Annual Review of Marine Science

Environmental DNA Metabarcoding: A Novel Method for Biodiversity Monitoring of Marine Fish Communities

Masaki Miya

Natural History Museum and Institute, Chiba, Chiba 260-8682, Japan;
email: masaki_miya@me.com

Annu. Rev. Mar. Sci. 2022. 14:161–85

First published as a Review in Advance on August 5, 2021

The *Annual Review of Marine Science* is online at marine.annualreviews.org

https://doi.org/10.1146/annurev-marine-041421-082251

Keywords

eDNA, marine conservation, fisheries management, fish ecology, Sustainable Development Goals, biodiversity

Abstract

Environmental DNA (eDNA) is genetic material that has been shed from macroorganisms. It has received increased attention as an indirect marker for biodiversity monitoring. This article reviews the current status of eDNA metabarcoding (simultaneous detection of multiple species) as a noninvasive and cost-effective approach for monitoring marine fish communities and discusses the prospects for this growing field. eDNA metabarcoding coamplifies short fragments of fish eDNA across a wide variety of taxa and, coupled with high-throughput sequencing technologies, allows massively parallel sequencing to be performed simultaneously for dozens to hundreds of samples. It can predict species richness in a given area, detect habitat segregation and biogeographic patterns from small to large spatial scales, and monitor the spatiotemporal dynamics of fish communities. In addition, it can detect an anthropogenic impact on fish communities through evaluation of their functional diversity. Recognizing the strengths and limitations of eDNA metabarcoding will help ensure that continuous biodiversity monitoring at multiple sites will be useful for ecosystem conservation and sustainable use of fishery resources, possibly contributing to achieving the targets of the United Nations' Sustainable Development Goal 14 for 2030.

1. INTRODUCTION

Following the Millennium Development Goals for 2015, the United Nations (UN) announced new Sustainable Development Goals for 2030, which comprise 17 specific goals for the environmental, political, and economic issues facing the world (Sachs 2012, UN 2021). Sustainable Development Goal 14 is related to the oceans and states that the marine environment and fishery resources should be conserved and used in a sustainable manner (UN 2021). It also sets 10 targets related to the goal, the first seven of which (14.1–7) are specific outcomes to be achieved, and the last three of which (14.a–c) are specific measures for achieving these outcomes. Five of the first seven targets are related to the conservation and restoration of the marine environment and fishery resources: preventing and significantly reducing marine pollution, sustainably managing and protecting marine ecosystems, reducing the impacts of ocean acidification, restoring fishery resources, and conserving marine areas (UN 2021).

To achieve the specific outcomes of these individual targets, we need to continuously monitor the status of the marine environment and fishery resources (Bhaduri et al. 2016, Ojha & Babu 2019). Among the abiotic components of the marine environment, some physical attributes (e.g., water temperature and salinity) are easily measurable with great accuracy, and an international team has constructed an observation network to continuously monitor such attributes (Riser et al. 2016). The biotic components are highly complex due to their diversification during the evolutionary process and the inclusion of a myriad of unknown species (Fujikura et al. 2010) and thus cannot be automatically monitored in the same way as the abiotic attributes. More than 16,700 marine fish species are known worldwide, and an average of approximately 100–150 new species are described annually (Eschmeyer et al. 2010). To investigate the species number and species composition in specific marine areas with highly diversified fishes, we have traditionally needed to conduct field surveys that utilize direct capture-based sampling methods (e.g., netting and fishing) or underwater visual censuses (e.g., diving and underwater video) (Oka et al. 2021). However, such field surveys are invasive, labor intensive, time consuming, and costly, and they require highly specialized taxonomic expertise in species identification (Thomsen & Willerslev 2015).

Moreover, marine ecosystems are vast, occupying 70.6% of the earth's surface (Costello et al. 2010) and constituting 96.5% of its water volume (Shiklomanov 1993). They are also diverse, as they range horizontally from coastal to oceanic areas with respect to the distance from a land mass, geographically from polar to equatorial regions, and vertically from the surface to deep waters exceeding 10 km (Miya et al. 2020). In general, rocky and sandy beaches alternate to form coastlines, where there are many locally distinct ecosystems—including coral reefs, mangroves, tidal wetlands, seagrass beds, barrier islands, kelp forests, estuaries, lagoons, salt marshes, and undersea caves—that each exhibit unique and diverse fish communities (Burke et al. 2001). However, recent shoreline developments (e.g., revetments and reclamations) in highly populated urbanized areas have modified these natural habitats into artificial coasts (Chee et al. 2017). In comparison with coastlines, oceanic areas outside the continental shelf are seemingly monotonous, although oceanic fronts between the major water masses and the ocean currents, seamounts, and deep-sea hydrothermal vents locally form unique biological communities (Jollivet 1996). In such diverse and vast marine ecosystems, it is often impossible to conduct traditional surveys for biodiversity monitoring, and thus most of these ecosystems have not been thoroughly surveyed (Costello & Chaudhary 2017). Therefore, although onetime biodiversity surveys can be conducted with limited resources in small and easily accessible areas, it is not practical to conduct continuous biodiversity monitoring in extensive, difficult-to-access areas that span several geographic regions.

For the conservation of marine environments, one of the strategies used despite the difficulty of continuous biodiversity monitoring is the selection of specific organisms as flagship species (Sergio et al. 2006). Such flagship species are often large animals with a strong public appeal (e.g.,

dugongs, dolphins, sea turtles, and ospreys) that can serve as umbrella species to allow the conservation of entire marine ecosystems (Monti et al. 2018). Similarly, one of the principal strategies for the conservation and management of fishery resources focuses on maximizing the catch of a single target species and estimating the maximum sustainable yield without depleting the fishery resources (Zabel et al. 2003). Both strategies target a limited number of focal species (flagship species or fishery target species), often ignoring the habitat, predators, and prey of the target species; other ecosystem components; and interactions (Heinen et al. 2020, Pikitch et al. 2014). Although global fisheries management is currently undergoing a paradigm shift from this single-species approach to an ecosystem approach (Pikitch et al. 2014), marine environment conservation still depends heavily on the flagship species approach (Monti et al. 2018). It appears that the emergence of a fast, cost-effective, and ideally noninvasive biodiversity monitoring method that targets biological communities instead of individual species (Fediajevaite et al. 2021, Port et al. 2016) is essential for breaking through the current stagnant situation in the conservation and management of marine environments and fishery resources.

In the past decade, environmental DNA (eDNA) has received increased attention as an indirect genetic marker for inferring species presence during biodiversity monitoring (Cristescu & Hebert 2018, Deiner et al. 2017). eDNA is defined here as extraorganismal genetic material that is shed from macroorganisms through feces, body mucus, blood, and sloughed tissue or scales and suspended in environmental samples such as water and sediment (Bohmann et al. 2014). After sampling a certain amount of water (**Figure 1*a***), filtration of the sampled water makes it possible to concentrate and capture eDNA on the filter membrane (**Figure 1*b***), from which it is extracted (**Figure 1*c***) and subjected to various molecular biology experiments for species detection (Miya et al. 2016). In particular, the eDNA metabarcoding approach enables the simultaneous detection of multiple species by using a high-throughput sequencing platform (Taberlet et al. 2012). This approach coamplifies a short fragment of eDNA from the target taxa (e.g., fish) using a set of universal primers through polymerase chain reaction (PCR). It appends various adapters and index sequences to both ends of the amplified fragments (amplicons) (**Figure 1*d***; for details, see **Figure 2**). Various combinations of different index sequences enable massively parallel sequencing to be performed using a high-throughput sequencing platform (e.g., Illumina MiSeq), with an output comprising tens of millions to billions of amplicons from dozens to hundreds of samples (**Figure 1*e***). After the data have been preprocessed and taxonomic assignments have been determined using a bioinformatics pipeline, a tentative taxonomic list is available for each sampling site (**Figure 1*f***) (Miya et al. 2015, Sato et al. 2018).

This article provides an overview of the current status of eDNA metabarcoding as a novel, noninvasive, and cost-effective approach for monitoring marine fish biodiversity and discusses the prospects for this rapidly growing field. For this purpose, a literature survey was conducted using Google Scholar (as of February 12, 2021) with the keywords "environmental DNA metabarcoding" and "marine fish." In all, 1,020 records from the search results were individually inspected, screened, and selected if the study (*a*) sampled eDNA from seawater in natural aquatic marine ecosystems (including estuarine ecosystems), (*b*) used an eDNA metabarcoding approach, and (*c*) analyzed the data at the species level or with an equivalent molecular operational taxonomic unit. Studies on an entire biological community that analyzed fish data solely at higher taxonomic levels (e.g., family) were excluded because the central issues of this review are associated with topics related to species diversity, such as species richness and species composition. Of the 1,020 search results, 50 papers fit all of these criteria, and those papers are reviewed in this article (see **Supplemental Table 1**). Deiner et al. (2017), Taberlet et al. (2018), and Ruppert et al. (2019) have provided extensive useful information on eDNA metabarcoding in general, including for nonfish and freshwater organisms; Hansen et al. (2018) and Gilbey et al. (2021) have discussed

Supplemental Material >

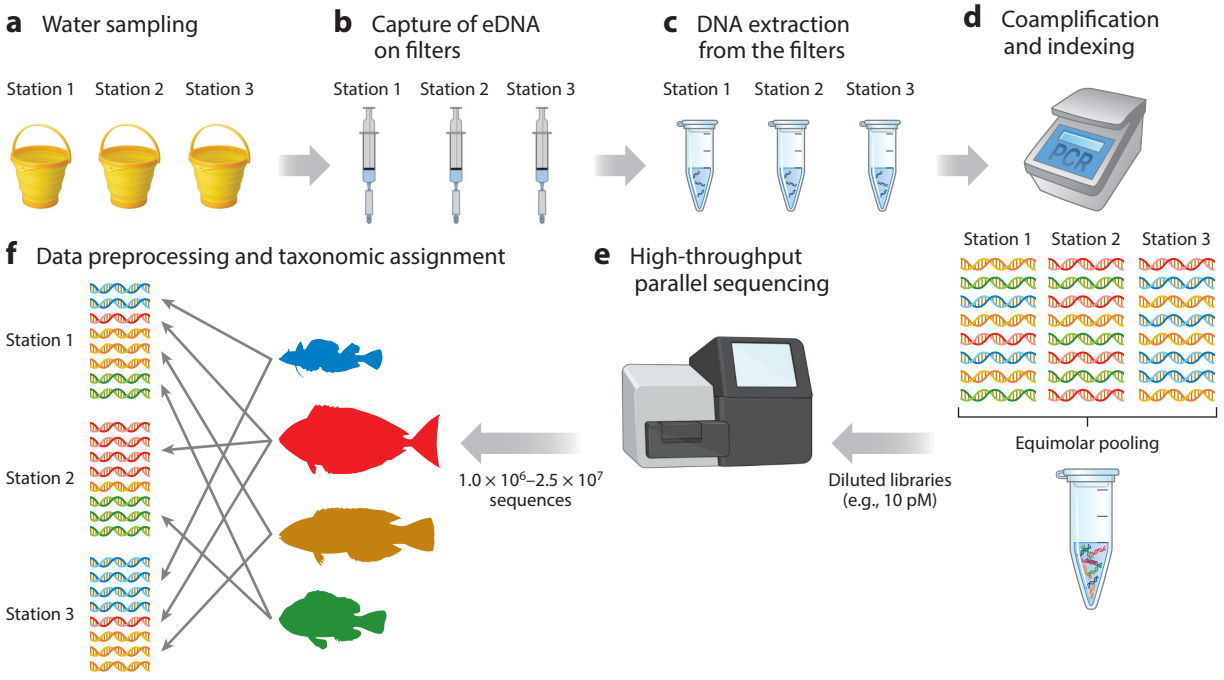

Figure 1

The eDNA metabarcoding workflow: (*a*) water sampling from three stations, (*b*) on-site filtration using a filter cartridge and syringe to concentrate and collect eDNA on the filter membrane, (*c*) eDNA extraction from the membrane, (*d*) coamplification of fish eDNA fragments using universal PCR primers and the addition of adapter and index sequences to both ends of the fragments, (*e*) high-throughput parallel sequencing, and (*f*) bioinformatic analysis of DNA sequences for taxonomic assignment. Abbreviations: eDNA, environmental DNA; PCR, polymerase chain reaction.

applications of eDNA metabarcoding to fishery resources; and Beng & Corlett (2020) have discussed applications for environmental conservation.

