Environmental DNA (eDNA) Metabarcoding as a Sustainable Tool of Coastal Biodiversity Assessment

Z. A. Danial Hariz and M. A. Noor Adelyna

Abstract Global biodiversity loss represents one of the most serious environmental crises of the 21st centuries, with substantial impact on both ecosystem services and the health of our environment. 52% of global biodiversity decline was recorded between 1970 and 2010, and this loss was even higher for freshwater populations than for marine or terrestrial ecosystems (WWF 2014). Undeniably, Malaysian coastal ecosystem harbour extraordinary biodiversity having this country is recognised as one of the megadiverse country. However, unsustainable development of the Malaysian coastal area has led to the decline of the aquatic biodiversity. There is limited knowledge on the extent of biodiversity and the genetic resources in the aquatic environment some of them could already be extinct before we could identify them. Therefore, by implementing the Living Lab concept accustomed to the university, a non-invasive alternative method called environmental DNA or eDNA metabarcoding with an aid of Next Generation Sequencing (NGS) was introduced to identify species diversity of fish in a coastal ecosystem. In this study, the eDNA metabarcoding tool is applied in assessing water samples for fish species detection. This new, time and cost-effective method enables the acquisition of large datasets, paving the way for a finer understanding of fish diversity in different river landscape, with major implications for sustainable fisheries management and conservation, in parallel to goal fourteen (14) of the United Nations Sustainable Development Goals—Life Below Water.

Keywords Biodiversity · Environmental DNA (eDNA) · Sustainable tool · Metabarcoding

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1 Introduction

Biodiversity epitomizes the diversity of different organisms with their functions and relationship within an ecosystem. In order to understand the biodiversity of a certain ecosystem, assessments are crucial in recognizing the key components of the ecosystem. Biodiversity assessments or surveys are critical to monitor and assess the health of ecosystems and the species within them. Biodiversity assessment usually is done using conventional identification methods which involves classifying morphological characters and utilizing taxonomic keys for species identification. The morphology-based biodiversity assessment can be invasive, time consuming and financially expensive. Additionally, the limitation of experienced taxonomist and a proper developed species identification keys are the major challenges in this method of assessment. It is impossible to develop conservation plans and long-term management of an ecosystem without knowing what species are involved.

Anthropogenic disturbances are causing ongoing decline in biodiversity globally (Schmeller et al. 2017). In the past five centuries, human-driven global change has started the sixth mass extinction, which is more severe than the previously feared and massive five mass extinctions recorded in Earth's history (Barnosky et al. 2011). The rapid loss of biodiversity that has occurred primarily affecting both human health and the sustainable future of the planet. Within the last century, the rate of global biodiversity continues to decline significantly with this loss being greatest in freshwater ecosystems despite efforts to prevent such loss (Collen et al. 2014; Turak et al. 2016). In order to prevent further declines, many jurisdictions have enacted regulation to protect species at risk and their habitats. This is being done through collecting information regarding the species distribution, diversity and biology through regular biological monitoring.

Presently, there are several commonly practiced biological monitoring methods which apply specific designed for a specific group of organisms which includes combination of both observational and invasive capture methods. However, these methods of assessments were restricted by high cost, time consuming and limited number of trained labour (Darling and Mahon 2011). Likewise, the invasive nature of the conventional capture-based surveys will increase the probability of predation risk to the organism despite probably damaging the entire ecosystem (Shaw et al. 2017). Furthermore, an ongoing decrease in the number of taxonomic expertise and the non-standardised skill levels of different taxonomist may also hinder the traditional morphological identification-based methodologies despite might introducing bias in the assessment (Shaw et al. 2016).

