eDNA in Fish Disease

Subjects: Agriculture, Dairy & Animal Science | Fisheries

Contributor: Kailash Bohara , Amit K. Yadav , Pabitra Joshi

Organisms release their nucleic acid in the environment, including the DNA and RNA, which can be used to detect their presence. Environmental DNA (eDNA)/eRNA techniques are being used in different sectors to identify organisms from soil, water, air, and ice. The advancement in technology led to easier detection of different organisms without impacting the environment or the organism itself. These methods are being employed in different areas, including surveillance, history, and conservation. eDNA and eRNA methods are being extensively used in aquaculture and fisheries settings to understand the presence of different fish species and pathogens in water.

eDNA eRNA fish disease surveillance disease bacteria virus detection qPCR

1. Introduction

Environmental DNA (eDNA) analysis is a new scientific technique for identifying species from materials that contain the cellular and extracellular DNA leached off all living organisms. The terminology for eDNA as extracellular DNA is noted by Pietramellara, et al., 2009 [1]. However, researchers are using different terminologies, such as exDNA (extracellular DNA) or cfDNA (cell-free DNA). The idea of obtaining DNA from the environmental sample was first demonstrated in 1986 [2] and called environmental DNA (eDNA). The identification of various eDNA from macro-organisms validated the method as important in a conservation context, and it has been demonstrated in a wide range of ancient and modern habitats, both terrestrial and aquatic [3][4][5][6]. Environmental DNA (eDNA) approaches are becoming more widely used in conservation biology, biodiversity research, and invasion ecology. The most significant benefits of eDNA sampling are the undemanding way of obtaining samples, as the target organism does not need to be isolated. The detection of parasites and diseases in water can also be performed using environmental DNA methods. There is a lot of evidence for the detection of several bacterial species from aquatic environments, including Aeromonas and Flavobacterium.

DNA is leached into the aquatic environment by different means, such as mucosal secretion, bodily fluids, tissues, scales, skin, microbial cells, and cell ruptures. This gives the researcher the potential to isolate DNA from different water sources without impacting the aquatic habitat. eDNA is not only being extracted from water samples, but it is also being extracted from different substrates, including soil, snow, and air as well. The extensive study of eDNA has led historians to identify new species and detect the presence of endangered species. Environmental nucleic acid, including eRNA for the recent infective SARS-CoV-2, has been successfully isolated from hospital air

sampling [7]. The advances in diagnostic techniques and instruments are the biggest reasons behind the success of environmental nucleic acid detection. Direct detection in water utilizing eDNA-based approaches eliminates the need to acquire and investigate diseased hosts, reducing disease monitoring efforts and costs dramatically. Eukaryotic micro- and macrobial communities and populations have been effectively detected and monitored using eDNA analysis. The advances in eDNA analysis have resulted in efficient identification and quantification of extracellular nucleic acids in different mediums. DNA metabarcoding, sequencing, quantitative polymerase chain reaction (qPCR), and digital droplet PCR (ddPCR) are different methods being used.

2. Bacteria

The ability of bacteria, archaea, and fungi cultures to release their genetic material into the extracellular medium, as well as in the context of multicellular microbial communities such as biofilms, has been reported. Bacteria release their DNA in water by different methods, including cell lysis and extrusion. The integrity of DNA released by cell lysis is usually higher, because the exonucleases cannot act quickly to degrade the DNA. Extrusion is used as a survival strategy by certain bacteria such as *Deinococcus radiodurans*, in which damaged DNA is released and new DNA is synthesized [8]. Many environmental bacteria including *Micrococcus*, *Acinetobacter*, *Bacillus*, *Flavobacterium*, *Azotobacter*, *Pseudomonas*, and *Alcaligenes* release their genetic material while growing in the media [9][10]. The amount of eDNA found depends on several factors, such as temperature, salinity, turbidity, and vegetation. In freshwater systems, the amount of DNA ranges from 1.74 to 7.77 µg/L [11]. There are many fish bacterial diseases affecting freshwater aquaculture, causing huge economic losses to the farmers. eDNA techniques might help them to predict bacterial load in their farms. Several research studies are being carried out to find an efficient method to detect those pathogens directly from the water samples. In most cases of *Flavobacterium columnare* infection, a gram-negative bacterium affecting different fish species, is found only externally in the skin, gills, and water samples before being systemic. Early and rigorous *F. columnare* diagnosis, as well as the implementation of practical preventive measures, are the only credible means of disease control. *F. psychrophilum* was found in different river water samples in Japan. They found a higher presence of *F. psychrophilum* during early summer and fall, and the presence of this bacteria depends on the water temperature [12]. In addition to that, *F. psychrophilum* and *Yersinia ruckeri* were also detected in salmon recirculatory aquaculture systems (RAS). Similarly, seven distinct species of Aeromonas were confirmed from coastal zones of river basins in Bangladesh. Over the 2-year study period, they also found that the number of bacteria changes with change in temperature using the eDNA method [13]. There are still many bacterial pathogens that are responsible for losses, which are yet to be studied.

