoring equipment, such as trawls [81]. Additionally, the complex topography of deep-sea environments—including steep slopes, rocky substrates, and areas inhabited by sensitive fauna such as corals and sponges—renders traditional trawling methods inappropriate [64,82]. Visual survey methods utilizing remotely operated vehicles (ROVs) may be compromised by light requirements and noise, potentially skewing species observations [152]. In contrast, eDNA metabarcoding methods are relatively noninvasive, adaptable to the intricacies of deep-sea environments, and require minimal effort for sampling. For example, Thomsen et al. [81] demonstrated that eDNA metabarcoding could detect the equivalent of 26 fish families compared to trawling, achieved through straightforward water sample collection at specified depths (188–918 m). Notably, eDNA metabarcoding revealed biodiversity levels approximately 3.9 and 10.1 times greater than those detected by vertical nets and trawls, respectively. Additionally, the community patterns (beta diversity) identified through eDNA analyses aligned with conventional net sampling for deep-ocean pelagic zooplankton and fish across depth ranges of approximately 500 to 2500 m [64]. Seamounts, recognized as biodiversity hotspots within deep-sea ecosystems, are increasingly threatened by climate change and human activities, including mineral resource exploitation. eDNA metabarcoding has been employed to monitor discrepancies in prokaryotic and eukaryotic communities across various contexts of seamounts and adjacent abyssal plains [153], as well as to assess benthic indicator organisms like foraminiferal communities [154]. These efforts provide a crucial biodiversity baseline for the protection and management of deep-sea environments. However, the current lack of knowledge regarding deep-sea organisms, coupled with the scarcity of relevant reference databases, remains a significant constraint to effective biodiversity monitoring in these ecosystems using eDNA metabarcoding methods [155].

5.2. Integrating Citizen Science with eDNA Metabarcoding: Regional/National to Global Biodiversity Monitoring

Citizen science programs are increasingly recognized for their ability to combine accessible methods with public engagement, facilitating environmental monitoring over broader spatial and temporal scales than traditional research allows. By lowering technical barriers to sampling, these initiatives have seen numerous successful implementations, such as the Reef Life Survey, which focuses on marine biodiversity [156]. In recent years, several citizen science projects employing eDNA metabarcoding have emerged at both national and international levels, targeting freshwater and marine ecosystems. These programs leverage public participation to enhance monitoring effectiveness. For instance, Miya et al. [99] engaged volunteers in a citizen science project across six temperate sites in Japan. Participants received training on eDNA collection and filtration techniques using syringes and filter cartridges, followed by the collection of water samples. Subsequent DNA extraction, eDNA metabarcoding, and data analysis revealed 66 families and 118 genera of fish, illustrating distinct spatial biodiversity patterns within the region. Similarly, Agersnap et al. [157] coordinated a national "BioBlitz" citizen science initiative utilizing eDNA metabarcoding to assess marine fish species diversity in Denmark's coastal zone. This project mobilized 360 citizen scientists to collect seawater samples from 100 sites, which were then frozen and dispatched to Aarhus University for analysis within 24 to 72 h. The project achieved a high sample return rate of 94% and identified a total of 52 fish species,

representing approximately 80% of Danish coastal fish diversity and 25% of all marine fish species. These initiatives demonstrate the advantages of integrating citizen science with eDNA methodologies, particularly in terms of the volume of samples that can be collected simultaneously—something often challenging for professional researchers. Moreover, eDNA metabarcoding has shown resilience to procedural failures, enabling citizens with varying levels of training to conduct biodiversity monitoring with considerable accuracy. This approach is poised to play an essential role in future large-scale species assessments. Beyond regional and national efforts, advancements in technology and decreasing costs have positioned eDNA metabarcoding within the framework of global conservation initiatives [158]. For example, the United Nations Educational, Scientific and Cultural Organization (UNESCO) has launched the Environmental DNA Expeditions in UNESCO World Heritage Marine Sites project (https://www.unesco.org/en/edna-expeditions, accessed on 27 August 2024) since 2022. This initiative trains local communities in eDNA sampling techniques, enabling them to monitor biodiversity over the course of a year. In summary, the outcomes of these citizen science programs underscore the efficacy of eDNA-based monitoring for biodiversity assessment at regional, national, and global scales. The findings generated hold significant potential for advancing large-scale biomonitoring efforts in the future.

6. Machine Learning (ML)-Assisted eDNA Metabarcoding for Large-Scale Marine Biodiversity Monitoring

Over the past decade, AI has significantly transformed our capacity to automatically, accurately, and reliably identify features and patterns within ecological datasets. Machine learning focuses on developing models from pre-processed training datasets by selecting suitable algorithms—such as classification, regression, and clustering. These models are evaluated using a test dataset, allowing for the optimization of the trained model before applying it to new datasets for accurate predictions [159]. eDNA metabarcoding typically generates vast amounts of sequencing data, and optimizing machine learning techniques for predictions from this data can substantially reduce the labor-intensive data processing typically required by researchers. For instance, Cordier et al. [16] employed supervised machine learning to analyze eDNA metabarcoding data to predict biological indices (BIs) that assess Ecological Quality Status. They obtained eDNA data from 144 sediment samples collected from five salmon farms in Norway, which included operational taxa units (OTUs) and OTU richness. Using Random Forest and Self-Organizing Map algorithms, they trained models on data from different samples designated as either the training or test dataset to predict four BIs related to alpha and beta diversity. Their comparative analysis revealed that the machine learning predictions closely aligned with results derived from traditional morphological identification methods. Subsequently, Cordier et al. [17] further extended their work by training eDNA data from five different molecular markers using a similar machine learning approach, demonstrating the broad applicability of supervised machine learning across various molecular markers, with all tested markers generating accurate predictive models. Similarly, Dully et al. [160] investigated the impact of sequencing depth on machine learning predictions derived from eDNA metabarcoding, utilizing K-means clustering and Random Forest algorithms. Their results indicated that a sequencing depth of 40,000 to 80,000 sequences per sample was necessary for the machine learning methods to achieve over 80% classification accuracy.

Machine learning techniques utilizing coding algorithms have also been applied in biodiversity monitoring [161]. In this approach, three coding methods were developed to encode the four nucleotide bases, along with genus and species names. A traditional three-layer perceptron neural network was then employed, where the encoded bases served as the input layer and the corresponding genus and species names as the output layer. Data from the database were fed into the neural network, which learned through backpropagation. Following training, the model was capable of automatically recognizing and classifying new eDNA sequencing data as they were input. These studies demonstrate

that machine learning can effectively manage large volumes of eDNA metabarcoding data, enhancing overall processing efficiency. The successful integration of machine learning into biodiversity assessments based on sequencing data alleviates researchers from labor-intensive tasks and highlights the necessity for further exploration of ML applications in eDNA metabarcoding for ecological monitoring and conservation. The effectiveness of machine learning-assisted eDNA metabarcoding and species identification relies heavily on high-quality training data, careful feature selection, and thorough model evaluation.

7. Centralized Environmental Database for Enhanced Marine Environmental Baseline and Impact Monitoring

Local biodiversity and species inventories serve as essential reference databases for the double verification of emerging monitoring techniques. Benefiting from long-term local monitoring efforts, a substantial volume of environmental data—including biodiversity, water quality, and land use—can be systematically organized into centralized environmental databases. Such Centralized Environmental Databases facilitate data analysis and visualization, enabling stakeholders to gain insights into the current state of the environment, assess potential risks, predict future changes, formulate regulations to address existing issues, and implement effective environmental protection and management strategies. The integration of environmental data with Geographic Information Systems (GIS) and analytical tools enhances the accessibility, comprehensiveness, and efficiency of environmental data management, potentially reducing the need for redundant environmental surveys. For instance, the Centralized Environmental Database (CED) developed by the Environmental Protection Department (EPD) of Hong Kong (https://eiaced.epd.gov.hk/, accessed on 27 August 2024) features a GIS-enabled mapping platform that encompasses various baseline environmental and ecological data, including historical water quality assessments derived from Environmental Impact Reports. The CED enhances the interpretative power of innovative noninvasive monitoring technologies by providing a reference database. This localized ecological dataset can be correlated with emerging noninvasive monitoring methods—such as underwater imaging and environmental DNA (eDNA) analysis—to validate findings and improve species detection accuracy. Conversely, data generated from these advanced monitoring techniques can be integrated into the CED, enriching the database and facilitating comprehensive trend analyses. Moreover, databases documenting historical local environmental data provide critical baseline information, including essential environmental indicators and variations in conditions across different regions [162]. This information is vital for prioritizing monitoring targets, optimizing resource allocation, and enhancing the efficiency of environmental oversight. By comparing current monitoring data with historical baseline data, deviations and trends can be rapidly identified, permitting the prediction of potential environmental risks through modeling and data analysis [163].

