Proc Zool Soc (July-Sept 2019) 72(3):211–228
https://doi.org/10.1007/s12595-018-0268-9

REVIEW ARTICLE

Environmental DNA (eDNA): A Promising Biological Survey Tool for Aquatic Species Detection

Debabrata Senapati[1] · Manojit Bhattacharya[2] · Avijit Kar[1] · Deep Sankar Chini[1] · Basanta Kumar Das[2] · Bidhan Chandra Patra[1]

Received: 24 September 2017 / Revised: 25 January 2018 / Accepted: 7 July 2018 / Published online: 30 August 2018

Abstract Aquatic species are facing at higher risk of extinction similar to that of any other living components of diversified ecosystem in present scenario. So that, the conservation of aquatic biodiversity is much more important to know about the accurate information regarding species composition and their biological community interactions. Generally, traditional survey methods depend on physical identification and characterization of species but it has some sorts of challenging chances due to the phenotypic plasticity, sibling species, different stages of life cycle and its invasiveness. To overcome such barriers one of the significant and promising tool likewise environmental DNA (eDNA), which way the collection of genetic materials from bulk environment (i.e. soil, water, sediment etc.) circuitously from organisms has been used to monitor and analyzed the biodiversity status, invasive species along with the species of conservation category. Recently, the real application of eDNA analysis based outcomes uphold the actual emerging know how practices in support of the population and community ecology, conservation biology as well as in the superior field of taxonomical research. Such scientific appraisal will be useful in understanding the brief history of aquatic eDNA and obviously its methodological considerations, gentle sources, collection and analysis process, physical form, its persistence and proper transport in aquatic ecosystem. Moreover, the fruitful drives for summarization the discoveries of eDNA application and method over traditional technique, its recent challenges and examine the current and future frontiers along with the appropriate practices of aquatic eDNA relevancy in aquatic ecosystem.

Keywords Biodiversity · Phenotypic plasticity · Sibling species · Invasive species · Population and Community ecology

Introduction

Biodiversity has forever been an integral component of the human experiences (Díaz et al. 2006) but, it has decline continuously in the 21st century as example freshwater population has declined 81% and marine population decline 36% between the year 1970 and 2012 (Barnosky et al. 2011). Although the knowledge of biodiversity is incomplete or even undescribed for numerous taxa and geographical regions, there is numerous political agreements to stop the progress of the current defeat in biodiversity (UNEP 2011). To protect and prosper biodiversity, the conservation efforts essentially depends on biological monitoring for obtaining the precise data on population size and species distribution on a relevant ecological and political time scale. Species monitoring has traditionally depend on physical identification and characterization of species by visual surveys and counting the individuals in the practical field (Brock 1982). However, in some cases this technique flawed with mistake due to the phenotypic plasticity, sibling species and closely related species with

Debabrata Senapati and Manojit Bhattacharya have contributed equally to this work.

✉ Bidhan Chandra Patra
patrabidhan1962@gmail.com

[1] Department of Aquaculture Management and Technology, Centre For Aquaculture Research, Extension and Livelihood, Vidyasagar University, Midnapore, West Bengal 721102, India

[2] ICAR-Central Inland Fisheries Research Institute, Barrackpore, KolkataWest Bengal, 700 120, India

very similar appearance in different life stages within natural habitat (Daan 2001). Additionally, several traditional monitoring methods have sometimes support to the invasive on the species or natural ecosystems under study, like in marine surveys that has reveal on highly destructive techniques (Jones 1992; Baldwin et al. 1996; Robertson and Smith-Vaniz 2008). Furthermore, phenotypic identification is fully dependent to a higher degree on taxonomist expertise, which is often drastically decreased (Hopkins and Freckleton 2002). For all of the above, such limitations of traditional biodiversity monitoring, researcher have hunts intended to several alternative approaches having significant application in modern environmental biology.

In addition to these questions one such substantial tool is the detection of an organism's environmental DNA (eDNA). The terminology of environmental DNA come up from microbiology (Ogram et al. 1987) i.e. genetic materials extracted from environment without isolating from the target organism, because every individual's leaves their clues of presence in inorganic components of the ecosystem either air, water or sediments. The eDNA is divided into two category based on its particulate size and origin. Collection of eDNA from aquatic ecosystem in aggregates greater than 0.2 μm associated with cells (e.g. microbial eDNA) is known particulate DNA (P-DNA), while eDNA smaller than this extent (e.g. viral DNA dissolved) is call dissolved DNA (D-DNA) (Paul et al. 1991). Alternatively, eDNA origin from epithelial cells released by organisms to the environment through skin, urine, faces or mucus are termed as cellular DNA and the eDNA in the environment resulting from cells death and successive destruction of cellular structure which is referred as extracellular DNA (Foote et al. 2012). Methodologically it may state that, eDNA detection requires the development of genetic markers specific to the target taxon. After genetic marker developed, eDNA fragments can be detected followed by different improvised molecular methods or tools such as end-point Polymerase Chain Reaction (PCR), Quantitative PCR (q-PCR), Real-Time PCR (RT-PCR), Sanger sequencing or recent Next-Generation Sequencing (NGS) (Taberlet et al. 2012a; Yoccoz 2012).

In eDNA approach, biodiversity monitoring or detection of species is fully depends on DNA metabarcoding or DNA barcoding. In general, traditional DNA barcoding containing the polymerase chain reaction (PCR) which is prepared of a short gene segment (500–800 bp) using widely conserved primers and sequenced (via Sanger sequencing) by commencing DNA extracted from a single individual. This process should be very species specific, which allowing taxonomic identification by compare with a public DNA databank such as the iBOL (International Barcode of Life), Consortium's Barcode Library (Barcode of Life Data System; http://www.boldsystems.org) (Ratnasingham and Hebert 2007). The most universally used sequence in DNA barcodes are the mitochondrial Cytochrome C Oxidase subunit type I gene i.e. COI for animal model (Hebert et al. 2003), it has some advantages to use in barcoding technique because, mitochondrial DNA has a haploid mode of inheritance, elevated rate of molecular evolution, lacks introns part and has limited recombination part (Clayton 1984; Wilson et al. 1985; Bowen et al. 1994; Piganeau et al. 2004; Tsaousis et al. 2005). Besides that, chloroplast contain ribulose bisphosphate carboxylase gene (rbcL) for plants (Group et al. 2009), the ribosomal Internal Transcribed Spacer (ITS) for fungi (Schoch et al. 2012) and 18s rRNA gene for nematode and other meiofaunal group (Blaxter et al. 2004), all of these are found in multiple copies in a cell (Powers 2004). For environmental sample application, require shorter fragments to analysis than the standard barcodes to overcome the DNA degradation troubles. Thus, researcher developed Mini-barcodes based species identification performance where minimum amounts of nucleotide sequence require typically a 90–250 bp long strand of primary barcoding genes (Hajibabaei et al. 2006; Meusnier et al. 2008; Little 2014).

Here, certain approaches is performed in order to summarize the contribution of eDNA in aquatic ecosystem by means a brief history, ecology and fruitful implications specially for biodiversity study, species detection as well as community analysis exclusively depends on eDNA in aquatic environment. Special attention also required in connection to the research design on eDNA from macroorganisms which are the key target in conservation aspects. In order to keep this focus, a sum up was done to the related studies of advantages and challenged or limitation over eDNA method in aquatic environmental system.

Backgrounds of Aquatic eDNA

Environmental DNA concept has been started practically since the year 1980 in marine sediments for detection and in field characterization of bacterial community (Ogram et al. 1987). First eDNA was determined in dissolved condition within the aquatic environments (DeFlaun et al. 1986) and it was quantified by advanced fluorescence method. After 10 years eDNA concentration was determined in seven small southern German lake (Siuda and Güde 1996), and it was started a new vista of biological species surveying method.

Freshwater Ecosystem

Freshwater and marine ecosystems represent grand reservoirs of eDNA (Beebee 1991). First, in freshwater

ecosystem, eDNA was applied to differentiate human, bovine, porcine and ovine sources in faecally contaminated surface water by the use of eukaryotic mitochondrial DNA (Martellini et al. 2005) in Quebec, Canada. Assessment of species distribution is the first phase for biodiversity studies and also it is necessary aspect for conservation biology, biogeography and ecology. Thus one of the first demonstrate species detection using environmental DNA targeted invasive species American bull frogs (*Rana catesbeiana*) from laboratory as well as field experiment in France (Ficetola et al. 2008). University of Notre Dame scientist Christopher L. Jerde showed the sight-unseen recognition of rare aquatic species i.e., Silver and Bighead Asian carp (*Hypophthalmichthys molitrix* and *H. nobilis*) which established the efficacy of eDNA as a significant tool for study of invasive species in freshwater ecosystems (Jerde et al. 2011). Seasonality on eDNA discovery of amphibians for federally endangered organisms was published in year 2011 to apply eDNA as a prolific detection tool (Goldberg et al. 2011). In this year, the first findings on eDNA persistence of amphibian species inhabiting freshwater ecosystems was published (Dejean et al. 2012). Initially freshwater biodiversity was monitored using eDNA tool explored by the Northern European scientist Thomsen (2012a, b) which is proved that the estimation of fish biomass is likely to possible in an aquatic ecosystem (Takahara et al. 2012). Tony Dejean applied eDNA barcoding method to improve the detection of alien invasive species in year 2012. Next year 2013, numerous articles are published regarding eDNA research as estimating occupancy and abundance of stream amphibians (Pilliod et al. 2013). Another group of researcher explored that early species detection is markedly possible by eDNA analysis method (Goldberg et al. 2013) in aquatic ecosystem.

Environmental conditions directly influence eDNA persistence (Barnes et al. 2014), particle size distribution and optimal capture of eDNA (Wilcox et al. 2015b), transport distance of invertebrate eDNA components (Deiner and Altermatt 2014), factor control the detection of eDNA (Pilliod et al. 2014), distance, flow, and PCR inhibition of eDNA dynamics (Jane et al. 2015) was at first examined in the year 2014. Quantification of eDNA detachment rates was done in USA from carp species by in field controlled condition. Besides that, experimental evidences explored and analysis eDNA and support that rivers are pass on belts of crucial biodiversity information (Deiner et al. 2016). After that, estimating species richness using eDNA (Olds et al. 2016), in silico assessment of primers for eDNA studies using primer tree probe and application to characterize the biodiversity within the specified riverine ecosystem (Cannon et al. 2016) was published.

Marine Ecosystem

Marine ecosystems hold eDNA for short time period than freshwater ecosystem (DeFlaun et al. 1987). Whereas eDNA degradation rate influences by adverse physio-chemical factors likewise water temperature, high salinity, and UV radiation in marine ecosystem. The first review work related to eDNA came in year 2012 (Taberlet et al. 2012b; Lodge et al. 2012; Yoccoz 2012). The foremost uses of eDNA detection in the marine environment was conducted by Andrew D Foote and his co-researchers (Foote et al. 2012) for fine genetic monitoring of marine mammals, consequently how to estimate marine fish biodiversity -its process and critique was published in 2012. Followed by aquatic eDNA analysis method by Kelly E. Willims, he also developed a process how to marine fishes census in a large mesocosm. Population characteristics of a large whale shark aggregation inferred from seawater eDNA was last come in 2016, that was conducted by Denmark scientist Sigsgaard (2016). Despite such successful applications, the detection of desired species or biodiversity estimation by examination of aquatic eDNA has to our knowledge never been reported from marine water samples.