2. PROOF OF CONCEPT

The 50 selected eDNA metabarcoding studies on marine fish communities had diverse objectives, habitats, and geographic locations and scales, and they used various mitochondrial eDNA fragments (**Figures 3** and **4**; **Supplemental Table 1**). Despite these differences, all studies sought to use eDNA detection to obtain information on when and where the source organisms were present, using the eDNA of the source organisms as a proxy for their presence (Harrison et al. 2019). However, eDNA detection does not necessarily indicate the presence of the source organism at the water sampling site and time (Goldberg et al. 2016). In aquatic environments, after eDNA is shed from the host organism, it is transported from the original location by ocean currents or tides, during which time its concentration decreases as it diffuses and decays until it can no longer be detected (Barnes & Turner 2016). Therefore, a lack of understanding of these processes makes it challenging to accurately interpret the results of eDNA metabarcoding studies (Goldberg et al. 2016). Furthermore, a number of known factors affect these processes, such as the biology of source organisms (life history, behavior, tissue characteristics), hydrology (site characteristics, current, tide, wind), the abiotic environment (temperature, salinity, pH, substrate, turbidity, UV radiation), and the biotic environment (microbial communities, biofilms, extracellular nucleases) (Harrison et al. 2019).

Supplemental Material >

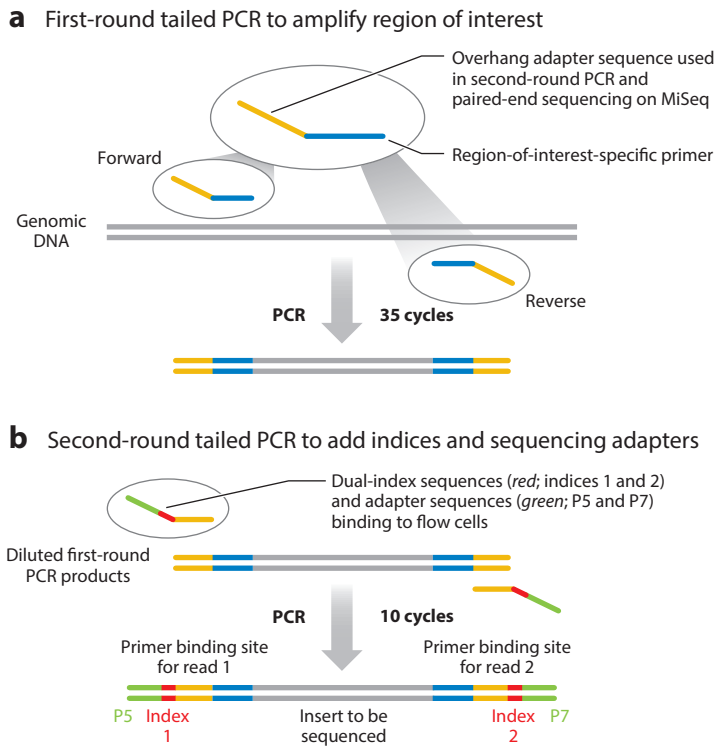

Figure 2

Schematic representation of paired-end library preparation using a two-step tailed PCR for MiSeq sequencing (**Figure 1*d***). (*a*) First-round PCR to amplify a region of interest using a pair of universal primers (*blue*) with overhang adapter sequences (*gold*). (*b*) Second-round PCR to add dual indices (*red*; indices 1 and 2) and sequencing adapters (*green*; P5 and P7). Abbreviation: PCR, polymerase chain reaction. Figure adapted from Miya et al. (2015).

Ideally, eDNA production, transport, diffusion, and decay models should be developed for each investigated species to fill the knowledge gap between eDNA detection and the presence of source organisms in space and time. Such proof-of-concept modeling allows for more accurate and robust estimates of the spatial and temporal dynamics of fish distributions based on eDNA detection, thereby alleviating the uncertainties of eDNA metabarcoding. A good example is a recent study that incorporated the production, transport, and degradation of eDNA using numerical hydrodynamic models: Fukaya et al. (2021) simulated the distribution of the eDNA concentration within an aquatic area based on these models and obtained reasonable estimates of the abundance of the target species (Japanese jack mackerel) in Maizuru Bay, Japan. Nevertheless, such a proof-of-concept study requires repeated laboratory experiments that measure the production and degradation rates of eDNA from each target species, as well as the renewed development of a hydrodynamic model for each aquatic area of interest to trace the movement of eDNA molecules.

eDNA metabarcoding studies often involve water sampling in a large area across several geographic regions over an extended period, with each sampling site having significantly different abiotic and biotic environments. Furthermore, eDNA metabarcoding studies target multiple fish species with diverse morphological and ecological characteristics, making it difficult to construct a simple model that is applicable to all component species without conducting laboratory

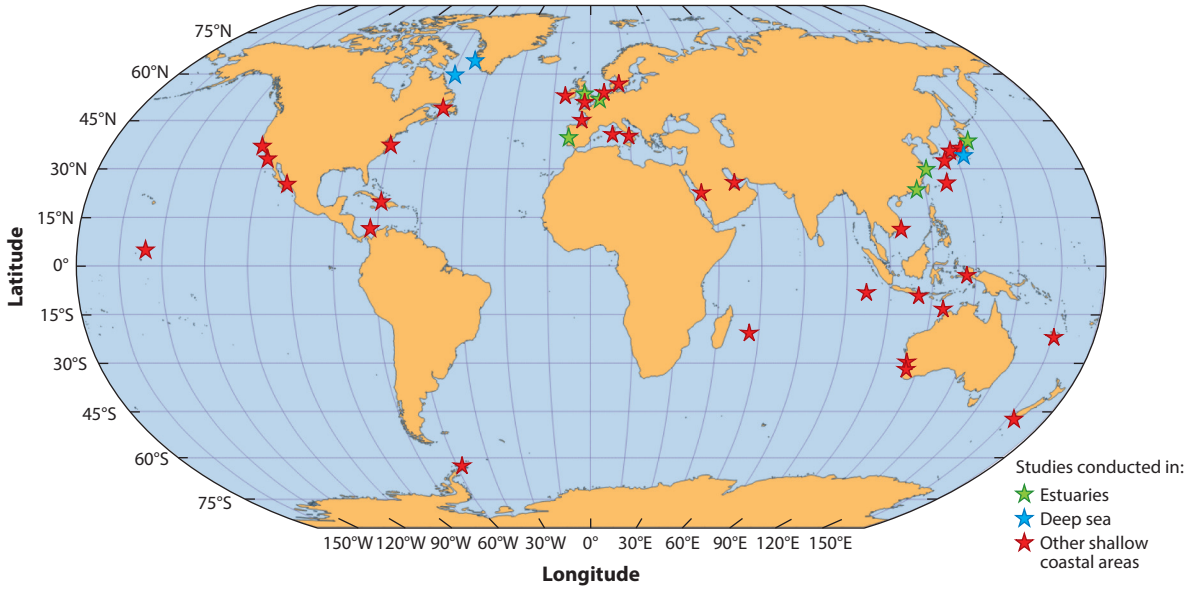

Figure 3

The approximate geographic locations of the survey sites from the 50 selected studies. Multiple studies conducted in the same area are indicated by a single star. Green, blue, and red stars represent studies conducted in estuaries, the deep sea, and other shallow coastal areas, respectively. For details on the references, see **Supplemental Table 1**.

Supplemental Material >

experiments. Given the difficulties of conducting proof-of-concept eDNA metabarcoding studies, controlled field experiments and empirical observations on the spatiotemporal resolution of species distributions may be useful for linking eDNA detection with the actual distributions of fish species.

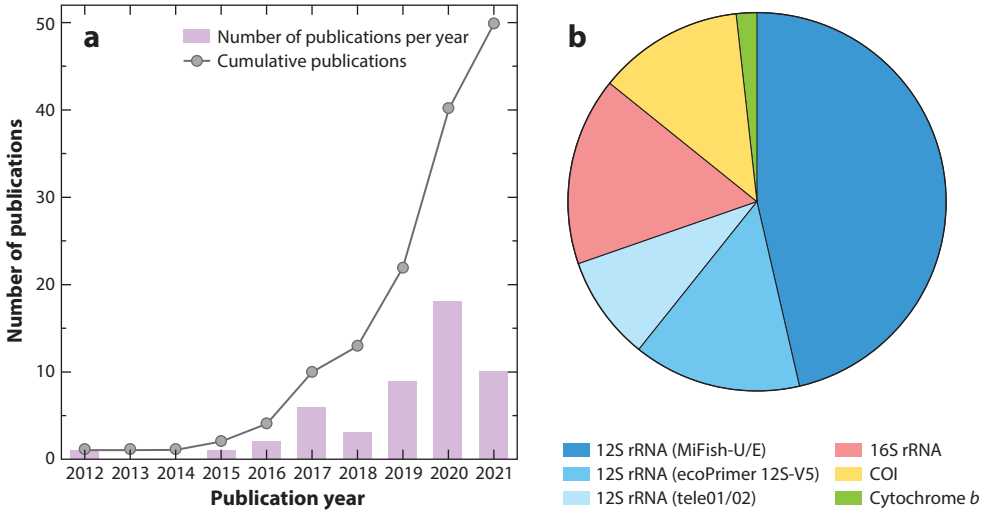

Figure 4

(*a*) Number of publications in each year (*bars*) and cumulatively (*line*) for the 50 selected studies. (*b*) Target mitochondrial genes in the 50 selected studies and the PCR primers used for the 12S gene. Abbreviation: PCR, polymerase chain reaction.

The only controlled field experiment that has been conducted in a marine environment was by Murakami et al. (2019). To investigate the dispersion and degradation processes of eDNA in the sea, they used caged fish (striped jack, an exogenous species in the study area) as a known source of eDNA and placed the cage in Maizuru Bay. They performed quantitative PCR using a species-specific primer and probe set and analyzed eDNA samples for seven distance intervals (1, 10, 30, 100, 300, 600, and 1,000 m) from the cage and six time intervals (0, 2, 4, 8, 24, and 48 h) after placing the cage and again after removing the cage. They found that most quantitative PCR detections (79%) fell within 30 m of the cage and that the target eDNA was detectable up to 1 h after removing the cage. They therefore suggested that the area of eDNA dispersion in the sea is as narrow as 30 m and that the duration of persistence could be as short as 1 h after release from a limited source. It should be noted that their study area was in a protected inner bay with low tidal differences (<30 cm) and no strong currents.

Empirical eDNA studies of marine fish communities have indicated remarkably high spatial turnover and site fidelity of the source communities (Harrison et al. 2019). An eDNA metabarcoding study in a kelp forest ecosystem in Monterey Bay, California, found spatial concordance between individual species' eDNA and visual survey trends, which consequently distinguished the vertebrate community assemblages from habitats that were separated by as little as ∼60 m (Port et al. 2016). Subsequently, a similar study in the oceanic ecosystem of the same bay found significantly different vertebrate community compositions at different sampling depths (0, 20, or 40 m) across all stations and significantly different communities at stations located on the continental shelf (<200-m bottom depth) compared with those at stations in the deeper waters of the canyons of Monterey Bay (>200-m bottom depth) (Andruszkiewicz et al. 2017). Additionally, Thomsen et al. (2016) collected seawater samples from the continental slope of southwest Greenland at depths of 188–918 m, performed eDNA metabarcoding analysis, and compared their results with trawl catches. They demonstrated that eDNA metabarcoding data could be used as a qualitative and quantitative proxy for marine fish assemblages in deep-water oceanic habitats. Yamamoto et al. (2017) conducted systematic grid sampling at the surface and near-bottom waters of 47 stations in Maizuru Bay, Japan, and performed an eDNA metabarcoding analysis. Based on the dissimilarity among the fish communities at the 47 stations, they found that the detected spatial autocorrelation of the fish communities fell within ∼800 m. More recently, Oka et al. (2021) found distinct differences between fish communities separated by ∼300 m along the offshore coral reef edge and shoreside seagrass beds. These findings are consistent with recent observations from eDNA metabarcoding analyses that tidal cycles do not significantly impact biological communities containing eukaryotic planktonic and benthic organisms in Hood Canal, Washington (Kelly et al. 2018), or the fish communities on intertidal sand flats at Palmyra Atoll in the central Pacific (Lafferty et al. 2021).