8. The Integration of Underwater Optical Imaging-Based Monitoring and eDNA Metabarcoding-Based Monitoring for Marine Environmental Conservation

As discussed in previous sections, both underwater optical imaging and eDNA metabarcoding methods possess distinct advantages. Integrating these two approaches for marine environmental monitoring can leverage their strengths while mitigating some limitations, providing more comprehensive data and enhancing monitoring efficiency. eDNA metabarcoding serves as a complementary method to optical monitoring, expanding the range of detectable species [164]. For instance, eDNA metabarcoding can effectively supplement underwater visual surveys in coral reef ecosystems, where cryptic fish and other species may obscure themselves within the complex structures of coral, making direct observation challenging. This synergy allows for the detection of a greater diversity of pelagic and benthic fishes [165]. Notably, the simultaneous use of eDNA metabarcoding and baited remote underwater video systems has resulted in over a 30% increase in the abundance of detected fish genera compared to either method used independently. Due to the inherent limitations and biases of each technique, one method frequently identifies taxa that the other does not [166]. Valdivia-Carrillo et al. [167] observed that the number

of fish species detected doubled when integrating underwater visual census with eDNA metabarcoding.

Overall, as described in Figure 3, eDNA metabarcoding methods facilitate extensive spatial and temporal monitoring on regional and global scales, owing to their standardizable nature across geographical boundaries. These methods can also be effectively combined with citizen science initiatives and artificial intelligence technologies. In contrast, underwater visual monitoring allows for the capture of detailed information about marine organisms and their environments, particularly when complemented by real-time data transmission and processing systems, which yield high-resolution image data. This capability compensates for the limitations and cost constraints associated with high-resolution underwater visual monitoring equipment. In summary, the combination of underwater optical imaging and eDNA metabarcoding methods creates a synergistic approach that validates and enhances each technique, offering more reliable baseline information. This integrated methodology provides a robust framework for marine environmental monitoring, yielding valuable insights into biodiversity, ecosystem health, and the impacts of human activities.

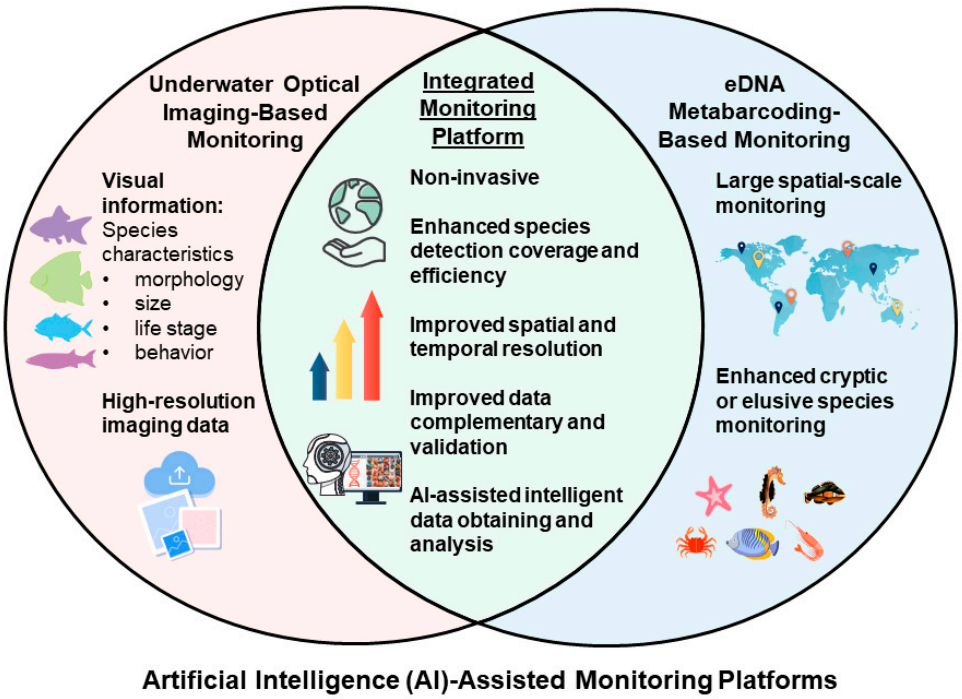

Figure 3. The integration of underwater optical imaging-based monitoring and eDNA metabarcoding-based monitoring methods for enhanced marine environmental monitoring and conservation.

9. Future Perspectives

Traditional biomonitoring has evolved from simple ecological observations aimed at identifying and assessing species diversity and abundance to a more comprehensive approach focused on understanding the underlying functions and interactions among species and communities. Contemporary biomonitoring is increasingly moving away from manual, visual, and capture-based methods, which are not only disruptive and unsustainable but also often fail to provide a holistic view of the ecosystem. The emergence of what we term "next-generation biomonitoring" may only be achievable through scalable approaches that harness large-scale data from next-generation sequencing and machine learning tools. These technologies enable the interpretation of visual data and the reconstruction of ecological networks through globally distributed, automated sampling stations and collaborative efforts [168]. Thomsen et al. [169] have proposed a global eDNA biomonitoring framework that leverages advancements in sequencing technologies—such as long-read and birdshot sequencing—alongside refined reference databases and artificial intelligence for

data analysis. This framework emphasizes local sampling, regional sequencing, and global information sharing, supported by autosamplers, drones, and large-scale citizen science initiatives to facilitate comprehensive eDNA-based monitoring. Concurrently, advancements in acoustic and optical technologies have paved the way for the simultaneous use of multiple monitoring tools to achieve global oversight. Besson et al. [168] advocate for the integration of diverse automated recording instruments—such as eDNA metabarcoding and digital cameras—to collect large-scale, high-resolution data. Machine learning can then be employed to detect, identify, and analyze this data, creating accessible pipelines for real-time, multidimensional, and continuous monitoring of various species. Hartig et al. [170] have introduced new sensing technologies, including eDNA, acoustic, and optical sensors, collectively termed the "Novel Community", to facilitate real-time and long-term monitoring of species and trophic interactions. Looking forward, we anticipate that future trends in next-generation biomonitoring will be characterized by (1) the integration and simultaneous deployment of multiple monitoring techniques; (2) the utilization of machine learning and artificial intelligence to enhance ecosystem-level understanding and connectivity; and (3) the implementation of automated monitoring equipment, citizen science projects, and data-sharing platforms to enable global, real-time, and high-resolution biomonitoring. This review highlights the urgent need for effective and strategic biomonitoring as a global imperative, necessitating public engagement and interdisciplinary collaboration. Only through the combined efforts of the scientific community, the public, and various stakeholders can we begin to better understand and manage our environment.

Author Contributions: Conceptualization, J.Y., C.L., H.Y. and J.C.; methodology and investigation, J.Y., C.L., X.Z., H.Y. and J.C.; writing—original draft preparation, J.Y., C.L., X.Z., H.Y. and J.C.; writing—review and editing, L.S.H.L., Z.C., C.U., Z.D., M.N., J.G., H.Y., J.Y. and J.C.; visualization, J.Y. and C.L.; supervision, H.Y. and J.C.; funding acquisition, J.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the General Research Fund and Early Career Scheme of the Research Grants Council (Project No. 16101821 & 28300024) and the Environment and Conservation Fund (Project No. 132/2022 & 163/2023) of the Hong Kong Special Administrative Region, China. This research was also supported by the FLASS Dean's Research Fund (Project 0400X) at the Education University of Hong Kong, and the State Key Laboratory of Marine Pollution (SKLMP) Seed Collaborative Research Fund (SKLMP/SCRF/0075), City University of Hong Kong. Any opinions, findings, conclusions, or recommendations expressed in this material/event do not necessarily reflect the views of the Government of Hong Kong Special Administrative Region and the Environment and Conservation Fund.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Visbeck, M. Ocean Science Research Is Key for a Sustainable Future. *Nat. Commun.* **2018**, *9*, 690. [CrossRef] [PubMed]
2. Inniss, L.; Simcock, A.; Ajawin, A.Y.; Alcala, A.C.; Bernal, P.; Calumpong, H.P.; Araghi, P.E.; Green, S.O.; Harris, P.; Kamara, O.K. The First Global Integrated Marine Assessment; Cambridge University Press, Cambridge, UK, 2017.
3. Sala, E.; Knowlton, N. Global Marine Biodiversity Trends. *Annu. Rev. Environ. Resour.* **2006**, *31*, 93–122. [CrossRef]
4. Environment, U.N. Annual Report 2023 | UNEP—UN Environment Programme. Available online: https://www.unep.org/resources/annual-report-2023 (accessed on 27 August 2024).
5. UNESCO-IOC The United Nations Decade of Ocean Science for Sustainable Development (2021–2030): Implementation Plan—UNESCO Digital Library. Available online: https://unesdoc.unesco.org/ark:/48223/pf0000377082 (accessed on 27 August 2024).
6. Unit, B. COP Decision. Available online: https://www.cbd.int/decision/cop?id=12268 (accessed on 27 August 2024).
7. Yuan, S.; Li, Y.; Bao, F.; Xu, H.; Yang, Y.; Yan, Q.; Zhong, S.; Yin, H.; Xu, J.; Huang, Z.; et al. Marine Environmental Monitoring with Unmanned Vehicle Platforms: Present Applications and Future Prospects. *Sci. Total Environ.* **2023**, *858*, 159741. [CrossRef] [PubMed]
8. Danovaro, R.; Carugati, L.; Berzano, M.; Cahill, A.E.; Carvalho, S.; Chenuil, A.; Corinaldesi, C.; Cristina, S.; David, R.; Dell'Anno, A. Implementing and Innovating Marine Monitoring Approaches for Assessing Marine Environmental Status. *Front. Mar. Sci.* **2016**, *3*, 213. [CrossRef]