Existing biodiversity assessment methods require improvement in order to monitor and protect the whole biodiversity in general especially for the future of freshwater biodiversity and resources. In turn to bear the improvement, more detailed, extensive and rapid surveys are crucial. Nevertheless, such advances cannot be made without increasing the costs and input time with effort in the conventional methods. Hence, a new, time and cost-effective tool has to be developed in order to support the lim-

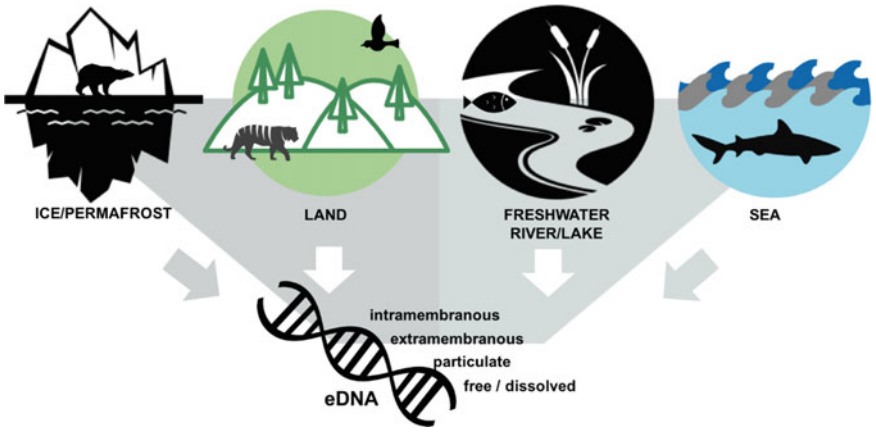

Fig. 1 Potential environments where presence of eDNA has been reported. Environmental DNA (eDNA) has been reported based on successful extraction and characterisation from basal glacier ice, terrestrial sediments, lake, rivers and lake sediments, and ocean water. Hypothetically, the eDNA came from animals' faeces, urine, epithelial cells, eggs, sperm, plants' pollen grains and other plant and animal associated micro- and macro- fossils and organisms that may be present extra-cellularly and/or intra-cellularly. Adapted and modified from Pedersen et al. (2015). Images were retrieved and modified from free-access and non-licenced Google images

itations of the conventional biodiversity assessment and provide enough taxonomic resolution using the new method.

The biodiversity assessment is the core aspect of conservation biology. Environmental metabarcoding is a tool used in species detection in the assessment with very minimal impact to the surrounding environment (Schnell et al. 2012; Bohmann et al. 2014). Metabarcoding is a relatively new molecular method that is used in characterising biological taxa from DNA within an environmental sample (Taberlet et al. 2012). While the concept of DNA barcoding in taxa identification is well established, the use of environmental DNA (eDNA) starts to gain popularity from its effectiveness in identifying taxa from environmental samples in bulk. DNA barcoding characterised short fragments of DNA that serve to identify a taxon or even to species level by comparing the standardised fragment to a reference database (Hebert et al. 2003). Environmental DNA (eDNA) metabarcoding extends the concept of DNA barcoding by analysing environmental samples to determine the species composition within a sample. Environmental DNA (eDNA) mixtures can consist of DNA from multiple taxa of all life stages, such as vertebrates, invertebrates, bacteria or algae, from a wide variety of sample types such as sediments, soil, faeces or marine and fresh waters (Fig. 1) (Taberlet et al. 2012).

2 University as Living Labs: Efforts in Revolutionising Biodiversity Assessment for Sustainable Development

The idea of a university as a Living Lab were firstly introduced in the early 2000s (Van Geenhuizen 2013). As for any emerging sustainability concept, many preliminary or pilot research reports have been published thus far, as well as many reviews highlighting the potential of the Living Lab concept in sustainable development (Evans and Karoven 2011; Dell'Era and Landoni 2014; Evans et al. 2015). Living Lab concept can be described as an ecosystem that catalyse action and collaboration in developing innovation. Living Lab concept has started to be practiced in Universiti Sains Malaysia (USM) recently, with the implementation of various knowledge transfer programs and holistic academic syllabus that focusing on sustainable development and conservation of the ecosystem. USM tries to combat global sustainability-related crisis by providing guidance and answer to students, staffs and academics through advance research infrastructures and practices. The Living Lab concept in USM also offers opportunities to all university stakeholders, researchers, industry, and policy makers to collaborate in initiating high-impact research and innovation for the sustainable development of socio-cultural and economic.