The results of these experiments and field observations suggest that eDNA rapidly diffuses and deteriorates after being shed from the host organisms and rapidly falls below the detection limit. The spatial and temporal scales of the detection limit range from several tens to hundreds of meters within 1 h (Andruszkiewicz et al. 2017, Lafferty et al. 2021, Murakami et al. 2019, Oka et al. 2021, Port et al. 2016). eDNA detection shows high spatial turnover and site fidelity of the source communities (Harrison et al. 2019), which can be considered circumstantial evidence for the presence of source organisms. However, caution should be taken, as eDNA can spill over from adjacent ecosystems (Lafferty et al. 2021), sewage and wastewater flowing from various sources (Yamamoto et al. 2016), feces of other predatory animals, or dead animals (Rees et al. 2014), all of which are sources of false positives in biodiversity monitoring of marine fish communities.

3. OVERVIEW OF THE 50 SELECTED STUDIES

3.1. Geographic and Habitat Coverage

The 50 selected eDNA metabarcoding studies on marine fish communities (**Supplemental Table 1**) came from around the world (**Figure 3**) and are cited in the text. The only published study from African waters was from the Red Sea of Saudi Arabia (DiBattista et al. 2017). Although the number of published papers varies depending on the continent, the latitudinal coverage of these studies is extensive, ranging from boreal (>60°N/S; 4 studies) through temperate (30–60°N/S; 30 studies) to tropical and subtropical (<30°N/S; 17 studies) zones (duplicate counts included; **Supplemental Table 1**). Habitat coverage in these studies is also extensive. Most studies were conducted in coastal areas (45 studies, including 8 and 9 studies in estuaries and coral reefs, respectively); the remainder (5 studies) targeted oceanic areas outside the continental shelf (**Supplemental Table 1**). It should be noted that an eDNA metabarcoding approach has even been employed for biodiversity monitoring of deep-sea benthic and pelagic fish communities down to 3,000-m depth (McClenaghan et al. 2020).

Supplemental Material >

3.2. Publication Year and Corresponding Authorship

Thomsen et al. (2012) were the first to apply an eDNA metabarcoding approach to biodiversity monitoring of marine fish communities. They designed region-specific universal primers and supplementary species-specific primers for the mitochondrial cytochrome *b* gene, with reference to the existing reference sequences for local fish. They successfully detected 15 fish species, including both commercially important species and species that were rarely or never before recorded by conventional capture-based methods in the coastal waters of Denmark (Thomsen et al. 2012). Three years later, Miya et al. (2015) published a new universal primer pair called MiFish for the eDNA metabarcoding of fishes, and they tested its performance in four aquarium tanks with known species compositions and a nearby coral reef lagoon in southern Japan, in which they detected more than 230 tropical and subtropical fish species. Since then, the number of published papers has gradually increased, culminating with 18 studies published in 2020 (**Figure 4*a***). Although 2021 has just begun at the time of writing, the number has already reached 10 (as of February 12) and is likely to significantly exceed 2020's record by the end of the year. The nationalities of the corresponding authors of these papers include 15 European, 13 Asian, 12 North American, 9 Oceanian, and 1 South American country (**Supplemental Table 1**), reflecting the distribution of eDNA research in advanced countries (Wang et al. 2021).

3.3. Marker Genes and Polymerase Chain Reaction (PCR) Primers

A recent shotgun sequencing study demonstrated that fish-derived eDNA fragments occupied only 0.004% (875 reads) of the total sequences (22.3 million reads) collected from natural seawater in Coral Bay, Western Australia (Stat et al. 2017). Thus, we need to amplify the trace quantity of target molecules exclusively from fish to an amount that can be analyzed using a high-throughput sequencing platform. Currently, PCR is the most established and cost-effective method for such gene amplification, and it requires universal primers that coamplify the gene fragments across a range of diverse types of fish. All 50 selected studies targeted mitochondrial genes, of which two ribosomal genes (12S and 16S) were most frequently used (**Figure 4*b***; **Supplemental Table 1**). The near-exclusive use of ribosomal genes (87.5%) is because it is relatively easy to design PCR primers for the two highly conserved regions (each primer is only 20–30 base pairs) that flank the intermediate hypervariable region of ~200 base pairs (Deagle et al. 2014). Of the major 12S primers used in these studies, MiFish primers (Miya et al. 2015, 2020) have been most frequently

used (46.4%), reflecting their high performance, which has been demonstrated in recent comparisons of competing primers in terms of their specificity to fishes, taxonomic discriminatory power, and taxonomic coverage in reference sequences (Bylemans et al. 2018, Collins et al. 2019, Shu et al. 2021, Zhang et al. 2020). It appears that the taxonomic coverage of the COI gene (the cytochrome *c* oxidase subunit I gene, which is widely used in the International Barcode of Life project; Ward et al. 2009) is far more comprehensive than the coverage of other mitochondrial genes (Collins et al. 2019). However, Collins et al. (2019) demonstrated that nonspecific amplification of prokaryotic and nonfish eukaryotic DNA with COI primers results in excessive volumes of wasted sequencing effort, making their use in fish eDNA metabarcoding complicated and difficult.

3.4. Number of Detected Species

The total number of detected species from each study area was available for 40 of the 50 selected studies (**Supplemental Table 1**), ranging from 15 to 310 species, with a mean of 86 species. **Figure 5** plots these numbers of species on a scattergram without correction for the sampling effort or sampling area. The number of detected species is generally high at tropical latitudes (<30°N/S), with exceptionally high diversity observed in coral reef habitats. The number of detected species gradually decreases from temperate (30–60°N/S) to boreal (>60°N/S) latitudes, a trend that reflects the previous knowledge of the latitudinal gradient in species richness observed in marine fish communities (Rohde et al. 1993). Two canals and a port in a highly urbanized area (34.7°N; Kamimura et al. 2018), as well as a fish landing station (8.4°S; Andriyono et al. 2019), show noticeably lower species numbers than those at the equivalent latitudes (**Figure 5**).

Supplemental Material >

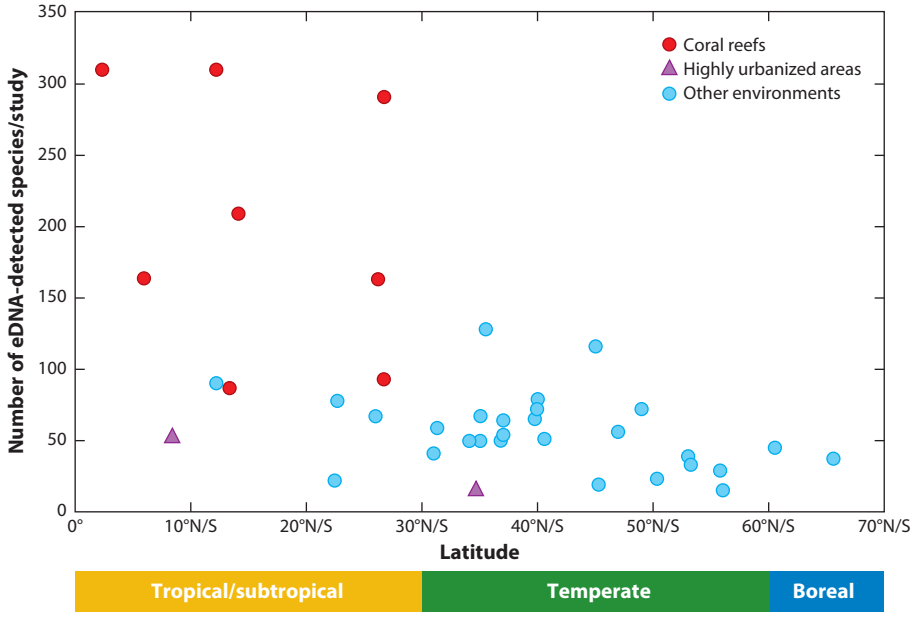

Figure 5

Scatter plot of the total numbers of detected species available from 40 eDNA metabarcoding studies (see **Supplemental Table 1**). The numbers were not corrected for the sampling effort or sampling area and are plotted against the latitudes of the study areas. Observations from coral reefs and highly urbanized areas are shown with red circles and purple triangles, respectively. Abbreviation: eDNA, environmental DNA.

3.5. Comparison of Monitoring Methods

In a pioneering study, Thomsen et al. (2012) investigated the detection efficiency of the eDNA approach and compared its performance with that of nine monitoring methods conventionally used in marine fish surveys. They found that eDNA captured fish diversity mostly better than or as well as any of the conventional methods. The high detection efficiency of the eDNA metabarcoding approach has been supported by subsequent studies that concurrently employed direct capture-based sampling (10 studies), underwater visual censuses through diving (9 studies), underwater video records (5 studies), and combinations of these monitoring methods (2 studies) (**Supplemental Table 1**). The 23 other studies compared eDNA detection with cumulative records (e.g., checklists) based on literature surveys.

Supplemental Material >

4. METHODOLOGICAL CHALLENGES

The major objective of eDNA metabarcoding is to accurately estimate the species number (species richness) and species composition (ideally with the relative species abundance) of the target biological community in a given area (Deiner et al. 2017). Therefore, it is of paramount importance to clarify the factors that are responsible for the accuracy of these biodiversity parameters and can lead to erroneous estimation in eDNA metabarcoding (Cristescu & Hebert 2018). Erroneous estimation sources include diverse types of false negatives and false positives resulting from each eDNA metabarcoding analysis step, including field sampling, laboratory experiments, and bioinformatics (**Figure 1**). Miya et al. (2020) extensively discussed the methodological challenges of eDNA metabarcoding in a review of MiFish metabarcoding. The following sections are generalized summaries that update the previous discussions in terms of the false negatives (Section 4.1) and false positives (Section 4.2). The two erroneous estimates resulting from the bioinformatics pipeline are treated separately (Section 4.3).

4.1. False Negatives

False negatives, which are failures to detect species that are actually present in a given area, exist in any ecological field survey (Doi et al. 2019). In eDNA metabarcoding, detection rates increase (or false negatives decrease) with an increased volume of filtered water and increased sampling effort (Bessey et al. 2020, Miya et al. 2016). However, study designs for optimal water sampling that avoid false negatives may be study specific under limited resources and time, and thus they require a preliminary survey for each study. This section provides an overview of the experimental methods that minimize the false negatives that are unique to eDNA metabarcoding, focusing on the workflow after eDNA extraction. Note that the experimental methods before this point, such as filtration (e.g., the choice of filters and nominal pore size) and the DNA extraction itself (e.g., the volume of reagents and collection of eDNA from the membrane), greatly affect the eDNA yield and may result in false negatives for minor species (Kawato et al. 2021, Miya et al. 2016, Spens et al. 2017, Wong et al. 2020).

Given the enormous biodiversity of marine fishes, which exceeds 16,700 species (Eschmeyer et al. 2010), no universal primer pairs can amplify each target amplicon across all of the diverse types of fishes (Miya et al. 2020). In fact, Miya et al. (2015) found that some large sharks and rays swimming in an aquarium tank were underrepresented in the results of preliminary eDNA metabarcoding that used the original MiFish primers (MiFish-U). Therefore, Miya et al. (2015) optimized the MiFish-U primers to accommodate sequence variations in sharks and rays (Elasmobranchii), using the two primer sets (MiFish-U/E) simultaneously in PCR amplification (multiplex PCR). The multiplex PCR approach successfully detected all 18 elasmobranch species in the tank.