Sources of eDNA from Aquatic Ecosystem

The sources of eDNA in aquatic environments are still now little known. But its origin may be deposition through skin flakes (Bunce et al. 2005), faeces (Poinar et al. 1998; Höss et al. 1992), urine (Valiere and Taberlet 2000), egg shells (Strausberger and Ashley 2001), saliva (Nichols et al. 2012), regurgitation pellets (Taberlet and Fumagalli 1996), or it can be deposited by living prokaryotes through the secretion of extra nuclear plasmid and chromosomal DNA (Meier and Wackernagel 2003). Alternative sources of eDNA in aquatic ambience are hair (Higuchi et al. 1988), insect exuviae (Hofreiter et al. 2012), feathers (Taberlet and Bouvet 1991), leaves (Trevors 1996; Poté et al. 2009), root cap cells and in rare cases pollen parts (Parducci et al. 2013; Levy-Booth et al. 2007). In the first instance, the production of eDNA (micrograms/liter per hours) was study by microbial populations in Florida reservoir (Paul et al. 1990). Although, its seems to likely that multicellular organisms discard their genetic clues into the aquatic environment first as sloughed and or entire tissues and subsequently it can be collapse by physical, chemical or mechanical ways, standard workflow of the processes described in Fig. 1 (Barnes and Turner 2016). During the biodiversity study in oligotrophic lake ecosystem reside at Snowdonia National Park, Wales, UK it is found that fish or amphibians skin cells and mucus are the probable

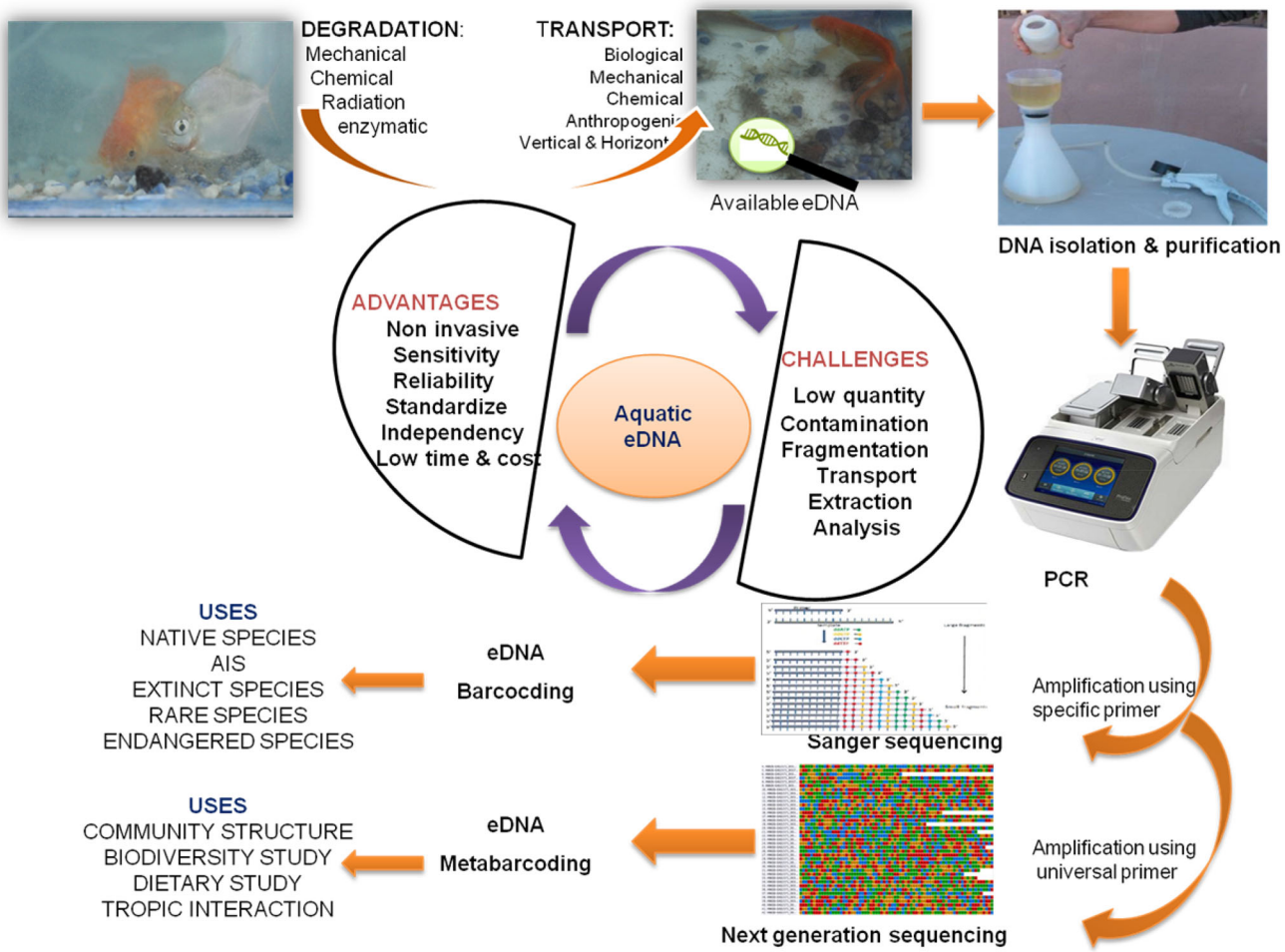

Fig. 1 Experimental workflow in aquatic environmental DNA (eDNA) barcoding or metabarcoding studies

primary sources of eDNA component in momentous amount. While aquatic invertebrates such as chironomids are individually much smaller in size and number, where the accumulated biomass of the community evidently produces required detectable and persistent amounts of eDNA from natural shedding, moulting and finally death. For the biodiversity assessment in significant ways it can be thoroughly dynamic nature with changeable seasonality of its natural habitat (Bista et al. 2017).

Before collection of eDNA for further analysis, it is more important to know about what factors are influencing eDNA production- it will unmasking the taxa and environment perception for which individual's eDNA represent as an dependable ecological survey tool and promising future weapon for conservation ecology. For example, how to collect eDNA, we must have require the accurate information about the relationship between eDNA production and accurate size of organisms, their age and/or their proper biological activity. As water temperature is identified having impact the real metabolism rate of fish and therefore could robustly affect eDNA discharge (Lacoursière-Roussel et al. 2016a, b; Robson et al. 2016). Apart that, the eDNA detection likelihood for the black warrior waterdog (*Necturus alabamensis*) and the flattened musk turtle (*Sternotherus depressus*) are significantly influenced by specified time of a year during sampling. The detection chances showed strongly highest for *N. alabamensis* in cold season and *S. depressus* in warm season which are consistent with the single place of both species (de Souza et al. 2016).

Methodology of eDNA Collection from Aquatic Ecosystem

It's a different experimental methods for collecting aquatic eDNA sample and analyzing including precipitation of small volumes (15–50 ml) (Dejean et al. 2012; Ficetola et al. 2008; Foote et al. 2012; Piaggio et al. 2014; Thomsen et al. 2012a, b) after that filtration of larger volume (> 50 ml) (Goldberg et al. 2011, 2013; Pilliod et al. 2013, 2014; Jerde et al. 2011). Filtration techniques

provides the suitible benefit of collecting DNA components from larger volumes of water, however, it loses dissolved DNA content, that may possibly provide increased detection (Deiner and Altermatt 2014). There are also variants on filtering methods, including 1.2 µm isopore membrane (Barnes et al. 2014), 0.45 µm cellulose nitrate filter (Goldberg et al. 2011), 1.5 µm glass fibre filter (Jerde et al. 2011), 0.22 µm durapore membrane filter (Williams et al. 2016), 3 µm isopore polycarbonate filter (Minamoto et al. 2012). However, a superior comparison is presence between filtering and extraction techniques and recommended to the filtering and extraction with the Qiagen DNeasy Blood and Tissue kit for proper detection of macroorganisms. But, there results also indicated that this method may not be the best method for characterizing the micro-organisms biodiversity (Deiner and Altermatt 2014).

Physical form of Aquatic eDNA

Pioneer microbial researcher showed that genetic materials (eDNA) in environment are present in both intracellular and extracellular forms (Ogram et al. 1987). Even though, there is no much more research exits on the different ecological processes driving to the eDNA transition (Levy-Booth et al. 2007). However, some studies have well thought-out of what physical state being collection during an eDNA survey. The collection of Common Carp eDNA from small lake and zoo pond by the serial filtration of water samples (Turner et al. 2014) through filters of decreasing pore size (180–0.2 µm). This finding concluded that aggregation of eDNA from cells of common carp collected on filters with larger pore sizes and extra membranous eDNA in the last precipitation is seems as particle sizes < 0.2 µm. After releasing genetic materials from sources, it has some possible consequences likewise; in aquatic ecosystem, naked genetic materials vulnerable to microbes or others having DNAse, with the former well-known believed to the key mechanisms for DNA degradation in the environment (Blum et al. 1997). Nuclease activity of eDNA can reduced by binding to environmental compounds such as clay minerals, larger organic molecule and others charged ionic particles (because DNA is negatively charges). More than 50% of extracellular DNA was recalcitrant by enzymatic degradation and DNA might play a non negligible role in Phosphorus Biogeochemical cycle (Dell'Anno et al. 2002).

Existence of eDNA in Aquatic Ecosystem

Current studies suggested that eDNA is much more stable in freshwater ecosystem than in marine ecosystem. But there is very little information about how environmental condition direct and indirectly reflects the existence of eDNA. Different laboratory protocol, freshwater and natural pond experimental result revealed that the fish species likewise Common carp (*Cyprinus carpio*), silver carp and bighead carp (*Hypophthalmichthys* sp.) eDNA collapse extremely soon, after 4 long days the accurate detection chances is $< 5\%$ even degradation rate is negatively related with the microbial activity and pH (Barnes et al. 2014). Furthermore, eDNA detection is rightly possible in pond sediments for > 130 days after species taking away (Turner et al. 2014; Merkes et al. 2014). Laboratory based freshwater mesocosms and small natural ponds experiment with the experimental species such as, American bullfrog (*Rana catesbeiana*), Siberian Sturgeon (*Acipenser baerii*), Idaho giant salamander (*Dicamptodon aterrimus*), Common spadefoot toad (*Pelobates fuscus*) and great crested newt (*Triturus cristatus*) having the chance of eDNA moreover 5% detection probability for 15–20 days it also much more stable in shaded environment than full-sunlight exposure higher temperature and Ultraviolet B interacted positively with eDNA degradation (Pilliod et al. 2014; Dejean et al. 2012; Strickler et al. 2015; Thomsen et al. 2012a, b). Generally, the open range of persistence period of the aquatic eDNA in the published literature indicated that there is still much additional research needed to know about real facts and consequences of eDNA degradation. Besides that, it helps us better and clears understanding to detect organism in spatiotemporal variation and political space support to the focus of prime objectives (Fig. 2).

Transportation of eDNA in Aquatic Ecosystem

eDNA moves through its aquatic environment, by the way of physically or chemically following being shed from an organism, which could influence the inferences of eDNA-based research design and biodiversity conservation. Previously, eDNA research work performed on genetically transgenic agricultural organism then transferring to bacterial and others organisms (Douville et al. 2007). Through present study, we screened and summarized the all pattern of the way of transportation of eDNA in aquatic ecosystem from different refereed research publications (Table 1).

eDNA Analysis: A Novel Application Method

In twentieth century, it may consider that the frontiers compare to the use of eDNA analysis method and traditional surveys technique for the species detection to ecological and ecosystem management study. Aquatic eDNA could be helpful for some following reasons which discuss in below.

Nondestructive

As collection of desired sample (i.e. genetic materials) obtained from target environment and not from direct physical contact of particular species or particular population which occurs in traditional surveys methods, so eDNA analysis method is noninvasive than conventional method. In conventional method the species of interest has to be disturbed or caught to get a positive recognition and encroaching on animal welfare (Martellini et al. 2005; Olson et al. 2013). In USA, study with two species such as, Asian Carp-Silver Carp (*H. molitrix*) Big headed Carp (*H. nobilis*) which is rare and threatened aquatic species could find through eDNA analysis. So, it is proved that eDNA have been occupy a powerful biological tool for survey in future as its nondestructive nature.