3. Fungi

Fungi are some of the common fish pathogens in aquaculture settings. The most common fungal disease that affect fish species is saprolegniasis, branchiomycosis, and aspergillosis. Because farmed animals are typically held in high densities and exposed to constant stress and various types of pollutants, the risk of infection and disease spread is higher in fish farms than in wild environments. There are only a few studies on fish fungal disease

identification using the eDNA method in aquaculture. Following high mortality outbreaks in the river Loue, for finding *S. parasitica* in water, a qPCR assay was designed. The pathogen was detected in river water but not in the tap water of surrounding villages [14]. There are other fungi from water identified by this approach that affect amphibians. *Batrachochytrium dendrobatidis* and *B. salamandrivorans*, two major fungal diseases of amphibians, were found in water samples in Spain using a qPCR assay [15]. DNA released from fungus cells has received less attention than DNA released from bacterial cells. Although fungi constitute more than 70% of the microbiome in soil, due to the fast rate of DNA degradation in dead fungal cells, the contribution of fungal DNA to the eDNA pool in soil should be insignificant. The fungus can spread to ponds and rivers via rainwater flow and water infiltration. Adequate and efficient methods to detect the presence of these fungal pathogens using eDNA will allow the farmer to predict the fungal disease outbreak, leading to timely management and control strategies.

4. Parasites

Environmental DNA (eDNA) sampling methods, in conjunction with different molecular methods, are well suited to quickly detecting the presence of pathogens in different fish farms, which helps the managers with valuable information that can be used to reduce disease threats. Parasites are the most common group of fish pathogens that are being detected easily using eDNA method. Standard fish parasite surveillance entails capturing and euthanizing fish before manually inspecting for the presence of parasites. Using this conventional method is both expensive and time-consuming, and it necessitates the sacrifice of many fish species. eDNA/eRNA fragments of several species in water samples have recently been established as an accurate, low-cost alternative to the traditional monitoring techniques, which require sampling the fish itself. A ddPCR assay was developed to detect eDNA in field samples, demonstrating the utility of eDNA detection in natural water systems for *G. salaris* [16]. eDNA of *Dactylogyrus* species was detected in a consignment of ornamental fish water and confirmed by sanger sequencing. Although there are some limitations regarding the use of the eDNA tool as a biosecurity and quarantine method. It detects eDNA from water and not directly from fish, and this might create a false positive even though the fish might not have the targeted parasite; the assay can show positive because of the source water used [17]. Chilodonella abundance was detected at varying levels across the year in the barramundi fish farm monitored in the study [18]. Another assay was developed that can detect low concentrations of parasites in tank water containing goldfish, presumably corresponding to an early stage of disease [19]. As a result, it could be a useful tool for monitoring and controlling ichthyophthiriasis in aquaculture.

5. Virus

There are mainly two different forms of viruses that infect fish species, which are DNA viruses and RNA viruses. Several studies have shown that DNA and RNA from viruses can be detected using eDNA or eRNA methods [20][21][22][23][24][25][26]. Since DNA is more stable than RNA, detection of eDNA is more practical and easier than detecting RNA from an environmental source. There are many common forms of virus that are found in freshwater aquaculture, including herpesvirus, viral hemorrhagic septicemia virus (VHSV), infectious hematopoietic necrosis

virus (IHNV), golden shiner virus (GSV), channel catfish virus (CCV), red seabream virus, tilapia tilapine virus, and salmon alphavirus.