USM realizes that the sustainability challenges are new and emerging and there is an urgent need to develop a new approach in tackling these circumstances. Through facilitating research, global environment situation, such as the challenges in documenting biodiversity and assessing environment health can be solved by developing practical solutions and embracing the digital technologies advancement. By utilising the existing and newly generated qualitative and quantitative data, refine solutions to these environment issues can be proposed. Collaborative Living Lab projects with multiple researchers, government agencies and natural resource managers would definitely enable more opportunities of initiating impactful research and catalyse sustainable innovation. For instance, with collaboration of USM's researchers, students and a government agency—Fisheries Research Institute (FRI), Malaysia, a pilot study has been proposed with a specific goal to develop a sustainable and cost-effective method in assessing fish diversity in selected ecosystem utilising the environmental DNA (eDNA) metabarcoding technique. Through the Living Lab concept, conventional method of biodiversity surveys can be revolutionised, and these advances will enable researchers and natural resource managers to better understand, manage, and conserve biodiversity globally.

3 eDNA Metabarcoding as a Sustainable Tool of Biodiversity Surveys

Routine biological surveys are crucial in managing the species population and monitoring the aquatic ecosystem especially in the important coastal environment. Netting approaches in capture-based method of biological surveys are invasive to the natural

ecosystem while increasing stress and predation risk to the animals itself (Rottmann et al. 1992). Fish survey in natural aquatic ecosystem covers multiple components which includes estimating the total area of the habitat, total fish numbers, and species composition (including identifying introduced species) (Hankin and Reeves 1988). In Malaysia specifically, there are a variety of methods that is used to sample fish in the coastal area which includes the rivers, streams and estuaries. Gill net, hook-and-line, bag net, trammel net, lift net, traps, dip net, hyke net, angling, visual census and electrofishing are the most common methods in conducting fish surveys (Fisheries 2011). While these common methods are traditionally used, the molecular approach has revolutionised a pathway by using environmental DNA (eDNA). Application of eDNA metabarcoding for biodiversity assessments/surveys has gained much attention because of its efficiency in ecosystem biomonitoring. A comprehensive monitoring program should account for knowledge of a species' distribution, their habitat and allowing adaptive management to be practiced. One of the earliest, successful eDNA surveys utilising water samples is done by Ficetola et al. (2008) where they utilise eDNA in detecting invasive species of the bullfrog, *Rana catesbeiana* in natural wetland ponds for the purpose of biomonitoring. This study marks the novel application of eDNA in conservation genetics to document occurrences of aquatic invasive species.

Environmental DNA (eDNA) has effectively detects the presence of fish species in both freshwater and marine ecosystem (Bohmann et al. 2014; Rees et al. 2014). Recent studies also have shown that eDNA is far more sensitive in detecting rare or uncommon species as compared to capture-based survey methods (Jerde et al. 2013; Laramie et al. 2015) and several researches have proven that eDNA is useful for detecting species that would be difficult to find using traditional methods due to either low density or trap shyness (Shaw et al. 2016; Simpfendorfer et al. 2016). Environmental DNA (eDNA) surveillance is not only essential for detection of invasive species but can also be useful in the discovery of endangered species. Research utilising eDNA in the field of endangered species has shown positive detection of both amphibians (Dejean et al. 2012; Ficetola et al. 2008; Pilliod et al. 2013; Rees et al. 2014) and freshwater fishes (Bylemans et al. 2017; Eva et al. 2016). A summary of several applications of eDNA in biodiversity monitoring, specifically in fish surveys is presented in Table 1.

4 General Methodologies of eDNA Metabarcoding

Generally, eDNA metabarcoding approach of biodiversity assessment begin with the collection of environment sample, extracting the DNA within the environmental sample, and developing a DNA library that either comprises of a random subset of the DNA present (by using shortgun sequencing) or targets specific and informative DNA fragments (amplicon sequencing) (Shaw et al. 2017). Next Generation Sequencing (NGS) platforms were then being used in generating the DNA sequences before the sequence reads is run through the bioinformatics analyses in taxonomic identification

Table 1 Some of the major findings in application of eDNA in biodiversity monitoring, specifically in fish detections

