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APPLICATION OF E-DNA FOR DETECTION OF FAUNAL DIVERSITY IN A LOTIC HABITAT: A REVIEW

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ABSTRACT

Decline in biodiversity is one of the greatest challenge faced by the world in the current times. The loss in biodiversity has led to extinction of many valuable species and has also affected the genetic variations appearing in the coming generation. This decreasing biodiversity has reduced the production of food grains and has declined the health of an individual. Fresh water ecosystems are source of rich biodiversity but unfortunately due to increasing pollution the ecosystems are undergoing decline in biodiversity in both lentic and lotic habitats. The first and foremost step in conservation of biodiversity is the introduction of a proper monitoring technique which is able to present a clear picture about the exact health status of the an ecosystem. Since, traditional and conventional techniques do not allow researchers to identify cryptic or immature life stages so, introduction of new and technologically equipped techniques for better assessment of the ecosystem is need of the hour. One such effective technique is the use of e-DNA for assessment of biodiversity, as the method is cheap, time saving and approachable it can be easily used for assessing any aquatic habitat. e-DNA actually refers to the genetic material obtained directly from environmental samples like soil, sediment and water without any signs of biological source material, it is an rapid, efficient, non-invasive and easy to standardize sampling approach. The paper is a genuine attempt to analyse the use of e-DNA for detecting faunal diversity of a lotic habitat, we have also discussed the use of e- DNA in detection of invasive species, studying trophic interactions and spawning ecology of different taxon and for monitoring the overall health status of a lotic ecosystem. Through this review researchers can better understand the potential advantages and future perspectives of using e-DNA for analyzing biodiversity of lotic ecosystems.

KEYWORDS: Faunal Diversity, Lotic Ecosystem, e-DNA, Biodiversity, Freshwater Ecosystem

In the 21st Century the world has experienced considerable changes at exponential levels and these changes are responsible for transforming the world into a new geological epoch. With, the rapidly changing world changes have been witnessed in the flora and fauna across the globe. Some of the main reasons which have changed the biodiversity of flora and fauna around the world include global warming, loss of natural habitat of the species and increased level of pollution across the world (Prakash & Verma 2022). Loss in flora & fauna of the earth has resulted in the loss of biodiversity which has not only been a critical challenge to the world, but has also been one of the major cause of ecological disturbance in the major ecosystems of the world (Dirzo *et al.*, 2014). Thus, conserving the diminishing biodiversity is an immediate need of the hour for which the foremost requirement is use of a proper monitoring system, so that biodiversity can be conserved in the best possible manner (Rihan *et al.*, 2023). Initially, traditional methods were used for monitoring biodiversity but with the growing time and changing era these conventional methods have proved to be invasive or destructive to the species. On, the other hand the use of taxonomic methods and techniques require trained and specialized skills (Daan 2001). In order to counter the shortcomings arising by use of traditional methods in bio-monitoring of biodiversity, the use of molecular techniques like analysis of e-DNA for analyzing biodiversity have revolutionized the world and have also served as powerful tools for studying biodiversity (Othman 2021). One such new and effective approach is the use of e-DNA which is define as a short fragment of DNA that organisms leave in non-living elements like soil, air, water etc. (Rodgers 2017). e-DNA can be gathered from mucus, egg feces, urine, roots, fruit, pollen and leaves in the ecosystem (Yang *et al.*, 2023 and Sahu *et al.*, 2022). e-DNA in other and more clear words can be define as genetic material directly obtained from environmental samples as soil, sediment, water etc. without any signs of biological source material (Biggs *et al.*, 2015). One of the major advantages of using e-DNA for assessment of Biodiversity is that it require less skilled professionals and can be easily applied in any habitat (Thomas *et al*, 2019). The current article is an serious attempt to understand the use of e-DNA in studying the fauna of a fresh water lotic habitat, detection of invasive species persisting in any lotic ecosystem,

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studying trophic interactions and spawning ecology of different taxon residing in a particular fresh water lotic and for monitoring the overall health status of an ecosystem. The paper is also an attempt to focus on approaches for obtaining genetic material from environment on which a number of techniques belonging to molecular methodology and analysis tools like DNA Meta bar-coding etc. can be applied in order to obtain knowledge about Paleontology, Ecology, and Conservation Biology.

