The eDNA Society
NEWSLETTER

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no. 4

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Greetings from the President of the Society

Michio Kondoh
(President of the Society, Professor, Graduate School of Life Sciences, Tohoku University)

Thank you so much for continually supporting The eDNA Society. Personally, there are many events at universities at the end of the year, such as guidance and examination of theses and preparation for various academic conferences. Time passes without breathing. At The eDNA Society, discussions on the next year's symposia and conference planning started after we finished the last conference in November. However, in the longer term, the situation surrounding eDNA should become even "busier."

As stated in the prospectus for the founding of The eDNA society, we aim to contribute to the well-being of all humankind through the conservation and sustainable use of ecosystems. Three and a half years have passed since its establishment. During that time, we have implemented several projects in which industry, government, academia, and the private sector collaborated to achieve. Recently, I have been increasingly reminded that this speed may not be enough and that eDNA technology needs to be developed faster. A reason for this "business" could be the growing awareness of nature and biodiversity and the increased activity of specific actions.

As the name "Anthropocene" that we hear so often these days suggests, it is widely recognized that humanity is gradually transforming the Earth into an "unlivable place." However, against the backdrop of this rapidly growing awareness of the issues surrounding the sustainability of society, 2021 saw several crucial developments in nature. One of the most important would be the launch of the Task Force on Nature-related Financial Disclosures (TNFD) in June. However, at the end of December, the Ministry of the Environment of Japan announced its participation in the TNFD forum.

To create a sustainable society in the Anthropocene, which TNFD aims for, we must accurately understand the present state of ecosystems supporting our lives, industrial activities, and culture and develop management and operation methods that effectively make necessary services sustainable. However, nature is a gigantic complex system comprising countless living and non-living elements, which would require sufficient information on quality and quantity that commensurate with the complexity of nature. This is a challenging task. Yet, we do not have much time left. At the G7 summit held in the UK in June, Nature Compact was adopted, declaring that we put the brakes on the destruction of nature and reverse the trend toward recovery by 2030. We have eight years left. In this international climate, eDNA technology expectations, which enable rapid and massive acquisition of biodiversity

information, will undoubtedly become even greater. Additionally, I believe this will mean that the community surrounding eDNA and The eDNA Society will bear a heavy responsibility.

How shall we accomplish this mission? While working hard and cooperating with members of the society, we want to run through the next year to achieve this important goal. Although this is not easy, it will lead to the well-being of all humankind in the future. I look forward to your continued support. Thank you.