Source of sample/environment	Application	Study summary	References
Fresh water/stream	Species detection and distribution	The study utilizes eDNA in detecting Arctic grayling in Missouri River basin	Carim et al. (2016)
Fresh water/pond	Species detection	The study is conducted to test eDNA persistence in water in order to confirm the presence of the focus species (Siberian sturgeon and Bullfrog tadpoles in freshwater ecosystems)	Dejean et al. (2011)
Fresh water/mesocosms	Species abundance quantification	The study determines whether environmental DNA (eDNA) sampling and metabarcoding analysis can be used to accurately measure species diversity (freshwater fishes and amphibians) in aquatic assemblages with differing structures	Evans et al. (2016)
Fresh water/natural lake	Invasive/rare species detection	This study discovers the use of eDNA in Asian Carp detection and promotes the implementation of eDNA in effective and sustainable surveillance program for invasive/rare species	Jerde et al. (2013)

(continued)

Table 1 (continued)

Source of sample/environment	Application	Study summary	References
Sea water/mesocosms	Multiple species detection	Fish community-wide assessment in marine environment using eDNA metabarcoding	Kelly et al. (2014)
Fresh water/river	Species detection and distribution	The study reveals the effectiveness of eDNA detection methods for determining landscape-level distribution of anadromous salmonids in large river systems	Laramie et al. (2015)
Fresh water/river-estuary	Multiple species detection	The study utilizes eDNA in temporal mapping of marine and freshwater fish populations	Stoeckle et al. (2017)
Fresh water/pond	Invasive/rare species detection and distribution	The study utilised eDNA in detecting the presence of invasive bluegill in natural ponds of the studies area	Takahara et al. (2013)
Sea water/ocean	Multiple species detection	The study demonstrates the potential of eDNA in biodiversity monitoring and fisheries as compared to conventional methods	Thomsen et al. (2012a)
Fresh water/mesocosms	Species abundance quantification	This study discovers the use of eDNA in fish and amphibians' detection in freshwater mesocosms	Thomsen et al. (2012b)

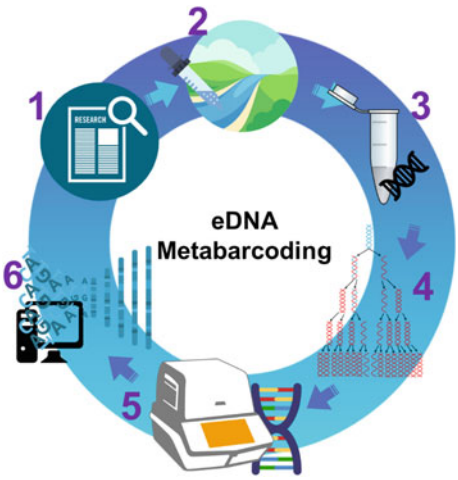

Fig. 2 The fundamental pipeline of an eDNA metabarcoding for biodiversity survey. (1) Research question is being identified in order to develop a proper experimental design. (2) Environmental samples are being collected from the studied site. (3) DNA is extracted from the environmental sample using conventional or commercial extraction kit. (4) The target gene region (selected genetic marker) is amplified by PCR by using tagged primers. (5) Multiple samples are pooled before parallel sequencing is done using NGS platform. (6) Sequences generated by the NGS platform is run through bioinformatics analyses in assigning MOTUs. Images were retrieved and modified from free-access and non-licenced Google images

by comparing them to the developed genetic reference databases. In this particular study, in order to identify fish diversity in a coastal ecosystem which includes an estuary and different landscapes of streams and rivers, three replicates of 1L of the fresh/brackish water are collected from multiple sampling points across the river and estuary. The water samples were immediately filtered using a sterile capsule filter before being dried and stored with silica beads that serve as a desiccator, preventing the DNA from degrading. The sample filters were then stored at −20 °C until extraction. DNA extraction were then performed using a commercial DNA extraction kit following the manufacture's protocol. Purified extracts were then assessed for DNA concentration using DNA spectrophotometer. In the next step, DNA is amplified using the selected metabarcoding primers' set. Commonly, a universal, validated primer set such as MiFishU (Miya et al. 2015) is used in identifying marine and freshwater fishes in such project. Generated amplicons were then being tagged and pooled before the NGS library is being developed. The sequences produced from the NGS platform were then being analysed using multiple bioinformatics tools in delimiting the MOTUs and taxonomic clustering. Figure 2 illustrates the simplified visualisation of the general methodologies of eDNA metabarcoding.