ANALYSIS OF E-DNA FOR DETECTION OF ANIMALS

Critical review of literature was carried out by using variety of online indexes (Nature, Science Direct, PubMed, Springer etc.), which will also be helpful to explain the use of environmental DNA along with the challenges and limitations it counters. A standard methodology for using e-DNA in detection of animals is followed which includes following steps-

Sample Collection

The volume of sample, the depth of sample, purpose of research, number of species and the condition of sampling region are all important factors which affect the collection of samples for the analysis of e-DNA. Samples of air, water, soil, silt ice and snow are used generally for the analysis of e-DNA. In case of sample collection from a water body, the sample volume should vary between 1.5 ml to 45 ml (Kumar *et al*., 2020) and at least three samples should be collected from the same location of the water body (Hinlo *et al.*, 2018).

Extraction of e-DNA

The first and the foremost important step in the extraction of DNA is the selection of filters that are used in extraction process. The filters made up of mixed cellulose nitrate, polycarbonate, nylon, glass, cellulose acetate and poly-ether sulfone etc. are used extensively as they have a pore size of about 0.45 um they become very convenient for use. However, cellulose filters are considered to be the best for isolation of e-DNA from water sample but in recent times capsule filters are also used (Spens *et al.*, 2017). For the extraction of e-DNA Dneasy Blood & Tissue Kit (Coaster *et al.*, 2021) and Power Water DNA isolation kit are used extensively. These kits are less expensive, easy to use and have efficient PCR amplification and also they do not contain any harmful chemical. Hence, they are regarded as best option for extracting e-DNA (Li *et al*., 2021,). It is evidently known that in both lentic and lotic habitats sediments present in water interfere with the detection of e-DNA from water samples (Goldberg *et al*., 2011).

Genetic Biomarkers for Detection of e-DNA

Mostly mitochondrial and nuclear genes are successfully used biomarkers for detection of e-DNA. Because, of their fast evolution and ability to provide description of biodiversity nuclear genes are considered to be the best genetic biomarkers for detection of e-DNA. On, the other hand mitochondrial genes have been successfully used for detecting vertebrate and specifically fish diversity (Freeland 2017). Cytochrome-b gene is also used for detecting species-species interaction and has also been implied for characterizing e-DNA from fish (Zhang *et al.*, 2020).

Detection of e-DNA using PCR

A number of e-DNA detection system are used in monitoring of Biodiversity. Most of the investigations feature on species-species interaction and detection of vulnerable species. Initial e-DNA detection of combined PCR utilizing cloning or Sanger sequencing technologies in obtaining target e-DNA for the purpose of species identification, Due to superior sensitivity and reproduciblty q-PCR is recommended over c-PCR for the detection of single species, at the same time q-PCR technique is also utilized to determine population and biomass of fish by quantifying sample's target DNA (Langolis *et al.*, 2021).

USE OF E-DNA FOR THE DETECTION OF VARIOUS SPECIES IN A LOTIC HABITAT

Aquatic sediments are considered to be one of the biggest reservoir of e-DNA in the world. Because of the anoxic conditions prevalent in the oceans, the nuclease degradation of e-DNA considerably reduced as a result of which e-DNA could be preserved for longer periods (Del and Corinaldesi 2004). Study on lake sediments in the year 2008 reveals the presence of macro-organism e-DNA in the samples taken from the lake. Later in 2011 amplified plant e-DNA from 4600 years old lake were obtained which further clarified that e-DNA gets preserved in anoxic conditions and this was the major concept which formed basis for the use of e-DNA as a potent source of understanding ecological and evolutionary consequences of environmental change (Matiso *et al.*, 2008).