This exemplifies the challenge of PCR dropouts, that is, the failure to amplify fish DNA in the eDNA extracts, inevitably resulting in false negatives during eDNA metabarcoding. Additional examples can be found for some species of sea sculpins, lampreys, and diadromous osmerid fishes in Japanese coastal waters (Miya & Sado 2019).

The second challenge is the lack of variation in the amplicons of closely related congeners, which inevitably leads to underestimation of the species number in a given sample, community, or area (i.e., species richness; Colwell 2009). For example, the amplicons from the MiFish primers exhibited little variation among species of the scombrid genus *Thunnus* (Miya et al. 2015), freshwater eel genus *Anguilla* (Takeuchi et al. 2019a), salmonid genus *Oncorhynchus* (Morita et al. 2019), puffer fish genus *Takifugu* (Yamanoue et al. 2009), and rockfish genus *Sebastes* (Min et al. 2020), all of which are commercially important groups of fishes that exhibit explosive radiation. Some of the authors of the studies investigating these species designed new group-specific primers on more variable mitochondrial genes, such as ND5 (tunas), ATP6 (freshwater eels), ND2 (salmonids), and cytochrome *b* (rockfishes). The multiplex PCR approach is also effective for these auxiliary primers if the amplicon sizes are similar to that of the MiFish primers (~170 base pairs) (Miya et al. 2015).

Note that some study groups have employed multiple markers from different mitochondrial genes to avoid false negatives and the underestimation of species diversity (Fraija-Fernández et al. 2020, Jeunen et al. 2021, McClenaghan et al. 2020). In a review of eDNA metabarcoding studies on species richness in fishes, McElroy et al. (2020) argued that multimarker assays are required to ensure robust species richness estimates in ecosystems with great biodiversity. Multimarker assays, however, depend on reference databases with different taxonomic coverage and accuracy, resulting in multiple taxonomic tables containing different taxa at various taxonomic levels. Therefore, combining such different taxonomic tables into a single table requires various assumptions, making species-level ecological inference difficult. Regardless of the primers used, information on the sequence variations within priming sites and amplicons is essential for accurately evaluating the species diversity of fish communities in a study area.

In addition to the PCR primers, the number of biological replicates (number of samples) and technical replicates (number of repeated PCRs for the same sample) affect the number of species detected by eDNA metabarcoding (O'Donnell et al. 2016, Yamamoto et al. 2017). Doi et al. (2019) examined the effects of the number of replicates during filtration and PCR (repeated extractions and amplification for the same samples) on the detection probabilities of species using water from an aquarium tank with a known species composition. Their analysis showed consistently high detection rates at the filtration step and relatively low and variable detection rates at the PCR step (**Figure 6**), which favored an increase in the number of PCR replicates rather than an increase in filter replicates. Therefore, in practice, it would be a good choice to perform PCR with multiple replicates (e.g., eight replicates, for ease of experimental manipulations) to maintain a relatively high detection probability of the minor species contained in eDNA extracts (Ficetola et al. 2015).

PCR dropouts due to primer–template mismatches, a lack of interspecific variation within the amplicons, or an insufficient number of filter and PCR replicates all lead to underestimation of species richness and diversity and should be optimized depending on the local fish fauna and purpose of the study. Thus, a preliminary survey should be conducted before initiating a full-scale survey in a study area to avoid false negatives in fish eDNA metabarcoding (Miya et al. 2020).

4.2. False Positives

For high-throughput sequencing, the current eDNA metabarcoding protocol involves PCR amplification of gene fragments from trace amounts of DNA in aquatic environments. Therefore,

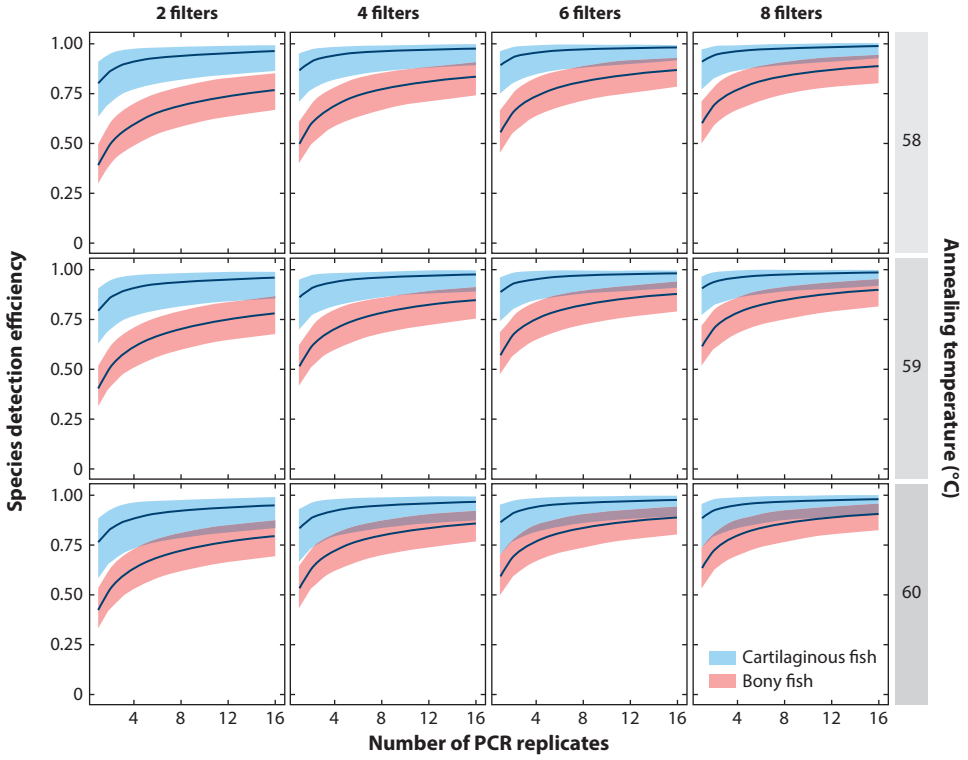

Figure 6

Changes in species detection efficiency versus number of PCR replicates for four filter replicates at three different annealing temperatures. Solid lines and colored bands indicate medians and 95% credible posterior distribution intervals, respectively. Abbreviation: PCR, polymerase chain reaction. Figure adapted from Doi et al. (2019).

contamination with exogenous DNA in a series of pre-PCR workflows in the field and laboratory causes serious false-positive detections and is virtually unavoidable (Miya et al. 2015). One of the requirements for eDNA metabarcoding is to set up an experimental environment that is less susceptible to exogenous DNA. To avoid adverse effects of exogenous DNA, Miya et al. (2020) listed the minimum requirements for laboratory facilities (e.g., at least one room dedicated to eDNA extraction, pre-PCR steps, and post-PCR steps, respectively, with each room having its own equipment), rules for the personnel in charge of experiments (e.g., the one-way rule to avoid carryover contamination from the post-PCR room to other rooms), and decontamination methods for laboratory equipment (e.g., UV sterilization) (for details, see eDNA Soc. 2019).

In addition to contamination inside the laboratory, there are several possible sources of exogenous DNA in the field. For example, sewage and wastewater flowing out of fishing ports, fishery processing factories, aquaculture ponds, and aquariums are sources of exogenous DNA stemming from catches, raw material processing, breeding fish, and fish feed, respectively (Yamamoto et al. 2016). Feces of piscivorous animals, such as migratory marine birds, mammals, and fish, may contain nonresident fishes, and a large amount of eDNA is also released from the bodies of dead fish (Rees et al. 2014). Therefore, water sampling sites should be carefully chosen to avoid collecting exogenous DNA from such sources in the field. Other than these exogenous DNA sources,

Lafferty et al. (2021) pointed out that habitat-specific analyses using eDNA need to account for eDNA spillover from one habitat to another due to tidal flows or ocean currents.

4.3. Bioinformatic Pipeline and Reference Database

It is generally known that high-throughput sequencing data include numerous erroneous sequences that differ from the true biological sequences by one or a few bases at random positions (Coissac et al. 2012, Edgar 2016). These errors may arise when DNA polymerase incorporates incorrect bases during eDNA amplification and/or sequencing (e.g., cluster amplification and sequencing reactions in the Illumina MiSeq platform) (Miya et al. 2020). Amplicon sequence variant methods have recently been developed in microbiology and have been used instead of the clustering method (grouping of DNA sequences whose similarity falls within a certain threshold—e.g., ≥97%), which lacks reusability, reproducibility, and comprehensiveness (Callahan et al. 2016). These methods infer the biological sequences in the sample before introducing amplification and sequencing errors, and they distinguish sequence variants that differ by as little as one nucleotide (Callahan et al. 2017). My research group currently incorporates this denoising process in a custom analysis pipeline, PMiFish (**https://github.com/rogotoh/PMiFish.git**), which has greatly improved the accuracy and reproducibility of taxon assignments (Miya et al. 2020).

Miya et al. (2015) pointed out that several methodological challenges must be addressed before the eDNA metabarcoding approach is likely to become mainstream technology in fish biodiversity research. One is the completeness and accuracy of the reference sequence database, as incomplete and inaccurate reference sequences result in false-negative and false-positive results. The current reference database is far from satisfactory, considering the enormous diversity of fish, which encompasses more than 32,000 known species in aquatic environments worldwide (Nelson et al. 2016). Indeed, as of March 13, 2021, my laboratory's custom reference sequence database (MiFish DB) included 15,584 sequences, covering approximately 8,523 fish species (25.3%) from 62 orders (100%), 489 families (95.7%), and 2,808 genera (54.8%). These taxonomic coverages are far from satisfactory for accurate taxonomic assignments, and database construction through international collaboration is needed. In this regard, it is worth noting that a joint initiative among universities and research institutes in the California Current region has built a reference database for MiFish eDNA metabarcoding, assembling reference sequences from 712 species among the 864 known species from the region (Gold et al. 2020). Stoeckle et al. (2020) recently reported that strengthening the reference sequences that cover most (74%) of the 341 fish species on the New Jersey checklist greatly improved eDNA metabarcoding detection, especially by including species that were overlooked by traditional surveys.

5. BIODIVERSITY ASSESSMENT IN SPACE AND TIME

5.1. Species Richness

Estimating the species richness of fish in marine ecosystems using traditional capture-based sampling or visual observation presents a number of difficulties (Oka et al. 2021). McElroy et al. (2020) synthesized 37 eDNA studies in aquatic ecosystems to compare estimates of fish community species richness between eDNA metabarcoding and conventional methods (e.g., netting, visual census, electrofishing). Based on critical comparisons, they argued that eDNA metabarcoding is reliable and provides a biodiversity assessment path that can outperform conventional methods for estimating species richness. In particular, eDNA metabarcoding detects small, cryptic, nocturnal, rare, or elusive species that are missed when conventional methods are used (Bessey et al. 2020, Closek et al. 2019, Mirimin et al. 2021), leading to a higher proportion of eDNA-only

detections in marine ecosystems (McElroy et al. 2020). Therefore, eDNA metabarcoding should play a significant role in the biodiversity assessment of highly diverse tropical fish communities, in which it is expected that more than 1,500 species occur, many of which do not have reference sequences for accurate species assignments (Juhel et al. 2020, Oka et al. 2021).