4th Annual Meeting of The eDNA Society

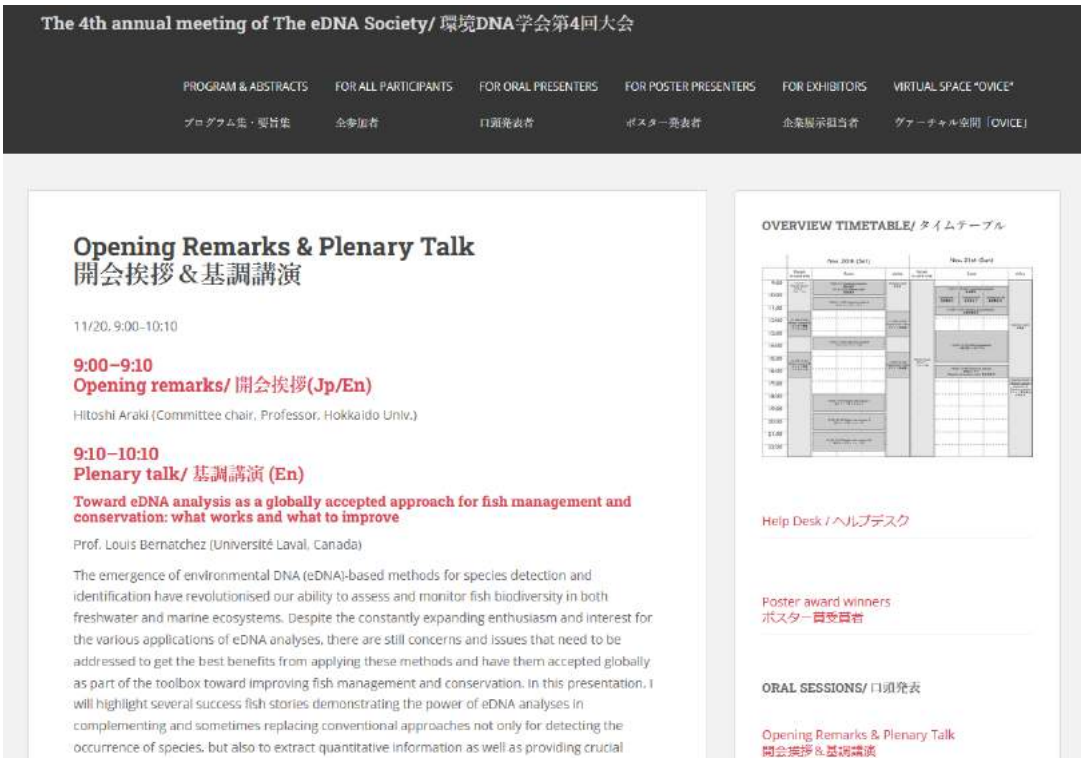
Meeting report of edna2021

Hitoshi Araki
(Chair of the edna2021 meeting committee, Professor, Hokkaido University)

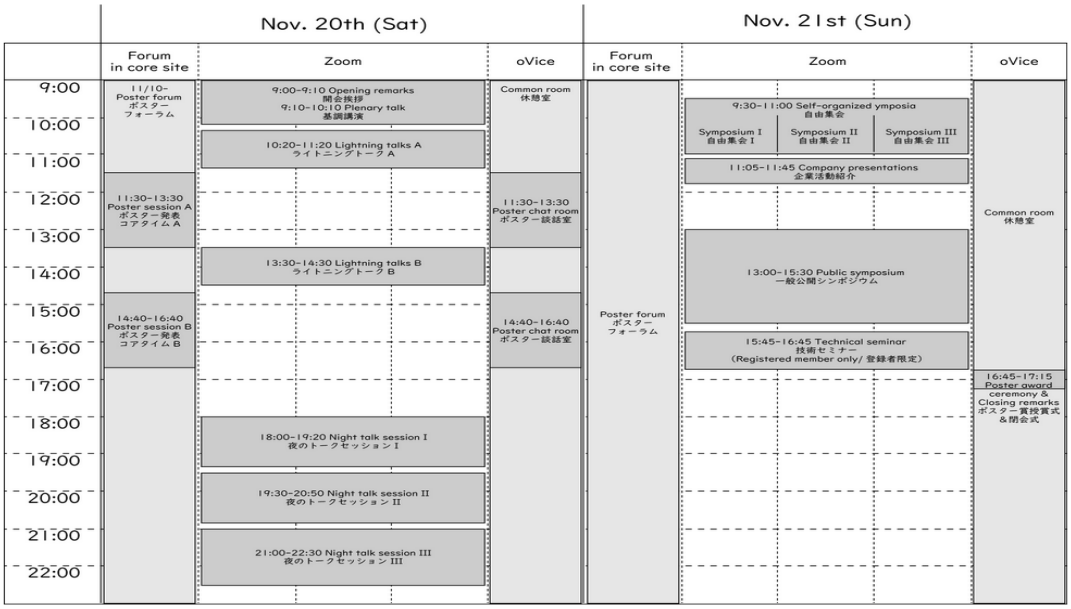
On behalf of the meeting committee, I report edna2021, the fourth annual meeting of The eDNA Society, held online on November 20–21, 2021. The main theme of edna2021 was "Species Distributions, and Beyond" (Fig. 1a). It reflects the various interests that the meeting attendees share, and we aimed to further improve the network of eDNA researchers and stakeholders globally. The meeting gathered more than 500 participants from at least 15 countries worldwide. They included ecologists, fishery scientists, geneticists, taxonomists, bioinformaticians, conservation managers, policymakers, groups of citizens, and more. The meeting had 11 sessions with themes ranging from new field/laboratory techniques related to eDNA/eRNA surveys, their applications and standardization, and their applicability to social implementations and promotions of environmental education.

The first session was a plenary talk by Dr. Louis Bernatchez (Université Laval, Canada) (Fig. 1b). His talk was entitled "Toward eDNA analysis as a globally accepted approach for fish management and conservation: what works and what to improve." In the session, Dr. Bernatchez first explained how eDNA analyses could contribute to worldwide fishery management and fish biodiversity conservation, causing a paradigm shift toward ecosystem-based management to sustain healthy ecosystems and the fisheries they support. His presentation covered various subjects related to fishery management, including habitat use, environmental determinants of community structure, Spatio-temporal dynamics, and abundance of target species. In the plenary talk, Dr. Louis Bernatchez mentioned that eDNA is still timidly implemented as a part of the toolbox for routine fish monitoring and fishery management, citing Pawlowski et al. (2021). Although his talk focused mainly on fish management, he mentioned that the skepticism and concerns about eDNA techniques had been expressed by stakeholders worldwide.