Use of e-DNA for the Detection of Various Species from Fresh Water

Water bodies containing freshwater are referred as freshwater bodiesor fresh water habitats and these fresh water habitats comprise of about less than 1% of world's total surface area yet they are house of about 10% of the known animals and 40% of the known fishes in the world. Freshwater Ecosystem persisting in any water

body is divided into two Lentic and Lotic habitat/ ecosystem respectively (Anderson *et al.,* 2021 and Giguet *et al.,* 2014). Lentic ecosystem refers to an ecosystem where water is still these include ponds, marshes, ditches, lakes and swamps. On, the other hand lotic ecosystem refers to an ecosystem which is characterized by running water and this includes rivers, streams, brooks and springs. One significant study retrieved e-DNA from freshwater and used it as a method to detect sources in fecal contaminated surface water (Martellini *et al.,* 2005). After, sometime retrieval of American Bull Frog native of North America from natural pond water sample in France initiated a series of studies for biodiversity conservation in an aquatic ecosystem (Table. 1). It has been clearly demonstrated that in comparison to traditional methods used initially e-DNA is a better mode for detecting species from water. It is clearly evident that fish, mammals, insects etc. can be monitored using e-DNA however, by the use of high-throughput sequencing entire lake fauna can also be studied (Thomsen *et al.,* 2012). In case of river system e-DNA has been transported from some meters to several kilometers have been reported in river system.

Table 1: Faunal Diversity Reported from different Lotic Ecosystems around the World

S. No.	Country	Water Body	Faunal Study	Application of e-DNA	Reference
1.	Czech Republic	Reservoir	Parasites	Detection of Freshwater Myxozoan Communities	(Langolis *et al.,* 2021)
2.	Australia	River	Parasites	Detection of Protozoan parasites from rivers	(Gomes *et al.,* 2017)
3.	China	River	Fish	Assessment of fish diversity in river system	(Xu *et al.,* 2022)
4.	Austria	River	Fish	Monitoring of fish Diversity	(Pont *et al.,* 2019)
5.	Egypt	River	Sharptooth Catfish	Tracking of Cat Fish	(Elberri *et al.,* 2012)
6.	United Kingdom	River	Fish	Spawn Distribution	(Harpes *et al.,* 2012)
7.	USA	River	Fish	e-DNA surveillance sensitivity for detection of fish	(Mahon *et al.,* 2013)
8.	Japan	River/Stream	Fish	Surveillance of Fish Species Composition	(Minimoto *et al.,* 2011)
9.	Japan	River	Fish	Estimation of Fish	(Takshara *et al.,* 2012)
10.	USA	Rivers/Steams	Fish & Ambhibians	Detection of Vertebrates	(Jerde *et al.,* 2011)
11.	Japan	River	Fish	Surveillance of Fish Species Composition using e-DNA	(Minimoto *et al.,* 2011)
12.	USA	River	Fish	Validation of e-DNA Surveillance sensitivity for detection of Asian Carps	(Mahon *et al.,* 2013)
13.	USA	Rivers	Mollusk	Environmental DNA as a new method for early detection of snails	(Turner 2015 *et al.,* 2012)
14.	USA	Rivers	Amphibians	Use of e-DNA to detect Amphibian	(Elison *et al.,* 2006)
15.	USA	Rivers/ Streams	Amphibia	State wise survey of Amphibians by using Salamanders	(Santas *et al.,* 2012)
16.	USA	Stream	Amphibia	Detection of Amphibia by use of DNA	(Golglier *et al*., 2011)
17.	Denmark	Streams	Fish	Fish Detection by use of e-DNA	(Piliord *et al.,* 2014)
18.	USA	Rivers	Fish	Detection of e-DNA from sediments of rivers	(Stickeries *et al.,* 2015)
19.	USA	Streams	Fish	e-DNA dynamics in head water streams	(Biggs *et al.,* 2015)

20.	USA	Stream	Amphibians	e-DNA is used to detect e-DNA in a stream	(Santas *et al.*, 2012)
21.	Switzerland	River	Crustacean, Mussel	Distance of invertebrate e-DNA in a natural river	(Piliord *et al.*, 2014)
22.	USA	Streams	Amphibian	Detection of e-DNA using samples from freshwater	(Piliord *et al.*, 2013)
23.	Northern Europe	Streams	Fish & Amphibia	Monitoring of Biodiversity by using e-DNA	(Thomsen . *et al.*, 2012)
24.	USA	River	Rusty Crayfish	Detection of Crayfishes	(Coaster *et al.*, 2021)
25.	USA	Rivers	Mussels	Quantitation of shielding rates of e-DNA	(Klymus *et al.*, 2021)
26.	USA	Rivers	Catfishes	Traking of Catfish	(Elberirri *et al.*, 2020)
27.	UK	Rivers	Fishes	Study of Spawn Distribution in Fishes	(Antognazza *et al.*, 2013)
28.	Switzerland	Rivers	Fishes	Detection of Invasive Fish Species	(Kalchhauser *et al.*, 2016)
29.	Switzerland	Rivers	Cruastacea & Insects	Use of e-DNA for assessment of invertebrate species from freshwater	(Deiner *et al.*, 2016)
30.	USA	River	Fishes	Invasive Fish Population	(Jerde *et al.*, 2011)

