The eDNA Society
NEWSLETTER

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no. 5

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Greetings from the President of The eDNA Society

Toshifumi Minamoto
(Professor, Graduate School of Human Development and Environment, Kobe University)

It is an honor to write my first message to you as the Second President of the eDNA Society. First, I would like to thank former President Dr. Michio Kondoh and all the members who contributed to the growth of the eDNA Society. I will try my best to further advance the society, and I appreciate your support and feedback.
Four and a half years have passed since the establishment of the eDNA Society in April, 2018. The society grew under the leadership of the first President. At the end of August 2022, there were 327 regular members and 31 supporting organizational members. Although eDNA science is still in its infancy, I am pleased to see the rapid and continuous growth of this new field, with an increasing number of people joining in.

Over the past few years, the state of eDNA research has changed dramatically. The eDNA Society has expended great efforts to standardize eDNA monitoring methods. In order to do so, we published the "Environmental DNA Sampling and Experiment Manual" (Japanese version) in 2019 and the English version in 2020; additionally, we hosted regular technical seminars to share these methods. Because of these efforts, eDNA analysis is now recognized as one of the most powerful and reliable biological monitoring methods. It is noteworthy that eDNA analysis is being used by central and local governments for biological monitoring projects in Japan. Cooperative surveys with citizens and companies is also progressing. Due to these efforts, eDNA science is being implemented publicly. I believe that the eDNA Society has played a major role in these achievements, and I would like to thank the many members who have contributed their efforts.

Although eDNA science has developed rapidly, we must continue to move forward to prove its true value. We need to promote international collaborations since it is crucial to establish standardized international methods in the current era of global biodiversity decline. At the same time, we must connect academia, industry, and government to facilitate the social implementation of eDNA methods. In the next few years, we will attempt to shape eDNA research towards this direction.

On the other hand, I would like to emphasize the importance of scientific excitement: eDNA is interesting! Many of you would be excited when you learn about eDNA for the first time. You might have thought "it's crazy a cup of water

tells what's there!". Some of you may even develop a habit of peering into the water of a lake, river, or sea. I think it is very important not to lose this kind of pure fun and excitement about scientific exploration, and to pass it on to the next generation.

The eDNA Society is currently facing a variety of challenges, and I would like to address these challenges and work hard to resolve them. I would greatly appreciate your continuous support of our activities.

Report on the Online Workshop Held in November 2022

Committee Meeting Report

Toshifumi Minamoto (Professor, Graduate School of Human Development and Environment, Kobe University)

The eDNA Society has held its annual meeting in the fall of previous years, but the Society's annual meeting for FY2022 (August 2022–July 2023) will be "The eDNA Society International Meeting 2023," which will be held in May 2023. Therefore, we decided to hold a workshop with a collection of poster presentations in November 2022, since it was thought that many members would like to present their research results in the fall and to present and discuss their research results mainly in Japanese. If possible, we would have liked to provide an on-site venue where people could hold discussions face-to-face, but due to the situation, we had to hold the workshop online.

We were a little concerned about how many presenters and participants we would be able to attract, given the online format that we thought members were getting tired of. However, we obtained a number of members, with 34 presentations (including 4 posters from high schools) and 86 participants in total. This engagement could be attributed to the fact that this was the third year of the pandemic and the participants' online skills had improved; the question-and-answer segment was very lively, and I would say that it was a great success. However, it is also true that it was not very different from other online conferences in that you could not enjoy the unique on-site experience of having discussions with people you happen to pass by or interact with when you stop in front of a poster that catches your eye. It is especially regrettable that some students graduate or complete their studies without ever presenting their research at a conference in person, which makes me wish that we could have managed to hold the on-site workshop. As of February 2023, our daily lives are returning to the way before the pandemic, both internationally and domestically, and conferences will probably be held in person in the near future. With this in mind, I would like to try my best so that the participants can feel the advantages of a face-to-face meeting.

In organizing this workshop, we asked young members to serve on the organizing committee, which placed a burden on them; however, they were able to make the most of their respective areas of expertise and ensure that the event went smoothly. In closing, I would like to express my gratitude to all committee members for their efforts in organizing the workshop.

