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# Role of e-DNA in aquatic animal health

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***Abstract***

Regular monitoring and sampling of fish and other aquatic animal for disease investigation is crucial step in aquaculture and conservation of endangered species. But it is difficult to take regular investigation and requires expertise skills. The utilization of e-DNA (environmental DNA) technique becomes a perfect solution for major problems associated with aquatic animal health. It identifies the pathogens like bacteria, virus and parasite in the water sample even the aquatic animal cannot exhibit any clinical signs. Therefore, this method prevents massive loss of stock in aquaculture due to pathogenic infection and mortality of endangered aquatic animal like sea turtle. This method works in low cost and high-throughput period.

**Introduction:**

Environmental DNA (e-DNA) is the DNA present in the environment and it released from living and dead organism in environmental samples such as water and soil. It has huge potential to monitor the diseases, water-borne virus and protozoan parasite. e-DNA can present in the environment in two forms, either intracellular (such as microorganisms, zooplankton and phytoplankton) or free form from the urine, faeces, saliva, gametes and epidermal cells. Once the DNA is distributed into environment, it can be persisted from hours to week in temperate water and month to year in soil, frozen areas and sediments (Baillie *et al. 2019*)

**Steps involved in e-DNA sequencing:**

(i) **Collection of samples:** The collection of samples from the ecosystem will depend on the target taxon abundance and total biomass of the ecosystem. Sample from the eutrophic water, sewage effluent and wastewater contain many algae, bacterial and viral species requires small volume of sample due to the high abundance and the high volume of sample is taken while it is collected from the extreme environmental conditions (shaw *et al. 2016*).


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(ii) **Processing of sample and extraction of e-DNA:** After the sample collection, the sample will be concentrated before DNA extraction. The concentration methods include filtration and centrifugation and any one method can be used (or) combination of these methods used at a time based on the sediment particles in the sample. The extraction of DNA is most widely done by the bead-based mechanical lysis of the cell. This method requires detergents like sodium dodecyl sulphate (SDS) (or) triton-X to breakup membrane structure and lysis and buffer contain salt like EDTA or tris-HCl to regulate osmolality and acidity of extracted solution. The bead beating speed will depend on the sample collected from which type of the environment (shaw *et al. 2016*).

(iii) **PCR amplification, design of primer and genetic marker:** It is the step that discriminate the DNA into different taxa group. The primer is designed so that it is equally complementary to any kind of DNA for the efficient and quality amplicons production. The genetic marker is incorporated with primer to discriminate the DNA as whether bacterial or eukaryotic etc. the genetic marker will not discriminate in generic and species level. It only classify into large taxa level like bacteria, fungi, eukaryotes etc. commonly used genetic marker include 16s rRNA for bacteria, 18s rRNA for eukaryotes and ITS for fungi and algae. After primer design and incorporation of genetic marker, amplification of DNA by PCR will be taking place (shaw *et al. 2016*).

(iv) **Next generation sequencing (NGS):** It is also known as high-throughput sequencing and non-sanger-based methods. It will sequence millions of DNA strands in parallelly. This sequence contains sample specific tag (genetic marker) and additionally the platform adaptor sequence is added. This amplified DNA sequence along with sample-specific tag and platform adaptor sequence will be pooled to form “DNA library” in the NGS (next generation sequencing) platform (shaw *et al. 2016*).

(v) **Bioinformatic analysis:** The first step include searching of sample-specific tag and separate these sample-specific tag sequence. Discard other sequence and then remove (trim) the sample-specific tag and platform-specific adaptor and then remove low quality sequence and then cluster the similar sequences (also known as operational taxonomic unit (OTU)) and finally compare this OTU with genetic database like genbank, greengenes (bacterial 16s rRNA), SILVA (eukaryotic 16s/18s rRNA) etc. to identify the taxa (shaw *et al. 2016*).

**Role of environmental DNA (e-DNA) in aquatic animal health**

The e-DNA based method is used for detecting the pathogenic parasite *Ribeiroia ondatrae* in the north American amphibians to evaluate disease risk. The DNA collected from the environment (e-DNA) is still detectable in lab after 21 days at 25ºC and this method have high advantage and accuracy over the traditional survey methods (Huver *et al.* 2015)

The key advantage of e-DNA is early detection of parasitic infection in water using e-DNA technique even the fish cannot exhibit any clinical signs in order to avoid disease outbreak (Gomes *et al.* 2017a).

Peters *et al.* (2018) develops a e-DNA metabarcoding tool using lon torrent sequencing for detecting the pathogens in salmonid aquaculture. They identify the two parasites *Lepeophtheirus salmonis* and *Paramoeba perurans* to species level.

Pawlowski *et al.* (2014) also used metabarcoding tool based on next-generation sequencing of e-DNA and RNA for detecting the impact of aquaculture and other industrial activities in marine ecosystem by comparing the foraminiferans species richness between fish farming environment and other environment.

Fong *et al.*(2016) uses environmental DNA to detect the seasonality and pathogenicity (virulence) of the *Aeromonas* bacterial strain from water samples of different waterways of Korea.

Farrell *et al*. (2021) uses the environmental DNA to detect the viral transmission in sea turtle. Chelonid herpes virus 5 (ChHV5) causes fibropapillomatosis in sea turtle and they identified that the transmission of ChHV5 through water column rather than marine leeches using e-DNA technique.

Gomes *et al*. (2017b) uses environmental DNA and water quality data to detect the protozoan parasite *Chilodonella hexasticha* as a model in *Lates calcarifer* fish farm and water was sampled in one year time interval and they identified that there is no correlation between water and parasite abundance.

Brannelly *et al*. (2020) uses e-DNA for detecting an amphibian pathogen *Batrachochytrium.* They examined the soil sample and water sample to compare the efficiency of e-DNA technique for pathogen detection in two different environments. They conclude that detection of e-DNA in water sample give better result than soil sample.

**Conclusion:**

Environmental DNA (e-DNA) is a novel approach to diagnose the pathogenic

infection in aquatic environment. It gives curate result that is similar to traditional methods. The e-DNA methods not only used in aquatic animal health, but also have huge applications including biodiversity monitoring, organism abundance, population genetics, sustainable aquaculture, stock assessment and detection of aquatic invasive species (Baillie *et al. 2019*).

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