Review: DNA metabarcoding approach for fisheries management

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Abstract. Increased publications on meta-barcoding promote interests in studies associated with fisheries biodiversity, diet, and biomonitoring. These studies primarily concentrated on an ecosystem that has implications for fisheries management with complex interpretation. Various theories have arisen to describe the interactions among aquatic species and eventually contribute to the roles they play in ecology. Identifying organisms is also a prerequisite for answering questions in the field of ecology, which shows that reliable and accurate knowledge is important and desirable. Therefore, as a modern molecular technique, metabarcoding provides a realistic approach that lets ecologists analyze organisms in complex environmental samples and rapidly solve the problem associated with the food web in the coral reef ecosystem. This research explores the use of metabarcoding in fisheries management research and highlights the drawback and value of the future process.

Keyword: diet; diversity; ecology; food web

1. Introduction
Fisheries represent a significant percentage of aquatic life containing a wide range of primary, secondary, and predatory groups of organisms, which interact with each other to affect the ecological function of the aquatic ecosystem. All organisms that describe fisheries' diversity and interactions are essential in science and management to maintain sustainable resources [1]. Therefore, to achieve this goal, an ecosystem approach in fisheries management is implemented [2]. Various new methods have emerged as an ecosystem approach to analyze the diversity and possibilities of interactions [3-5]. Currently, new techniques in the molecular field have been created and developed to rapidly explore diversity and complex interactions, known as DNA metabarcoding. According to [6], DNA metabarcoding is an emerging method used to identify various species from a sample pool using Next-Generation Sequencing (NGS). This method, which is also called High Throughput Sequencing (HTS), is different from the Sanger sequencing, usually applied in DNA barcodes [7]. Sanger only sequenced the DNA of a species' specimens, while HTS or NGS allowed multiple species' sequencing from a bulk sample [8]. Therefore, sequencing using NGS produces many taxonomies of species, which is determined in a short time [9].
The quick result of many taxonomic assignments is a cost-effective method used to reveal unpredictable interactions in coral reefs [10] and aquatic ecosystems. This method also improves the resolution of the morphological identification of organisms related to biodiversity [11].

2. **Guideline of metabarcoding workflow**

As a new modern method, DNA metabarcoding is becoming popular with ecologists capable of understanding the ecosystem. The sampling activity used to describe the ecosystem analysis is as follows:

1) **Sampling for DNA metabarcoding**

A bulk sample in DNA metabarcoding is obtainable from water, soil or sediment, excretion/ feces, and gut content [11-13]. The research aims to define the sample that needs to be taken. For example, this research focuses on analyzing the food web of coral reef ecosystems, which is traceable through predator-prey relationships [14]. Choosing the right key species helps to investigate its relationship. The gut content of key species is identified to determine the research aim, with metabarcoding samples derived from a mixture of various organisms from key species. A small amount of sample was preserved by DNA shield to keep it undegraded. Sterilization and aseptic principal need to be applied in apparatus and fieldwork with the sampling protocol clearly defined.

2) **DNA extraction**

The requirement of DNA fragments in metabarcoding is short and able to display a variable region [9]. Therefore, in order to obtain DNA in smaller pieces, the isolated ones are fragmented using physical or enzymatic methods. Nowadays, many extraction kits provide a quick guide to break the cell. Therefore, DNA needs to be extracted first in order to isolate the nucleic acid. The quantity of extracted DNA needs to be sufficient to enter the next step (amplification), which is checked using qubit.

3) **NGS preparation (Library preparation)**

One of the major strategies in determining the NGS workflow is preparing the amplifying for sequencing using the target selection, addition of adapter sequences, and library quantification steps [15].

a. **Target selection.** The specific region of DNA in this research need to be amplified using PCR techniques. For example, DNA with selected regions such as COI, which has 300bp length of the sequence, was amplified using the PCR method to produce an amplicon. A primer containing overhang and Taq polymerase is the important materials used to strengthen the interest region and determine the three basics of PCR, namely denaturation, annealing, and extension. Primer and overhang need to be designed based on the researchers’ target and ordered from a third party. In NGS, this step is called the first PCR, with the cycle repeated multiple times until the copy of region interest reaches twice from the previous amount. After this step, the PCR clean-up purification is carried out to remove free primers that do not attach to the amplicon [15].

