# ENVIRONMENTAL DNA (eDNA) METABARCODING - BASED ESTIMATION OF MARINE STOCKS

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### Abstract

Information on species composition and biomass/abundance of exploited species in coastal fisheries is vital in management of resources. One of the most important mandates of the leading institution is judicious management of coastal and deep sea fishery resources. Traditional methods of identifying species and estimating biomass/abundance have inherent drawbacks which could be ameliorated by DNA marker based approach. Environmental DNA (eDNA) can be obtained from the skin, mucous, gamates, faeces, blood and other cells that are constantly being shed into the immediate environment by the organism. Analysis of this eDNA can give us information on the organisms, their abundance and biomass. Recent advances in next generation sequencing enable simultaneous sequencing of DNA from whole communities known as metabarcoding. Studies carried out in aquaria, large lakes, rivers and marine environment consistently suggest that eDNA metabarcoding outperforms traditional survey methods in terms of non-invasive sampling, sensitivity and cost incurred.

### Introduction

Traditional marine fish stock assessment is largely carried out using visual surveys, trawls, seines and tissue biopsies, while they serve as critical sources of data, these monitoring methods are expensive, time consuming, invasive, environmentally destructive and highly prone to misidentification. Use of more efficient, sensitive, non-invasive and cost effective methods is desirable for assessment of the ecosystem as well as in improving baseline ecological data about marine ecosystems. In aquatic environments the eDNA can persist for a day and up to 21 days depending on the environmental conditions. Analysis of this eDNA can give us information on the organisms, their abundance and biomass. Recent advances in next generation sequencing enable simultaneous sequencing of DNA from whole communities known as metabarcoding. There is now increased interest in using eDNA to supplement existing survey methods.



Since 2012 there has been a plethora of studies on eDNA metabarcoding as applied in biodiversity conservation, fish community identification, fisheries management, invasive species, as well as in fish biomass/abundance estimations. eDNA approach became popular because of its non-invasive nature, relatively economy and better results. Thomsen et al (2012) have used eDNA for detection of marine fauna. Pilliod et al (2013) have described eDNA applications in amphibians and fish. The tool has found wide applications in both marine and freshwater environments (Ferguson and Moyer 2014). Miya et al (2015) have developed MiFish primers, which were used to detect more than 230 subtropical species. Largest fish on earth, whale shark was detected from eDNA in water samples (Sigsgaard et al., 2016). Jiang and Yang (2017) have used Scientometric methods have been used to quantitatively assess the current global research status in the eDNA field based on SCI-EXPANDED and Social Sciences Citation Index databases during the period 1992–2016. eDNA can also be used for estimating fish biomass/abundance, and in marine census (Takahara et al., 2012; Kelly et al., 2014; Klymus et al., 2015; Doi et al., 2015; Thomsen et al., 2016; Yamamoto et al., 2016; Roussel and Bernatchez, 2016).

A total 25 research papers related to eDNA metabarcoding/metagenomics by Indian authors have been cited. They are predominantly pertaining to the study of microbial biodiversity from food, soil and deep sea sediments (Jiang and Yang, 2017 for review). Not a single publication related to such study in fish has been cited.

## Gap in Knowledge

Metabarcoding is constrained by factors like PCR efficiency, primer tags and sequencing efficacy. Another limitation is lack of comprehensively cured reference databases for certain metazoans for assigning taxon to the OTUS. Future studies are needed to improve sampling strategies (selection of season, sampling location within habitat, etc.) and to understand the relationship between sequence reads and species density. Still there are gaps in knowledge about the dynamic mechanisms relating to shedding of tissue into the environment, metabolism related processes which could also affect quantity of DNA released by an organism into the water. Dynamics of eDNA under field conditions, such as patterns of release, degradation, and diffusion should be taken into consideration to get a better estimate of fish distribution and biomass/abundance based on eDNA.

## **Technical Approaches**

Methodology includes seawater filtration, quantitative real-time PCR, Library preparation, Next Gen Sequencing (NGS) and statistical analysis. Copy number of DNA could be quantitatively interpreted in terms of fish abundance. High throughput sequencing data analysis using the state-of-the art tools could throw light on family level abundance in general and species level abundance of fish in particular.



## **Expected Utility of Research**

Research on eDNA can generated eDNA signatures of exploited pelagic and demersal fish species from Indian coastal fisheries, which would facilitate for accurate estimation of biomass/abundance of fish. Further, India-specific eDNA-linked database on exploited marine species from coastal fisheries could be generated.