APPLICATION OF E-DNA FOR STUDYING FAUNAL DIVERSITY OF LOTIC ECOSYSTEM

In olden time the identification of species was based on traditional methods like study of external appearance or morphology which was done by capturing the animal in nets etc. However, with the growing time and increased technological approaches new methods of identification of different groups have been developed which include scuba diving, bottom walk etc. (Beng *et al.*, 2020, Rosel 2021, Minioto 2022). Use of e-DNA is one such method which provides better idea of sessile and migratory species in a freshwater ecosystem. It is reported that species distribution and biomass of a water body are correlated with the concentration of e-DNA obtained from environment. Several groups of animals belonging to phylum invertebrates and vertebrates have been isolated from freshwater ecosystem these include Parasites, Fishes, Amphibians, Crustaceans, Insects etc (Table-1). Some of the major studies in this field show the detection of parasites from rivers of Czech Republic and Austria (Langolis *et al.*, 2021, Gomes *et al.*, 2017), detection of fish species from China, Australia, Egypt, UK. Japan and USA (Jerde *et al.*, 2011, Elison *et al.*, 2021, Santas *et al.*, 2021,), detection of Mollusk from USA (Tuener *et al.*, 2015, Deiner 2014) and detection of Crustacean and insects from streams of Switzerland (Deiner *et al.*, 2017). This suggest that e-DNA can be easily used for the detection of various taxon/ groups from freshwater ecosystem.

APPLICATION OF E-DNA FOR ANALYZING INVASIVE SPECIES PRESENT IN AN ECOSYSTEM

Invasive Species are one of the biggest threats to any aquatic ecosystem and so, it becomes necessary to detect and remove such invaders as soon as possible otherwise they will harm the biodiversity by competing with the existing species in an ecosystem (Ferguson and Moyar 2014). During early days techniques like oocyte collection, electro shocking and use of nets were common for collection of such species. Nowadays, newer technology with more equipped methods like e-DNA have been successfully used for the detection of American Bull Frog in France (Attista *et al.*, 2022), Blurgill Sunfish in Japan (Takasharar *et al.*, 2012), Asian Carp in reservoir of US (Jerder *et al.*, 2013), mud snail from Portneuf river of New Zealand (Hunter *et al.*, 2019), invasive golden mussel from rivers and streams of China (*Limnoperna fortune*) (Zhou *et al.*, 2022) and Cray Fish from Europe and China (*Paufastacus linusculus*) (Mauveisseau *et al.*, 2019). In 2015, e-DNA was used for the first time for the detection of invasive plants (Scriver *et al.*, 2015) this formed the basis for the detection of invasive species by using e-DNA.

APPLICATION OF E-DNA TO ANALYZE HEALTH OF FRESHWATER ECOSYSTEM

Growing human interventions and antheropogenic activities, changes in land use, increased industrial pollution, and concentration of algal /blooms have disturbs the stability of any ecosystem (Lacy *et al.,* 2022). The above stated factors may deteriorate health of any fresh water ecosystem leading to the introduction of pathogenic species in an ecosystem (Billah and Rehman 2017), this further poses a threat to the biodiversity of the ecosystem affecting the overall health of that particular ecosystem. e-DNA have been successfully used to monitor the growing microbes in an aquatic system so that they can be controlled and the ecosystem can be restored to its original state. To, add a step further e-DNA has also been used to study shift in community structure and sharp decline in species diversity (Strayer *et al.*, 2010). Loss in biodiversity changes the entire composition of the ecosystem by lowering the water quality of that particular ecosystem (Didham *et al.,* 2005). Researchers indicate the potent use of e-DNA in detection of population dynamics, assessment of threatened biological organism and analysis of organism present in an ecosystem. It is also recommended that in future e-DNA will become a major tool for analysis of risk factor in an ecosystem.