Messages from the best presentation award winners

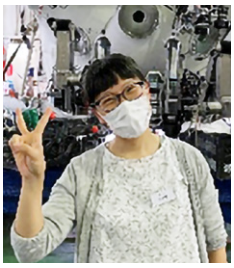
Exploring phylogeography from water

Tetsu Yatsuyanagi
(JSPS Research Fellow, DC2, Graduate School of Agriculture, Hokkaido University)

Understanding phylogeographic structures is of fundamental importance in preserving biodiversity. However, large-scale phylogeographic information is difficult to obtain because thousands of individual samples from large geographic areas are required. Recent advances in environmental DNA (eDNA) techniques have enabled the identification of intra-specific genetic variations in aquatic organisms which can also be applied to estimate phylogeographic patterns of freshwater fish without collecting individual fish samples. In this study, we explored the phylogeographic structures of a primary-freshwater fish, *Barbatula toni*, which is a widely distributed species in Hokkaido.

Using a newly-developed *Barbatula*-specific eDNA detection system, we detected well-screened 50 mtDNA haplotypes in 51 rivers. The phylogeography of *B. toni* was composed of six genetic groups with close correspondence to their distributed regions, which were divided into two strongly divergent clades. Their genetic differentiation is well explained by isolation by distance. However, spatial demography estimated by mismatch distribution analysis was largely different among geographic regions, suggesting geology-associated divergences of this species in Hokkaido. Our findings demonstrate the advantages of eDNA techniques as an innovative population genetics method to assess biodiversity at extensive and fine scales.

A journey to the deep sea using environmental metabarcoding analysis



Qianqian Wu
(Post-doc, Graduate School of Human Development and Environment, Kobe University)

After receiving my Ph.D., I got the chance to switch from researching freshwater shrimp to researching deep-sea invertebrates with which I was unfamiliar. However, instead of developing detection technology for a single species, I had to deal with a method for recognizing multiple species. Taking on such a project was

Fig. 1 JAMSTEC's research vessel "Kaimei"


Fig. 2 Water samples were collected using Niskin samplers.

both an opportunity and a challenge for me as a new post-doctoral researcher.
In search of deep-sea organisms, we sailed out to sea on a research vessel (Kaimei; Fig. 1) provided by JAMSTEC, and collected deep seawater (350–2050 m deep) samples using Niskin samplers (Fig. 2). Because we had to collect samples from the deep sea and filter a substantial amount of water on the ship, the task of sampling and filtering was very time-consuming and laborious.

Data analysis was the most exciting step in this study. As is well known, eDNA metabarcoding analysis provides a very large number of sequences. By comparing the sequences in the INSDC database, we can determine the species that have been detected. As a researcher specializing in freshwater shrimp, I had little knowledge of the formal name of the species and its biology initially. Nevertheless, I treasured the opportunity to spend time analyzing the data because it allowed me to learn more about a variety of deep-sea organisms. From our sampling sites, the DNA of a variety of species was discovered, suggesting that the deep sea is not a lonesome world. Looking at these results, I felt as if I had completed a trip to the deep sea myself.

It is impossible to conduct deep-sea research alone through individual efforts. Each survey requires substantial funding and the cooperation of different professionals. I would like to thank my post-doctoral study supervisor as well as the people who participated in the deep-sea survey. I am grateful for this project, which introduced me to a new world of deep-sea ecology.

Introduction of the Laboratory of Animal Ecology of Hokkaido University

Masayuki Sakata
(Assistant Professor, Research Faculty of Agriculture, Hokkaido University)

The Laboratory of Animal Ecology, Faculty of Agriculture, Hokkaido University, is dedicated to understanding the ecology and evolutionary mechanisms of wild animals through field research and genetic analysis (Laboratory website: https://animalecologystaff.wixsite.com/hgs-lae). Led by Professor Hitoshi Araki, many members of the laboratory (22 members in total in FY2022) conduct research to elucidate the evolution and ecology of wild animals using field surveys and eDNA analysis.