9. Andrade, H.; Massabuau, J.-C.; Cochrane, S.; Ciret, P.; Tran, D.; Sow, M.; Camus, L. High Frequency Non-Invasive (HFNI) Bio-Sensors As a Potential Tool for Marine Monitoring and Assessments. *Front. Mar. Sci.* **2016**, *3*, 187. [CrossRef]
10. Beuchel, F.; Gulliksen, B.; Carroll, M.L. Long-Term Patterns of Rocky Bottom Macrobenthic Community Structure in an Arctic Fjord (Kongsfjorden, Svalbard) in Relation to Climate Variability (1980–2003). *J. Mar. Syst.* **2006**, *63*, 35–48. [CrossRef]
11. Shen, Y.; Zhao, C.; Liu, Y.; Wang, S.; Huang, F. Underwater Optical Imaging: Key Technologies and Applications Review. *IEEE Access* **2021**, *9*, 85500–85514. [CrossRef]
12. Stat, M.; Huggett, M.J.; Bernasconi, R.; DiBattista, J.D.; Berry, T.E.; Newman, S.J.; Harvey, E.S.; Bunce, M. Ecosystem Biomonitoring with eDNA: Metabarcoding across the Tree of Life in a Tropical Marine Environment. *Sci. Rep.* **2017**, *7*, 12240. [CrossRef] [PubMed]
13. Zhang, Y.; Pavlovska, M.; Stoica, E.; Prekrasna, I.; Yang, J.; Slobodnik, J.; Zhang, X.; Dykyi, E. Holistic Pelagic Biodiversity Monitoring of the Black Sea via eDNA Metabarcoding Approach: From Bacteria to Marine Mammals. *Environ. Int.* **2020**, *135*, 105307. [CrossRef]
14. Lecun, Y.; Bengio, Y.; Hinton, G. Deep Learning. *Nature* **2015**, *521*, 436–444. [CrossRef]
15. Khanzode, K.C.A.; Sarode, R.D. Advantages and Disadvantages of Artificial Intelligence and Machine Learning: A Literature Review. *Int. J. Libr. Inf. Sci. (IJLIS)* **2020**, *9*, 3.
16. Cordier, T.; Esling, P.; Lejzerowicz, F.; Visco, J.; Ouadahi, A.; Martins, C.; Cedhagen, T.; Pawlowski, J. Predicting the Ecological Quality Status of Marine Environments from eDNA Metabarcoding Data Using Supervised Machine Learning. *Environ. Sci. Technol.* **2017**, *51*, 9118–9126. [CrossRef]
17. Cordier, T.; Forster, D.; Dufresne, Y.; Martins, C.I.M.; Stoeck, T.; Pawlowski, J. Supervised Machine Learning Outperforms Taxonomy-based Environmental DNA Metabarcoding Applied to Biomonitoring. *Mol. Ecol. Resour.* **2018**, *18*, 1381–1391. [CrossRef] [PubMed]
18. Ma, A.; Lu, X.; Gray, C.; Raybould, A.; Tamaddoni-Nezhad, A.; Woodward, G.; Bohan, D.A. Ecological Networks Reveal Resilience of Agro-Ecosystems to Changes in Farming Management. *Nat. Ecol. Evol.* **2019**, *3*, 260–264. [CrossRef] [PubMed]
19. Stefanni, S.; Mirimin, L.; Stanković, D.; Chatzievangelou, D.; Bongiorni, L.; Marini, S.; Modica, M.V.; Manea, E.; Bonofiglio, F.; del Rio Fernandez, J.; et al. Framing Cutting-Edge Integrative Deep-Sea Biodiversity Monitoring via Environmental DNA and Optoacoustic Augmented Infrastructures. *Front. Mar. Sci.* **2022**, *8*, 797140. [CrossRef]
20. Duan, Z.; Yuan, Y.; Lu, J.C.; Wang, J.L.; Li, Y.; Svanberg, S.; Zhao, G.Y. Underwater Spatially, Spectrally, and Temporally Resolved Optical Monitoring of Aquatic Fauna. *Opt. Express* **2020**, *28*, 2600–2610. [CrossRef]
21. Zhang, L.; Li, C.; Sun, H. Object Detection/Tracking toward Underwater Photographs by Remotely Operated Vehicles (ROVs). *Future Gener. Comput. Syst.* **2022**, *126*, 163–168. [CrossRef]
22. Tang, J.; Chen, Z.; Fu, B.; Lu, W.; Li, S.; Li, X.; Ji, X. ROV6D: 6D Pose Estimation Benchmark Dataset for Underwater Remotely Operated Vehicles. *IEEE Robot. Autom. Lett.* **2024**, *9*, 65–72. [CrossRef]
23. Ma, Y.; Wu, Y.; Li, Q.; Zhou, Y.; Yu, D. ROV-Based Binocular Vision System for Underwater Structure Crack Detection and Width Measurement. *Multimed. Tools Appl.* **2023**, *82*, 20899–20923. [CrossRef]
24. Borković, G.; Fabijanić, M.; Magdalenić, M.; Malobabić, A.; Vuković, J.; Zieliński, I.; Kapetanović, N.; Kvasić, I.; Babić, A.; Mišković, N. Underwater ROV Software for Fish Cage Inspection. In Proceedings of the 2021 44th International Convention on Information, Communication and Electronic Technology (MIPRO), Opatija, Croatia, 27 September–1 October 2021; pp. 1747–1752.
25. Lakshmi, K.; Muralikrishna, P.; Soman, K.P. Compressive Estimation of UWA Channels for OFDM Transmission Using Iterative Sparse Reconstruction Algorithms. In Proceedings of the 2013 International Mutli-Conference on Automation, Computing, Communication, Control and Compressed Sensing (iMac4s), Kottayam, India,, 22–23 March 2013; pp. 847–851.
26. Han, J.; Zhang, L.; Leus, G. Partial FFT Demodulation for MIMO-OFDM over Time-Varying Underwater Acoustic Channels. *IEEE Signal Process. Lett.* **2015**, *23*, 282–286. [CrossRef]
27. Lu, H.; Li, Y.; Zhang, Y.; Chen, M.; Serikawa, S.; Kim, H. Underwater Optical Image Processing: A Comprehensive Review. *Mob. Netw. Appl.* **2017**, *22*, 1204–1211. [CrossRef]
28. Kindsvater, H.K.; Dulvy, N.K.; Horswill, C.; Juan-Jordá, M.-J.; Mangel, M.; Matthiopoulos, J. Overcoming the Data Crisis in Biodiversity Conservation. *Trends Ecol. Evol.* **2018**, *33*, 676–688. [CrossRef] [PubMed]
29. Farley, S.S.; Dawson, A.; Goring, S.J.; Williams, J.W. Situating Ecology as a Big-Data Science: Current Advances, Challenges, and Solutions. *BioScience* **2018**, *68*, 563–576. [CrossRef]
30. Ahn, J.; Yasukawa, S.; Sonoda, T.; Nishida, Y.; Ishii, K.; Ura, T. An Optical Image Transmission System for Deep Sea Creature Sampling Missions Using Autonomous Underwater Vehicle. *IEEE J. Ocean. Eng.* **2018**, *45*, 350–361. [CrossRef]
31. Dinakaran, R.; Zhang, L.; Li, C.-T.; Bouridane, A.; Jiang, R. Robust and Fair Undersea Target Detection with Automated Underwater Vehicles for Biodiversity Data Collection. *Remote Sens.* **2022**, *14*, 3680. [CrossRef]
32. Rosli, M.S.A.B.; Isa, I.S.; Maruzuki, M.I.F.; Sulaiman, S.N.; Ahmad, I. Underwater Animal Detection Using YOLOV4. In Proceedings of the 2021 11th IEEE International Conference on Control System, Computing and Engineering (ICCSCE), Penang, Malaysia, 27–28 August 2021; pp. 158–163.
33. Coro, G.; Walsh, M.B. An Intelligent and Cost-Effective Remote Underwater Video Device for Fish Size Monitoring. *Ecol. Inform.* **2021**, *63*, 101311. [CrossRef]
34. Zhang, K.; Yang, M.; Lang, S.D.J.; McInnes, A.M.; Sherley, R.B.; Burghardt, T. Diving with Penguins: Detecting Penguins and Their Prey in Animal-Borne Underwater Videos via Deep Learning. *arXiv* **2023**, arXiv:2308.07267.