Nevertheless, his talk and following presentations in the meeting convinced us of the potential of eDNA and its applications to various subjects ranging from large-scale biodiversity monitoring to citizen Science. The attendees also realized what should be improved in the near future, including on-site DNA detection and more. Additionally, we realized that the pace of technical improvements became higher, with more experts involved in eDNA surveys worldwide. We appreciate all attendees of edna2021 for fruitful discussions, and we are looking forward to meeting them in the next eDNA meeting, where more improvements and developments of eDNA techniques and their applications will be discussed. (For more detail: Meeting report in Environmental DNA, https://doi.org/10.1002/edn3.278)



Clip of the meeting core-site



The schedule of the meeting

Meeting flyer and some captures of sessions

Night Talk Sessions

Hiroki Yamanaka
(Associate Professor, Faculty of Advanced Science and Technology, Ryukoku University)

On November 20, the first day of the conference, "Night Talk Sessions" were held; the first session was a talk by Neil Gemmell (University of Otago, New Zealand) entitled "The Science of Monster Hunting." He reported the scientific results and related side stories of the world-famous "Loch Ness Project." More than 3000 species of DNA were detected in this project, covered in more than 7000 articles in various media. The media repercussion was great due to the project's purpose: detecting the Loch Ness monster, and, simultaneously, the project was built on a scientific context. Furthermore, the project results raised expectations of the great impact that environmental DNA analysis can have on society as a Science and technology.

The second session was a talk by Jordana Meyer (Stanford University, USA.) on "Connecting the dots in the web of life with eDNA and Networks." The focus of her presentation was a study on the Jasper Ridge Biological Reserve in California, using metabarcoding technology on fecal samples to analyze networks of interspecies relationships to reveal species interactions. It was reported that by analyzing prey from samples derived from pumas, coyotes, and bobcats in the reserve, and by further molecular ecological network analysis, they successfully showed the complex food web of 212 interrelated species and the strength of their ties. Apart from this

scientific topic, she talked about her career path and introduced her work experience in different research institutes before starting her master's and doctoral programs. This was a stimulating and attractive career path for students and young researchers in Japan, where many students go straight from undergraduate to master's and doctoral programs.

The third session, organized by Hideyuki Doi (University of Hyogo) and others, was unique, where young researchers and students shared their concerns about their career development and the future of environmental DNA research. However, the session was not limited to that, and discussions were held in many fields, such as practical ways to avoid contamination in the laboratory and information on currently open positions at universities and companies.

Public Symposium "Environmental DNA Citizen Sciences –Keeping our Eyes on Our Local Ecosystems"

Satoquo Seino
(Associate Professor, Graduate School of Engineering, Kyushu University)

At the Public Symposium of the 4th Annual Meeting of The eDNA Society, citizen science based on eDNA was taken up. By observing changes in familiar organisms, it is possible to grasp and monitor the phenomena that are thought to have an impact on the local ecosystem caused by environmental changes such as global warming. As one of the methods, the eDNA of "just collecting water on site" has begun to be introduced into citizen surveys and education. In addition, observations at multiple sites have been started, centered on The eDNA Society, and expectations are rising as a new field of ecology.

In the planning, we considered a common perspective for this symposium based on the practices in each local region. We have created planning teams centered on academic members at three locations in Sapporo, Minami-Sanriku, and Fukuoka, and the nature and social characteristics of the local ecosystem (rivers / sea areas, cities / fishing villages), and collaborative projects between citizens and researchers were introduced. At the symposium using remote technology, discussions were possible at multiple location at the same time. We obtained 334 participants via the internet and discussed the ideas and responses of community-based practical ecosystem monitoring activities. Information sharing in regional ecosystem surveys enabled the participation of diverse people and multiple generations, especially from areas other than cities where there were few opportunities to participate in conferences. This was a revolutionary advance in ecological research.

As prospects, "eDNA Citizen Science" and information sharing via the internet have been steadily developing as research, education, and dissemination and enlightenment of regional ecosystems. The results of biodiversity and multipoint simultaneous observations can contribute to consensus building and decision-making for

conservation and utilization of regional ecosystems. The nation-wide observation cannot be possible without the accumulation of information obtained in regional ecosystems. Facilitation of the cooperation among active regions by implementing any mechanisms to connect them will give us a further development in this field.