The longest-running research using eDNA is the monthly Chitose River water-sampling survey. This survey was initiated in April 2014, and since then, at least one survey per month has been conducted on an ongoing basis throughout the year. One of the advantages of eDNA samples is that they can be preserved in DNA form for long periods. By conducting continued surveys, we can not only accumulate long-term data from the past to the present, but also go back in time to obtain information on target organisms when new detection systems are developed. For example, since salmon migrate up the Chitose River, many individuals can be observed during fall and winter. In 2022, 580,000 salmon were observed to return to the Chitose River (https://chitose-aq.jp/data/captureinformation.html), the largest number ever recorded, and a very large number of salmon were observed at the survey sites (Fig. 1). If long-term observational data are available, it may be possible to clarify the reasons for such fluctuations in salmon populations by combining them with recorded environmental changes and other data. It is also very important to conduct eDNA analysis going back to three or four years before they were born, especially in years with high salmon abundance.

Fig. 1 Large number of salmons running up the Chitose River (Photo by Masayuki Sakata)

Hokkaido is home to many salmonids. The MiFish primer (Miya et al. 2015 Royal Society Open Science) is the definitive

eDNA metabarcoding primer for fish and is used as a standard not only in Japan, but also worldwide. However, it is sometimes difficult to identify salmonid species because of intraspecific polymorphisms. Therefore, our laboratory has been researching and developing universal primers specifically for salmonids. We believe that the use of these primers will enable us to identify salmonids with resolution higher than ever before and will greatly contribute to the study of salmonids, which are useful as a fishery resource. The paper on this primer is currently being submitted for publication; therefore, it will be made publicly available in the future and can be used by many people.

Various studies using eDNA analysis have been conducted in our laboratory, not only on organisms that live only in water, but also on wildlife in general, including those that live on land. The taxonomic groups covered included fish, mammals, birds, amphibians, crustaceans, flatworms (parasites), and many others. For example, the Southern Asian Dolly Varden (*Salvelinus curilus*) is a salmonid fish found in Hokkaido. It is established that in the Shiretoko Peninsula, mitochondrial gene infiltration has occurred in the past because of interspecific hybridization with White-spotted char (*Salvelinus leucomaenis leucomaenis*) and an attempt is being made to estimate the proportion of such individuals based on eDNA. A study in Hokkaido is also underway to determine the geographic distribution of intraspecific polymorphisms in the indigenous species of stone loach (*Barbatula barbatula*) using eDNA to reveal the geographic distribution of such polymorphisms throughout the island. Thus, research on intraspecific polymorphisms in fish species is not limited to distribution monitoring. In addition to native species, a study on the breeding site of the toad (*Bufo japonicus formosus*), a domestic invasive species, was conducted by detecting eDNA in river water, and the results were used to estimate the breeding site of the toad (Mizumoto et al. 2021 Biological Invasions). In addition to eDNA analysis using water samples, research is being conducted on the use of lake sediments to reconstruct the recent past of fish species.

Fig. 2 Survey scene in a snow field
(Photo by Hitoshi Araki)

As you all know, Hokkaido winters are very cold and snowfall is heavy. Therefore, there are cases where roads to the water's edge are blocked by snow, making it impossible to reach

Fig. 3 Left: Drone water sampling. Right: enlarged image (Photo by Masayuki Sakata)

Fig. 4 Water sampling on the frozen lake
(Photo by Hitoshi Araki)

the water's edge by car or other means. In such cases, it is necessary to march through the snow with snowshoes or other equipment to approach the water surface (Fig. 2). Drone water sampling is also effective when water sampling is desired at inaccessible survey sites or at the center of a stream or lake (Fig. 3). A water sampling bottle was connected to the drone, and the bottle could reach the sampling point directly, making it useful for a variety of surveys. In some cases, when the water surface was frozen, a hole was drilled and water was collected under the ice (Fig. 4). One of the major advantages of eDNA is that it can be used to survey underwater organisms even during periods when it is difficult to conduct conventional sampling surveys using nets or other methods.

Easy and Compact: Gravity Filtration of Environmental DNA

Shin-ichiro Oka
(Manager and Senior Research Scientist, Zoological Laboratory, Okinawa Churashima Foundation)

Two years ago, I introduced the "Parallel Filtering System", which was created with familiar materials, in the Environmental DNA Newsletter No. 3[1]. In this system, multiple sample bags are connected to a unit consisting of vinyl chloride pipes, and aspirators are used for simultaneous vacuum suction. However, two months later, we developed an ultra-compact filtration system that requires no external forces[2]. Although this new system is not as fast as vacuum filtration, it has been highly praised by some researchers for its convenience, which outweighs its shortcomings. I am pleased to have the opportunity to introduce it.