References

1. Pietramellara, G.; Ascher, J.; Borgogni, F.; Ceccherini, M.T.; Guerri, G.; Nannipieri, P. Extracellular DNA in Soil and Sediment: Fate and Ecological Relevance. Biol. Fertil. Soils 2009, 45, 219–235.
2. Ogram, A.; Sayler, G.S.; Barkay, T. The Extraction and Purification of Microbial DNA from Sediments. J. Microbiol. Methods 1987, 7, 57–66.
3. Haile, J.; Froese, D.; Macphee, R.; Roberts, R.; Arnold, L.; Reyes, A.; Rasmussen, M.; Nielsen, R.; Brook, B.; Robinson, S.; et al. Ancient DNA Reveals Late Survival of Mammoth and Horse in Interior Alaska. Proc. Natl. Acad. Sci. USA 2009, 106, 22352–22357.
4. Epp, L.S.; Boessenkool, S.; Bellemain, E.P.; Haile, J.; Esposito, A.; Riaz, T.; Erséus, C.; Gusarov, V.I.; Edwards, M.E.; Johnsen, A.; et al. New Environmental Metabarcodes for Analysing Soil DNA: Potential for Studying Past and Present Ecosystems. Mol. Ecol. 2012, 21, 1821–1833.
5. Bhadury, P.; Austen, M.; Bilton, D.; Lambshead, P.J.; Rogers, A. Molecular Detection of Marine Nematodes from Environmental Samples: Overcoming Eukaryotic Interference. Aquat. Microb. Ecol. 2006, 44, 97–103.
6. Andersen, K.; Bird, K.L.; Rasmussen, M.; Haile, J.; Breuning-Madsen, H.; Kjaer, K.H.; Orlando, L.; Gilbert, M.T.P.; Willerslev, E. Meta-Barcoding of “dirt” DNA from Soil Reflects Vertebrate Biodiversity. Mol. Ecol. 2012, 21, 1966–1979.
7. Lednicky, J.A.; Lauzard, M.; Fan, Z.H.; Jutla, A.; Tilly, T.B.; Gangwar, M.; Usmani, M.; Shankar, S.N.; Mohamed, K.; Eiguren-Fernandez, A.; et al. Viable SARS-CoV-2 in the Air of a Hospital Room with COVID-19 Patients. Int. J. Infect. Dis. 2020, 100, 476–482.
8. Battista, J.R. Against All Odds: The Survival Strategies of Deinococcus Radiodurans. Annu. Rev. Microbiol. 1997, 51, 203–224.
9. Paget, E.; Simonet, P. On the Track of Natural Transformation in Soil. FEMS Microbiol. Ecol. 1994, 15, 109–117.
10. Lorenz, M.G.; Wackernagel, W. Bacterial Gene Transfer by Natural Genetic Transformation in the Environment. Microbiol. Rev. 1994, 58, 563–602.
11. DeFlaun, M.F.; Paul, J.H.; Davis, D. Simplified Method for Dissolved DNA Determination in Aquatic Environments. Appl. Environ. Microbiol. 1986, 52, 654–659.
12. Tenma, H.; Tsunekawa, K.; Fujiyoshi, R.; Takai, H.; Hirose, M.; Masai, N.; Sumi, K.; Takihana, Y.; Yanagisawa, S.; Tsuchida, K.; et al. Spatiotemporal Distribution of Flavobacterium Psychrophilum

and Ayu Plecoglossus Altivelis in Rivers Revealed by Environmental DNA Analysis. Fish. Sci. 2021, 87, 321–330.