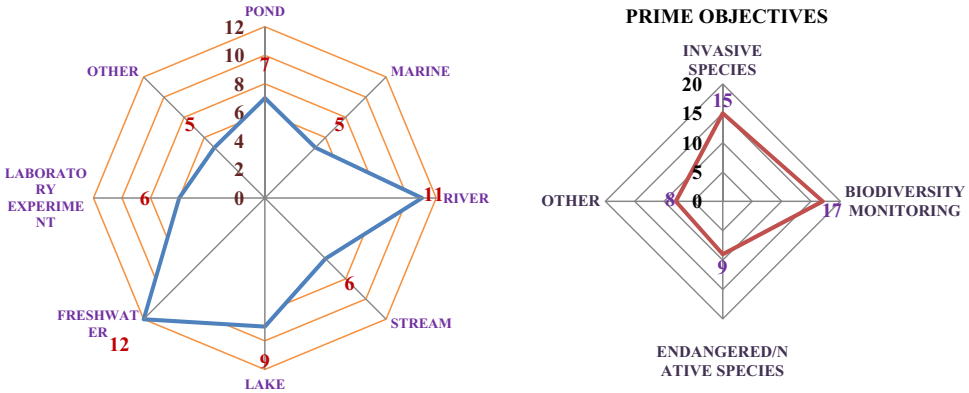

Fig. 2 Number of scientific articles published on aquatic eDNA to detect the presence of aquatic organism according to their ecosystem type (left panel) and interest for detection (right panel). A total number of 46 relevant articles were found and when more than one prime objectives (or more than one ecosystem type) were included in an article, the study was counted as many times as the numbers of interest for detection (or ecosystem type) reported to create these diagrams

Table 1 Summary of transport pattern of aquatic eDNA

Environment	Geographic location	Target eDNA	Physical move	References
River	Richelieu and ST. Lawrence rivers	*cry1Ab* gene from Bt corn (*Bacillus thuringiensis*)	82 km downstream from the corn cultivation plot	Douville et al. (2007)
Groundwater	Fountains of Geneva Champagne Basin	18s rDNA of plant, DNA including *Vitis rupestris*, *Vitis berlandieri*, *Polygonum* sp., *Boopis graminea*, and *Sinapis alba*	From an agricultural field into a nearby waterway at a depth of 3.2 m	Poté et al. (2009)
Two small brooks (Strijbeekse Beek and the Merkske)	Netherlands	Synthetic DNA	Detectable at the furthest sampling site 1192 m downstream	Foppen et al. (2011)
River	Study area within Switzerland	*Daphnia longispina* and *Unio tumidus*	*D. longispina* at all locations hereas with *U. tumidus*, and a decreased detection rate and did not detect (i.e. horizontally downstream) its eDNA after 9.1 km	Deiner and Altermatt (2014)
Experimental ponds and natural rivers, the University of Kansas Field Station (KUFS)	Lawrence, Kansas, USA	Bigheaded Asian carp (*Hypophthalmichthys* sp.)	Vertical transport from surface water to sediment	Turner et al. (2014)
Stream water	A tributary of the Avery Brook system in Conway, Massachusetts	Trout	239.5 m downstream from the source	Jane et al. (2015)

Sensitivity

Investigation also showed that the eDNA of interest species were detected in each aquatic body. Even eDNA was detected where the targeted species are not observed in particular time scale and political space (Martellini et al. 2005). On the other hand, where species are important for conservation that have different life stages, cryptic life and sibling species that are difficult to confirm species recognition, still taxonomic expertise have lack of knowledge and often inadequate information, here eDNA analysis method can strongly superior than traditional survey method to detect species (Dejean et al. 2012; Biggs et al. 2015).

Reliability

Many studies showed that the eDNA research approaches could be more functional for studying cautious aquatic and semi aquatic environment (Ficetola et al. 2008). For occurrences the traditional sampling method, for the species detection the Chucky madtom (*Noturus crypticus*) a united stated federal endangered species was last observed in year 2004, as the federal stated decide for this species as supposed to extinct, but in eDNA survey methods showed the occupancy of density of the target organism (Goldberg et al. 2011; Takahara et al. 2012). eDNA survey method show much better detection chance over traditional survey or historical data and reliability of this method was cautiously validated in vitro, in silico and in situ situation (Valentini et al. 2016).

Time and Cost Effectiveness

eDNA method give us more information than traditional method (Deiner et al. 2016). For instance, classical sampling only detect a fraction of actual local diversity due in parts to spatial auto co-relation, where as eDNA sampling permit an estimate of catchment level diversity jointly both aquatic and terrestrial taxa. As next generation sequencing (i.e.-NGS method) method and price of sequencing per base pair decline continues (Schuster 2008), so eDNA definitely become superior when using metabarcoding approach to some traditional method.

Standardized

In general, eDNA as a survey tool have still downside in water body (Roussel et al. 2015; Ebach and Holdrege 2005), but it obtaining an environmental sample and result appear from examination of environmental sample can be conceded in a very standardized way across localities in a certain type of territory (Deiner et al. 2016) rather than depends on personnel carrying out of surveys.

Independency

Several traditional survey methods like visual, auditory etc. are deeply belief on taxonomist or experienced persons which are fast decline (Bacher et al. 2012; Hopkins and Freckleton 2002). Furthermore, some species not found throughout the year, if we want to detection or know their existence, we able to wait for particular season. However, in eDNA survey method there is no dependency over taxonomy (or partly dependence on taxonomy) because here, species exposure occur by molecular bioinformatics, sequence comparison to existing databases (DNA barcoding and DNA meta barcoding) which is stored as web materials and easy to get access.

Prospects of Aquatic eDNA

Among the huge benefits of eDNA as here describe that its noninvasiveness, sensitivity, reliability, time and cost effectiveness, standardized, independency, eDNA has been uses in diverse scientific area as well as ecology, conservation biology in aquatic ecosystem (Table 2).

eDNA Barcoding

Several studies confirm from concept to practice of species detection with eDNA are possible and its starts from the species detection take up by the environmental DNA from water samples (Ficetola et al. 2008) to detect the presence of a frog (*Rana catesbeiana*), where mitochondrial DNA sequences utilized (Fig. 1) out of natural pond as well as controlled condition then it exuberant in many other aquatic ecosystem (Fig. 2, right panel).

Alien Invasive Species (AIS) and Native Species

eDNA sampling help for detecting invasive as well as native aquatic species co-occur with each other with narrowly linked taxa. Following all aquatic eDNA case studies only 18% research occur on native species and 31% finding happened for AIS identification (Fig. 2, left panel) over 46 article. Depending qPCR marker based approach distinguish Westslope cutthroat trout (*Oncorhynchus clarkii lewsi*), Yellowstone cutthroat trout (*O. clarkii bouvieri*), and Rainbow trout (*O. mykiss*), was well studies in Montana, United States of America (Wilcox et al. 2015a). Another current study in Missoula, MT, USA investigated fast detection with the experimental invasive species

Table 2 Examples of recognized species aquatic eDNA case studies by scientific article publication worldwide

S. no.	Environment	Prime objective	Species	Uses of target Sequence	Method	References
1.	Pond	Pathogen detection	*Batrachochytrium dendrobatidis* (Bd)	Fungal DNA	PCR	Kirshtein et al. (2007)
2.	Natural pond and laboratory method	Detection of invasive species	American bullfrog (*Rana catesbeiana* = *Lithobates catesbeianus*)	Mitochondrial *cyt-b*	Standard PCR	Ficetola et al. (2008)
3.	Bight	Dietary analysis	Diverse taxa	Mitochondrial 16S ribosomal RNA gene	Standard PCR	Braley et al. (2010)
4.	Marine	Measuring biodiversity	Diverse taxa	Mitochondrial COI gene and nuclear ribosomal RNA genes	Standard PCR	Creer et al. (2010)
5.	Marine	Measuring biodiversity	Diverse	rRNA gene	Standard PCR	Fonseca et al. (2010)
6.	River	Measuring biodiversity	Benthic macro invertebrate taxa	COI	Standard PCR	Hajibabaei et al. (2011)
7.	Stream	Measuring biodiversity	Rocky Mountain tailed frogs (*Ascaphus montanus*) and Idaho Giant Salamanders (*Dicamptodon aterrimus*)	A small region of the mitochondrial DNA (mtDNA) cytochrome *b* gene	Standard PCR	Goldberg et al. (2011)
8.	River and canal complex	Detection of invasive species	Asian carps, Silver carp (*Hypophthalmichthys molitrix*) and Bighead carp (*H. nobilis*)	Mitochondrial d-loop region	Standard PCR	Jerde et al. (2011)
9.	Lake	Detection of invasive species	Zebra mussel (*Dreissena polymorpha*)	18S rDNA gene	Standard PCR	Lance and Carr (2012)
10.	Pond	Detection of invasive species	American bullfrog (*Rana catesbeiana* = *Lithobates catesbeianus*)	COI	Standard PCR	Dejean et al. (2012)
11.	Marine	Detection of native species	Harbor porpoise (*Phocoena phocoena*)	12S region mtDNA sequences	qPCR	Foote et al. (2012)
12.	Pond	Pathogen detection	Fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*)		qPCR	Hyman and Collins (2012)
13.	Pond and laboratory method	Detection of fish species composition	Five fish species	Mitochondrial cytochrome *b* gene	Standard PCR	Minamoto et al. (2012)
14.	Lake and laboratory experiment	Estimation of fish species biomass	*Cyprinus carpio*	Mitochondrial cytochrome *b* gene	RT-PCR	Takahara et al. (2012)
15.	Ponds, lakes and streams	Measuring biodiversity	Diverse taxa	Mitochondrial genes (cytochrome oxidase I and cytochrome b)	qPCR	Thomsen et al. (2012a, 2012b)
16.	Marine	Diverse fish fauna	Diverse taxa	Mitochondrial gene cytochrome b (*cytb*) in fish	Standard PCR	Thomsen et al. (2012a, 2012b)
17.	Freshwater	Detection of invasive species	Zebrafish or zebra danio (*Danio rerio*)	Cytochrome c oxidase subunit I (COI)	Standard PCR	Collins et al. (2013)