I was motivated to develop a new filtration system when I found a description of gravity filtration in an eDNA study, but the paper did not mention the details of the system[3,4]. I came up with the idea that gravity would allow for filtration even in places where a power source is unavailable and relieve us from preparing heavy equipment or hand-pushing syringe plungers. This can also relieve us from the stress brought by equipment malfunctioning during filtration in the field. I mastered 3D printer technologies as a hobby around the time which assisted me in developing this system about one month after the conception. Herein, I introduce a new filtration system that was published last year[2].

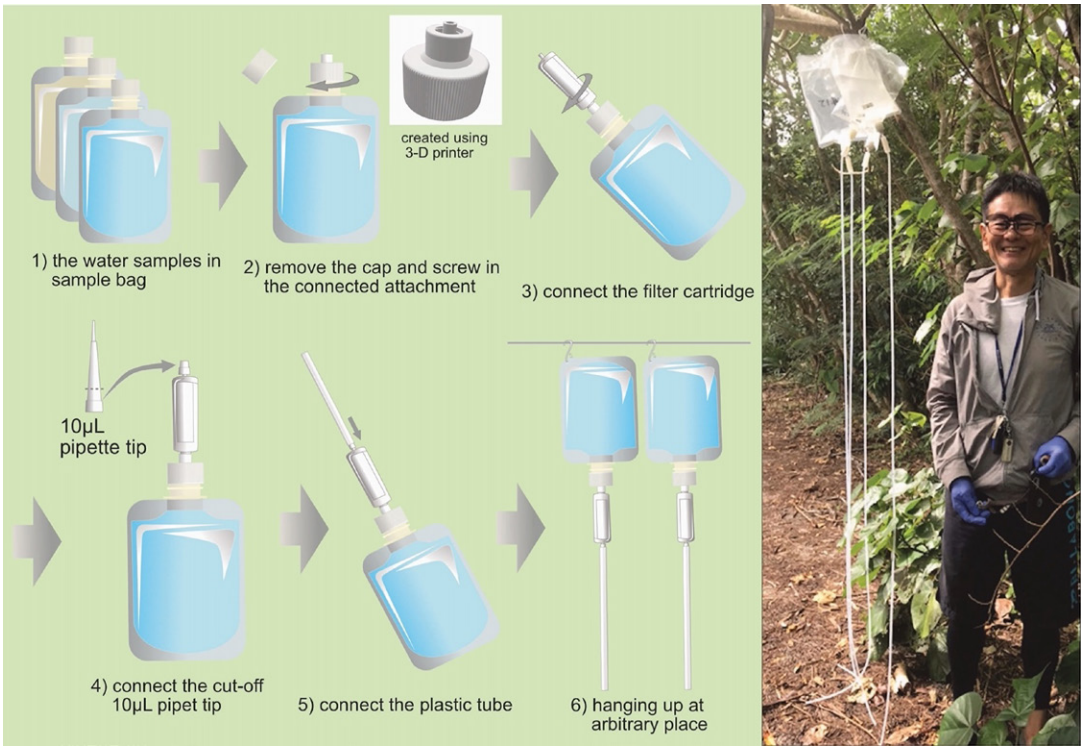


Fig. 1 Protocol set-up of the novel gravity filtration system for eDNA water samples. The photograph shows the filtration system hanging at about 2 m height in the field by Dr. Miya. [This figure is adopted from reference 2]