[Date and time] November 21, 2021 (Sun) 13:00-15:30
[Method] Zoom Webinar and hybrid
[Participation fee] Free

"Participating in environmental DNA surveys and public symposium"

Yuzuki Ito (Junior High School Student in Fukuoka City)

In the environmental DNA survey in the autumn of 2020, I investigated Hakata Pier in Fukuoka Prefecture, Japan. The survey detected fish eDNA of 29 species. I was excited to find that some eDNA of fish are not normally caught, such as *Omobranchus punctatus*, in the sea. I was happy to find fish eDNA of 99 species in the nine groups of the participants and feel the richness of the local sea.

At this symposium, various topics in each local area were fun, and I learned a lot. For example, in addition to the increase in yellowtail moving north and the decrease in salmon due to the effects of global warming, the feeding damage by spine foot of southern species in the Minami-Sanriku region was impressive. There was also a southern species of sardine in my results. Thus, by continuing such surveys, I think there will be more opportunities to learn about the current situation in the sea, which is difficult to observe.

Also, I learned that the survey kit was packed with an ingenuity so that our kids would be happy. Though the analysis work is difficult and the accuracy seems to be an issue, we hope that the time will come when such analytical study can be performed easily, as in such a survey kit.

In the future, when water samples from all over Japan are collected, the amount of data will be enormous, and the realization that there are various organisms may be an opportunity to be interested in nature and environmental conservation. I want to investigate whether the fish species change in the same area but in different seasons and how much fish eDNA is detected in Okinawa, where there are many fish species.

"Encouraging meta-analysis and data simulation for eDNA analysis"

Toshiaki Jo
(Research assistant, Faculty of Advanced Science and Technology, Ryukoku University)

Our symposium focused on a meta-analysis that integrates previous findings and data simulation that mirror phenomena of interest on a computer. The first presenter, Dr. Toshiaki Jo, presented two meta-analytical findings; the relationship between eDNA concentration and species abundance is greatly affected by eDNA source and state, and the downstream transport distance of eDNA is strongly influenced by the discharge rate in riverine environments. The second presenter, Dr. Ryosuke Nakadai (National Institute for Environmental Studies), performed a meta-analysis using open data (raw data publicly published) and discussed the similarities and differences in the patterns observed in marine microbial and tree communities. The third presenter, Mr. Tatsuya Saito (University of Hyogo), performed a simulation based on a meta-analysis of eDNA studies and predicted the decay rate of eDNA of a given fragment length under a given temperature. Lastly, Mr. Kanta Kobayashi (Yamaguchi University) simulated the downstream transport of eDNA using river ecosystem models and examined the correlation between eDNA spatial distribution and fish abundance among multiple environmental conditions.

Many people may be unfamiliar with meta-analysis and simulation and could feel that there are barriers to using these approaches. However, it is no doubt that combining various approaches, including a meta-analysis and data simulation, is important to develop the eDNA analysis and establish the Science of eDNA in the future. Therefore, I believe that this symposium provided valuable information for participants.

"Current status and prospects of environmental DNA analysis in various taxonomic groups"

Masayuki K. Sakata
(Postdoctoral Researcher, Graduate School of Human Development and Environment, Kobe University)

This self-organized symposium was organized to discuss the current status and prospects of environmental DNA analysis for taxa other than fish, often targeted in environmental DNA studies. We decided to focus on designing and selecting universal primers and reference databases, which are essential for environmental DNA metabarcoding.

The first speaker and organizer, Dr. Sakata, specified the points to be considered in designing new universal primers and reported the actual development of universal primers for amphibians and their field application. The second speaker, Dr. Qianqian Wu (Kobe University), reported that the current development status of environmental DNA metabarcoding for cephalopods and its application results in the deep sea. The

third speaker, Dr. Natsuko Kondo (National Institute for Environmental Studies), focused on environmental DNA metabarcoding in insects, especially on barcoding DNA regions. She presented the advantages and problems of the COI barcode region commonly used in insects and the potential of the 16S rRNA gene to overcome these problems. Finally, the fourth speaker, Dr. Masatoshi Nakamura (IDEA Consultants, Inc.), multifacetedly discussed environmental DNA metabarcoding in plants from the perspective of multiple factors, such as target region, taxonomic resolution, and the degree of database enrichment.

In this symposium, every presenter mentioned the importance of selecting functional barcode regions and the difficulty in constructing reference databases. As environmental DNA metabarcoding is expected to develop for various taxa in the future, it was meaningful to share the current status, challenges, and prospects. I hope that this symposium provided the participants with a good opportunity to share the latest information on environmental DNA metabarcoding.