35. Cai, L.; McGuire, N.E.; Hanlon, R.; Mooney, T.A.; Girdhar, Y. Semi-Supervised Visual Tracking of Marine Animals Using Autonomous Underwater Vehicles. *Int. J. Comput. Vis.* **2023**, *131*, 1406–1427. [CrossRef]
36. Bosch, J.; Gracias, N.; Ridao, P.; Ribas, D. Omnidirectional Underwater Camera Design and Calibration. *Sensors* **2015**, *15*, 6033–6065. [CrossRef]
37. Li, C.; Guo, C.; Ren, W.; Cong, R.; Hou, J.; Kwong, S.; Tao, D. An Underwater Image Enhancement Benchmark Dataset and Beyond. *IEEE Trans. Image Process.* **2020**, *29*, 4376–4389. [CrossRef]
38. Han, M.; Lyu, Z.; Qiu, T.; Xu, M. A Review on Intelligence Dehazing and Color Restoration for Underwater Images. *IEEE Trans. Syst. Man Cybern. Syst.* **2018**, *50*, 1820–1832. [CrossRef]
39. López-Vázquez, J.; Pérez-Mayán, L.; Fernández-Fernández, V.; Cela, R.; Rodríguez, I. Direct, Automated and Sensitive Determination of Glyphosate and Related Anionic Pesticides in Environmental Water Samples Using Solid-Phase Extraction on-Line Combined with Liquid Chromatography Tandem Mass Spectrometry. *J. Chromatogr. A* **2023**, *1687*, 463697. [CrossRef] [PubMed]
40. He, K.; Sun, J.; Tang, X. Single Image Haze Removal Using Dark Channel Prior. *IEEE Trans. Pattern Anal. Mach. Intell.* **2010**, *33*, 2341–2353. [PubMed]
41. Chen, Z.; Liu, C.; Zhang, K.; Chen, Y.; Wang, R.; Shi, X. Underwater-Image Super-Resolution via Range-Dependency Learning of Multiscale Features. *Comput. Electr. Eng.* **2023**, *110*, 108756. [CrossRef]
42. Yang, Y.; Wang, C.; Liu, R.; Zhang, L.; Guo, X.; Tao, D. Self-Augmented Unpaired Image Dehazing via Density and Depth Decomposition. In Proceedings of the IEEE/CVF Conference on Computer Vision and Pattern Recognition, New Orleans, LA, USA, 18–24 June 2022; pp. 2037–2046.
43. Kaiyan, Z.; Xiang, L.; Weibo, S. Underwater Object Detection Using Transfer Learning with Deep Learning. In Proceedings of the 2020 International Conference on Computers, Information Processing and Advanced Education, Ottawa, ON, Canada, 16–18 October 2020; Association for Computing Machinery: New York, NY, USA, 2020; pp. 157–160.
44. Yang, H.; Tian, F.; Qi, Q.; Wu, Q.M.J.; Li, K. Underwater Image Enhancement with Latent Consistency Learning-Based Color Transfer. *IET Image Process.* **2022**, *16*, 1594–1612. [CrossRef]
45. Kim, G.; Park, J.; Kwon, J. Deep Dehazing Powered by Image Processing Network. In Proceedings of the IEEE/CVF Conference on Computer Vision and Pattern Recognition, Vancouver, BC, Canada, 18–22 June 2023; pp. 1209–1218.
46. Guo, A.; Dian, R.; Li, S. A Deep Framework for Hyperspectral Image Fusion between Different Satellites. *IEEE Trans. Pattern Anal. Mach. Intell.* **2022**, *45*, 7939–7954. [CrossRef]
47. Kim, J. U-Gat-It: Unsupervised Generative Attentional Networks with Adaptive Layer-Instance Normalization for Image-to-Image Translation. *arXiv* **2019**, arXiv:1907.10830.
48. Ma, H.; Wang, Y.; Li, Y.; Zhao, Y. Desert Seismic Low-Frequency Noise Attenuation Using Low-Rank Decomposition-Based Denoising Convolutional Neural Network. *IEEE Trans. Geosci. Remote Sens.* **2022**, *60*, 5900809. [CrossRef]
49. Chen, R.; Fu, Z.; Huang, Y.; Cheng, E.; Ding, X. A Robust Object Segmentation Network for Underwater Scenes. In Proceedings of the ICASSP 2022-2022 IEEE International Conference on Acoustics, Speech and Signal Processing (ICASSP), Virtual, 7–13 May 2022; Singapore, 22–27 May 2022. pp. 2629–2633.
50. Yeh, C.-H.; Lin, C.-H.; Kang, L.-W.; Huang, C.-H.; Lin, M.-H.; Chang, C.-Y.; Wang, C.-C. Lightweight Deep Neural Network for Joint Learning of Underwater Object Detection and Color Conversion. *IEEE Trans. Neural Netw. Learn. Syst.* **2022**, *33*, 6129–6143. [CrossRef] [PubMed]
51. Liu, R.; Fan, X.; Zhu, M.; Hou, M.; Luo, Z. Real-World Underwater Enhancement: Challenges, Benchmarks, and Solutions Under Natural Light. *IEEE Trans. Circuits Syst. Video Technol.* **2020**, *30*, 4861–4875. [CrossRef]
52. Thapa, S.; Li, N.; Ye, J. Learning To Remove Refractive Distortions From Underwater Images. In Proceedings of the IEEE/CVF International Conference on Computer Vision 2021, Virtual, 11–17 October 2021; pp. 5007–5016.
53. Iakushkin, O.; Pavlova, E.; Pen, E.; Frikh-Khar, A.; Terekhina, Y.; Bulanova, A.; Shabalin, N.; Sedova, O. Automated Marking of Underwater Animals Using a Cascade of Neural Networks. In Proceedings of the Computational Science and Its Applications—ICCSA 2021, Cagliari, Italy, 13–16 September 2021; Gervasi, O., Murgante, B., Misra, S., Garau, C., Blečić, I., Taniar, D., Apduhan, B.O., Rocha, A.M.A.C., Tarantino, E., Torre, C.M., Eds.; Lecture Notes in Computer Science. Springer International Publishing: Cham, Switzerland, 2021; Volume 12956, pp. 460–470, ISBN 978-3-030-87009-6.
54. Jiang, J.; Ye, T.; Bai, J.; Chen, S.; Chai, W.; Jun, S.; Liu, Y.; Chen, E. Five A^+ Network: You Only Need 9K Parameters for Underwater Image Enhancement. *arXiv* **2023**, arXiv:2305.08824.
55. Zhao, C.; Cai, W.; Dong, C.; Zeng, Z. Toward Sufficient Spatial-Frequency Interaction for Gradient-Aware Underwater Image Enhancement. In Proceedings of the ICASSP 2024—2024 IEEE International Conference on Acoustics, Speech and Signal Processing (ICASSP), Seoul, Republic of Korea, 14–19 April 2024; pp. 3220–3224.
56. Qi, H.; Dong, X. Physics-Aware Semi-Supervised Underwater Image Enhancement. *arXiv* **2024**, arXiv:2307.11470.
57. De Langis, K.; Sattar, J. Realtime Multi-Diver Tracking and Re-Identification for Underwater Human-Robot Collaboration. In Proceedings of the 2020 IEEE International Conference on Robotics and Automation (ICRA), Paris, France, 31 May–31 August 2020; pp. 11140–11146.
58. Le, M.-Q.; Le, T.-N.; Nguyen, T.; Echizen, I.; Tran, M.-T. Multimodal-Based Scene-Aware Framework for Aquatic Animal Segmentation. *arXiv* **2021**, arXiv:2112.06193. [CrossRef]
59. Liu, H.; Song, P.; Ding, R. Towards Domain Generalization In Underwater Object Detection. In Proceedings of the 2020 IEEE International Conference on Image Processing (ICIP), Virtual, 25–28 October 2020; pp. 1971–1975.