13. Sadique, A.; Neogi, S.B.; Bashar, T.; Sultana, M.; Johura, F.-T.; Islam, S.; Hasan, N.A.; Huq, A.; Colwell, R.R.; Alam, M. Dynamics, Diversity, and Virulence of Aeromonas Spp. in Homestead Pond Water in Coastal Bangladesh. Front. Public Health 2021, 9, 902.
14. Rocchi, S.; Tisserant, M.; Valot, B.; Laboissière, A.; Frossard, V.; Reboux, G. Quantification of Saprolegnia Parasitica in River Water Using Real-Time Quantitative PCR: From Massive Fish Mortality to Tap Drinking Water. Int. J. Environ. Health Res. 2017, 27, 1–10.
15. Gonzalez, R.; Curtis, K.; Bivins, A.; Bibby, K.; Weir, M.H.; Yetka, K.; Thompson, H.; Keeling, D.; Mitchell, J.; Gonzalez, D. COVID-19 Surveillance in Southeastern Virginia Using Wastewater-Based Epidemiology. Water Res. 2020, 186, 116296.
16. Rusch, J.C.; Hansen, H.; Strand, D.A.; Markussen, T.; Hytterød, S.; Vrålstad, T. Catching the Fish with the Worm: A Case Study on EDNA Detection of the Monogenean Parasite Gyrodactylus Salaris and Two of Its Hosts, Atlantic Salmon (Salmo Salar) and Rainbow Trout (Oncorhynchus Mykiss). Parasites Vectors 2018, 11, 333.
17. Trujillo-González, A.; Edmunds, R.C.; Becker, J.A.; Hutson, K.S. Parasite Detection in the Ornamental Fish Trade Using Environmental DNA. Sci. Rep. 2019, 9, 5173.
18. Bastos Gomes, G.; Hutson, K.S.; Domingos, J.A.; Chung, C.; Hayward, S.; Miller, T.L.; Jerry, D.R. Use of Environmental DNA (EDNA) and Water Quality Data to Predict Protozoan Parasites Outbreaks in Fish Farms. Aquaculture 2017, 479, 467–473.
19. Jousson, O.; Pretti, C.; Di Bello, D.; Cognetti-Varriale, A.M. Non-Invasive Detection and Quantification of the Parasitic Ciliate Ichthyophthirius Multifiliis by Real-Time PCR. Dis. Aquat. Organ. 2005, 65, 251–255.
20. Minamoto, T.; Yamanaka, H.; Takahara, T.; Honjo, M.N.; Kawabata, Z. Surveillance of Fish Species Composition Using Environmental DNA. Limnology 2012, 13, 193–197.
21. Haramoto, E.; Kitajima, M.; Katayama, H.; Ohgaki, S. Detection of Koi Herpesvirus DNA in River Water in Japan. J. Fish Dis. 2007, 30, 59–61.
22. Kawato, M.; Yoshida, T.; Miya, M.; Tsuchida, S.; Nagano, Y.; Nomura, M.; Yabuki, A.; Fujiwara, Y.; Fujikura, K. Optimization of Environmental DNA Extraction and Amplification Methods for Metabarcoding of Deep-Sea Fish. MethodsX 2021, 8, 101238.
23. Taengphu, S.; Kayansamruaj, P.; Kawato, Y.; Delamare-Deboutteville, J.; Mohan, C.; Dong, H.; Senapin, S. Concentration and Quantification of Tilapia Tilapinevirus from Water Using a Simple Iron Flocculation Coupled with Probe-Based RT-QPCR. bioRxiv 2021.

24. Vilaça, S.T.; Grant, S.A.; Beaty, L.; Brunetti, C.R.; Congram, M.; Murray, D.L.; Wilson, C.C.; Kyle, C.J. Detection of Spatiotemporal Variation in Ranavirus Distribution Using EDNA. Environ. DNA 2020, 2, 210–220.
25. Miaud, C.; Arnal, V.; Poulain, M.; Valentini, A.; Dejean, T. EDNA Increases the Detectability of Ranavirus Infection in an Alpine Amphibian Population. Viruses 2019, 11, 526.
26. Weli, S.C.; Bernhardt, L.-V.; Qviller, L.; Myrmel, M.; Lillehaug, A. Development and Evaluation of a Method for Concentration and Detection of Salmonid Alphavirus from Seawater. J. Virol. Methods 2021, 287, 113990.