ASSESSMENT OF BIODIVERSITY BY USING E-DNA

Loss of Biodiversity is one of the biggest problem on earth. Increased level of pollution has adversely affected biodiversity of the world (Thomsen *et al.,* 2015). e-DNA from last some decades has being a promising tool for assessing the threats of biodiversity in a particular ecosystem. The period of identifying multiple species by use of e-DNA samples is referred as DNA metabarcoding (Tabertland *et al.,* 2012). e-DNA has proved to be a time saving and cost effective method for analyzing biodiversity (Ashton *et al.,* 2013) it is also used to study assemblage pattern of groups and is also used to draw a conclusion about diversity of taxa in an ecosystem (Stat *et al.,* 2019).

USE OF E-DNA TO STUDY TROPHIC INTERACTION

Ecological Research involves studying relationships between predators and prey, host and parasites, tropical niches and food web etc. DNA metabarcoding has been successful to study wildlife feeding pattern by flowing invertebrates (Ando et al., 2020). By use of DNA bar code procedures researchers are able to analyse the species by analyzing food from stomach. e-DNA has been successfully used for nutritional & trophic investigation using e-DNA fragments or metabarcoding approach by using gastrointestinal material as target DNA (Cristescu 2014). At the same time DNA is also used in investigating interaction between plants and animals and finding out significance of these relationship in maintaining ecosystem function (Thomsen *et. al.*, 2022).

USE OF E-DNA IN SPAWNING ECOLOGY

Spawning is an important part of reproduction, it helps in conservation or population management. Collection of larvae & adult in spawning phase has attracted researchers to great extent for advancing their knowledge in material reproductive biology of aquatic organisms (Chen *et al.,* 2020). Traditional Techniques have posted a threat to survival of species as they are time consuming & difficult. This gave rise to technique like DNA bar coding which can evaluate species composition of egg & make prediction on spawning cycle of species (Lian *et al.,* 2013). If the egg is determined accurately by bar coding method then it can be used to determine fish egg in streams. DNA bar code is used to determine first ever species level identification of fish in Danube river, Austria (Meulenbroek *et al.,* 2018). Bar Coding is also used to determine spawning area & viability of egg from fish (Lian *et al.,* 2013).

FUTURE PERSPECTIVES OF E-DNA

e-DNA based research has proved to be an important tool for future. In this technological era and with the advancing technology the use of e-DNA in biological research will increase in the near future. DNA sequencing technology and expanded technologies have increased the use e-DNA in future perspectives. The paper is an attempt to review the use of e-DNA in studying species diversity, detecting invasive species and analyzing health of ecosystem. In the near future e-DNA technique might concentrate on following:

(1) e-DNA technology is successfully used to analyse the effect of toxic chemicals on environment, e-DNA is used for the characterization of population in water, soil, sediments, samples etc.

(2) In future e-DNA will be successfully used for conservation of different species however, the method of conservation will depend on genetic diversity of that species. By use of e-DNA techniques researchers can figure out species diversity and the exact location of a particular species in a habitat.

(3) In the coming era, e-DNA will soon become a tool for analyzing public health surveillance and will also be used in the study of different deadly diseases.

(4) In the coming era e-DNA will also be used to assess health of an ecosystem by analyzing the biological and functional component of soil and water.

CONCLUSION

e-DNA has been extensively used to increase our capabilities for scientific study, protection & conservation of biodiversity. e-DNA is comparatively simple, cheap and standardized technique for collection and study of the population size. It is evidently seen that e-DNA provides valuable information on species diversity, identification of hot spots, presence of invasive species etc. e-DNA has played a crucial role in exploring ecosystem. The paper is an attempt to to analyse the use of e-DNA for detecting faunal diversity of a lotic habitat, we have also discussed the use of e- DNA in detection of invasive species, studying trophic interactions, spawning ecology of different taxon and for monitoring the overall health status of a lotic ecosystem. We have tried to analyze the studies till date on the use of e-DNA for detection of species diversity in lotic fresh water habitats. Which has also opened door for finding stable methods for the detection of invasive species and maintenance of health of an ecosystem. However, the method of using e-DNA has some limitations as it cannot replace the skilled techniques employed by the researchers for the assessment biodiversity further more research is required in this direction so that e-DNA becomes an effective tool for assessing faunal diversity of an fresh water ecosystem in the coming future.

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