60. Jiang, L.; Quan, H.; Xie, T.; Qian, J. Fish Recognition in Complex Underwater Scenes Based on Targeted Sample Transfer Learning. *Multimed. Tools Appl.* **2022**, *81*, 25303–25317. [CrossRef]
61. Pietramellara, G.; Ascher, J.; Borgogni, F.; Ceccherini, M.T.; Guerri, G.; Nannipieri, P. Extracellular DNA in Soil and Sediment: Fate and Ecological Relevance. *Biol. Fertil. Soils* **2009**, *45*, 219–235. [CrossRef]
62. Ficetola, G.F.; Miaud, C.; Pompanon, F.; Taberlet, P. Species Detection Using Environmental DNA from Water Samples. *Biol. Lett.* **2008**, *4*, 423–425. [CrossRef] [PubMed]
63. Aylagas, E.; Borja, Á.; Irigoien, X.; Rodríguez-Ezpeleta, N. Benchmarking DNA Metabarcoding for Biodiversity-Based Monitoring and Assessment. *Front. Mar. Sci.* **2016**, *3*, 96. [CrossRef]
64. Cote, D.; McClenaghan, B.; Desforges, J.; Fahner, N.A.; Hajibabaei, M.; Chawarski, J.; Roul, S.; Singer, G.; Aubry, C.; Geoffroy, M. Comparing eDNA Metabarcoding and Conventional Pelagic Netting to Inform Biodiversity Monitoring in Deep Ocean Environments. *ICES J. Mar. Sci.* **2023**, *80*, 2545–2562. [CrossRef]
65. Balint, M.; Pfenninger, M.; Grossart, H.-P.; Taberlet, P.; Vellend, M.; Leibold, M.A.; Englund, G.; Bowler, D. Environmental DNA Time Series in Ecology. *Trends Ecol. Evol.* **2018**, *33*, 945–957. [CrossRef] [PubMed]
66. Zhao, Y.; Chen, J.; Dong, L.; Ma, X.; Bai, J.; Tian, K. Advances in the Application of Environmental DNA in Aquatic Ecosystems. *J. Agro- Environ. Sci.* **2021**, *40*, 2057–2065. [CrossRef]
67. Littlefair, J.E.; Rennie, M.D.; Cristescu, M.E. Environmental Nucleic Acids: A Field-Based Comparison for Monitoring Freshwater Habitats Using eDNA and eRNA. *Mol. Ecol. Resour.* **2022**, *22*, 2928–2940. [CrossRef] [PubMed]
68. Foote, A.D.; Thomsen, P.F.; Sveegaard, S.; Wahlberg, M.; Kielgast, J.; Kyhn, L.A.; Salling, A.B.; Galatius, A.; Orlando, L.; Gilbert, M.T.P. Investigating the Potential Use of Environmental DNA (eDNA) for Genetic Monitoring of Marine Mammals. *PLoS ONE.* **2012**, *7*, e41781. [CrossRef]
69. Deiner, K.; Bik, H.M.; Mächler, E.; Seymour, M.; Lacoursière-Roussel, A.; Altermatt, F.; Creer, S.; Bista, I.; Lodge, D.M.; De Vere, N.; et al. Environmental DNA Metabarcoding: Transforming How We Survey Animal and Plant Communities. *Mol. Ecol.* **2017**, *26*, 5872–5895. [CrossRef]
70. West, K.M.; Adam, A.A.S.; White, N.; Robbins, W.D.; Barrow, D.; Lane, A.T.; Richards, Z. The Applicability of eDNA Metabarcoding Approaches for Sessile Benthic Surveying in the Kimberley Region, North-Western Australia. *Environ. DNA* **2022**, *4*, 34–49. [CrossRef]
71. Rivera, S.F.; Rimet, F.; Vasselon, V.; Vautier, M.; Domaizon, I.; Bouchez, A. Fish eDNA Metabarcoding from Aquatic Biofilm Samples: Methodological Aspects. *Mol. Ecol. Resour.* **2022**, *22*, 1440–1453. [CrossRef] [PubMed]
72. Clarke, L.J.; Suter, L.; Deagle, B.E.; Polanowski, A.M.; Terauds, A.; Johnstone, G.J.; Stark, J.S. Environmental DNA Metabarcoding for Monitoring Metazoan Biodiversity in Antarctic Nearshore Ecosystems. *PeerJ* **2021**, *9*, e12458. [CrossRef]
73. Gelis, E.R.E.; Kamal, M.M.; Subhan, B.; Bachtiar, I.; Sani, L.M.I.; Madduppa, H. Environmental Biomonitoring of Reef Fish Community Structure with eDNA Metabarcoding in the Coral Triangle. *Environ. Biol. Fish.* **2021**, *104*, 887–903. [CrossRef]
74. Tagliabue, A.; Matterson, K.O.; Ponti, M.; Turicchia, E.; Abbiati, M.; Costantini, F. Sediment and Bottom Water eDNA Metabarcoding to Support Coastal Management. *Ocean Coast. Manag.* **2023**, *244*, 106785. [CrossRef]
75. Pawlowski, J.; Apothéloz-Perret-Gentil, L.; Mächler, E.; Altermatt, F. *Environmental DNA Applications in Biomonitoring and Bioassessment of Aquatic Ecosystems: Guidelines*; Federal Office for the Environment: Bern, Switzerland, 2020; 71p, (Environmental Studies. no. 2010). [CrossRef]
76. Thomsen, P.F.; Kielgast, J.; Iversen, L.L.; Møller, P.R.; Rasmussen, M.; Willerslev, E. Detection of a Diverse Marine Fish Fauna Using Environmental DNA from Seawater Samples. *PLoS ONE* **2012**, *7*, e41732. [CrossRef]
77. Nichols, P.K.; Marko, P.B. Rapid Assessment of Coral Cover from Environmental DNA in Hawai'i. *Environ. DNA* **2019**, *1*, 40–53. [CrossRef]
78. Truelove, N.K.; Patin, N.V.; Min, M.; Pitz, K.J.; Preston, C.M.; Yamahara, K.M.; Zhang, Y.; Raanan, B.Y.; Kieft, B.; Hobson, B.; et al. Expanding the Temporal and Spatial Scales of Environmental DNA Research with Autonomous Sampling. *Environ. DNA* **2022**, *4*, 972–984. [CrossRef]
79. Andres, K.J.; Lambert, T.D.; Lodge, D.M.; Andrés, J.; Jackson, J.R. Combining Sampling Gear to Optimally Inventory Species Highlights the Efficiency of eDNA Metabarcoding. *Environ. DNA* **2023**, *5*, 146–157. [CrossRef]
80. von Ammon, U.; Pochon, X.; Casanovas, P.; Trochel, B.; Zirngibl, M.; Thomas, A.; Witting, J.; Joyce, P.; Zaiko, A. Net Overboard: Comparing Marine eDNA Sampling Methodologies at Sea to Unravel Marine Biodiversity. *Mol. Ecol. Resour.* **2023**, *23*, 440–452. [CrossRef]
81. Thomsen, P.F.; Møller, P.R.; Sigsgaard, E.E.; Knudsen, S.W.; Jørgensen, O.A.; Willerslev, E. Environmental DNA from Seawater Samples Correlate with Trawl Catches of Subarctic, Deepwater Fishes. *PLoS ONE* **2016**, *11*, e0165252. [CrossRef]
82. McClenaghan, B.; Fahner, N.; Cote, D.; Chawarski, J.; McCarthy, A.; Rajabi, H.; Singer, G.; Hajibabaei, M. Harnessing the Power of eDNA Metabarcoding for the Detection of Deep-Sea Fishes. *PLoS ONE* **2020**, *15*, e0236540. [CrossRef]
83. Pawlowski, J.; Bruce, K.; Panksep, K.; Aguirre, F.I.; Amalfitano, S.; Apothéloz-Perret-Gentil, L.; Baussant, T.; Bouchez, A.; Carugati, L.; Cermakova, K.; et al. Environmental DNA Metabarcoding for Benthic Monitoring: A Review of Sediment Sampling and DNA Extraction Methods. *Sci. Total Environ.* **2022**, *818*, 151783. [CrossRef]
84. Geraldi, N.R.; Krause-Jensen, D.; Ørberg, S.B.; Frühe, L.; Sejr, M.K.; Hansen, J.L.S.; Lund-Hansen, L.; Duarte, C.M. Environmental Drivers of Arctic Communities Based on Metabarcoding of Marine Sediment eDNA. *Proc. R. Soc. B* **2024**, *291*, 20231614. [CrossRef] [PubMed]