Recently, Juhel et al. (2020) collected 92 seawater samples from the center of the Coral Triangle, a diversity hot spot in the Indo-Pacific region, and performed a fish eDNA metabarcoding analysis. Although they were unable to assign species names to many molecular operational taxonomic units, they used the number of units to model accumulation curves and estimate the species richness of the region. They found that the asymptotic curve was saturated at 1,531, which was in concordance with the 1,611 species supposedly occurring in the area (Juhel et al. 2020). Concurrently, Sigsgaard et al. (2020) investigated the potential of eDNA metabarcoding for characterizing marine vertebrate communities at large spatial scales by comparing species compositions among six different habitats off Qatar in the Arabian Gulf. They detected 163 fish species, and they suggested that a few hundred eDNA samples could potentially capture more than 90% of the entire community, although the species accumulation curve analyses showed that the number of sample replicates was insufficient for some sampling sites. Subsequently, Oka et al. (2021) confirmed 217 fish species in a small lagoon near Okinawa Island, Japan, during 16 capture-based surveys, and they also detected 291 fish species in a subsequent eDNA metabarcoding study based on 11 water samples, identifying a total of 410 species. They found that two different approaches for estimating species richness based on eDNA data yielded values close to 410 species. Bessey et al. (2020) reported remarkable concordance between the species number found in cumulative fish species lists (~200 species) and through eDNA metabarcoding (209 species) from tropical coral reef ecosystems on a remote island off the west coast of Australia. However, the discrepancies between the two data sets were much larger than that of Oka et al. (2021): 127 species versus 395 species from 25 eDNA samples at Palmyra Atoll in the central Pacific (Lafferty et al. 2021) and 242 species versus 602 species from 242 eDNA samples from the Cocos Islands in the eastern North Indian Ocean (West et al. 2020).

These studies suggest that eDNA data have inherent predictability for species richness even in highly diverse tropical fish communities. However, the accuracy of the prediction should be verified by considering the size of the study area on different geographic scales and the corresponding sample size (Barneche et al. 2018).

5.2. Habitat Segregation

A local marine ecosystem consists of multiple patchy or adjacent habitats (Costello 2009). Fish can utilize these different habitats in diverse manners depending on their life stage and the season, such as the periods of reproduction, feeding, and nursing, which results in there being different fish communities in each habitat (Perry et al. 2018). For example, coral reefs and seagrass beds are often located adjacent to one another (Dorenbosch et al. 2005), and fish communities change along the coral reef–seagrass bed gradient (Fabricius et al. 2005). A number of empirical eDNA metabarcoding studies have detected such habitat signals from the component fish species, indicating high spatial turnover and site fidelity of eDNA to the source communities (Harrison et al. 2019, Oka et al. 2021) (**Figure 7**).

Port et al. (2016) were the first to detect such habitat segregation using an eDNA metabarcoding approach. They found that fish communities associated with seagrass microhabitats, kelp forests, rocky reefs, and open water were separated by as little as ~60 m. Subsequently, Yamamoto et al. (2017), Andruszkiewicz et al. (2017), and Stoeckle et al. (2017) reported habitat signals in a range of diverse fish communities in different marine ecosystems, including temperate coastal fish

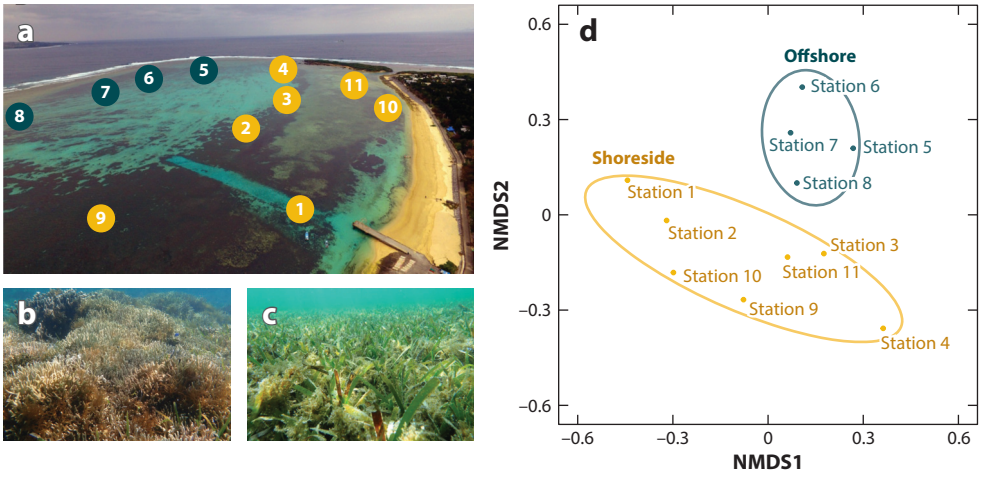

Figure 7

(*a–c*) The locations of 11 sampling stations (panel *a*) in a coral reef lagoon at Bise, Okinawa, Japan. Stations 5–8 (*green numbers*) were located along the offshore reef edge on a coral-dominated area (panel *b*), while stations 1–4 and 9–11 (*gold numbers*) were located on the shoreside seagrass beds (panel *c*). (*d*) Nonmetric multidimensional scaling (NMDS) of the fish communities. Figure adapted from Oka et al. (2021).

communities in a protected inner bay (seagrass beds versus other habitats), midwater fish communities in oceanic waters (horizontal and vertical zonation), and estuarine fish communities in an urban area (two adjacent river mouths), respectively.

Since then, many authors have reported the spatial turnover and site fidelity of eDNA to source fish communities in different marine ecosystems, including coral reefs (Bessey et al. 2020, Cole et al. 2021, Lafferty et al. 2021, Oka et al. 2021, Sigsgaard et al. 2020, Stat et al. 2018, West et al. 2020); temperate coastal waters (Cheang et al. 2020; Gold et al. 2021; Jeunen et al. 2019, 2021; Nester et al. 2020; van Bleijswijk et al. 2020); and offshore benthic, pelagic, and deep waters (Afzali et al. 2021, Fraija-Fernández et al. 2020, McClenaghan et al. 2020). In addition to these examples for horizontal habitat segregation of marine fish communities, Jeunen et al. (2020) detected two distinct fish communities separated by near-permanent water column stratification in the form of a strong halocline at ~3 m.

In marine ecosystems, however, ocean currents, water density, wind, and tides all combine to build a complex fabric of interacting currents (Harrison et al. 2019), likely leading to spillover of eDNA from adjacent habitats or ecosystems (Lafferty et al. 2021). Oka et al. (2021) performed eDNA metabarcoding in a small coral reef lagoon and found eDNA from a few lantern fish species that were undergoing diurnal vertical migration (Kaartvedt et al. 2019). These deep-sea midwater fishes cannot survive in shallow water during the daytime in hot temperatures (≥30°C), and eDNA from these fishes likely spills over into the lagoon at night when they migrate to the upper layer along the steep outer-reef slope. Lafferty et al. (2021) also noticed that some reef eDNA spilled over into adjacent sand flats, though the overwhelming signals were site specific.

5.3. Biogeographic Pattern

In addition to the detection of fish community changes across small spatial scales such as adjacent habitats, eDNA metabarcoding has been applied to detect a major marine biogeographic break and transitions that have been established over evolutionary timescales. West et al. (2021)

collected seawater samples from 71 sites along the coastline of northwestern Australia, where a marine biogeographic break is purported to occur between two regions. They performed eDNA metabarcoding analysis, confirming the biogeographic break and finding previously unrecognized subregional differentiation. They then argued that further applications of this technique could be used to potentially reveal marine biogeographic breaks in other regions, such as the marine Wallace's line found in the phylogeographic structure in mantis shrimp between the Indian and Pacific Ocean regions (Barber et al. 2000).

5.4. Spatiotemporal Dynamics

Unlike sessile organisms, fish move in response to daylight, temperature, capture, and predation (Stoeckle et al. 2017). Fish also undergo occasional, diurnal, and seasonal migrations for foraging, growth, and spawning, often involving long distances spanning a few thousand kilometers (Secor 2015). Monitoring fish movements using conventional capture-based methods is challenging, but eDNA metabarcoding has greatly improved the situation. Sigsgaard et al. (2017) and Stoeckle et al. (2017) collected seasonal eDNA samples from coastal and urban estuarine waters, respectively. They performed eDNA metabarcoding analyses with these samples, successfully capturing the seasonal changes in the fish communities, which were concordant with previous knowledge. Subsequently, using 52 weekly eDNA samples from an inner bay, Ushio et al. (2018a) estimated the relative abundances of the 10 most dominant fish species by developing a quantification method for eDNA metabarcoding (for details of the quantification method, see Section 5.7). They observed that the seasonal changes in the quantified eDNA were synchronous with the number of individuals estimated using underwater visual observations by scuba divers. eDNA metabarcoding analysis revealed seasonal changes in fish communities in estuaries (Jia et al. 2020, Zhang et al. 2019, Zou et al. 2020) and coastal waters (Stoeckle et al. 2020, 2021; van Bleijswijk et al. 2020). Since the species-specific detection method was able to identify the spawning grounds of Japanese eels in the oceanic water of the western North Pacific (Takeuchi et al. 2019b), the eDNA metabarcoding approach may be used in the future to search for spawning grounds and migratory routes (Takeuchi et al. 2019a), which are largely unknown ecological events for many fish species (Secor 2015).

5.5. Anthropogenic Impact

Anthropogenic disturbances, including intensive habitat modification, urbanization, and climate change, all have the potential to lead not only to altered species diversity within a community but also to an increased similarity in species compositions among communities (Iacarella et al. 2018). eDNA metabarcoding can detect such anthropogenic impacts on marine fish communities. As shown in **Figure 5**, two highly urbanized areas exhibit noticeably lower species numbers than nonurban areas at equivalent latitudes (Andriyono et al. 2019, Kamimura et al. 2018). Ahn et al. (2020) performed fish eDNA metabarcoding analyses for five estuaries, noting lowered species diversity in the two estuaries that were located in a metropolitan area (but see Kume et al. 2021). Bakker et al. (2017) and Boussarie et al. (2018) investigated tropical shark diversity using eDNA metabarcoding and demonstrated that geographic patterns of the diversity coincided with anthropogenic pressure levels and conservation effort. Clementi et al. (2021) investigated fish species diversity in the greater Caribbean using eDNA metabarcoding and observed that moray eels were more common in coral reefs subjected to high human pressure (e.g., due to the depletion of large predatory sharks by fishing activity). Together, these observations indicate the usefulness of eDNA metabarcoding for detecting various anthropogenic impacts on fish community structures in a range of diverse marine ecosystems.

5.6. Functional Diversity

Although most biodiversity surveys using eDNA metabarcoding have focused on taxonomic diversity, the diversity of ecological roles (functional diversity) is also essential to understand how ecosystems respond to natural and anthropogenic perturbations (Aglieri et al. 2021, D'agata et al. 2014). Aglieri et al. (2021) recently investigated both taxonomic and functional diversity using eDNA metabarcoding in Mediterranean fish communities and compared its efficiency with that of three traditional survey methods. They showed that the choice of the survey method can influence ecological conclusions from biodiversity studies and that eDNA is well suited for capturing most of the functional fish diversity in coastal marine environments. Besides the advantages of ease of sampling and freedom from taxonomic expertise, this new monitoring tool appears to boost the collection of complex information from marine environments, including their functional dimensions (Aglieri et al. 2021).

5.7. Quantification

The results from eDNA metabarcoding analysis can provide taxonomic tables with read abundances. However, read abundances are greatly affected by the amplification efficiency of the PCR primers (Kelly et al. 2019), making it difficult to compare read abundances between different samples and studies. Ushio et al. (2018a) recently attempted to quantify fish eDNA copy numbers during eDNA metabarcoding by adding four internal standard DNA sequences with known concentrations to the eDNA samples. Using this method, they demonstrated the temporal dynamics of the 10 most abundant fish species in an inner bay based on 53 weekly samples, while acknowledging that their results required the correction of species-specific PCR amplification biases for between-species comparisons. More recently, the quantitative sequencing technique (Hoshino & Inagaki 2017) has been applied to fish eDNA metabarcoding, and Hoshino et al. (2021) demonstrated its effectiveness using five freshwater fish species kept in a mesocosm.