Table 2 continued

S. no.	Environment	Prime objective	Species	Uses of target Sequence	Method	References
18.	Lake	Detection of invasive species	Zebra mussel, (*Dreissena polymorpha*)	universal invertebrate mtDNA COI gene	Standard PCR	Mahon et al. (2013)
19.	River and laboratory method	Detection of invasive species	Zealand mudsnail (*Potamopyrgus antipodarum*)	COI	qPCR	Goldberg et al. (2013)
20.	Streams	Measuring/monitoring biodiversity	Rocky Mountain tailed frogs (*Ascaphus montanus*) Idaho giant salamanders (*Dicamptodon aterrimus*)	Mitochondrial DNA		Pilliod et al. (2013)
21.	Pond	Detection of invasive species	Bluegill sunfish (*Lepomis macrochiru*)	Mitochondrial cytochrome *b* gene	qPCR	Takahara et al. (2013)
22.	Lake and stream	Detection of rare species	Brook trout (*Salvelinus fontinalis*) and bull trout (*S. confluentus*)	*Cytochrome b* gene (*cyt b*	qPCR	Wilcox et al. (2013)
23.	Freshwater	Measuring biodiversity	Diverse taxa	The hypervariable V4 region of the nuclear small subunit ribosomal DNA (V4-nSSU)	Standard PCR	Zhan et al. (2013)
24.	Freshwater and river	Rare and indicator species	Diverse macro invertebrate taxa	Cytochrome oxidase I	Standard PCR	Mächler et al. (2014)
25.	Lake	Detection of invasive species	Diverse fish species	Mitochondrial COI	qPCR	Mahon et al. (2013)
26.	Freshwater	Detection of invasive species	Burmese python (*Python bivittatus*)	Mitochondrial cyt *b*	Standard PCR	Piaggio et al. (2014)
27.	Mesocosm experiment	Estimate fish abundance and biomass	Common carp, (*Cyprinus carpio* L)	Mitochondrial cytochrome *b* gene	qPCR and ddPCR	Doi et al. (2015)
28.	Freshwater	Detection native and invasive species	Westslope cutthroat trout (*Oncorhynchus clarkii lewsi*), Yellowstone cutthroat trout (*O. clarkiibouvieri*) and rainbow trout (*O. mykiss*)	*NADH* region of the mitogenome	qPCR	Wilcox et al. (2015a)
29.	Freshwater and Mesocosm experiment	Particle size distribution	Brook Trout (*Salvelinus fontinalis*)	Mitochondrial gene	qPCR	Wilcox et al. (2015b)
30.	Freshwater	Detection of native species	Arctic grayling (*Thymallus arcticus*)	Cytochrome C oxidase 1 (COI) mitochondrial gene	qPCR	Carim et al. (2016a)
31.	Freshwater	Detection of introduce species	Brown trout (*Salmo trutta*)	Cytochrome b (cytb) region of the mitochondrial genome	qPCR	Carim et al. (2016b)
32.	Freshwater and lake	Detection of invasive species	*Mysis diluviana* (Crustacean)	Cytochrome C oxidase subunit I (COI) sequences	qPCR	Carim et al. (2016a)
33.	River	Measuring biodiversity	Diverse taxa	Cytochrome C oxidase 1 COI	Standard PCR	Deiner et al. (2016)
34.	River	Detection of endangered species	Loach minnow (*Rhinichthys cobitis*) and spikedace (*Meda fulgida*)	Cytochrome b (cytb) mitochondrial gene	qPCR	Dysthe et al. (2016)

Table 2 continued

S. no.	Environment	Prime objective	Species	Uses of target Sequence	Method	References
35.	River and lake	Detection of endangered species and measuring herpetofauna	Wood turtle (*Glyptemys insculpta*) and diverse taxa of herpetofauna	Mitochondrial *Cytochrome C oxidase subunit I* (COI), cytochrome *b* (cytb), 12S, 16S, and 18S ribosomal subunits	Standard PCR and qPCR	Lacoursière-Roussel et al. (2016a, b)
36.	Freshwater and aquarium experiment	Estimating fish biomass and abundance	Brook Charr fingerlings	*Cytochrome b* gene (cyt b)	qPCR	Lacoursière-Roussel et al. (2016a, b)
37.	Stream	Detection of rare species	Bull trout (*Salvelinus confluentus*)	–	qPCR	McKelvey et al. (2016)
38.	River	Measuring fish species richness	Six Indo-Pacific marine fishes	*Cytochrome b* gene, two sections of the 12s gene, and 16s rDNA	Standard PCR	Olds et al. (2016)
39.	Freshwater	Detection of invasive species	Mozambique tilapia (*Oreochromis mossambicus*) and spotted tilapia (*Tilapia mariae*)	Mitochondrial 16S rRNA	qPCR	Robson et al. (2016)
40.	Seawater	Detection of endangered species	Whale shark (*Rhincodon typus*)	Mitochondrial (mtDNA) (two polymorphic regions; DL1: 412 bp and DL2: 476–493 bp)	qPCR	Sigsgaard et al. (2016)
41.	River and lake	Measuring biodiversity	Diverse taxa	mtDNA and 12s rRNA	Standard PCR	Valentini et al. (2016)
42.	Stream	Estimate eDNA production and detection probabilities	Brook trout (*Salvelinus fontinalis*)	Mitochondrial DNA	qPCR and Standard PCR	Wilcox et al. (2016)
43.	Freshwater	Measuring biodiversity	Diverse taxa	COI	Standard PCR and qPCR	Bista et al. (2017)
44.	River and laboratory experiment	Detection of native and endangered species	Salmonidae	16S rRNA gene	Standard PCR	Clusa et al. (2017)
45.	Estuary	Detection of fish population	Diverse fish species	COI and 12S sequences	Standard PCR	Stoeckle et al. (2017)
46.	River	Detection of invasive fish	Zebra mussels (*Dreissena polymorpha*).	Mitochondrial gene: *Cytochrome C oxidase subunit I* (COI), *cytochrome b* (Cyt b), and 16S rRNA	qPCR	Gingera et al. (2017)

Brown trout (*Salmo trutta*), which inhabitant in Germany and Scotland and arctic grayling native in the upper Missouri river basin in North America are detected well and incredibly prompt through eDNA survey (Carim et al. 2016a, b). The quick detection of AIS allowing managers intended for reduce spreading and settlement of the invaders. As study on Opossum shrimp (*Dysis diluviana*) in USA, proved that eDNA is a strongly preference and reliable method for species detection (Carim et al. 2016a, b, c). Furthermore, eDNA method also could help to tone down in the spread of invasive species by monitoring ornamental aquatic organisms (Collins et al. 2013).

Extinct and Endangered Species

The use of eDNA has proliferated for the detection of rare species, threatened species or endangered taxa on the basic of large and local geographic area in recent scenario. Detection of bull trout (*Salvelinus confluentus*) which is rare species as declare by the US Fish and Wildlife Service (1999) in USA are detected fast, reliable and sensitive by eDNA survey method (McKelvey et al. 2016) than electrofishing. Another example, Loach minnow (*Rhinichthys cobitis*) and Spikedace (*Meda fulgida*) well studies with eDNA which is protected Endangered under the U.S. Endangered Species Act and are endemic to the Gila River basin of Arizona and New Mexico (Dysthe et al. 2016). Not only eDNA detect rare species but it also be an evidence for in extinct species as provide evolutionary history (Bunce et al. 2005) in new Zealand with the experimental species giant eagle (*Harpogornis moorei*).

Species Composition and Biomass Estimation

Detection and quantification of aquatic eDNA can be say as a preference and or indirect measurement of biomass evaluation. There are very little research work done relating to species composition and biomass estimation indices to aquatic eDNA (Fig. 2). A laboratory experiment at the Research Institute for Humanity and Nature (RIHN), Kyoto Prefecture, Japan and field experiment with the experimental species *Cuprinus carpio* found that a highly positive correlation between the concentration of eDNA and Carp biomass and among the number of Carp fish and its biomass (Takahara et al. 2012). So, thus it not only use to perceive invasive species but also it facilitate approximation of invasive species (Takahara et al. 2013). Another similar study utilized in fishes (Mahon et al. 2013) and in amphibian population distribution in France. Antoinette J Piaggio, estimated reptiles species successfully in Florida by the highly sensitive eDNA method (Piaggio et al. 2014).

In marine environment, till now no studies have been conducted to determine the relationship between eDNA concentration and species biomass determination. Additionally, the microbial community composition would be one of the chief factors for the DNA degradation and accumulation. Therefore, more study is essential in future to weigh up the relationships linking the microbial composition and eDNA concentration in natural surroundings.

eDNA Metabarcoding

Although, just it could acknowledged and focused on eDNA applications targeting single species, but stay many researchers have ongoing to find various species concurrently by implementing universal primers paired with cloning and after that Sanger sequencing (Minamoto et al. 2012) or high throughput sequencing (i.e.-HTS)—these address as metagenomics, metabarcoding, metagenetics, metasystematics (Taberlet et al. 2012b). Using eDNA survey within a single sampling may serves to identify multiple species with more economical, sensitive and efficient (Dejean et al. 2012; Biggs et al. 2015). The description of intact communities or groups of organisms in aquatic environment through eDNA manner have begun to start from monitoring endangered fresh water biodiversity where amphibians, fish, mammals, insects and crustaceans taxons are studies in Northern Europe in 2012 (Thomsen et al. 2012a, b). Another study have been fully done (Minamoto et al. 2012; Pilliod et al. 2013) combining river, dammed pool, artificial containers and streams water. First fresh water benthic macro invertebrate community access all the way through nondestructive eDNA technique in the Humber River in Southern Ontario, Canada, in order that, result indicate that the effectiveness of eDNA (ethanol based DNA extraction) providing sequence information is about 87% of taxa identified individually from mixture, as compared to 89% in conventional tissue extracted DNA (Hajibabaei et al. 2011).

As freshwater fishes and amphibians are more sensitive to particular environmental alteration and disturbance (Brander 2007) and it incorporated many taxa that are highly threatened and may globally declines (Stuart et al. 2004). So, fish and amphibian biodiversity (regionally) serves as a indicator of changes ecosystem health. Focus on this view mesocosm fish and amphibian species diversity are quantified via eDNA metabarcoding method (Evans et al. 2016). In water body, herpetological survey consequences empirically support the effectiveness of the eDNA method (Lacoursière-Roussel et al. 2016a, b). Italian scientist Valentini (2016), experiment with the amphibians and bony fishes in different aquatic ecosystem (ponds, ditches, streams, lakes and rivers) in Netherlands and in France show next generation monitoring of aquatic biodiversity is more significant via eDNA method.

Tropic Interaction and Dietary Studies

Understanding of food webs is a fundamental key to our understanding of aquatic ecosystem structure, health, stability and function (Hindell et al. 2003). As direct observation of tropic interactions and prey predator relationship is difficult in the aquatic environment as well as marine body, so that diet determination has been typically restricted through morphological identification of prey which remains in stomach content, faeces or fecal pellets (Symondson 2002). When observing small or elusive animals with cryptic food-web ecology there have not possibility to track tropic interactions connecting predators and prey by direct observation (Sheppard and Harwood 2005). In traditional method, observation or identification of the prey in the stomach or in the fecal material often is difficult and can reduce the taxonomic resolution or introduce bias (Braley et al. 2010). Thus eDNA meta-barcoding approach can be used for tropic and dietary studies devoid of the identification of the prey.

Historical Species Distribution Patterns

Environmental DNA sequences can persist for long time periods mainly permafrost (Kochkina et al. 2012) and temperate sediments (Turner et al. 2015) and DNA may preserved in the absence of obvious macrofossils (Willerslev et al. 2003). Aquatic sediments have also proven to be rich in eDNA, in fact in oceans the largest reservoir of extracellular DNA is marine sediments (Dell'Anno and Corinaldesi 2004). Especially nuclease degradation is inhibited by marine anoxic condition that favors strengthen the preservation of DNA (Corinaldesi et al. 2011). Thus, ancient eDNA in sediment or other environmental sources could allow us to gain the information about evolutionary documentation, reconstruct community structure and historical ecology in future experiments.

Critical Considerations of eDNA Application in Aquatic Environment

Aqueous eDNA sources from macro organisms usually spread heterogeneously all over in water body and it occurs at incredibly low concentration for example about e.g. < 200 picogram (pg)/liter (Takahara et al. 2012; Pilliod et al. 2013). Consequently eDNA sampling for species uncovering is contingent upon genetic sign detection probability (MacKenzie et al. 2002). This chance depends on capture efficacy, extraction efficacy, sample interference (e.g. inhibition) and analyze sensitivity. As example, eDNA had reasonable tackle chances at principally low animal densities (e.g. 0.18% detection chance at densities of single fish per stream kilometer) and very high detection probabilities at densities level is high (e.g. detection probability is > 0.99 at fish densities is ≥ 3 per 100 m) (Wilcox et al. 2016). Due to high sensitivity of eDNA methods, sample collection procedure should reduce the probability of contamination and studies should account details of precautions.