85. Laramie, M.B.; Pilliod, D.S.; Goldberg, C.S. Characterizing the Distribution of an Endangered Salmonid Using Environmental DNA Analysis. *Biol. Conserv.* **2015**, *183*, 29–37. [CrossRef]
86. Bruce, K.; Blackman, R.C.; Bourlat, S.J.; Hellström, M.; Bakker, J.; Bista, I.; Bohmann, K.; Bouchez, A.; Brys, R.; Clark, K.; et al. A Practical Guide to DNA-Based Methods for Biodiversity Assessment; Pensoft Advanced Books. 2021. Available online: https://ab.pensoft.net/article/68634/ (accessed on 27 August 2024).
87. Goldberg, C.S.; Strickler, K.M.; Fremier, A.K. Degradation and Dispersion Limit Environmental DNA Detection of Rare Amphibians in Wetlands: Increasing Efficacy of Sampling Designs. *Sci. Total Environ.* **2018**, *633*, 695–703. [CrossRef]
88. Thomas, A.C.; Howard, J.; Nguyen, P.L.; Seimon, T.A.; Goldberg, C.S. eDNA Sampler: A Fully Integrated Environmental DNA Sampling System. *Methods Ecol. Evol.* **2018**, *9*, 1379–1385. [CrossRef]
89. Doi, H.; Akamatsu, Y.; Watanabe, Y.; Goto, M.; Inui, R.; Katano, I.; Nagano, M.; Takahara, T.; Minamoto, T. Water Sampling for Environmental DNA Surveys by Using an Unmanned Aerial Vehicle. *Limnol. Ocean Methods* **2017**, *15*, 939–944. [CrossRef]
90. Nishitsuji, K.; Nagahama, S.; Narisoko, H.; Shimada, K.; Okada, N.; Shimizu, Y.; Satoh, N. Possible Monitoring of Mesophotic Scleractinian Corals Using an Underwater Mini-ROV to Sample Coral eDNA. *R. Soc. Open Sci.* **2024**, *11*, 221586. [CrossRef] [PubMed]
91. Yamahara, K.M.; Preston, C.M.; Birch, J.; Walz, K.; Marin, R.; Jensen, S.; Pargett, D.; Roman, B.; Ussler, W.; Zhang, Y.; et al. *In Situ* Autonomous Acquisition and Preservation of Marine Environmental DNA Using an Autonomous Underwater Vehicle. *Front. Mar. Sci.* **2019**, *6*, 373. [CrossRef]
92. Preston, C.; Yamahara, K.; Pargett, D.; Weinstock, C.; Birch, J.; Roman, B.; Jensen, S.; Connon, B.; Jenkins, R.; Ryan, J.; et al. Autonomous eDNA Collection Using an Uncrewed Surface Vessel over a 4200-Km Transect of the Eastern Pacific Ocean. *Environ. DNA* **2024**, *6*, e468. [CrossRef]
93. Formel, N.; Enochs, I.C.; Sinigalliano, C.; Anderson, S.R.; Thompson, L.R. Subsurface Automated Samplers for eDNA (SASe) for Biological Monitoring and Research. *HardwareX* **2021**, *10*, e00239. [CrossRef]
94. Govindarajan, A.F.; McCartin, L.; Adams, A.; Allan, E.; Belani, A.; Francolini, R.; Fujii, J.; Gomez-Ibañez, D.; Kukulya, A.; Marin, F. Improved Biodiversity Detection Using a Large-Volume Environmental DNA Sampler with In Situ Filtration and Implications for Marine eDNA Sampling Strategies. *Deep Sea Res. Part I Oceanogr. Res. Pap.* **2022**, *189*, 103871. [CrossRef]
95. Hendricks, A.; Mackie, C.M.; Luy, E.; Sonnichsen, C.; Smith, J.; Grundke, I.; Tavasoli, M.; Furlong, A.; Beiko, R.G.; LaRoche, J. Compact and Automated eDNA Sampler for In Situ Monitoring of Marine Environments. *Sci. Rep.* **2023**, *13*, 5210. [CrossRef]
96. Bessey, C.; Neil Jarman, S.; Simpson, T.; Miller, H.; Stewart, T.; Kenneth Keesing, J.; Berry, O. Passive eDNA Collection Enhances Aquatic Biodiversity Analysis. *Commun. Biol.* **2021**, *4*, 236. [CrossRef]
97. van der Heyde, M.; Alexander, J.; Nevill, P.; Austin, A.D.; Stevens, N.; Jones, M.; Guzik, M.T. Rapid Detection of Subterranean Fauna from Passive Sampling of Groundwater eDNA. *Environ. DNA* **2023**, *5*, 1706–1719. [CrossRef]
98. Chen, X.; Kong, Y.; Zhang, S.; Zhao, J.; Li, S.; Yao, M. Comparative Evaluation of Common Materials as Passive Samplers of Environmental DNA. *Environ. Sci. Technol.* **2022**, *56*, 10798–10807. [CrossRef]
99. Miya, M. Environmental DNA Metabarcoding: A Novel Method for Biodiversity Monitoring of Marine Fish Communities. *Annu. Rev. Mar. Sci.* **2022**, *14*, 161–185. [CrossRef] [PubMed]
100. Nelson, J.S.; Grande, T.C.; Wilson, M.V.H. *Fishes of the World*; John Wiley & Sons: Hoboken, NJ, USA, 2016; ISBN 978-1-119-22082-4.
101. Shanlin, L. DNA Barcoding and Emerging Reference Construction and Data Analysis Technologies. *Biodivers. Sci.* **2019**, *27*, 526. [CrossRef]
102. Kasmi, Y.; Eschbach, E.; Hanel, R. Mare-MAGE Curated Reference Database of Fish Mitochondrial Genes. *BMC Genom. Data* **2023**, *24*, 18. [CrossRef] [PubMed]
103. Gold, Z.; Curd, E.E.; Goodwin, K.D.; Choi, E.S.; Frable, B.W.; Thompson, A.R.; Walker, H.J.; Burton, R.S.; Kacev, D.; Martz, L.D.; et al. Improving Metabarcoding Taxonomic Assignment: A Case Study of Fishes in a Large Marine Ecosystem. *Mol. Ecol. Resour.* **2021**, *21*, 2546–2564. [CrossRef]
104. Chen, Z.; Cai, X.; Zhang, Q.; Li, G.; Ma, C.; Shen, Z. Preliminary Construction and Comparative Analysis of Environmental DNA Metabarcoding Reference Database of Freshwater Fishes in Hainan Island. *South China Fish. Sci.* **2022**, *18*, 1–12. [CrossRef]
105. Jiang, P.; Li, M.; Zhang, S.; Chen, Z.; Xu, S. Construction of DNA meta-barcode database of fish in Pearl River Estuary based on mitochondrial cytochrome COI and 12S rDNA gene. *South China Fish. Sci.* **2022**, *18*, 13–21. [CrossRef]
106. Vaulot, D.; Sim, C.W.H.; Ong, D.; Teo, B.; Biwer, C.; Jamy, M.; Lopes dos Santos, A. metaPR: A Database of Eukaryotic 18S rRNA Metabarcodes with an Emphasis on Protists. *Mol. Ecol. Resour.* **2022**, *22*, 3188–3201. [CrossRef] [PubMed]
107. Takahashi, M.; Saccò, M.; Kestel, J.H.; Nester, G.; Campbell, M.A.; van der Heyde, M.; Heydenrych, M.J.; Juszkiewicz, D.J.; Nevill, P.; Dawkins, K.L.; et al. Aquatic Environmental DNA: A Review of the Macro-Organismal Biomonitoring Revolution. *Sci. Total Environ.* **2023**, *873*, 162322. [CrossRef] [PubMed]
108. Cannon, M.V.; Hester, J.; Shalkhauser, A.; Chan, E.R.; Logue, K.; Small, S.T.; Serre, D. In Silico Assessment of Primers for eDNA Studies Using PrimerTree and Application to Characterize the Biodiversity Surrounding the Cuyahoga River. *Sci. Rep.* **2016**, *6*, 22908. [CrossRef]
109. Ardura, A.; Zaiko, A.; Martinez, J.L.; Samulioviene, A.; Semenova, A.; Garcia-Vazquez, E. eDNA and Specific Primers for Early Detection of Invasive Species–A Case Study on the Bivalve Rangia Cuneata, Currently Spreading in Europe. *Mar. Environ. Res.* **2015**, *112*, 48–55. [CrossRef] [PubMed]