5.8. New Biological Sampler

Filtration of large water volumes (e.g., >1 L) would be a simple and effective way to collect more eDNA from aquatic environments with scarce fish abundance and biomass, such as in open-ocean and deep-sea ecosystems (Miya et al. 2016). Mariani et al. (2019) used sponges (phylum Porifera) as natural eDNA samplers, as they are known as the most effective water filterers since they can sift up to 10,000 L of water per day (Kahn et al. 2015). They collected five Antarctic and four Mediterranean sponge samples, from which eDNA was extracted and subjected to eDNA metabarcoding. The Antarctic samples comprised five typical Antarctic notothenioid fishes (icefishes), while the Mediterranean samples comprised common fishes of the region. Subsequently, Turon et al. (2020) confirmed the utility of sponges as natural samplers in eutrophic and well-preserved coral reefs in a tropical bay by detecting the differentiation of fish community structures between the two habitats.

6. CONCLUDING REMARKS

This article has reviewed the current status of eDNA metabarcoding studies conducted on fish in diverse marine ecosystems around the world. It was based on 50 selected studies, and these studies commonly consider the detection of the source organisms' eDNA to be a proxy for their presence. As such, I have assembled circumstantial evidence based on a controlled field experiment (Murakami et al. 2019) and detection of microhabitat segregation (e.g., Port et al. 2016) that links the eDNA detections with the actual presence of fish species. These lines of evidence

strongly indicate the site fidelity of eDNA to the source fish communities in different marine environments, justifying the use of eDNA metabarcoding for the study of marine fish communities. Nevertheless, proof-of-concept research should be continued to fill the knowledge gap between eDNA detection and the presence of source organisms in various marine ecosystems. It should also be recognized that eDNA represents indirect, extraorganismal genetic markers shed from macroorganisms and that any eDNA-based estimations necessarily involve errors such as false negatives and false positives. These errors can be introduced at any stage of the eDNA metabarcoding workflow, from water sampling through molecular experiments to bioinformatic analyses (**Figure 1**), and should be mitigated by future technical developments of eDNA metabarcoding.

Despite these inherent limitations, eDNA metabarcoding of marine fishes can (*a*) predict the species richness in a given area with a sufficient number of samples (e.g., Juhel et al. 2020), (*b*) detect the habitat segregation of fish communities within a range of diverse local ecosystems (e.g., Port et al. 2016), (*c*) find the known and unknown biogeographic breaks in an extended spatial scale across several geographic regions (West et al. 2021), and (*d*) track temporal changes in fish communities (e.g., Sigsgaard et al. 2017, Stoeckle et al. 2017) and migration routes and spawning grounds (e.g., Takeuchi et al. 2019a); in addition to these taxonomic diversity assessments at various spatiotemporal scales, it can (*e*) detect the anthropogenic impact on fish communities (e.g., Bakker et al. 2017) and (*f*) reveal the functional roles of component fish species (i.e., functional diversity; Aglieri et al. 2021). Furthermore, two quantification methods can allow the read abundances from eDNA metabarcoding studies to be compared between samples (Hoshino et al. 2021, Ushio et al. 2018a). Quantitative eDNA metabarcoding represents a breakthrough, although many challenges need to be overcome to correlate the eDNA concentration with absolute abundance or biomass of the multiple target fish species (Rees et al. 2014). This is partly because eDNA itself does not contain any information on the size, number, age, weight, life-history stages, or (in most cases) sex of individuals, all of which are available from specimens collected by traditional capture-based sampling (Hansen et al. 2018). Finally, a new biological sampler (sponges) allows the filtration of a large volume of water and would be expected to enable comprehensive detection of highly diverse fish communities (Mariani et al. 2019).

It appears that eDNA metabarcoding demonstrates its strength when continuous biodiversity monitoring is performed at multiple sites. In this way, we can quickly detect spatial and temporal changes in ecosystems, such as biodiversity loss or invasion of alien species, and take immediate measures to resolve such environmental issues. However, several technical challenges remain before eDNA biodiversity monitoring can be performed on a scale comparable to the monitoring of physical attributes. For example, it is essential to further develop environmental sample processors (Scholin et al. 2017), autonomous robotic devices that can be programmed to automate water sample filtration and preservation of the captured material for immediate analyses in situ (Fukuba et al. 2019, Yamahara et al. 2019). Furthermore, such instrumentation should be cost effective when installed in large numbers over a wide area. Instead, it may be possible to perform such frequent, multisite eDNA sampling with citizens (Deiner et al. 2017, Larson et al. 2020, Meyer et al. 2021).

By combining on-site eDNA sampling and sample processing automation with the rapid developments in molecular analysis technology and quantitative eDNA metabarcoding (Ushio et al. 2018a), one could generate a large quantitative data set to capture the fluctuating interspecific interactions among component species in fish communities (Ushio et al. 2018b). If such complex and dynamic interspecific interactions can be modeled using a nonlinear method (e.g., empirical dynamic modeling), it may be possible to forecast fish community dynamics from time series data (Chang et al. 2017). Such a forecast would be useful for marine ecosystem conservation and

the sustainable use of marine fishery resources, which would help meet the individual targets of Sustainable Development Goal 14 (UN 2021).

DISCLOSURE STATEMENT

The author is not aware of any affiliations, memberships funding, or financial holdings that might affect the objectivity of this review.

ACKNOWLEDGMENTS

I would like to thank Stephen J. Giovannoni and the other members of the *Annual Review of Marine Science* Editorial Committee for inviting me to write this review. I would not have had such an opportunity without support from the Core Research for Evolutional Science and Technology (CREST) program (JPMJCR13A2) of the Japan Science and Technology Agency; the Canon Foundation; the Environment Research and Technology Development Fund (4-1602, JPMEERF20S20720) of the Japanese Ministry of the Environment; the Grant-in-Aid for Scientific Research (KAKENHI) program (JP19H03291) of the Japan Society for the Promotion of Science; and the Ocean Resource Use Promotion Technology Development Program (JPMXD0618068274) of the Japanese Ministry of Education, Culture, Sports, Science, and Technology. K. Fukaya, H. Murakami, S. Oka, M. Sakata, and M. Ushio kindly read an earlier version of this article and provided useful comments. A number of comments and edits from an anonymous reviewer and Jim Duncan were very helpful in improving the manuscript.

LITERATURE CITED

Afzali SF, Bourdages H, Laporte M, Mérot C, Normandeau E, et al. 2021. Comparing environmental metabarcoding and trawling survey of demersal fish communities in the Gulf of St. Lawrence, Canada. *Environ. DNA* 3:22–42

Aglieri G, Baillie C, Mariani S, Cattano C, Calò A, et al. 2021. Environmental DNA effectively captures functional diversity of coastal fish communities. *Mol. Ecol.* 31:3127–39

Ahn H, Kume M, Terashima Y, Ye F, Kameyama S, et al. 2020. Evaluation of fish biodiversity in estuaries using environmental DNA metabarcoding. *PLOS ONE* 15:e0231127

Andriyono S, Alam MJ, Kim H-W. 2019. Environmental DNA (eDNA) metabarcoding: diversity study around the Pondok Dadap fish landing station, Malang, Indonesia. *Biodiversitas* 20:3772–81

Andruszkiewicz EA, Starks HA, Chavez FP, Sassoubre LM, Block BA, Boehm AB. 2017. Biomonitoring of marine vertebrates in Monterey Bay using eDNA metabarcoding. *PLOS ONE* 12:e0176343

Bakker J, Wangensteen OS, Chapman DD, Boussarie G, Buddo D, et al. 2017. Environmental DNA reveals tropical shark diversity in contrasting levels of anthropogenic impact. *Sci. Rep.* 7:16886

Barber P, Palumbi S, Erdmann M, Moosa M. 2000. A marine Wallace's line? *Nature* 406:692–93

Barneche DR, Rezende EL, Parravicini V, Maire E, Edgar GJ, et al. 2018. Body size, reef area and temperature predict global reef-fish species richness across spatial scales. *Glob. Ecol. Biogeogr.* 28:315–27

Barnes MA, Turner CR. 2016. The ecology of environmental DNA and implications for conservation genetics. *Conserv. Genet.* 17:1–17

Beng KC, Corlett RT. 2020. Applications of environmental DNA (eDNA) in ecology and conservation: opportunities, challenges and prospects. *Biodivers. Conserv.* 29:2089–121

Bessey C, Jarman SN, Berry O, Olsen YS, Bunce M, et al. 2020. Maximizing fish detection with eDNA metabarcoding. *Environ. DNA* 2:493–504

Bhaduri A, Bogardi J, Siddiqi A, Voigt H, Vörösmarty C, et al. 2016. Achieving Sustainable Development Goals from a water perspective. *Front. Environ. Sci.* 4:64

Bohmann K, Evans A, Gilbert MTP, Carvalho GR, Creer S, et al. 2014. Environmental DNA for wildlife biology and biodiversity monitoring. *Trends Ecol. Evol.* 29:358–67

Boussarie G, Bakker J, Wangensteen OS, Mariani S, Bonnin L, et al. 2018. Environmental DNA illuminates the dark diversity of sharks. *Sci. Adv.* 2:e23297

Burke L, Kura Y, Kassem K, Revenga C, Spalding M, McAllister D. 2001. *Coastal ecosystems*. Rep., World Resour. Inst., Washington, DC

Bylemans J, Gleeson DM, Lintermans M, Gilligan DM, Hardy CM, et al. 2018. Monitoring riverine fish communities through eDNA metabarcoding: determining optimal sampling strategies along an altitudinal and biodiversity gradient. *Metabarcoding Metagenom*. 2:e30457

Callahan BJ, McMurdie PJ, Holmes SP. 2017. Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. *ISME J.* 11:2639–43

Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. 2016. DADA2: high-resolution sample inference from Illumina amplicon data. *Nat. Methods* 13:581–83

Chang C-W, Ushio M, Hsieh C. 2017. Empirical dynamic modeling for beginners. *Ecol. Res.* 32:785–96

Cheang C, Lee B, Ip BH, Yiu W, Tsang L, Ang PO. 2020. Fish and crustacean biodiversity in an outer maritime estuary of the Pearl River Delta revealed by environmental DNA. *Mar. Pollut. Bull.* 161:111707

Chee SY, Othman AG, Sim YK, Adam ANM, Firth LB. 2017. Land reclamation and artificial islands: walking the tightrope between development and conservation. *Glob. Ecol. Conserv.* 12:80–95

Clementi GM, Bakker J, Flowers KI, Postaire BD, Babcock EA, et al. 2021. Moray eels are more common on coral reefs subject to higher human pressure in the greater Caribbean. *iScience* 24:102097

Closek CJ, Santora JA, Starks HA, Schroeder ID, Andruszkiewicz EA, et al. 2019. Marine vertebrate biodiversity and distribution within the central California Current using environmental DNA (eDNA) metabarcoding and ecosystem surveys. *Front. Mar. Sci.* 6:732

Coissac E, Riaz T, Puillandre N. 2012. Bioinformatic challenges for DNA metabarcoding of plants and animals. *Mol. Ecol.* 21:1834–47

Cole VJ, Harasti D, Lines R, Stat M. 2021. Estuarine fishes associated with intertidal oyster reefs characterized using environmental DNA and baited remote underwater video. *Environ. DNA.* **https://doi.org/10.1002/edn3.190**

Collins RA, Bakker J, Wangensteen OS, Soto AZ, Corrigan L, et al. 2019. Non-specific amplification compromises environmental DNA metabarcoding with COI. *Methods Ecol. Evol.* 10:1985–2001

Colwell RK. 2009. Biodiversity: concepts, patterns, and measurement. In *The Princeton Guide to Ecology*, ed. SA Levin, SR Carpenter, HCJ Godfray, AP Kinzig, M Loreau, et al., pp. 257–63. Princeton, NJ: Princeton Univ. Press

Costello MJ. 2009. Distinguishing marine habitat classification concepts for ecological data management. *Mar. Ecol. Prog. Ser.* 397:253–68