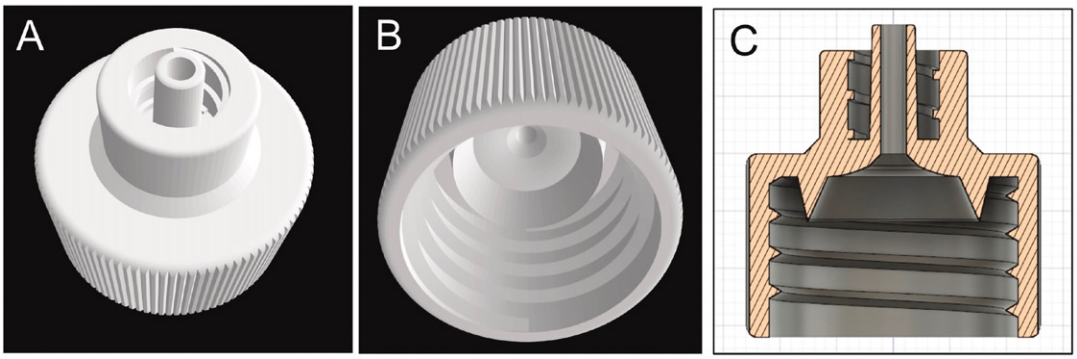


Fig. 2 Attachment (designed with 3D CAD software) connected with a sample bag and filter cartridge[2]. Top (A), bottom (B), and cross-section (C) views. The 3D design files (STL) are available in the supplementary files of Oka et al. (2022)[2]. [This figure is adopted from reference 2]

As shown in Fig. 1, the system consists of four parts: (1) a plastic bag (1 L, DP16-TN1000, COWPACK LTD; airtight, general-purpose product for gel or liquid foods with an attached screw cap) containing the water sample, (2) a self-made connecting attachment (Fig. 2) created with a 3D printer, (3) a Sterivex filter cartridge (Merck, Rahway, NJ, USA), and (4) a plastic tube (inner diameter: 4 mm) with an adjuster (cut-off 10 μL pipette tip base).

The printed attachment (Fig. 2) was designed to fit the plastic bag and Sterivex filter cartridge using 3D CAD software and printed using a stereolithography 3D-printer (ELEGOO Mars Pro 2), which is an affordable and widely available model. The printer could print the attachment within 3 h in a normal setting. The number of pieces produced depends on the size of the platform build. The cost of the resin is less than 0.5 USD per piece. The 3D design files of the attachment are available for downloading as supplementary files from Oka et al. (2022)[2].

The filtration is performed by hanging the system at an arbitrary height (Fig. 1). The water in the bag was passed through a filter cartridge and discharged

automatically through a plastic tube. The only forces required for filtration are the pushing and pulling forces of the water weight in the sample bag and plastic tube, respectively. Increasing the bag height increases the water weight in the plastic tube, consequently increasing filtration speed. A hanging height of 9 m yields the same performance as vacuum filtration. However, a height of approximately 2 m is suitable for practical use because of its maneuverability and compactness, although it takes more than twice as long as vacuum filtration. With this gravity system, filtration can be performed anywhere which has a hanging place, even outdoors or in a hotel bathroom, with care for contamination.

The following are "notes for use" for this method:

- The connecting attachment and plastic tube can be reused after decontamination with bleach.
- Tight joints are required at each connection point to avoid air leaks to avoid filtration speed reduction.
- A plastic tube with an inner diameter of 3 mm can be attached directly to the outlet port of the Sterivex filter cartridge (outer diameter, 3.5 mm) without a cut-off pipette tip adjuster. However, the filtration speed may be reduced slightly.
- Making a hole in the upper part of the hanged sample bag produces air influx and prevents a decrease in the filtration speed owing to the negative air pressure in the bag.
- Turbid water may take several hours to filter. To prevent degradation of eDNA over time, benzalkonium chloride (BAC) should be added immediately after water sampling.

The proposed system has several advantages. First, because it requires no external force such as an aspirator, mechanical problems can be avoided. Moreover, the start-up costs are low because they consist of inexpensive components. In addition, it enables rapid sampling because there is no on-site filtration and only water sampling is required for field protocols.

Many people who see this gravity filtration system might be skeptical of its performance. In fact, I created this system just for fun, and I never expected to publish it as a research paper. Contrary to my initial expectations, this system was very useful for eDNA sampling. Several researchers who have tested this system have expressed positive feedback regarding its value. Some user comments can be found below.

Visit the following link for a video of the gravity filtration system. Note that the link may become invalid without notice.

https://www.youtube.com/watch?v=lD1FKBISYGc

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User's comments

Gravity filtration greatly improved eDNA sampling efficiency!