5 Integrating eDNA Metabarcoding in Intensive Coastal Biodiversity Assessment

The Sustainable Development Goals or SDGs was formally adopted in September 2015 and it addresses the three inter-related elements of sustainable development, they are, economic, growth social inclusion and environmental sustainability. Parallel to this goal, this research will address SDG 14 which is life below water. One of the many challenges to achieving Agenda 2030 is how do we work across these three elements in order to achieve sustainable development. For this purpose, we are relating this study of eDNA metabarcoding as a method to expose sustainable practice of coastal biodiversity assessment.

As mentioned earlier, the coastal ecosystems are under a lot of pressure from anthropogenic stressors including climate change, pollution and overexploitation (WWF 2014). As a result, health degradation in freshwater and marine ecosystem globally is observed, especially in terms of biodiversity loss (Butchart et al. 2010), species invasions (Strayer et al. 2006) and water quality (Foley et al. 2005). Traditional diversity sampling methods for coastal biodiversity assessment include nets, visual observation, hooks and lines and many more could prove to be a disaster to many sensitive taxa. In many countries, monitoring commercially important fish stocks are done by using bottom trawl surveys to get data for fish abundance, their distribution and diversity. Much to disappointment, these bottom trawlers damage the marine environment and slowly it will have detrimental effect on the fishing industries. This will have negative impacts to the livelihood and the socio-economics of the people who depend on it. Therefore, sustainable method in the monitoring and management is required in sustaining the health of the ecosystem.

Under the implementation of the university as a Living Lab, the eDNA metabarcoding method is proposed to be a new technique in achieving large-scale species diversity acquisition without harming the environment. This method will stress on the important of SDG 14—life below water in highlighting the importance of species documentation in order to assess the health and socio-economics value of an ecosystem, especially in sustainable fisheries management and conservation to achieve food security. This is imperative because in 2013, fish accounted for about 17% of the global population's intake of animal protein and 6.7% of all protein consumed and global total of capture fishery production in 2014 was 93.4 million tonnes, of which 81.5 million tonnes from marine waters and 11.9 million tonnes from inland waters (FAO 2016). Therefore, species documentation is necessary in keeping track of biodiversity values and resources to a country's social and economic development. The eDNA metabarcoding methods can speed up species documentation by building an inventory for species diversity and it is able to catalogue and map species distribution and associations, indirectly, linking these habitat distributions with the natural and anthropogenic processes influencing them.

Recently, a large scale eDNA metabarcoding study was done by looking at vertebrate biodiversity in Monterey Bay, California (Closek et al. 2016). The fluctuation of dominance between *Sardinops sagax*, pacific sardine and *Engraulis mordax*, north-

ern anchovy has been documented in the California Coastal Ecosystem for more than 100 years and these two species are some strong drivers of trophic interactions in the region. In the experiment that was done over a span of eight years (2008–2015), collecting sea water, they found that more than 20 fish genera were detected in the area where *Engraulis mordax* served as the dominant fish. Both *Engraulis mordax* and *Megaptera novaeangliae*, humpback whale was also detected present in temporal patterns and this is similar to visual observations reported during the years from 2013 to 2015 compared to other years. This study is important because it establishes a building capacity in biodiversity monitoring at the global scale using eDNA method.

The application of eDNA metabarcoding was also successful in an assessment done in Coral Bay, Australia. In this study they focused on assessing the broad potential of eDNA for auditing marine taxa and successfully analysed over 23 million sequences originating from 9L of filtered seawater. Using metabarcoding, data was successfully assigned to 434 eukaryotic taxa (from the kingdom Animalia to Protozoa) with 38 phyla, 88 classes, 186 orders and 287 families (Stat et al. 2017).

As this method can update species inventory from time to time, it can also be applied as an early detection system in the detection of invasive species before it spread and reduce the biodiversity of a certain area. For example, the invasion of two species of Asian silver carp, *Hypophthalmichthys molitrix* and the bighead carp, *Hypophthalmichthys nobilis* in the the Missouri and Mississippi River systems (Klymus et al. 2015). By focusing on eDNA method, they were able to detect the early presence of the two invasive species, locate their habitat and estimate the biomass and timing as well as location of spawning events. Therefore, this can help in the management and conservation of the river.