Costello MJ, Chaudhary C. 2017. Marine biodiversity, biogeography, deep-sea gradients, and conservation. *Curr. Biol.* 27:R511–27

Costello MJ, Cheung A, Hauwere ND. 2010. Surface area and the seabed area, volume, depth, slope, and topographic variation for the world's seas, oceans, and countries. *Environ. Sci. Technol.* 44:8821–28

Cristescu ME, Hebert PDN. 2018. Uses and misuses of environmental DNA in biodiversity science and conservation. *Annu. Rev. Ecol. Evol. Syst.* 49:209–30

D'agata S, Mouillot D, Kulbicki M, Andréfouët S, Bellwood DR, et al. 2014. Human-mediated loss of phylogenetic and functional diversity in coral reef fishes. *Curr. Biol.* 24:555–60

Deagle BE, Jarman SN, Coissac E, Pompanon F, Taberlet P. 2014. DNA metabarcoding and the cytochrome *c* oxidase subunit I marker: not a perfect match. *Biol. Lett.* 10:20140562

Deiner K, Bik HM, Mächler E, Seymour M, Roussel AL, et al. 2017. Environmental DNA metabarcoding: transforming how we survey animal and plant communities. *Mol. Ecol.* 26:5872–95

DiBattista JD, Coker DJ, Sinclair-Taylor TH, Stat M, Berumen ML, Bunce M. 2017. Assessing the utility of eDNA as a tool to survey reef-fish communities in the Red Sea. *Coral Reefs*. 36:1245–52

Doi H, Fukaya K, Oka S, Sato K, Kondoh M, Miya M. 2019. Evaluation of detection probabilities at the water-filtering and initial PCR steps in environmental DNA metabarcoding using a multispecies site occupancy model. *Sci. Rep.* 9:3581

Dorenbosch M, Grol M, Nagelkerken I, van der Velde G. 2005. Distribution of coral reef fishes along a coral reef-seagrass gradient: edge effects and habitat segregation. *Mar. Ecol. Prog. Ser.* 299:277–88

Edgar RC. 2016. UNOISE2: improved error-correction for Illumina 16S and ITS amplicon sequencing. bioRxiv 081257. **https://doi.org/10.1101/081257**

eDNA Soc. 2019. *Environmental DNA Sampling and Experiment Manual: Version 2.1*. Otsu, Jpn.: eDNA Soc. **http://ednasociety.org/en/manual**

Eschmeyer WN, Fricke R, Fong JD, Polack DA. 2010. Marine fish diversity: history of knowledge and discovery (Pisces). *Zootaxa* 2525:19–50

Fabricius K, De'ath G, McCook L, Turak E, Williams DM. 2005. Changes in algal, coral and fish assemblages along water quality gradients on the inshore Great Barrier Reef. *Mar. Pollut. Bull.* 51:384–98

Fediajevaite J, Priestley V, Arnold R, Savolainen V. 2021. Meta-analysis shows that environmental DNA outperforms traditional surveys, but warrants better reporting standards. *Ecol. Evol.* 11:4803–15

Ficetola GF, Pansu J, Bonin A, Coissac E, Giguet-Covex C, et al. 2015. Replication levels, false presences and the estimation of the presence/absence from eDNA metabarcoding data. *Mol. Ecol. Resour.* 15:543–56

Fraija-Fernández N, Bouquieaux M, Rey A, Mendibil I, Cotano U, et al. 2020. Marine water environmental DNA metabarcoding provides a comprehensive fish diversity assessment and reveals spatial patterns in a large oceanic area. *Ecol. Evol.* 10:7560–84

Fujikura K, Lindsay D, Kitazato H, Nishida S, Shirayama Y, Schnur J. 2010. Marine biodiversity in Japanese waters. *PLOS ONE* 5:e11836

Fukaya K, Murakami H, Yoon S, Minami K, Osada Y, et al. 2021. Estimating fish population abundance by integrating quantitative data on environmental DNA and hydrodynamic modelling. *Mol. Ecol.* 30:3057–67

Fukuba T, Sano Y, Yamamoto H, Miwa T, Fujii T. 2019. Development, deployment and improvement of miniaturized in situ bio/biochemical analysis systems towards multi-modal ocean sensing. In *2019 IEEE Underwater Technology*. Piscataway, NJ: IEEE. **https://doi.org/10.1109/UT.2019.8734416**

Gilbey J, Carvalho G, Castilho R, Coscia I, Coulson MW, et al. 2021. Life in a drop: sampling environmental DNA for marine fishery management and ecosystem monitoring. *Mar. Policy* 124:104331

Gold Z, Choi E, Kacev D, Frable B, Burton R, et al. 2020. FishCARD: fish 12S California Current specific reference database for enhanced metabarcoding efforts. Authorea 159136805.55528691. **https://doi.org/10.22541/au.159136805.55528691**

Gold Z, Sprague J, Kushner DJ, Zerecero Marin E, Barber PH. 2021. eDNA metabarcoding as a biomonitoring tool for marine protected areas. *PLOS ONE* 16:e0238557

Goldberg CS, Turner CR, Deiner K, Klymus KE, Thomsen PF, et al. 2016. Critical considerations for the application of environmental DNA methods to detect aquatic species. *Methods Ecol. Evol.* 7:1299–307

Hansen BK, Bekkevold D, Clausen LW, Nielsen EE. 2018. The sceptical optimist: challenges and perspectives for the application of environmental DNA in marine fisheries. *Fish Fish*. 19:751–68

Harrison JB, Sunday JM, Rogers SM. 2019. Predicting the fate of eDNA in the environment and implications for studying biodiversity. *Proc. R. Soc. B* 286:20191409

Heinen JH, Rahbek C, Borregaard MK. 2020. Conservation of species interactions to achieve self-sustaining ecosystems. *Ecography* 43:1603–11

Hoshino T, Inagaki F. 2017. Application of stochastic labeling with random-sequence barcodes for simultaneous quantification and sequencing of environmental 16S rRNA genes. *PLOS ONE* 12:e0169431

Hoshino T, Nakao R, Doi H, Minamoto T. 2021. Simultaneous absolute quantification and sequencing of fish environmental DNA in a mesocosm by quantitative sequencing technique. *Sci. Rep.* 11:4372

Iacarella JC, Adamczyk E, Bowen D, Chalifour L, Eger A, et al. 2018. Anthropogenic disturbance homogenizes seagrass fish communities. *Glob. Change Biol.* 24:1904–18

Jeunen G-J, Knapp M, Spencer HG, Lamare MD, Taylor HR, et al. 2019. Environmental DNA (eDNA) metabarcoding reveals strong discrimination among diverse marine habitats connected by water movement. *Mol. Ecol. Resour.* 19:426–38

Jeunen G-J, Lamare MD, Knapp M, Spencer HG, Taylor HR, et al. 2020. Water stratification in the marine biome restricts vertical environmental DNA (eDNA) signal dispersal. *Environ. DNA* 2:99–111

Jeunen G-J, Urban L, Lewis R, Knapp M, Lamare M, et al. 2021. Marine environmental DNA (eDNA) for biodiversity assessments: a one-to-one comparison between eDNA and baited remote underwater video (BRUV) surveys. Authorea 160278512.26241559. **https://doi.org/10.22541/au.160278512.26241559/v1**

Jia H, Wang Y, Yoshizawa S, Iwasaki W, Li Y, et al. 2020. Seasonal variation and assessment of fish resources in the Yangtze Estuary based on environmental DNA. *Water* 12:2874
Jollivet D. 1996. Specific and genetic diversity at deep-sea hydrothermal vents: an overview. *Biodivers. Conserv.* 5:1619–53
Juhel J-B, Utama RS, Marques V, Vimono IB, Sugeha HY, et al. 2020. Accumulation curves of environmental DNA sequences predict coastal fish diversity in the coral triangle. *Proc. R. Soc. B* 287:20200248
Kaartvedt S, Langbehn TJ, Aksnes DL. 2019. Enlightening the ocean's twilight zone. *ICES J. Mar. Sci.* 76:803–12
Kahn AS, Yahel G, Chu JWF, Tunnicliffe V, Leys SP. 2015. Benthic grazing and carbon sequestration by deep-water glass sponge reefs. *Limnol. Oceanogr.* 60:78–88
Kamimura S, Kozuki Y, Otani S, Hirakawa R, Iwami K, et al. 2018. Fish diversity detection at port and urban canal area using environmental DNA metabarcoding. *J. Jpn. Soc. Civil Eng. B3* 74:474–79
Kawato M, Yoshida T, Miya M, Tsuchida S, Nagano Y, et al. 2021. Optimization of environmental DNA extraction and amplification methods for metabarcoding of deep-sea fish. *MethodsX* 8:101238
Kelly RP, Gallego R, Jacobs-Palmer E. 2018. The effect of tides on nearshore environmental DNA. *PeerJ* 6:e4521
Kelly RP, Shelton AO, Gallego R. 2019. Understanding PCR processes to draw meaningful conclusions from environmental DNA studies. *Sci. Rep.* 9:12133
Kume M, Lavergne E, Ahn H, Terashima Y, Kadowaki K, et al. 2021. Factors structuring estuarine and coastal fish communities across Japan using environmental DNA metabarcoding. *Ecol. Indic.* 121:107216
Lafferty KD, Garcia-Vedrenne AE, McLaughlin JP, Childress JN, Morse MF, Jerde CL. 2021. At Palmyra Atoll, the fish-community environmental DNA signal changes across habitats but not with tides. *J. Fish Biol.* 98:415–25
Larson ER, Graham BM, Achury R, Coon JJ, Daniels MK, et al. 2020. From eDNA to citizen science: emerging tools for the early detection of invasive species. *Front. Ecol. Environ.* 18:194–202
Mariani S, Baillie C, Colosimo G, Riesgo A. 2019. Sponges as natural environmental DNA samplers. *Curr. Biol.* 29:R401–2
McClenaghan B, Fahner N, Cote D, Chawarski J, McCarthy A, et al. 2020. Harnessing the power of eDNA metabarcoding for the detection of deep-sea fishes. *PLOS ONE* 15:e0236540
McElroy ME, Dressler TL, Titcomb GC, Wilson EA, Deiner K, et al. 2020. Calibrating environmental DNA metabarcoding to conventional surveys for measuring fish species richness. *Front. Ecol. Evol.* 8:276
Meyer R, Ramos M, Lin M, Schweizer T, Gold Z, et al. 2021. The CALeDNA program: Citizen scientists and researchers inventory California's biodiversity. *Calif. Agric.* 75:20–32
Min M, Barber P, Gold Z. 2020. MiSebastes: an eDNA metabarcoding primer set for rockfishes (genus *Sebastes*). bioRxiv 2020.10.29.360859. **https://doi.org/10.1101/2020.10.29.360859**
Mirimin L, Desmet S, Romero DL, Fernandez SF, Miller DL, et al. 2021. Don't catch me if you can – using cabled observatories as multidisciplinary platforms for marine fish community monitoring: an in situ case study combining underwater video and environmental DNA data. *Sci. Total Environ.* 773:145351
Miya M, Gotoh RO, Sado T. 2020. MiFish metabarcoding: a high-throughput approach for simultaneous detection of multiple fish species from environmental DNA and other samples. *Fish. Sci.* 86:939–70
Miya M, Minamoto T, Yamanaka H, Oka S, Sato K, et al. 2016. Use of a filter cartridge for filtration of water samples and extraction of environmental DNA. *J. Vis. Exp.* 117:e54741
Miya M, Sado T. 2019. Multiple species detection using MiFish primers. See eDNA Soc. 2019, pp. 55–92
Miya M, Sato Y, Fukunaga T, Sado T, Poulsen JY, et al. 2015. 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.* 2:150088
Monti F, Duriez O, Dominici J-M, Sforzi A, Robert A, et al. 2018. The price of success: integrative long-term study reveals ecotourism impacts on a flagship species at a UNESCO site. *Anim. Conserv.* 21:448–58
Morita K, Sahashi G, Miya M, Kamada S, Kanbe T, Araki H. 2019. Ongoing localized extinctions of stream-dwelling white-spotted charr populations in small dammed-off habitats of Hokkaido Island, Japan. *Hydrobiologia* 840:207–13
Murakami H, Yoon S, Kasai A, Minamoto T, Yamamoto S, et al. 2019. Dispersion and degradation of environmental DNA from caged fish in a marine environment. *Fish. Sci.* 85:327–37