Environmental DNA start to degradation immediately after shedding (Thomsen et al. 2012a, b), even this process continues in subsequent sample collection caused by mechanical forces, microbial activity (nucleases) and spontaneous chemical reactions (oxygenation) (Lindahl 1993; Nielsen et al. 2007). For that reason, sampled should be defended using a standardized protocol immediate following the collection. Water samples have been kept on ice for up to 24 h devoid of reducing eDNA detection (Pilliod et al. 2013), and result from USA scientist, Katherine M. Stickler point to that keeping samples cool significantly reduces eDNA degradation rate (Strickler et al. 2015). Biological conservation scientist Teruhiko Takahara took this likewise a profound step and established that, freezing and then thawing samples might be drastically shrink (Takahara et al. 2015).

One of the major challenges concerned with the eDNA sample extraction, which is performed from the rest of the environment are still also contained in the experimental sample. Even, several of these substances are detached during extraction (and potentially filtering) events, some are co-extracted and possibly will inhibit the PCR reaction. If, the same trouble are concealed, samples perhaps classified as negative, when they in actually contain the DNA of the target organism. McKee et al. (2015), demonstrate that a silica-based inhibitor removal kit may offer higher rates of detection while compared with 5–10-fold dilutions, although a quantity of eDNA (approximately 25%) is lost in the trial process (McKee et al. 2015).

Another consideration is that most of research mostly focused on vertebrate species (primarily fishes and amphibians) and it seems early to indicate that eDNA will be a proficient survey tool for detecting all the species inhabited within the aquatic environment. Even detection of eDNA in water sample is effective for studying secretive aquatic and semi-aquatic species, which release DNA components into the surroundings through mucus, faeces, urine (Ficetola et al. 2008).

In case of survey, threatened species or detecting extinct species on the basic of local or geographical region there have difficulties for eDNA analysis. For instance, eDNA can transport verticality or horizontality, so the species still detected by eDNA method where target species not remain. So, one of the major disadvantage of eDNA method is that it affected by false positive detection, which introduces

together complexity in occupancy models using eDNA survey results (Moyer et al. 2014).

Discussions

Ecosystem function is fully dependant on key constituent of biodiversity (Risser 1995) and species richness is a basic measure of that biodiversity that is underlying by many ecological and models concepts (Gotelli and Colwell 2011). So, proper knowledge of natural ecosystem, biodiversity is fundamental aspects to accessing the real function of ecosystem and the prediction of impactful outcomes due to the environmental changes (Butchart et al. 2010). Over 5900 freshwater and marine animals' species are listed as endangered world-wide (IUCN Red List 2012) (Nature 2012). So, estimation of species richness in aquatic environments can be special challenging aspect due to the rareness of some species (Gu and Swihart 2004); therefore the development and validation of techniques are rising sensibility in the management and conservation of these species. Here, Environmental DNA technique is an impetuous becoming a significant tool in the study of aquatic species. On the other way, eDNA technique is a rapid method for the detection of single species or target species which is vital for positive ecosystem management. The technique also could provide itself to use a mobile sampling until permit for fast on site detection of target species in a specific way.

In eDNA method sequencing is a fundamental way for biological recording, sequence comparison, for detection target species and monitoring biodiversity. Presently, the advance in DNA sequencing technologies has notably overgrown the probabilities of using eDNA and is expected to continue improving in the upcoming state. If we see the first studies using eDNA build on cloning and following Sanger sequencing of PCR products and many experiments remain do. So, it is to be sure that the new emerging sequencing technologies will have great impact on eDNA studies (Shokralla et al. 2012), subsequently it become a fully integrated part of ecologists' toolbox (Baird and Hajibabaei 2012; Taberlet et al. 2012b; Valentini et al. 2009). Furthermore, new generations of powerful technologies such as real-time laser transmission spectroscopy (Egan et al. 2013; Li et al. 2011), novel real-time sequencing techniques e.g. PacBio RS by Pacific Bioscience or Nanopore-based sequencing by Oxford Nanopore Technologies, carbon nanotube chips (Mahon et al. 2011) and next generation sequencing technologies like Roche 454 Genome Sequencer (Roche Diagnostics Corp., Branford, CT, USA), HiSeq 2000 (Illumina Inc., San Diego, CA, USA), AB SOLiD^{TM} System (Life Technologies Corp., Carlsbad, CA, USA) moreover Ion Personal Genome Machine (Life Technologies, South San Francisco, CA, USA) (Shokralla et al. 2012) are ongoing and/or waiting for their promising potential in eDNA approaches. Thus, it is expected that the use of eDNA in conservation and biological monitoring will move from single-marker analyses of species or communities to meta-genomic surveys of entire ecosystems for predicting spatial and temporary biodiversity patterns (Davies 2012; Baird and Hajibabaei 2012).

The international barcode of life data systems portal (http://www.boldsystems.org/) has over 3.5 million geo referenced DNA sequences stored and is climbing-up gradually. The Barcode of Life Data System (BOLD) which is a bioinformatics workbench aiding the gaining, storage, analyzing and publication of DNA barcode records (http://www.barcodinglife.org). By assembling molecular, morphological and distributional data in computational way, it bridges with a traditional bioinformatics chasm. Researcher have freely access BOLD with interests in DNA barcoding (Ratnasingham and Hebert 2007). Another online tool is BLAST (Basic Local Alignment Search Tool), which finds regions of similarity linking biological (DNA nucleotides) sequences databases and calculated the measurable statistical significances. Also MEGAN (METaGenome Analyzer), a computer assistance program which permits the optimized analysis of large metagenomic datasets (Huson et al. 2007). So, all of these bioinformatics tool very effectively help to support in species detection and measuring biodiversity.

It might be highlight that eDNA approaches will complement rather than replace traditional monitoring in aquatic environment. This is evident from the literature on aquatic body, where American bullfrog Rana catesbeiana (*Lithobates catesbeianus*) (Ficetola et al. 2008), biomass estimation of *Cyprinus carpio* (Takahara et al. 2012), estimation of fish species composition (Minamoto et al. 2012) are all more or less complements with eDNA method (Parducci et al. 2013; Pedersen et al. 2013) and rational detection probabilities of eDNA for some freshwater taxa (Thomsen et al. 2012a, b). Furthermore, eDNA have huge potential to integrate with the postulated "ecogenomic sensors" (Scholin 2010) by which not only we estimate biodiversity but we are able to know the different parameters of micro label in aquatic ecosystem.

Currently, the eDNA research advances have afford many valuable insights to the support of earliest environments and it is already proven as the useful for monitoring the contemporary biodiversity in terrestrial as well as the aquatic ecosystems. In upcoming days, it may definitely anticipate the eDNA- based drive to move from single-marker analyses of the experimental species or communities to the meta-genomic surveys of whole ecosystems to predict and characterize the spatial and temporal

biodiversity patterns. That modern tool have greater applications for a wider range of geological, biological and environmental sciences. For time being such accomplishments gained throughout the analyses of eDNA from targated organisms in a conservation context and better sustaining its latent limitations and advantages specially for biodiversity monitoring.

Conclusions

Consequences from the investigation it may undoubtedly say that, a closer attention required for the most comprehensive way of utilizing the aquatic eDNA for the superior benefit of biological monitoring and conservation aspect of aquatic ecosystem. Although environmental DNA will not only the absolute way of the species detection but it could be facilitate and surely having positive impacts to nursing and increasing of the biodiversity. Parallely, such tools also offers the most technically fastest and scientifically efficient insights on the distribution of species, along with the species abundance and ultimately detection of whole population size, these information provides the significant knowledge for taking proper conservation and management action plans. However, it will never directly mitigate the challenges of biological survey method, so some sorts of competent approaches are essential for better innovative technique in upcoming future in connection with the eDNA analysis based research protocol.

Acknowledgements Authors are highly thankful to University Grants Commission (Ref. No.831 344156), Science and Engineering Research Board (Project file No Ref. No. PDF/2016/001776), Department of Science and Technology, Government of India for financial assistance to carried out the research work.

References

Bacher, Sven. 2012. Still not enough taxonomists: Reply to Joppa, et al. *Trends in Ecology & Evolution* 27(2): 65–66.

Baird, Donald J., and Mehrdad Hajibabaei. 2012. Biomonitoring 2.0: A new paradigm in ecosystem assessment made possible by next-generation DNA sequencing. *Molecular Ecology* 21(8): 2039–2044.

Baldwin, C. C., B. B. Collette, L. R. Parenti, D. G. Smith, and V. G. Springer. 1996. Collecting Fishes. pp. 11-33 in: Lang, M. A. and C. C. Baldwin (eds.). Methods and Techniques of Underwater Research. Proceedings of the 16th Annual Scientific Diving Symposium, American Academy of Underwater Sciences.

Barnes, Matthew A., Cameron R. Turner, Christopher L. Jerde, Mark A. Renshaw, W. Lindsay Chadderton, and David M. Lodge. 2014. Environmental conditions influence eDNA persistence in aquatic systems. *Environmental Science and Technology* 48(3): 1819–1827.

Barnes, Matthew A., and Cameron R. Turner. 2016. The ecology of environmental DNA and implications for conservation genetics. *Conservation Genetics* 17(1): 1–17.

Barnosky, Anthony D., Nicholas Matzke, Susumu Tomiya, Guinevere O.U. Wogan, Brian Swartz, Tiago B. Quental, Charles Marshall, Jenny L. McGuire, Emily L. Lindsey, and Kaitlin C. Maguire. 2011. Has the Earth's sixth mass extinction already arrived? *Nature* 471(7336): 51–57.

Beebee, Trevor J.C. 1991. Analysis, purification and quantification of extracellular DNA from aquatic environments. *Freshwater Biology* 25(3): 525–532.

Biggs, Jeremy, Naomi Ewald, Alice Valentini, Coline Gaboriaud, Tony Dejean, Richard A. Griffiths, Jim Foster, John W. Wilkinson, Andy Arnell, and Peter Brotherton. 2015. Using eDNA to develop a national citizen science-based monitoring programme for the great crested newt *(Triturus cristatus)*. *Biological Conservation* 183: 19–28.

Bista, Iliana, Gary R. Carvalho, Kerry Walsh, Mathew Seymour, Mehrdad Hajibabaei, Delphine Lallias, Martin Christmas, and Simon Creer. 2017. Annual time-series analysis of aqueous eDNA reveals ecologically relevant dynamics of lake ecosystem biodiversity. *Nature Communications* 8:14087. https://doi.org/10.1038/ncomms14087. https://www.nature.com/articles/ncomms14087#supplementary-information.

Blaxter, Mark, Robin Floyd, Mark Dorris, A. Eyualem, and Paul De Ley. 2004. Utilising the new nematode phylogeny for studies of parasitism and diversity. *Nematology Monographs and Perspectives (ed. R. Cook & DJ Hunt)* 2: 615–632.

Blum, Stephanie A.E., Michael G. Lorenz, and Wilfried Wackernagel. 1997. Mechanism of retarded DNA degradation and prokaryotic origin of DNases in nonsterile soils. *Systematic and Applied Microbiology* 20(4): 513–521.

Bowen, Brian W., Naoki Kamezaki, Colin J. Limpus, George R. Hughes, Anne B. Meylan, and John C. Avise. 1994. Global phylogeography of the loggerhead turtle (*Caretta caretta*) as indicated by mitochondrial DNA haplotypes. *Evolution* 48(6):1820–1828.

Braley, Michelle, Simon D. Goldsworthy, Brad Page, Mike Steer, and Jeremy J. Austin. 2010. Assessing morphological and DNA-based diet analysis techniques in a generalist predator, the arrow squid *Nototodarus gouldi*. *Molecular Ecology Resources* 10(3): 466–474.