Examples of above studies were some of the benefit of eDNA metabarcoding in highlighting species biomonitoring of many taxa. All of the studies were able to provide the fundamental for a practical large-scale monitoring program operating across the full range of commercialise, endangered, cryptic and invasive species for sustainable management and conservation of coastal ecosystem.

6 Challenges of eDNA Metabarcoding in Operational Biodiversity Research and Monitoring

Over this last decade, tremendous effort has been made toward implementation of sustainable development in managing the biodiversity and conserving the ecosystem. Environmental DNA (eDNA) metabarcoding has the potential to provide massive resource of biodiversity inventory, but it has its limitation. One of the challenges is the requirement of multiple and disparate skills. However, this limitation can be solved by the implementation of the Living Lab concepts by the organisation conducting the project. The Living Lab concepts enable the ecosystem manager to have a platform in exchanging resources and expertise. If being applied in an academic institution, for instance in a university, students and managers can be trained to

effectively integrate this tool into academic and operational biodiversity research and monitoring. Undoubtedly, with the proper implementation, eDNA metabarcoding will become the key solution of sustainable biodiversity assessment in the near future.

7 Conclusion

Biodiversity assessment do take up a lot of time and resources for example, budget and skilled labour, thus it may impede conservation effort especially with the current rapid rate of loss of biodiversity. Addressing this issue, by the implementation of the Living Lab concept, the eDNA metabarcoding method is proposed to contribute in rapid assessment of biological diversity so preservation and management of ecosystems can begin early. The advances of eDNA analyses has developed a sustainable tool that can assist ecosystem managers in assessing the diversity and distribution of fishes. Environmental DNA should be applied as a time and cost-effective bioassessment tool that can complement the conventional method of biological surveys. These advances will enable researchers and natural resource managers to better understand, manage, and conserve global biodiversity. Carrying through the Living Lab concept, specifically by the university, collaborative effort among academics, researchers, students, and ecosystem managers could be reinforced to apply the newly proposed sustainable method in assessing biodiversity in a larger scale and even in a different ecosystem.

Glossary

Amplicon	A fragment of DNA or RNA derived from replication process or amplification, either naturally or artificially, through for example a PCR process.
Biomonitoring	Biodiversity assessment that is done in repeats across space and time that may focus on a target organism such as invasive or at-risk species.
DNA Barcoding	The use of a short, standardised DNA fragments in taxonomic identification by comparing the DNA sequence to a reference database.

DNA Sequencing	A process whereby the order of nucleotides within a DNA sequence is determined.
Environmental DNA (eDNA)	Trace of DNA in environmental samples—water, soil or faeces. eDNA is a mixture of potentially degraded DNA from many different organisms and be in different physical states (intraorganismal, intramembranous, particulate, adsorbed or free) (Taberlet et al. 2012).
Marker	A gene or a region of DNA with a known location in the genome and can utilised in identifying individuals or species.
Metabarcoding	identification of taxon from DNA extracted from a sample containing many different organisms.
Molecular Operational Taxonomic Unit (MOTU)	The working proxy for "species" in molecular ecology. It is the taxonomic level of sampling defined by the researcher in a study. MOTUs are generated by comparing sequences against each other to form a distance matrix, followed by clustering groups of sequences with a specified amount of variability allowed within each MOTU (Bohmann et al. 2014).
Next Generation Sequencing	Parallel sequencing technologies which produce thousands to billions of DNA sequences in a single sequencing run—e.g. Illumina Genome Analyser Series, Roche 454, Life Technologies SOLiD, Ion Torrent and PacBio.
PCR	Polymerase chain reaction is a method used to amplify a single copy or a few copies of a fragment of DNA generating thousands to millions of copies of a particular DNA sequence.

Primers	Short oligonucleotides that are complimentary to a particular region of the genome and are the starting point of DNA replication in PCR process.
Taxon	An organism identified to any taxonomic rank; from kingdom to species level.

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