Nelson JS, Grande TC, Wilson MV. 2016. *Fishes of the World*. Hoboken, NJ: Wiley & Sons. 5th ed.
Nester GM, Brauwer MD, Koziol A, West KM, DiBattista JD, et al. 2020. Development and evaluation of fish eDNA metabarcoding assays facilitate the detection of cryptic seahorse taxa (family: Syngnathidae). *Environ. DNA* 2:614–26
O'Donnell JL, Kelly RP, Lowell NC, Port JA. 2016. Indexed PCR primers induce template-specific bias in large-scale DNA sequencing studies. *PLOS ONE* 11:e0148698
Ojha SN, Babu SC. 2019. Why convergence of fisheries co-management with agricultural technology management agency is significant. In *Agricultural Extension Reforms in South Asia: Status, Challenges, and Policy Options*, ed. SC Babu, PK Joshi, pp. 329–47. San Diego, CA: Academic
Oka S, Doi H, Miyamoto K, Hanahara N, Sado T, Miya M. 2021. Environmental DNA metabarcoding for biodiversity monitoring of a highly diverse tropical fish community in a coral reef lagoon: estimation of species richness and detection of habitat segregation. *Environ. DNA* 3:55–69
Perry D, Staveley TAB, Gullström M. 2018. Habitat connectivity of fish in temperate shallow-water seascapes. *Front. Mar. Sci.* 4:440
Pikitch EK, Santora C, Babcock EA, Bakun A, Bonfil R, et al. 2014. Ecosystem-based fishery management. *Science* 305:346–47
Port JA, O'Donnell JL, Romero-Maraccini OC, Leary PR, Litvin SY, et al. 2016. Assessing vertebrate biodiversity in a kelp forest ecosystem using environmental DNA. *Mol. Ecol.* 25:527–41
Rees HC, Maddison BC, Middleditch DJ, Patmore JRM, Gough KC. 2014. The detection of aquatic animal species using environmental DNA—a review of eDNA as a survey tool in ecology. *J. Appl. Ecol.* 51:1450–59
Riser SC, Freeland HJ, Roemmich D, Wijffels S, Troisi A, et al. 2016. Fifteen years of ocean observations with the global Argo array. *Nat. Clim. Change* 6:145–53
Rohde K, Heap M, Heap D. 1993. Rapoport's rule does not apply to marine teleosts and cannot explain latitudinal gradients in species richness. *Am. Nat.* 142:1–16
Ruppert KM, Kline RJ, Rahman MS. 2019. Past, present, and future perspectives of environmental DNA (eDNA) metabarcoding: a systematic review in methods, monitoring, and applications of global eDNA. *Glob. Ecol. Conserv.* 17:e00547
Sachs JD. 2012. From Millennium Development Goals to Sustainable Development Goals. *Lancet* 379:2206–11
Sato Y, Miya M, Fukunaga T, Sado T, Iwasaki W. 2018. MitoFish and MiFish pipeline: a mitochondrial genome database of fish with an analysis pipeline for environmental DNA metabarcoding. *Mol. Biol. Evol.* 10:421–23
Scholin CA, Birch J, Jensen S, Marin R III, Massion E, et al. 2017. The quest to develop ecogenomic sensors: a 25-year history of the environmental sample processor (ESP) as a case study. *Oceanography* 30(4):100–13
Secor DH. 2015. *Migration Ecology of Marine Fishes.* Baltimore, MD: John Hopkins Univ. Press
Sergio F, Newton I, Marchesi L, Pedrini P. 2006. Ecologically justified charisma: preservation of top predators delivers biodiversity conservation. *J. Appl. Ecol.* 43:1049–55
Shiklomanov IA. 1993. World fresh water resources. In *Water in Crisis: A Guide to the World's Fresh Water Resources*, ed. PH Gleick, pp. 13–24. New York: Oxford Univ. Press
Shu L, Ludwig A, Peng Z. 2021. Environmental DNA metabarcoding primers for freshwater fish detection and quantification: in silico and in tanks. *Ecol. Evol.* 11:8281–94
Sigsgaard EE, Nielsen IB, Carl H, Krag MA, Knudsen SW, et al. 2017. Seawater environmental DNA reflects seasonality of a coastal fish community. *Mar. Biol.* 164:128
Sigsgaard EE, Torquato F, Frøslev TG, Moore ABM, Sørensen JM, et al. 2020. Using vertebrate environmental DNA from seawater in biomonitoring of marine habitats. *Conserv. Biol.* 34:697–710
Spens J, Evans AR, Halfmaerten D, Knudsen SW, Sengupta ME, et al. 2017. Comparison of capture and storage methods for aqueous macrobial eDNA using an optimized extraction protocol: advantage of enclosed filter. *Methods Ecol. Evol.* 8:635–45
Stat M, Huggett MJ, Bernasconi R, DiBattista JD, Berry TE, et al. 2017. Ecosystem biomonitoring with eDNA: metabarcoding across the tree of life in a tropical marine environment. *Sci. Rep.* 7:12240
Stat M, John J, DiBattista JD, Newman SJ, Bunce M, Harvey ES. 2018. Combined use of eDNA metabarcoding and video surveillance for the assessment of fish biodiversity. *Conserv. Biol.* 33:196–205

Stoeckle MY, Adolf J, Charlop-Powers Z, Dunton KJ, Hinks G, VanMorter SM. 2021. Trawl and eDNA assessment of marine fish diversity, seasonality, and relative abundance in coastal New Jersey, USA. *ICES J. Mar. Sci.* 78:293–304

Stoeckle MY, Mishu MD, Charlop-Powers Z. 2020. Improved environmental DNA reference library detects overlooked marine fishes in New Jersey, United States. *Front. Mar. Sci.* 7:226

Stoeckle MY, Soboleva L, Charlop-Powers Z. 2017. Aquatic environmental DNA detects seasonal fish abundance and habitat preference in an urban estuary. *PLOS ONE* 12:e0175186

Taberlet P, Bonin A, Coissac E, Zinger L. 2018. *Environmental DNA: For Biodiversity Research and Monitoring*. Oxford, UK: Oxford Univ. Press

Taberlet P, Coissac E, Hajibabaei M, Rieseberg LH. 2012. Environmental DNA. *Mol. Ecol.* 21:1789–93

Takeuchi A, Sado T, Gotoh RO, Watanabe S, Tsukamoto K, Miya M. 2019a. New PCR primers for metabarcoding environmental DNA from freshwater eels, genus *Anguilla*. *Sci. Rep*. 9:7977

Takeuchi A, Watanabe S, Yamamoto S, Miller M, Fukuba T, et al. 2019b. First use of oceanic environmental DNA to study the spawning ecology of the Japanese eel *Anguilla japonica*. *Mar. Ecol. Prog. Ser.* 609:187–96

Thomsen PF, Kielgast J, Iversen LL, Møller PR, Rasmussen M, Willerslev E. 2012. Detection of a diverse marine fish fauna using environmental DNA from seawater samples. *PLOS ONE* 7:e41732

Thomsen PF, Møller PR, Sigsgaard EE, Knudsen SW, Jørgensen OA, Willerslev E. 2016. Environmental DNA from seawater samples correlate with trawl catches of subarctic, deepwater fishes. *PLOS ONE* 11:e0165252

Thomsen PF, Willerslev E. 2015. Environmental DNA—an emerging tool in conservation for monitoring past and present biodiversity. *Biol. Conserv.* 183:4–18

Turon M, Angulo-Preckler C, Antich A, Præbel K, Wangensteen OS. 2020. More than expected from old sponge samples: a natural sampler DNA metabarcoding assessment of marine fish diversity in Nha Trang Bay (Vietnam). *Front. Mar. Sci.* 7:605148

UN. 2021. Sustainable development. *UN Department of Economic and Social Affairs*. **https://sustainabledevelopment.un.org**

Ushio M, Hiroaki M, Masuda R, Sado T, Miya M, et al. 2018a. Quantitative monitoring of multispecies fish environmental DNA using high-throughput sequencing. *Metabarcoding Metagenom*. 2:e23297

Ushio M, Hsieh C, Masuda R, Deyle ER, Ye H, et al. 2018b. Fluctuating interaction network and time-varying stability of a natural fish community. *Nature* 554:360–63

van Bleijswijk JDL, Engelmann JC, Klunder L, Witte HJ, Witte JIJ, Veer HW. 2020. Analysis of a coastal North Sea fish community: comparison of aquatic environmental DNA concentrations to fish catches. *Environ. DNA* 2:429–45

Wang S, Yan Z, Hänfling B, Zheng X, Wang P, et al. 2021. Methodology of fish eDNA and its applications in ecology and environment. *Sci. Total Environ.* 755:142622

Ward RD, Hanner R, Hebert PD. 2009. The campaign to DNA barcode all fishes, FISH-BOL. *J. Fish Biol.* 74:329–56

West KM, Stat M, Harvey ES, Skepper CL, DiBattista JD, et al. 2020. eDNA metabarcoding survey reveals fine-scale coral reef community variation across a remote, tropical island ecosystem. *Mol. Ecol.* 29:1069–86

West KM, Travers MJ, Stat M, Harvey ES, Richards ZT, et al. 2021. Large-scale eDNA metabarcoding survey reveals marine biogeographic break and transitions over tropical north-western Australia. *Divers. Distrib.* 27:1942–57

Wong MK-S, Nakao M, Hyodo S. 2020. Field application of an improved protocol for environmental DNA extraction, purification, and measurement using Sterivex filter. *Sci. Rep.* 10:21531

Yamahara KM, Preston CM, Birch J, Walz K, Marin R, et al. 2019. In situ autonomous acquisition and preservation of marine environmental DNA using an autonomous underwater vehicle. *Front. Mar. Sci.* 6:373

Yamamoto S, Masuda R, Sato Y, Sado T, Araki H, et al. 2017. Environmental DNA metabarcoding reveals local fish communities in a species-rich coastal sea. *Sci. Rep.* 7:40368

Yamamoto S, Minami K, Fukaya K, Takahashi K, Sawada H, et al. 2016. Environmental DNA as a 'snapshot' of fish distribution: a case study of Japanese jack mackerel in Maizuru Bay, Sea of Japan. *PLOS ONE* 11:e0149786

Yamanoue Y, Miya M, Matsuura K, Miyazawa S, Tsukamoto N, et al. 2009. Explosive speciation of *Takifugu*: another use of fugu as a model system for evolutionary biology. *Mol. Biol. Evol.* 26:623–29

Zabel RW, Harvey CJ, Katz SL, Good TP, Levin PS. 2003. Ecologically sustainable yield. *Am. Sci.* 91:150–57

Zhang H, Yoshizawa S, Iwasaki W, Xian W. 2019. Seasonal fish assemblage structure using environmental DNA in the Yangtze Estuary and its adjacent waters. *Front. Mar. Sci.* 6:671

Zhang S, Zhao J, Yao M. 2020. A comprehensive and comparative evaluation of primers for metabarcoding eDNA from fish. *Methods Ecol. Evol.* 11:1609–25

Zou K, Chen J, Ruan H, Li Z, Guo W, et al. 2020. eDNA metabarcoding as a promising conservation tool for monitoring fish diversity in a coastal wetland of the Pearl River Estuary compared to bottom trawling. *Sci. Total Environ.* 702:134704

Annual Review of Marine Science

Volume 14, 2022

Contents

Errata

An online log of corrections to *Annual Review of Marine Science* articles may be found at http://www.annualreviews.org/errata/marine