Brander, Keith M. 2007. Global fish production and climate change. *Proceedings of the National Academy of Sciences* 104(50): 19709–19714.

Brock, Richard E. 1982. A critique of the visual census method for assessing coral reef fish populations. *Bulletin of Marine Science* 32(1): 269–276.

Bunce, Michael, Marta Szulkin, Heather R.L. Lerner, Ian Barnes, Beth Shapiro, Alan Cooper, and Richard N. Holdaway. 2005. Ancient DNA provides new insights into the evolutionary history of New Zealand's extinct giant eagle. *PLoS Biology* 3(1): e9.

Butchart, Stuart H.M., Matt Walpole, Ben Collen, Arco Van Strien, Jörn P.W. Scharlemann, Rosamunde E.A. Almond, Jonathan E.M. Baillie, Bastian Bomhard, Claire Brown, and John Bruno. 2010. Global biodiversity: Indicators of recent declines. *Science* 328(5982): 1164–1168.

Cannon, M.V., James Hester, Amanda Shalkhauser, Ernest R. Chan, Kyle Logue, Scott T. Small, and David Serre. 2016. In silico assessment of primers for eDNA studies using PrimerTree and application to characterize the biodiversity surrounding the Cuyahoga River. *Scientific Reports* 6: 22908.

Carim, Kellie J., Kyle R. Christianson, Kevin M. McKelvey, William M. Pate, Douglas B. Silver, Brett M. Johnson, Benjamin T. Galloway, Michael K. Young, and Michael K. Schwartz. 2016a. Correction: Environmental DNA marker development with

sparse biological information: A case study on opossum shrimp (*Mysis diluviana*). *PLoS ONE* 11(10):e0165573.

Carim, K.J., J.C.S. Dysthe, M.K. Young, K.S. McKelvey, and M.K. Schwartz. 2016b. An environmental DNA assay for detecting Arctic grayling in the upper Missouri River basin, North America. *Conservation Genetics Resources* 8(3): 197–199.

Carim, K.J., T.M. Wilcox, M. Anderson, D.J. Lawrence, M.K. Young, K.S. McKelvey, and M.K. Schwartz. 2016c. An environmental DNA marker for detecting nonnative brown trout (*Salmo trutta*). *Conservation Genetics Resources* 8(3): 259–261.

Clayton, David A. 1984. Transcription of the mammalian mitochondrial genome. *Annual Review of Biochemistry* 53(1): 573–594.

Clusa, L., Ardura, A., Fernández, S., Roca, A.A. and García-Vázquez, E. 2017. An extremely sensitive nested PCR-RFLP mitochondrial marker for detection and identification of salmonids in eDNA from water samples. *PeerJ* 5: 3045.

Collins, Rupert A., Karen F. Armstrong, Andrew J. Holyoake, and Suzanne Keeling. 2013. Something in the water: Biosecurity monitoring of ornamental fish imports using environmental DNA. *Biological Invasions* 15(6): 1209–1215.

Corinaldesi, Cinzia, Marco Barucca, Gian Marco Luna, and A. Dell'Anno. 2011. Preservation, origin and genetic imprint of extracellular DNA in permanently anoxic deep-sea sediments. *Molecular Ecology* 20(3): 642–654.

Creer, S., Fonseca, V.G., Porazinska, D.L., Giblin Davis, R.M., Sung, W., Power, D.M., Packer, M., Carvalho, G.R., Blaxter, M.L., Lambshead, P.J.D. and Thomas, W.K. 2010. Ultrasequencing of the meiofaunal biosphere: practice, pitfalls and promises. *Molecular Ecology* 19: 4–20.

Daan, Niels. 2001. *The IBTS database: a plea for quality control*. ICES CM 2001/T:03. 19.

Davies, Richard Gareth. 2012. *Computer programming in quantitative biology*. New York City: Elsevier.

de Souza, Lesley S., James C. Godwin, Mark A. Renshaw, and Eric Larson. 2016. Environmental DNA (eDNA) detection probability is influenced by seasonal activity of organisms. *PLoS ONE* 11(10): e0165273.

DeFlaun, Mary F., John H. Paul, and Dean Davis. 1986. Simplified method for dissolved DNA determination in aquatic environments. *Applied and Environmental Microbiology* 52(4): 654–659.

DeFlaun, Mary F., John H. Paul, and Wade H. Jeffrey. 1987. Distribution and molecular weight of dissolved DNA in subtropical estuarine and oceanic environments. *Marine Ecology-Progress Series* 38(1): 65.

Deiner, Kristy, and Florian Altermatt. 2014. Transport distance of invertebrate environmental DNA in a natural river. *PLoS ONE* 9(2): e88786.

Deiner, Kristy, Emanuel A. Fronhofer, Elvira Mächler, Jean-Claude Walser, and Florian Altermatt. 2016. Environmental DNA reveals that rivers are conveyer belts of biodiversity information. Nature Communications 7: 12544. https://doi.org/10.1038/ncomms12544.

Dejean, Tony, Alice Valentini, Christian Miquel, Pierre Taberlet, Eva Bellemain, and Claude Miaud. 2012. Improved detection of an alien invasive species through environmental DNA barcoding: The example of the American bullfrog *Lithobates catesbeianus*. *Journal of Applied Ecology* 49(4): 953–959.

Dell'Anno, Antonio, and Cinzia Corinaldesi. 2004. Degradation and turnover of extracellular DNA in marine sediments: Ecological and methodological considerations. *Applied and Environmental Microbiology* 70(7): 4384–4386.

Dell'Anno, Antonio, Bompadre Stefano, and Roberto Danovaro. 2002. Quantification, base composition, and fate of extracellular DNA in marine sediments. *Limnology and Oceanography* 47(3): 899–905.

Díaz, Sandra, F. Joseph Fargione, I.I.I. Stuart Chapin, and David Tilman. 2006. Biodiversity loss threatens human well-being. *PLoS Biology* 4(8): e277.

Doi, H., Uchii, K., Takahara, T., Matsuhashi, S., Yamanaka, H. and Minamoto, T. 2015. Use of droplet digital PCR for estimation of fish abundance and biomass in environmental DNA surveys. *PloS one* 10(3): 0122763.

Douville, M., F. Gagné, C. Blaise, and C. Andre. 2007. Occurrence and persistence of *Bacillus thuringiensis* (Bt) and transgenic Bt corn cry1Ab gene from an aquatic environment. *Ecotoxicology and Environmental Safety* 66(2): 195–203.

Dysthe, Joseph C., Kellie J. Carim, Yvette M. Paroz, Kevin S. McKelvey, Michael K. Young, and Michael K. Schwartz. 2016. Quantitative PCR assays for detecting loach minnow (*Rhinichthys cobitis*) and spikedace (*Meda fulgida*) in the southwestern United States. *PLoS ONE* 11(9): e0162200.

Ebach, Malte C., and Craig Holdrege. 2005. DNA barcoding is no substitute for taxonomy. *Nature* 434(7034): 697.

Egan, Scott P., Matthew A. Barnes, Ching-Ting Hwang, Andrew R. Mahon, Jeffery L. Feder, Steven T. Ruggiero, Carol E. Tanner, and David M. Lodge. 2013. Rapid invasive species detection by combining environmental DNA with light transmission spectroscopy. *Conservation Letters* 6(6): 402–409.

Evans, Nathan T., Brett P. Olds, Mark A. Renshaw, Cameron R. Turner, Yiyuan Li, Christopher L. Jerde, Andrew R. Mahon, Michael E. Pfrender, Gary A. Lamberti, and David M. Lodge. 2016. Quantification of mesocosm fish and amphibian species diversity via environmental DNA metabarcoding. *Molecular Ecology Resources* 16(1): 29–41.

Ficetola, Gentile Francesco, Claude Miaud, François Pompanon, and Pierre Taberlet. 2008. Species detection using environmental DNA from water samples. *Biology Letters* 4(4): 423–425.

Fonseca, V.G., Carvalho, G.R., Sung, W., Johnson, H.F., Power, D.M., Neill, S.P., Packer, M., Blaxter, M.L., Lambshead, P.J.D., Thomas, W.K. and Creer, S. 2010. Second-generation environmental sequencing unmasks marine metazoan biodiversity. *Nature communications* 1: 98.

Foote, Andrew D., Philip Francis Thomsen, Signe Sveegaard, Magnus Wahlberg, Jos Kielgast, Line A. Kyhn, Andreas B. Salling, Anders Galatius, Ludovic Orlando, M. Thomas, and P. Gilbert. 2012. Investigating the potential use of environmental DNA (eDNA) for genetic monitoring of marine mammals. *PLoS ONE* 7(8): e41781.

Foppen, J.W., Orup, C., Adell, R., Poulalion, V. and Uhlenbrook, S. 2011. Using multiple artificial DNA tracers in hydrology. *Hydrological Processes* 25(19): 3101–3106.

Gingera, T.D., Bajno, R., Docker, M.F. and Reist, J.D. 2017. Environmental DNA as a detection tool for zebra mussels *Dreissena polymorpha* (Pallas, 1771) at the forefront of an invasion event in Lake Winnipeg, Manitoba, Canada. *Management of Biological Invasions* 8(3): 287–300.

Goldberg, Caren S., David S. Pilliod, Robert S. Arkle, and Lisette P. Waits. 2011. Molecular detection of vertebrates in stream water: A demonstration using Rocky Mountain tailed frogs and Idaho giant Salamanders. *PLoS ONE* 6(7): e22746.

Goldberg, C.S., A. Sepulveda, A. Ray, J. Baumgardt, and L.P. Waits. 2013. Environmental DNA as a new method for early detection of New Zealand mudsnails (*Potamopyrgus antipodarum*). *Freshwater Science* 32: 792–800.

Gotelli, Nicholas J., and Robert K. Colwell. 2011. Estimating species richness. *Biological Diversity: Frontiers in Measurement and Assessment* 12: 39–54.

Group, CBOL Plant Working, Peter M. Hollingsworth, Laura L. Forrest, John L. Spouge, Mehrdad Hajibabaei, Sujeevan Ratnasingham, Michelle van der Bank, Mark W. Chase, Robyn S. Cowan, and David L. Erickson. 2009. A DNA barcode for land

plants. *Proceedings of the National Academy of Sciences* 106(31): 12794–12797.

Gu, Weidong, and Robert K. Swihart. 2004. Absent or undetected? Effects of non-detection of species occurrence on wildlife–habitat models. *Biological Conservation* 116(2): 195–203.

Hajibabaei, Mehrdad, Shadi Shokralla, Xin Zhou, Gregory A.C. Singer, and Donald J. Baird. 2011. Environmental barcoding: a next-generation sequencing approach for biomonitoring applications using river benthos. *PLoS ONE* 6(4): e17497.

Hajibabaei, Mehrdad, M. Smith, Daniel H. Janzen, Josephine J. Rodriguez, James B. Whitfield, and Paul D.N. Hebert. 2006. A minimalist barcode can identify a specimen whose DNA is degraded. *Molecular Ecology Notes* 6(4): 959–964.

Hebert, Paul D.N., Sujeevan Ratnasingham, and Jeremy R. de Waard. 2003. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society of London B: Biological Sciences* 270(Suppl 1): S96–S99.

Higuchi, Russell, Cecilia H. von Beroldingen, George F. Sensabaugh, and Henry A. Erlich. 1988. DNA typing from single hairs. *Nature* 332(6164): 543–546.