Kei Wakimura (Assistant Professor, Faculty of Pharmacy, Osaka Ohtani University)

Gravity filtration, which can be performed at a hotel by hanging sample bags from a shower curtain rail, enables efficient sampling and filtration because on-site procedures are not required and filtration is completed the next morning. We confirmed that overnight filtration at room temperature did not affect eDNA concentration in the presence of 0.01% BAC. Importantly, gravity filtration does not damage the Sterivex filter cartridges as filtration gradually progresses after the filters are clogged. Consequently, an equal or greater volume of water than that used in other methods (e.g., by a peristaltic pump) can be filtered overnight. Therefore, this method is highly recommended.

Gravity filtration at a hotel bathroom

No need for electricity and manpower, yet powerful!

Tetsu Yatsuyanagi (JSPS Research Fellow DC2, Graduate School of Agriculture, Hokkaido University)

Among the various eDNA filtration methods, gravity filtration is truly innovative in many respects. This system enables multi-sample filtration without electric power, which makes a sampling trip much easier. Initially, I was a bit skeptical of its performance, but after using it, I realized the power of gravity. Surprisingly, the gravity filtration system completed 500 mL × 2 filtration of very turbid water in 3–4 h, whereas syringe filtration (as shown in the photo) of the same water by hand stopped at 250 mL. Gravity filtration is practical for unifying the filtering volume across sampling sites, regardless of turbidity. Why don't you give it a try?

Syringe filtration by hand

Editor's Note / Newsletter Editorial Team

Editor's Note

Kimiko Uchii (Editor-in-Chief, The eDNA Society Newsletter)

Hello and welcome to our newsletter! This issue began with a message from the new President, Prof. Minamoto, and I am sure you felt the warmth and sincerity in his message. In the online workshop report, two young researchers who won the best presentation awards provided brief summaries of their research. We could feel their motivation and passion for tackling new challenges. The Laboratory of Animal Ecology at Hokkaido University was featured in the Frontiers of Environmental DNA Study, a series of articles from the 1st issue of the Newsletter. Many of you may have been impressed by the diverse research being conducted amid the great nature of northern Japan. At my request, Dr. Oka contributed an article on the gravity filtration system, a new filtration method that was published last year. In our laboratory, we almost completely switched to gravity filtration because it is very efficient. I am 100% convinced that sharing this information would benefit many people. As you can see, I would like to use the newsletter as a platform to share knowledge, information, and whatever else. If you have something you would like to share in the newsletter, please freely inform the members of the editorial team.

The Covid-19 pandemic and the resultant restrictions have prevented many opportunities for face-to-face academic interactions, especially international research exchanges. While virtual alternatives have rapidly advanced, many people may be eager to gather in person to share, discuss, and communicate. In May, the International Meeting will be held in person in Otsu, Japan. I look forward to seeing you there.

Newsletter Editorial Team

We welcomed two new members to the editorial team. Please find their profiles below.

Masayuki Sakata

(Assistant Professor, Research Faculty of Agriculture, Hokkaido University).

Short CV:

Ph.D. in Science from the Graduate School of Human Development and Environment, Kobe University, in 2021, followed by a post-doctoral research position at Kobe University. The current position has been held since July 2022. Started working on eDNA research since undergraduate. A member of the eDNA Society, The Ecological Society of Japan, The Japanese Society of Limnology, and British Ecological Society.

Message:

Thank you for letting me join the editorial team. Although I am young, I would like to do my best. Originally a mushroom lover, I found myself involved in eDNA since my undergraduate days, but I also like to catch fish in rivers and ponds. These days, I exclusively collected lake sediment. My main research area is inland water, but I like the ocean.

Noriko Uchida

(Assistant Professor, International Research Institute of Disaster Science, Tohoku University)

Short CV:

Ph.D. in Engineering (Tohoku University, Japan, 2020), and then the current position. From August 2022 to March 2023, I stayed at ETH Zurich (Switzerland) as a guest professor, that was promoted by the grant of Tohoku university. A member of The eDNA Society, Japan Society of Civil Engineering, Ecology and Civil Engineering Society,

Message:

The eDNA research has opened the door to the wonderful world of insects for me. I sincerely hope more people will have this kind of experience and expand their world through exposure to eDNA. With such ambitions in mind, I will do my best in my new role as a member of the editorial team.

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