CHAPTER

05

e DNA - A New Concept in Fisheries Research

P. JAYASANKAR
ICAR-Central Marine Fisheries Research Institute

Introduction

Environmental DNA (eDNA) is defined as the genetic material obtained from a water sample containing no distinguishing signs of source macro-organisms. The method utilizes DNA which is continuously excreted by organisms into the surrounding environment through mucus, gametes, faeces, blood and other cells, and captures, analyses and obtains the nucleotide sequence of this DNA based on an environmental sample. eDNA analysis has emerged as a potentially powerful tool to access aquatic community structures. The inherent drawbacks in traditional approaches to monitor fish biomass/abundance in exploited waters can be overcome by employing eDNA techniques. Analysis of this eDNA can give us information on the organisms, their abundance and biomass through two approaches – eDNA barcoding and eDNA metabarcoding. In the former, specific species are targeted in samples using standard or quantitative PCR, and using traditional Sanger sequencing method. In the latter, the whole community is screened using multiple conserved primers and Next Gen Sequencing (NGS) is done. Studies suggest that eDNA metabarcoding outperforms traditional survey methods in terms of non-invasive sampling, sensitivity and cost incurred. There is now increased interest in using eDNA to supplement existing survey methods.

Status of research

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 has been reviewed by Hansen *et al*. (2018 *Fish and Fisheries*, 1-18). A total 25 research papers related to eDNA metabarcoding/ metagenomics by Indian authors are predominantly pertaining to the study of microbial biodiversity from food, soil and deep sea sediments (Jiang and Yang, 2017 *Current Science* 112(8): 1659-1664). Not a single publication related to such study in fish has been cited from the Indian context.

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 (Operational Taxonomic Units). 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. Gaps in knowledge about the dynamic mechanisms relating to shedding of tissue into the environment and metabolism related processes which could also affect quantity of DNA released by an organism into the water have to be filled. Dynamics of eDNA under field conditions, such as patterns of release, degradation, and diffusion will have to be taken into consideration to get estimates of fish distribution and biomass/abundance based on eDNA.

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 and biomass. 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. However, the strength of the relationship depends on environmental parameters, such as water temperature, and technical parameters, such as the filter being used for capturing eDNA. Species biology, environment and filtration methods and other factors (e.g. extraction and fish ecology and spatial distribution) likely to interact and significantly influence eDNA concentration variation. Caution is needed when interpreting the patterns of eDNA concentration in practical contexts. Parameters such as detection limits in water samples, influence of microbial activities on eDNA degradation, sampling design, seasonal conditions, nature of eDNA and fish ecology should be considered in future studies before predicting fish abundance from eDNA in natural conditions.

A basic study design and sampling strategies are essential for estimation of biomass using eDNA surveys. The decision on sample number and density within various habitats is an important aspect whiling developing a statistical sampling strategy. Further, the relationship between fish density and eDNA abundance depends on, e.g. taxon- and age-specific shedding rates, specific eDNA degradation rates in the given environment, and non-local eDNA transported with sea currents; the effect of these factors has to be measured and taken into account while analyzing the data.

Potential advantages of eDNA over conventional approaches

Continuous ship-borne monitoring surveys are time-consuming and expensive. Generally they are invasive, selective and rely on some degree of subjectivity related to the taxonomic expertise of the monitoring personnel; further, problematic due to a general decline in taxonomic expertise and related difficulties associated with correct species identification especially across egg and juvenile life stages. On the other hand, collection and analyses of water samples for eDNA more cost-effective, sensitive and non-invasive for presence/absence surveys of species, in contrast to established monitoring techniques relying on catching whole organisms. As all organisms continuously shed DNA through their metabolic waste products (and gametes), the method has the potential to objectively identify either individual species using qPCR; or entire biological communities across taxonomic groups using NGS platforms. Moreover, species-specific DNA concentrations could positively correlate with biomass and abundance thus pointing to a large potential for many different quantitative monitoring applications.

Factors which control eDNA presence in a given environmental sample

Environmental effects on the production, persistence and transport of eDNA, especially in marine ecosystems, are keys to establish robust and reliable temporal and spatial relationships between recorded DNA and qualitative/quantitative monitoring data. With reference to body surface area and metabolism small adult/juvenile fish are likely to produce more eDNA than large adult fish. Temporal persistence of eDNA particles in water depends on whether it's in free state or encapsulated and the external biotic and abiotic factors. Persistence time of eDNA can be highly variable, such as from 1 day to 58 days; being shorter in marine and brackish environments when

compared to freshwater, presumably due to difference in environmental factors or osmoregulation between fresh- and marine species. In sea eDNA particles are estimated to travel more than 600 km in a week, and are less affected in the near coastal areas than in high seas.

Main challenges

Five principal challenges which affect eDNA concentration and its applications include: (i) to find what we are looking for, (ii) spatial origin, (iii) relationship between eDNA and biomass/numbers, (iv) application in fisheries management and (v) other sources of eDNA. Concern on 'false negative' and 'false positive' is common. 'False positive' can occur from empty fishing nets, bottom sediments, discards and fish carcases. Low fish density in marine environment compared to freshwater poses challenge in presence/absence detections in the former; this entails relatively larger volume of seawater to be sampled for eDNA analyses. In marine environment currents dissipate eDNA from the source; hence chances of detection diminish depending on the distance from the fish sampling was done. Faster degradation of DNA and dilution further blockade effective utility. In open water system relation between eDNA and biomass/numbers is obscure; there is need for more understanding of fundamental biological and environmental processes related to eDNA, and statistical modelling framework to make quantification more feasible in future. eDNA does not provide direct information on the size, number, age, weight, life stage or fecundity – cannot be a standalone tool for stock assessment paradigm.

Improving eDNA analyses

Fish metabolism and eDNA production could be correlated, though such studies are lacking. Influence of physical, chemical and biological environment on eDNA of source organism need to be studied to improve quantitative aspects of eDNA-based monitoring. Oceanographic modelling of eDNA transport and detection is a promising area of research to improve our understanding of the complex interactions and dynamics of eDNA in marine systems. Best estimates of eDNA dynamics are currently from freshwater environment. Research focus should be more on understanding the basic processes of eDNA in marine environments, rather than the present focus on direct application.

Novel applications

eDNA analysis can be applied in ecosystem monitoring, assessment of life history and migration patterns, stock structure analysis, and diet and processed fish product analysis.

Way forward

There has been amazing advancement in technology from quantitative real time PCR to smart phone-powered sequencer, which would minimize many of the classical logistical and practical challenges of handling, storing and transport of environmental samples. Finally technological advancement has reached in automated real time DNA measurements as in Environmental Sample Processor (ESP), which is set to monitor a specific geographic location ranging from coastal to deep sea, and does everything right from regular water sampling and storing to real-time molecular analysis. ESP may be costly, but cost-competitive compared to extensive ship time for visual monitoring or to continuous collection of water samples. eDNA is under the influence of many

physical, chemical and biological parameters, which need to be analysed. Its role in direct quantitative assessment is still challenging. Current focus of research in this field should be around relative strengths on detection of presence/absence, migration patterns and life history events, broad ecological understanding, taxonomic coverage and providing basis for ecosystem-based management. Despite the caveats, eDNA-based monitoring will continue to develop to have profound impact on futuristic fisheries research and management.