Hindell, Mark A., Corey J.A. Bradshaw, Michael D. Sumner, Kelvin J. Michael, and Harry R. Burton. 2003. Dispersal of female southern elephant seals and their prey consumption during the austral summer: Relevance to management and oceanographic zones. *Journal of Applied Ecology* 40(4): 703–715.

Hofreiter, Michael, Matthew Collins, and John R. Stewart. 2012. Ancient biomolecules in Quaternary palaeoecology. *Quaternary Science Reviews* 33: 1–13.

Hopkins, G.W., and R.P. Freckleton. 2002. Declines in the numbers of amateur and professional taxonomists: Implications for conservation. Animal Conservation 5(3): 245–249.

Höss, Matthias, Michael Kohn, Svante Pääbo, Felix Knauer, and Wolfgang Schröder. 1992. Excrement analysis by PCR. *Nature* 359(6392): 199.

Huson, Daniel H., Alexander F. Auch, Ji Qi, and Stephan C. Schuster. 2007. MEGAN analysis of metagenomic data. *Genome Research* 17(3): 377–386.

Hyman, O.J. and Collins, J.P. 2012. Evaluation of a filtration-based method for detecting *Batrachochytrium dendrobatidis* in natural bodies of water. *Diseases of Aquatic Organisms* 97(3): 185–195.

International Union for Conservation of Nature. 2012. *IUCN red list of threatened species. Version 2011.1.*

Jane, Stephen F., Taylor M. Wilcox, Kevin S. McKelvey, Michael K. Young, Michael K. Schwartz, Winsor H. Lowe, Benjamin H. Letcher, and Andrew R. Whiteley. 2015. Distance, flow and PCR inhibition: eDNA dynamics in two headwater streams. *Molecular Ecology Resources* 15(1): 216–227.

Jerde, Christopher L., Andrew R. Mahon, W. Lindsay Chadderton, and David M. Lodge. 2011. "Sight-unseen" detection of rare aquatic species using environmental DNA. *Conservation Letters* 4(2): 150–157.

Jones, J.B. 1992. Environmental impact of trawling on the seabed: A review. *New Zealand Journal of Marine and Freshwater Research* 26(1): 59–67.

Kirshtein, J.D., Anderson, C.W., Wood, J.S., Longcore, J.E. and Voytek, M.A. 2007. Quantitative PCR detection of *Batrachochytrium dendrobatidis* DNA from sediments and water. *Diseases of aquatic organisms* 77(1): 11–15.

Kochkina, Galina, Natalya Ivanushkina, Svetlana Ozerskaya, Nadezhda Chigineva, Oleg Vasilenko, Sergey Firsov, Elena Spirina, and David Gilichinsky. 2012. Ancient fungi in Antarctic permafrost environments. *FEMS Microbiology Ecology* 82(2): 501–509.

Lacoursière-Roussel, Anaïs, Yohann Dubois, Eric Normandeau, and Louis Bernatchez. 2016a. Improving herpetological surveys in eastern North America using the environmental DNA method 1. *Genome* 59(11): 991–1007.

Lacoursière-Roussel, Anaïs, Maikel Rosabal, and Louis Bernatchez. 2016b. Estimating fish abundance and biomass from eDNA concentrations: Variability among capture methods and environmental conditions. *Molecular Ecology Resources* 16(6): 1401–1414.

Lance, R.F. and Carr, M.R., 2012. Detecting eDNA of Invasive Dreissenid Mussels, Report on Capital Investment Project. *ERDC Aquatic Nuisance Species Research Program Technical Note ANSRP* 12–2.

Levy-Booth, David J., Rachel G. Campbell, Robert H. Gulden, Miranda M. Hart, Jeff R. Powell, John N. Klironomos, K. Peter Pauls, Clarence J. Swanton, Jack T. Trevors, and Kari E. Dunfield. 2007. Cycling of extracellular DNA in the soil environment. *Soil Biology & Biochemistry* 39(12): 2977–2991.

Li, Frank, Andrew R. Mahon, Matthew A. Barnes, Jeffery Feder, David M. Lodge, Ching-Ting Hwang, Robert Schafer, Steven T. Ruggiero, and Carol E. Tanner. 2011. Quantitative and rapid DNA detection by laser transmission spectroscopy. *PLoS ONE* 6(12): e29224.

Lindahl, Tomas. 1993. Instability and decay of the primary structure of DNA. *Nature* 362(6422): 709–715.

Little, Damon P. 2014. A DNA mini-barcode for land plants. *Molecular Ecology Resources* 14(3): 437–446.

Lodge, David M., Cameron R. Turner, Christopher L. Jerde, Matthew A. Barnes, Lindsay Chadderton, Scott P. Egan, Jeffrey L. Feder, Andrew R. Mahon, and Michael E. Pfrender. 2012. Conservation in a cup of water: Estimating biodiversity and population abundance from environmental DNA. *Molecular Ecology* 21(11): 2555–2558.

MacKenzie, Darryl I., James D. Nichols, Gideon B. Lachman, J. Sam Droege, Andrew Royle, and Catherine A. Langtimm. 2002. Estimating site occupancy rates when detection probabilities are less than one. *Ecology* 83(8): 2248–2255.

Mahon, Andrew R., Matthew A. Barnes, Satyajyoti Senapati, Jeffrey L. Feder, John A. Darling, Hsueh-Chia Chang, and David M. Lodge. 2011. Molecular detection of invasive species in heterogeneous mixtures using a microfluidic carbon nanotube platform. *PLoS ONE* 6(2): e17280.

Mahon, Andrew R., Christopher L. Jerde, Matthew Galaska, Jennifer L. Bergner, W. Lindsay Chadderton, David M. Lodge, Margaret E. Hunter, and Leo G. Nico. 2013. Validation of eDNA surveillance sensitivity for detection of Asian carps in controlled and field experiments. *PLoS ONE* 8(3): e58316.

Martellini, Anouk, Pierre Payment, and Richard Villemur. 2005. Use of eukaryotic mitochondrial DNA to differentiate human, bovine, porcine and ovine sources in fecally contaminated surface water. *Water Research* 39(4): 541–548.

McKee, Anna M., Stephen F. Spear, and Todd W. Pierson. 2015. The effect of dilution and the use of a post-extraction nucleic acid purification column on the accuracy, precision, and inhibition of environmental DNA samples. *Biological Conservation* 183: 70–76.

McKelvey, K.S., M.K. Young, W.L. Knotek, K.J. Carim, T.M. Wilcox, T.M. Padgett-Stewart, and M.K. Schwartz. 2016. Sampling large geographic areas for rare species using environmental DNA: A study of bull trout *Salvelinus confluentus* occupancy in western Montana. *Journal of fish biology* 88(3): 1215–1222.

Meier, Petra, and Wilfried Wackernagel. 2003. Mechanisms of homology-facilitated illegitimate recombination for foreign DNA acquisition in transformable Pseudomonas stutzeri. *Molecular Microbiology* 48(4): 1107–1118.

Merkes, Christopher M., S. Grace McCalla, Nathan R. Jensen, Mark P. Gaikowski, and Jon J. Amberg. 2014. Persistence of DNA in

carcasses, slime and avian feces may affect interpretation of environmental DNA data. *PLoS ONE* 9(11): e113346.

Meusnier, Isabelle, Gregory A.C. Singer, Jean-François Landry, Donal A. Hickey, Paul D.N. Hebert, and Mehrdad Hajibabaei. 2008. A universal DNA mini-barcode for biodiversity analysis. *BMC Genomics* 9(1): 214.

Minamoto, Toshifumi, Hiroki Yamanaka, Teruhiko Takahara, Mie N. Honjo, and Z.I. Kawabata. 2012. Surveillance of fish species composition using environmental DNA. *Limnology* 13(2): 193–197.

Moyer, Gregory R., Edgardo Diaz-Ferguson, Jeffrey E. Hill, and Colin Shea. 2014. Assessing environmental DNA detection in controlled lentic systems. *PLoS ONE* 9(7): e103767.

Nichols, Ruth V., H.E.L.E.N.A. KOeNIGSSON, K. Danell, and G. Spong. 2012. Browsed twig environmental DNA: Diagnostic PCR to identify ungulate species. *Molecular Ecology Resources* 12(6): 983–989.

Nielsen, Kaare M., Pål J. Johnsen, Douda Bensasson, and Daniele Daffonchio. 2007. Release and persistence of extracellular DNA in the environment. *Environmental Biosafety Research* 6(1–2): 37–53.

Ogram, Andrew, Gary S. Sayler, and Tamar Barkay. 1987. The extraction and purification of microbial DNA from sediments. *Journal of Microbiological Methods* 7(2–3): 57–66.

Olds, Brett P., Christopher L. Jerde, Mark A. Renshaw, Yiyuan Li, Nathan T. Evans, Cameron R. Turner, Kristy Deiner, Andrew R. Mahon, Michael A. Brueseke, and Patrick D. Shirey. 2016. Estimating species richness using environmental DNA. *Ecology and Evolution* 6(12): 4214–4226.

Olson, Deanna H., David M. Aanensen, Kathryn L. Ronnenberg, Christopher I. Powell, Susan F. Walker, Jon Bielby, Trenton W.J. Garner, George Weaver, Group The Bd Mapping, and Matthew C. Fisher. 2013. Mapping the global emergence of *Batrachochytrium dendrobatidis*, the amphibian chytrid fungus. *PLoS One* 8(2): e56802. https://doi.org/10.1371/journal.pone.0056802.

Parducci, Laura, Irina Matetovici, Sonia L. Fontana, Keith D. Bennett, Yoshihisa Suyama, James Haile, Kurt H. Kjær, Nicolaj K. Larsen, Andreas D. Drouzas, and Eske Willerslev. 2013. Molecular- and pollen-based vegetation analysis in lake sediments from central Scandinavia. *Molecular Ecology* 22(13): 3511–3524.

Paul, J.H., W.H. Jeffrey, and J.P. Cannon. 1990. Production of dissolved DNA, RNA, and protein by microbial populations in a Florida reservoir. *Applied and Environmental Microbiology* 56(10): 2957–2962.

Paul, John H., Sunny C. Jiang, and Joan B. Rose. 1991. Concentration of viruses and dissolved DNA from aquatic environments by vortex flow filtration. *Applied and Environmental Microbiology* 57(8): 2197–2204.

Pedersen, Mikkel Winther, Aurélien Ginolhac, Ludovic Orlando, Jesper Olsen, Kenneth Andersen, Jakob Holm, Svend Funder, Eske Willerslev, and Kurt H. Kjær. 2013. A comparative study of ancient environmental DNA to pollen and macrofossils from lake sediments reveals taxonomic overlap and additional plant taxa. *Quaternary Science Reviews* 75: 161–168.

Piaggio, Antoinette J., Richard M. Engeman, Matthew W. Hopken, John S. Humphrey, Kandy L. Keacher, William E. Bruce, and Michael L. Avery. 2014. Detecting an elusive invasive species: A diagnostic PCR to detect Burmese python in Florida waters and an assessment of persistence of environmental DNA. *Molecular Ecology Resources* 14(2): 374–380.

Piganeau, Gwenaël, Michael Gardner, and Adam Eyre-Walker. 2004. A broad survey of recombination in animal mitochondria. *Molecular Biology and Evolution* 21(12): 2319–2325.

Pilliod, David S., Caren S. Goldberg, Robert S. Arkle, and Lisette P. Waits. 2013. Estimating occupancy and abundance of stream amphibians using environmental DNA from filtered water samples. *Canadian Journal of Fisheries and Aquatic Sciences* 70(8): 1123–1130.

Pilliod, David S., Caren S. Goldberg, Robert S. Arkle, and Lisette P. Waits. 2014. Factors influencing detection of eDNA from a stream-dwelling amphibian. *Molecular Ecology Resources* 14(1): 109–116.