110. Xiong, F.; Shu, L.; Zeng, H.; Gan, X.; He, S.; Peng, Z. Methodology for Fish Biodiversity Monitoring with Environmental DNA Metabarcoding: The Primers, Databases and Bioinformatic Pipelines. *Water Biol. Secur.* **2022**, *1*, 100007. [CrossRef]
111. Jo, T.; Arimoto, M.; Murakami, H.; Masuda, R.; Minamoto, T. Particle Size Distribution of Environmental DNA from the Nuclei of Marine Fish. *Environ. Sci. Technol.* **2019**, *53*, 9947–9956. [CrossRef] [PubMed]
112. Wang, S.; Yan, Z.; Hänfling, B.; Zheng, X.; Wang, P.; Fan, J.; Li, J. Methodology of Fish eDNA and Its Applications in Ecology and Environment. *Sci. Total Environ.* **2021**, *755*, 142622. [CrossRef] [PubMed]
113. Xing, Y.; Gao, W.; Shen, Z.; Zhang, Y.; Bai, J.; Cai, X.; Ouyang, J.; Zhao, Y. A Review of Environmental DNA Field and Laboratory Protocols Applied in Fish Ecology and Environmental Health. *Front. Environ. Sci.* **2022**, *10*, 725360. [CrossRef]
114. Miya, M.; Sato, Y.; Fukunaga, T.; Sado, T.; Poulsen, J.Y.; Sato, K.; Minamoto, T.; Yamamoto, S.; Yamanaka, H.; Araki, H.; et al. MiFish, a Set of Universal PCR Primers for Metabarcoding Environmental DNA from Fishes: Detection of More than 230 Subtropical Marine Species. *R. Soc. Open Sci.* **2015**, *2*, 150088. [CrossRef] [PubMed]
115. Valentini, A.; Taberlet, P.; Miaud, C.; Civade, R.; Herder, J.; Thomsen, P.F.; Bellemain, E.; Besnard, A.; Coissac, E.; Boyer, F.; et al. Next-Generation Monitoring of Aquatic Biodiversity Using Environmental DNA Metabarcoding. *Mol. Ecol.* **2016**, *25*, 929–942. [CrossRef]
116. Evans, N.T.; Olds, B.P.; Renshaw, M.A.; Turner, C.R.; Li, Y.; Jerde, C.L.; Mahon, A.R.; Pfrender, M.E.; Lamberti, G.A.; Lodge, D.M. Quantification of Mesocosm Fish and Amphibian Species Diversity via Environmental DNA Metabarcoding. *Mol. Ecol. Resour.* **2016**, *16*, 29–41. [CrossRef] [PubMed]
117. Zhang, S.; Zhao, J.; Yao, M. A Comprehensive and Comparative Evaluation of Primers for Metabarcoding eDNA from Fish. *Methods Ecol. Evol.* **2020**, *11*, 1609–1625. [CrossRef]
118. Leray, M.; Yang, J.Y.; Meyer, C.P.; Mills, S.C.; Agudelo, N.; Ranwez, V.; Boehm, J.T.; Machida, R.J. A New Versatile Primer Set Targeting a Short Fragment of the Mitochondrial COI Region for Metabarcoding Metazoan Diversity: Application for Characterizing Coral Reef Fish Gut Contents. *Front. Zool.* **2013**, *10*, 34. [CrossRef]
119. Holman, L.E.; de Bruyn, M.; Creer, S.; Carvalho, G.; Robidart, J.; Rius, M. Detection of Introduced and Resident Marine Species Using Environmental DNA Metabarcoding of Sediment and Water. *Sci. Rep.* **2019**, *9*, 11559. [CrossRef] [PubMed]
120. Shi, X.; Jiang, Y.; Cao, L.; Zeng, C. Development of Environmental DNA Metabarcoding Primers for Marine Mollusks and Comparison with Published Primers. *BMC Ecol. EvoL.* **2024**, *24*, 73. [CrossRef] [PubMed]
121. Liu, J.; Zhang, H. Combining Multiple Markers in Environmental DNA Metabarcoding to Assess Deep-Sea Benthic Biodiversity. *Front. Mar. Sci.* **2021**, *8*, 684955. [CrossRef]
122. Shea, M.M.; Kuppermann, J.; Rogers, M.P.; Smith, D.S.; Edwards, P.; Boehm, A.B. Systematic Review of Marine Environmental DNA Metabarcoding Studies: Toward Best Practices for Data Usability and Accessibility. *PeerJ* **2023**, *11*, e14993. [CrossRef] [PubMed]
123. Cristescu, M.E.; Hebert, P.D.N. Uses and Misuses of Environmental DNA in Biodiversity Science and Conservation. *Annu. Rev. Ecol. Evol. Syst.* **2018**, *49*, 209–230. [CrossRef]
124. Yang, J.; Zhang, X.; Jin, X.; Seymour, M.; Richter, C.; Logares, R.; Khim, J.S.; Klymus, K. Recent Advances in Environmental DNA-Based Biodiversity Assessment and Conservation. *Divers. Distrib.* **2021**, *27*, 1876–1879. [CrossRef]
125. Zeng, Y.; Chen, Z.; Cao, J.; Li, S.; Xia, Z.; Sun, Y.; Zhang, J.; He, P. Revolutionizing Early-Stage Green Tide Monitoring: eDNA Metabarcoding Insights into *Ulva Prolifera* and Microecology in the South Yellow Sea. *Sci. Total Environ.* **2024**, *912*, 169022. [CrossRef]
126. Sigsgaard, E.E.; Torquato, F.; Frøslev, T.G.; Moore, A.B.M.; Sørensen, J.M.; Range, P.; Ben-Hamadou, R.; Bach, S.S.; Møller, P.R.; Thomsen, P.F. Using Vertebrate Environmental DNA from Seawater in Biomonitoring of Marine Habitats. *Conserv. Biol.* **2020**, *34*, 697–710. [CrossRef]
127. He, X.; Jeffery, N.W.; Stanley, R.R.E.; Hamilton, L.C.; Rubidge, E.M.; Abbott, C.L. eDNA Metabarcoding Enriches Traditional Trawl Survey Data for Monitoring Biodiversity in the Marine Environment. *ICES J. Mar. Sci.* **2023**, *80*, 1529–1538. [CrossRef]
128. Bakker, J.; Wangensteen, O.S.; Baillie, C.; Buddo, D.; Chapman, D.D.; Gallagher, A.J.; Guttridge, T.L.; Hertler, H.; Mariani, S. Biodiversity Assessment of Tropical Shelf Eukaryotic Communities via Pelagic eDNA Metabarcoding. *Ecol. Evol.* **2019**, *9*, 14341–14355. [CrossRef]
129. Pikitch, E.K. A Tool for Finding Rare Marine Species. *Science* **2018**, *360*, 1180–1182. [CrossRef]
130. Bakker, J.; Wangensteen, O.S.; Chapman, D.D.; Boussarie, G.; Buddo, D.; Guttridge, T.L.; Hertler, H.; Mouillot, D.; Vigliola, L.; Mariani, S. Environmental DNA Reveals Tropical Shark Diversity in Contrasting Levels of Anthropogenic Impact. *Sci. Rep.* **2017**, *7*, 16886. [CrossRef] [PubMed]
131. Boussarie, G.; Bakker, J.; Wangensteen, O.S.; Mariani, S.; Bonnin, L.; Juhel, J.-B.; Kiszka, J.J.; Kulbicki, M.; Manel, S.; Robbins, W.D.; et al. Environmental DNA Illuminates the Dark Diversity of Sharks. *Sci. Adv.* **2018**, *4*, eaap9661. [CrossRef] [PubMed]
132. Juhel, J.-B.; Marques, V.; Fernández, A.P.; Borrero-Pérez, G.H.; Martinezguerra, M.M.; Valentini, A.; Dejean, T.; Manel, S.; Loiseau, N.; Velez, L. Detection of the Elusive Dwarf Sperm Whale (Kogia Sima) Using Environmental DNA at Malpelo Island (Eastern Pacific, Colombia). *Ecol. Evol.* **2021**, *11*, 2956. [CrossRef] [PubMed]
133. Alter, S.E.; King, C.D.; Chou, E.; Chin, S.C.; Rekdahl, M.; Rosenbaum, H.C. Using Environmental DNA to Detect Whales and Dolphins in the New York Bight. *Front. Conserv. Sci.* **2022**, *3*, 820377. [CrossRef]
134. Mariani, S.; Fernandez, C.; Baillie, C.; Magalon, H.; Jaquemet, S. Shark and Ray Diversity, Abundance and Temporal Variation around an Indian Ocean Island, Inferred by eDNA Metabarcoding. *Conserv. Sci. Pract.* **2021**, *3*, e407. [CrossRef]