b. **Addition of adapter sequences.** The next step is adding a compatible adapter sequence with the overhang from the previous step (first PCR). The adapter is also known as an index, with the Illumina example of the P5 and P7 index. The adapter flanks the interest region by attaching to the overhang of the primer, with the downstream adapter comprising of a unique index for each sample. Amplifying the amplicon primer with index was run for several cycles using the second stage of the PCR method with the addition of a library index. PCR clean-up is also carried out after the second stage with the validation library run on Bioanalyzer to determine the size of the final library. For example, in the final library size estimation, COI as a region of interest has 300bp, with primer and overhang of 100 bp, and the index of 100bp. So, the total final library size is 500bp.

c. **Library quantification and pooling**
Library qualifications need to be calculated to obtain the same quality when pooling the DNA amplicon [16]. The fluorometric quantification method was recommended to quantify the final library, and the last step is associated with pooling all the samples for sequential processing.

4) Mi-Seq sample loading
In running samples, the library which has been pooled was run together in the sequencer to produce raw reads. Each run includes low diversity libraries such as pHi-X as a positive control, which is an indicator that the library can be sequenced. In addition, raw reads from sequencers are in fastq format.

5) Diversity estimation
Fastq format as a raw read is analyzed using many platforms to obtain OTU or operational taxonomic units. OTU determines diversity in sequence, and the general output from this workflow is a classification that reads at several taxonomic levels. Various analyses of ecology are performed to summarize nature and ecosystems.

Metabarcoding workflow from upstream to downstream is used to present a snapshot of the ecosystem briefly, as shown in figure 1.

![Figure 1. Metabarcoding approach for fisheries management.](image)

3. Research on metabarcoding
In aquatic ecosystems, some authors used metabarcoding to explore food habits, food web, predator-prey relationships, parasites, mock community, biodiversity, etc. [16-18]. These studies were applied in coastal, mangrove, freshwater, and river ecosystem, as shown in figure 2. The first research on food habit using metabarcoding was examined in lionfish [13]. The metabarcoding research also focuses on biodiversity to monitor the marine sediment [19-21].

DNA metabarcoding is a developing approach that identifies multiple species from a mixed sample, such as the gut content of fish (1), isolation and amplification from each sample (2), Library preparation (3), Next Generation Sequencing (NGS) (4), Analysis of raw sequence data using barcode database (5), Taxonomic information by particular platform (6), Ecological analysis with taxa information used to interpret the interaction in the ecosystem (7), and Snapshot ecosystem as a consideration in fisheries management (8). Step no 1 until 6 is a modification of Hariz and Adeylina 2020 research [22].
Figure 2. Schematic diagram of an aquatic ecosystem with DNA metabarcoding (modification from Ruppert et al. [23]).

4. Metabarcoding approach in fisheries management
Metabarcoding in fisheries expects to increase the welfare community, which is achieved through the following activities [24]:
1) Monitoring important species and all the biotic components in waters.
2) Improving the domestication or sea farming of economic fish such as groupers, snappers, etc.
3) Mapping the conservation area.
4) Rehabilitating endangered species.
A better understanding of species’ ecological role provides proper consideration in the management and decision-making of fisheries [25].

5. Future and challenges of DNA metabarcoding
The prospect of DNA metabarcoding is a modern method of determining fisheries management comprehensively because it informs more precise diversity as fishery source data [26]. The DNA metabarcoding is a quickly identification method in ecosystems, therefore, it offers cost-effective monitoring of environment studies and allows cost-cutting for an impractical large-scale sampling of ecosystems [23]. Furthermore, metabarcoding is used to provide holistic information on ecosystems through the data generated by the final step of the method. Data related to unidentified sequence matches are the main challenges of DNA metabarcoding [27]. As a mega biodiversity country, Indonesia’s marine and freshwater species are different from other regions of the world. Therefore, building a local reference database to enrich the list of species diversity in specific areas could solve this problem.
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