Poinar, Hendrik N., W. Michael Hofreiter, Geoffrey Spaulding, Paul S. Martin, B. Artur Stankiewicz, Helen Bland, Richard P. Evershed, Göran Possnert, and Svante Pääbo. 1998. Molecular coproscopy: Dung and diet of the extinct ground sloth *Nothrotheriops shastensis*. *Science* 281(5375): 402–406.

Poté, John, Rafaël Ackermann, and Walter Wildi. 2009. Plant leaf mass loss and DNA release in freshwater sediments. *Ecotoxicology and Environmental Safety* 72(5): 1378–1383.

Powers, Tom. 2004. Nematode molecular diagnostics: From bands to barcodes. *Annual review of Phytopathology* 42: 367–383.

Ratnasingham, Sujeevan, and Paul D.N. Hebert. 2007. bold: The barcode of life data system (http://www.barcodinglife.org). *Molecular Ecology Notes* 7(3):355–364. https://doi.org/10.1111/j.1471-8286.2007.01678.x.

Risser, Paul G. 1995. Biodiversity and ecosystem function. *Conservation Biology* 9(4): 742–746.

Robertson, D.Ross, and William F. Smith-Vaniz. 2008. Rotenone: An essential but demonized tool for assessing marine fish diversity. *BioScience* 58(2): 165–170.

Robson, Heather L.A., Tansyn H. Noble, Richard J. Saunders, Simon K.A. Robson, Damien W. Burrows, and Dean R. Jerry. 2016. Fine-tuning for the tropics: Application of eDNA technology for invasive fish detection in tropical freshwater ecosystems. *Molecular Ecology Resources* 16(4): 922–932.

Roussel, Jean-Marc, Jean-Marc Paillisson, Anne Treguier, and Eric Petit. 2015. The downside of eDNA as a survey tool in water bodies. *Journal of Applied Ecology* 52(4): 823–826.

Schoch, Conrad L., Keith A. Seifert, Sabine Huhndorf, Vincent Robert, John L. Spouge, C. André Levesque, Wen Chen, Elena Bolchacova, Kerstin Voigt, and Pedro W. Crous. 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proceedings of the National Academy of Sciences* 109(16): 6241–6246.

Scholin, Christopher A. 2010. What are " ecogenomic sensors?" A review and thoughts for the future. *Ocean Science* 6(1): 51–60.

Schuster, Stephan C. 2008. Next-generation sequencing transforms today's biology. *Nature Methods* 5(1): 16.

Sheppard, S.K., and J.D. Harwood. 2005. Advances in molecular ecology: Tracking trophic links through predator–prey foodwebs. *Functional Ecology* 19(5): 751–762.

Shokralla, Shadi, Jennifer L. Spall, Joel F. Gibson, and Mehrdad Hajibabaei. 2012. Next-generation sequencing technologies for environmental DNA research. *Molecular Ecology* 21(8): 1794–1805.

Sigsgaard, Eva Egelyng, Ida Broman Nielsen, Steffen Sanvig Bach, Eline D. Lorenzen, David Philip Robinson, Steen Wilhelm Knudsen, Mikkel Winther Pedersen, Mohammed Al Jaidah, Ludovic Orlando, and Eske Willerslev. 2016. Population characteristics of a large whale shark aggregation inferred from seawater environmental DNA. *Nature Ecology & Evolution* 1: 0004.

Siuda, W., and H. Güde. 1996. Determination of dissolved deoxyribonucleic acid concentration in lake water. *Aquatic Microbial Ecology* 11(2): 193–202.

Stoeckle, M.Y., Soboleva, L. and Charlop-Powers, Z. 2017. Aquatic environmental DNA detects seasonal fish abundance and habitat preference in an urban estuary. *PloS one* 12(4): 0175186.

Strausberger, B.M., and Mary V. Ashley. 2001. Eggs yield nuclear DNA from egg-laying female cowbirds, their embryos and offspring. *Conservation Genetics* 2(4): 385–390.

Strickler, Katherine M., Alexander K. Fremier, and Caren S. Goldberg. 2015. Quantifying effects of UV-B, temperature, and pH on eDNA degradation in aquatic microcosms. *Biological Conservation* 183: 85–92.

Stuart, Simon N., Janice S. Chanson, Neil A. Cox, Bruce E. Young, Ana S.L. Rodrigues, Debra L. Fischman, and Robert W. Waller. 2004. Status and trends of amphibian declines and extinctions worldwide. *Science* 306(5702): 1783–1786.

Symondson, W.O.C. 2002. Molecular identification of prey in predator diets. *Molecular Ecology* 11(4): 627–641.

Taberlet, Pierre, and Jean Bouvet. 1991. A single plucked feather as a source of DNA for bird genetic studies. *Auk* 108(4): 959–960.

Taberlet, Pierre, and Luca Fumagalli. 1996. Owl pellets as a source of DNA for genetic studies of small mammals. *Molecular Ecology* 5(2): 301–305.

Taberlet, Pierre, Eric Coissac, Mehrdad Hajibabaei, and Loren H. Rieseberg. 2012a. Environmental DNA. *Molecular Ecology* 21(8): 1789–1793. https://doi.org/10.1111/j.1365-294X.2012.05542.x.

Taberlet, Pierre, Eric Coissac, Francois Pompanon, Christian Brochmann, and Eske Willerslev. 2012b. Towards next-generation biodiversity assessment using DNA metabarcoding. *Molecular Ecology* 21(8): 2045–2050.

Takahara, Teruhiko, Toshifumi Minamoto, Hiroki Yamanaka, Hideyuki Doi, and Zen'ichiro Kawabata. 2012. Estimation of fish biomass using environmental DNA. *PLoS ONE* 7(4): e35868.

Takahara, Teruhiko, Toshifumi Minamoto, and Hideyuki Doi. 2013. Using environmental DNA to estimate the distribution of an invasive fish species in ponds. *PLoS ONE* 8(2): e56584.

Takahara, Teruhiko, Toshifumi Minamoto, and Hideyuki Doi. 2015. Effects of sample processing on the detection rate of environmental DNA from the Common Carp (*Cyprinus carpio*). *Biological Conservation* 183: 64–69.

Thomsen, Philip Francis, Jos Kielgast, Lars Lønsmann Iversen, Peter Rask Møller, Morten Rasmussen, and Eske Willerslev. 2012a. Detection of a diverse marine fish fauna using environmental DNA from seawater samples. *PLoS ONE* 7(8): e41732.

Thomsen, Philip, Jos Kielgast, Lars L. Iversen, Carsten Wiuf, M. Morten Rasmussen, Thomas P. Gilbert, Ludovic Orlando, and Eske Willerslev. 2012b. Monitoring endangered freshwater biodiversity using environmental DNA. *Molecular Ecology* 21(11): 2565–2573.

Trevors, Jack T. 1996. Nucleic acids in the environment. *Current Opinion in Biotechnology* 7(3): 331–336.

Tsaousis, Anastasios D., D.P. Martin, E.D. Ladoukakis, D. Posada, and E. Zouros. 2005. Widespread recombination in published animal mtDNA sequences. *Molecular Biology and Evolution* 22(4): 925–933.

Turner, Cameron R., Matthew A. Barnes, Charles C.Y. Xu, Stuart E. Jones, Christopher L. Jerde, and David M. Lodge. 2014. Particle size distribution and optimal capture of aqueous macrobial eDNA. *Methods in Ecology and Evolution* 5(7): 676–684.

Turner, Cameron R., Karen L. Uy, and Robert C. Everhart. 2015. Fish environmental DNA is more concentrated in aquatic sediments than surface water. *Biological Conservation* 183: 93–102.

US Fish and Wildlife Service. 1999. Endangered and threatened wildlife and plants; determination of threatened status for bull trout in the coterminous United States. *Federal Register* 64(210): 58909–58933.

Valentini, Alice, Christian Miquel, Muhammad Ali Nawaz, E.V.A. Bellemain, Eric Coissac, FranCOis Pompanon, Ludovic Gielly, Corinne Cruaud, Giuseppe Nascetti, and Patrick Wincker. 2009. New perspectives in diet analysis based on DNA barcoding and parallel pyrosequencing: The trnL approach. *Molecular Ecology Resources* 9(1): 51–60.

Valentini, Alice, Pierre Taberlet, Claude Miaud, Raphaël Civade, Jelger Herder, Philip Francis Thomsen, Eva Bellemain, Aurélien Besnard, Eric Coissac, and Frédéric Boyer. 2016. Next-generation monitoring of aquatic biodiversity using environmental DNA metabarcoding. *Molecular Ecology* 25(4): 929–942.

Valiere, Nathaniel, and Pierre Taberlet. 2000. Urine collected in the field as a source of DNA for species and individual identification. *Molecular Ecology* 9(12): 2150–2152.

Warning, United Nations Environment Programme. Division of Early, and Assessment. 2011. *UNEP year book: Emerging issues in our global environment*. Nairobi: UNEP/Earthprint.

Wilcox, T.M., McKelvey, K.S., Young, M.K., Jane, S.F., Lowe, W.H., Whiteley, A.R. and Schwartz, M.K. 2013. Robust detection of rare species using environmental DNA: the importance of primer specificity. *PloS one* 8(3): 59520.

Wilcox, Taylor M., Kellie J. Carim, Kevin S. McKelvey, Michael K. Young, and Michael K. Schwartz. 2015a. The Dual Challenges of Generality and Specificity When Developing Environmental DNA Markers for Species and Subspecies of Oncorhynchus. PLoS ONE 10(11): e0142008.

Wilcox, Taylor M., Kevin S. McKelvey, Michael K. Young, Winsor H. Lowe, and Michael K. Schwartz. 2015b. Environmental DNA particle size distribution from Brook Trout (*Salvelinus fontinalis*). *Conservation Genetics Resources* 7(3): 639–641.

Wilcox, Taylor M., Kevin S. McKelvey, Michael K. Young, Adam J. Sepulveda, Bradley B. Shepard, Stephen F. Jane, Andrew R. Whiteley, Winsor H. Lowe, and Michael K. Schwartz. 2016. Understanding environmental DNA detection probabilities: A case study using a stream-dwelling char *Salvelinus fontinalis*. *Biological Conservation* 194: 209–216.

Willerslev, Eske, Anders J. Hansen, Jonas Binladen, Tina B. Brand, M. Thomas, P. Gilbert, Beth Shapiro, Michael Bunce, Carsten Wiuf, David A. Gilichinsky, and Alan Cooper. 2003. Diverse plant and animal genetic records from Holocene and Pleistocene sediments. *Science* 300(5620): 791–795.

Williams, Kelly E., Kathryn P. Huyvaert, and Antoinette J. Piaggio. 2016. No filters, no fridges: A method for preservation of water samples for eDNA analysis. *BMC Research Notes* 9(1): 298.

Wilson, Allan C., Rebecca L. Cann, Steven M. Carr, Matthew George, Ulf B. Gyllensten, Kathleen M. Helm-Bychowski, Russell G. Higuchi, Stephen R. Palumbi, Ellen M. Prager, and Richard D. Sage. 1985. Mitochondrial DNA and two perspectives on evolutionary genetics. *Biological Journal of the Linnean Society* 26(4): 375–400.

Yoccoz, Nigel G. 2012. The future of environmental DNA in ecology. *Molecular Ecology* 21(8): 2031–2038.

Zhan, A., Hulák, M., Sylvester, F., Huang, X., Adebayo, A.A., Abbott, C.L., Adamowicz, S.J., Heath, D.D., Cristescu, M.E. and MacIsaac, H.J. 2013. High sensitivity of 454 pyrosequencing for detection of rare species in aquatic communities. *Methods in Ecology and Evolution* 4(6): 558–565.