135. Boyse, E.; Robinson, K.P.; Beger, M.; Carr, I.M.; Taylor, M.; Valsecchi, E.; Goodman, S.J. Environmental DNA Reveals Fine-scale Spatial and Temporal Variation of Marine Mammals and Their Prey Species in a Scottish Marine Protected Area. *Environ. DNA* **2024**, *6*, e587. [CrossRef]
136. De La Hoz Schilling, C.; Jabado, R.W.; Veríssimo, A.; Caminiti, L.; Sidina, E.; Gandega, C.Y.; Serrão, E.A. eDNA Metabarcoding Reveals a Rich but Threatened and Declining Elasmobranch Community in West Africa's Largest Marine Protected Area, the Banc d'Arguin. *Conserv. Genet.* **2024**, *25*, 805–821. [CrossRef]
137. Albonetti, L.; Maiello, G.; Cariani, A.; Carpentieri, P.; Ferrari, A.; Sbrana, A.; Shum, P.; Talarico, L.; Russo, T.; Mariani, S. DNA Metabarcoding of Trawling Bycatch Reveals Diversity and Distribution Patterns of Sharks and Rays in the Central Tyrrhenian Sea. *ICES J. Mar. Sci.* **2023**, *80*, 664–674. [CrossRef]
138. Jeunen, G.; Knapp, M.; Spencer, H.G.; Lamare, M.D.; Taylor, H.R.; Stat, M.; Bunce, M.; Gemmell, N.J. Environmental DNA (eDNA) Metabarcoding Reveals Strong Discrimination among Diverse Marine Habitats Connected by Water Movement. *Mol. Ecol. Resour.* **2019**, *19*, 426–438. [CrossRef]
139. Brandl, S.J.; Goatley, C.H.R.; Bellwood, D.R.; Tornabene, L. The Hidden Half: Ecology and Evolution of Cryptobenthic Fishes on Coral Reefs. *Biol. Rev.* **2018**, *93*, 1846–1873. [CrossRef]
140. Mathon, L.; Marques, V.; Mouillot, D.; Albouy, C.; Andrello, M.; Baletaud, F.; Borrero-Pérez, G.H.; Dejean, T.; Edgar, G.J.; Grondin, J.; et al. Cross-Ocean Patterns and Processes in Fish Biodiversity on Coral Reefs through the Lens of eDNA Metabarcoding. *Proc. R. Soc. B Biol. Sci.* **2022**, *289*, 20220162. [CrossRef] [PubMed]
141. Ip, Y.C.A.; Chang, J.J.M.; Tun, K.P.P.; Meier, R.; Huang, D. Multispecies Environmental DNA Metabarcoding Sheds Light on Annual Coral Spawning Events. *Mol. Ecol.* **2023**, *32*, 6474–6488. [CrossRef] [PubMed]
142. Shinzato, C.; Narisoko, H.; Nishitsuji, K.; Nagata, T.; Satoh, N.; Inoue, J. Novel Mitochondrial DNA Markers for Scleractinian Corals and Generic-Level Environmental DNA Metabarcoding. *Front. Mar. Sci.* **2021**, *8*, 758207. [CrossRef]
143. Alexander, J.B.; Bunce, M.; White, N.; Wilkinson, S.P.; Adam, A.A.S.; Berry, T.; Stat, M.; Thomas, L.; Newman, S.J.; Dugal, L.; et al. Development of a Multi-Assay Approach for Monitoring Coral Diversity Using eDNA Metabarcoding. *Coral Reefs* **2020**, *39*, 159–171. [CrossRef]
144. West, K.M.; Stat, M.; Harvey, E.S.; Skepper, C.L.; DiBattista, J.D.; Richards, Z.T.; Travers, M.J.; Newman, S.J.; Bunce, M. eDNA Metabarcoding Survey Reveals Fine-Scale Coral Reef Community Variation across a Remote, Tropical Island Ecosystem. *Mol. Ecol.* **2020**, *29*, 1069–1086. [CrossRef] [PubMed]
145. Dugal, L.; Thomas, L.; Wilkinson, S.P.; Richards, Z.T.; Alexander, J.B.; Adam, A.A.S.; Kennington, W.J.; Jarman, S.; Ryan, N.M.; Bunce, M.; et al. Coral Monitoring in Northwest Australia with Environmental DNA Metabarcoding Using a Curated Reference Database for Optimized Detection. *Environ. DNA* **2022**, *4*, 63–76. [CrossRef]
146. Dugal, L.; Thomas, L.; Meenakshisundaram, A.; Simpson, T.; Lines, R.; Colquhoun, J.; Jarman, S.; Meekan, M. Distinct Coral Reef Habitat Communities Characterized by Environmental DNA Metabarcoding. *Coral Reefs* **2023**, *42*, 17–30. [CrossRef]
147. Gösser, F.; Schweinsberg, M.; Mittelbach, P.; Schoenig, E.; Tollrian, R. An Environmental DNA Metabarcoding Approach versus a Visual Survey for Reefs of Koh Pha-ngan in Thailand. *Environ. DNA* **2023**, *5*, 297–311. [CrossRef]
148. Gold, Z.; Sprague, J.; Kushner, D.J.; Zerecero Marin, E.; Barber, P.H. eDNA Metabarcoding as a Biomonitoring Tool for Marine Protected Areas. *PLoS ONE* **2021**, *16*, e0238557. [CrossRef]
149. Pratomo, A.; Bengen, D.G.; Zamani, N.P.; Lane, C.; Humphries, A.T.; Borbee, E.; Subhan, B.; Madduppa, H. Diversity and Distribution of Symbiodiniaceae Detected on Coral Reefs of Lombok, Indonesia Using Environmental DNA Metabarcoding. *PeerJ* **2022**, *10*, e14006. [CrossRef] [PubMed]
150. Madduppa, H.; Cahyani, N.K.D.; Anggoro, A.W.; Subhan, B.; Jefri, E.; Sani, L.M.I.; Arafat, D.; Akbar, N.; Bengen, D.G. eDNA Metabarcoding Illuminates Species Diversity and Composition of Three Phyla (Chordata, Mollusca and Echinodermata) across Indonesian Coral Reefs. *Biodivers. Conserv.* **2021**, *30*, 3087–3114. [CrossRef]
151. Byrne, I.; Riginos, C.; Uthicke, S.; Brookes, D.; Popovic, I. DNA Metabarcoding as a Tool for Characterising the Spatio-Temporal Distribution of Planktonic Larvae in the Phylum Echinodermata. *Coral Reefs* **2024**, *43*, 717–731. [CrossRef]
152. Merten, V.; Bayer, T.; Reusch, T.B.H.; Puebla, O.; Fuss, J.; Stefanschitz, J.; Lischka, A.; Hauss, H.; Neitzel, P.; Piatkowski, U.; et al. An Integrative Assessment Combining Deep-Sea Net Sampling, *in situ* Observations and Environmental DNA Analysis Identifies Cabo Verde as a Cephalopod Biodiversity Hotspot in the Atlantic Ocean. *Front. Mar. Sci.* **2021**, *8*, 760108. [CrossRef]
153. Kim, E.-B.; Ju, S.-J.; Suh, Y.J. Biodiversity and Community Structures across the Magellan Seamounts and Abyssal Plains in the Western Pacific Ocean Revealed by Environmental DNA Metabarcoding Analysis. *Front. Mar. Sci.* **2024**, *11*, 1412678. [CrossRef]
154. Maeda, A.; Nishijima, M.; Iguchi, A.; Ota, Y.; Suzumura, M.; Suzuki, A. Environmental DNA Metabarcoding of Foraminifera for Biological Monitoring of Bottom Water and Sediments on the Takuyo-Daigo Seamount in the Northwestern Pacific. *Front. Mar. Sci.* **2024**, *10*, 1243713. [CrossRef]
155. Sinniger, F.; Pawlowski, J.; Harii, S.; Gooday, A.J.; Yamamoto, H.; Chevaldonné, P.; Cedhagen, T.; Carvalho, G.; Creer, S. Worldwide Analysis of Sedimentary DNA Reveals Major Gaps in Taxonomic Knowledge of Deep-Sea Benthos. *Front. Mar. Sci.* **2016**, *3*, 92. [CrossRef]
156. Stuart-Smith, R.D.; Brown, C.J.; Ceccarelli, D.M.; Edgar, G.J. Ecosystem Restructuring along the Great Barrier Reef Following Mass Coral Bleaching. *Nature* **2018**, *560*, 92–96. [CrossRef]

157. Agersnap, S.; Sigsgaard, E.E.; Jensen, M.R.; Avila, M.D.P.; Carl, H.; Møller, P.R.; Krøs, S.L.; Knudsen, S.W.; Wisz, M.S.; Thomsen, P.F. A National Scale "BioBlitz" Using Citizen Science and eDNA Metabarcoding for Monitoring Coastal Marine Fish. *Front. Mar. Sci.* **2022**, *9*, 824100. [CrossRef]
158. Sutherland, W.J.; Aveling, R.; Brooks, T.M.; Clout, M.; Dicks, L.V.; Fellman, L.; Fleishman, E.; Gibbons, D.W.; Keim, B.; Lickorish, F.; et al. A Horizon Scan of Global Conservation Issues for 2014. *Trends Ecol. Evol.* **2014**, *29*, 15–22. [CrossRef]
159. Li, F.; Yang, J.; Yang, Y.; Zhang, X. Using Environmental DNA Metabarcoding to Monitor the Changes and Health Status of Aquatic Ecosystems. *Environ. Monit. China* **2018**, *34*, 37–46.
160. Dully, V.; Wilding, T.A.; Mühlhaus, T.; Stoeck, T. Identifying the Minimum Amplicon Sequence Depth to Adequately Predict Classes in eDNA-Based Marine Biomonitoring Using Supervised Machine Learning. *Comput. Struct. Biotechnol. J.* **2021**, *19*, 2256–2268. [CrossRef] [PubMed]
161. Kimura, M.; Yamanaka, H.; Nakashima, Y. Application of Machine Learning to Environmental DNA Metabarcoding. *IEEE Access* **2022**, *10*, 101790–101794. [CrossRef]
162. Kolios, S.; Maurodimou, O.; Stylios, C. Integrated Large-Scale Environmental Information Systems: A Short Survey. In *Proceedings of the Collaborative Systems for Reindustrialization*; Camarinha-Matos, L.M., Scherer, R.J., Eds.; Springer: Berlin/Heidelberg, Germany, 2013; pp. 164–171.
163. Matějíček, L.; Engst, P.; Jaňour, Z. A GIS-Based Approach to Spatio-Temporal Analysis of Environmental Pollution in Urban Areas: A Case Study of Prague's Environment Extended by LIDAR Data. *Ecol. Model.* **2006**, *199*, 261–277. [CrossRef]
164. Muenzel, D.; Bani, A.; De Brauwer, M.; Stewart, E.; Djakiman, C.; Halwi; Purnama, R.; Yusuf, S.; Santoso, P.; Hukom, F.D.; et al. Combining Environmental DNA and Visual Surveys Can Inform Conservation Planning for Coral Reefs. *Proc. Natl. Acad. Sci. USA* **2024**, *121*, e2307214121. [CrossRef]
165. Rey, A.; Viard, F.; Lizé, A.; Corre, E.; Valentini, A.; Thiriet, P. Coastal Rocky Reef Fish Monitoring in the Context of the Marine Strategy Framework Directive: Environmental DNA Metabarcoding Complements Underwater Visual Census. *Ocean Coast. Manag.* **2023**, *241*, 106625. [CrossRef]
166. Stat, M.; John, J.; DiBattista, J.D.; Newman, S.J.; Bunce, M.; Harvey, E.S. Combined Use of eDNA Metabarcoding and Video Surveillance for the Assessment of Fish Biodiversity. *Conserv. Biol.* **2019**, *33*, 196–205. [CrossRef] [PubMed]
167. Valdivia-Carrillo, T.; Rocha-Olivares, A.; Reyes-Bonilla, H.; Domínguez-Contreras, J.F.; Munguia-Vega, A. Integrating eDNA Metabarcoding and Simultaneous Underwater Visual Surveys to Describe Complex Fish Communities in a Marine Biodiversity Hotspot. *Mol. Ecol. Resour.* **2021**, *21*, 1558–1574. [CrossRef]
168. Bohan, D.A.; Vacher, C.; Tamaddoni-Nezhad, A.; Raybould, A.; Dumbrell, A.J.; Woodward, G. Next-Generation Global Biomonitoring: Large-Scale, Automated Reconstruction of Ecological Networks. *Trends Ecol. Evol.* **2017**, *32*, 477–487. [CrossRef] [PubMed]
169. Thomsen, P.F.; Jensen, M.R.; Sigsgaard, E.E. A Vision for Global eDNA-Based Monitoring in a Changing World. *Cell* **2024**, *187*, 4444–4448. [CrossRef]
170. Hartig, F.; Abrego, N.; Bush, A.; Chase, J.M.; Guillera-Arroita, G.; Leibold, M.A.; Ovaskainen, O.; Pellissier, L.; Pichler, M.; Poggiato, G. Novel Community Data in Ecology-Properties and Prospects. *Trends Ecol. Evol.* **2024**, *39*, 280–293. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.