"Social implementation of eDNA: toward efficient biodiversity monitoring"

Hideyuki Doi

(Associate Professor, Graduate School of Information Science, University of Hyogo)

Environmental DNA (eDNA) techniques have made remarkable progress in the past decade. Furthermore, social implementation has been promoted by establishing the protocol manual of the eDNA Society. However, we still have various issues for social implementation. Primarily, in monitoring rare and invasive species, existing techniques cannot estimate the extinction risk of endangered species based on declining genetic diversity and population dynamics from the past to the present. In this symposium, we presented practical examples of social implementation of eDNA techniques and advanced studies to improve the practicality of eDNA techniques, i.e., eDNA methods for analyzing intraspecific variations using high-throughput sequencing and long-term population dynamics using sedimentary eDNA analysis.

We had five talks; Hitoshi Araki (Hokkaido University) spoke about the application of eDNA to managements of rare/invasive species, emphasizing how new eDNA-related technologies can improve wildlife management. Kimiko Uchii (Osaka Ohtani University) presented the use of the environmental DNA approach to evaluate genetic variation within species and suggested its application for the conservation of endangered fish and management of invasive fish. Hideyuki Doi spoke of the sedimentary DNA analysis for long-term reconstruction of biomass dynamics. It was stressed that the sedimentary DNA could be used to restore the fish population dynamics spanning the last several decades. Takahiro Okamura (KANSO TECHNOS CO., LTD.) presented their practices of social implementation of eDNA analysis. He mentioned the expectation and issues from on-site as a user of this technology, including actual case studies. Finally, Hiroki Yamanaka (Ryukoku University)

introduced the latest “Lake Biwa Challenge,” which included 100 sites eDNA survey along the shores of Lake Biwa by citizens and discussed its future perspectives and knowledge transfer to future projects in other areas.

In response to these talks, many questions were raised by the audience, especially on the aspect of social implementation. We discussed future approaches toward social implementation, including the certification system by the eDNA Society. This symposium was co-sponsored by the Environmental Restoration and Conservation Agency (4-2004) and Center for Biodiversity Science, Ryukoku University.

List of winners of Poster Presentation Award

High school students

First prize

Takeshi MORI, Toshinari OZAWA (Gifu High School),

“Estimating the amount of larval Ayu descent on the Nagara River by environmental DNA quantitative analysis,” SP001.

Outstanding performance award

Haruto SHIMBA, Ren MORISHITA, Seiya MATSUMURA (Kakegawanishi High School, Nature, and Science club),

“Aiming to identify the habitat of the rare species Libellula angelina :Designing Primers with Specificity,” SP005.

Maser students

First prize

Kai NAKANE (CMES. Ehime Univ.), **Hideyuki DOI** (Grad. Sch. Info. Sci. Univ. Hyogo), **Natsuki OCHI, Michinobu KUWAE** (CMES. Ehime Univ.), **Narumi TSUGEKI** (Fac. Law. Matsuyama Univ.),

“Reconstruction of 100-year dynamics in zooplankton spawning activity revealed by sedimentary DNA,” PP052.

Outstanding performance award

Takahito IKEDA, Takashi KANBE, Hitoshi ARAKI (Hokkaido Univ.),

“Seasonally differentiated distributions of the two smelt species in Hokkaido,” PP054.

Doctoral students and higher

First prize

Nozomu ONAKA, Manami INABA, Ryohei NAKAO, Yoshihisa AKAMATSU (Grad. Sch. Sci. Tech. Inno. Yamaguchi Univ),

"Study on air sampling method suitable for environmental DNA analysis in the air," PP024.

Outstanding performance award

Masayuki K. SAKATA, Mone U. KAWATA (Kobe Univ.), **Atsushi KURABAYASHI** (Nagahama Institute of Bio-Science and Technology, Hiroshima Univ.), **Takaki KURITA** (Natural History Museum and Institute Chiba), **Masatoshi NAKAMURA, Tomoyasu SHIRAKO** (IDEA Consultants Inc.), **Ryosuke KAKEHASHI** (Nagahama Institute of Bio-Science and Technology), **Kanto NISHIKAWA** (Kyoto Univ.), **Mohamad Yazid Hossman** (Forest Department Sarawak), **Takashi NISHIJIMA, Junichi KABAMOTO** (Ministry of Agriculture Forestry and Fisheries), **Masaki MIYA** (Natural History Museum and Institute Chiba), **Toshifumi MINAMOTO** (Kobe Univ.),

"Development and evaluation of PCR primers for environmental DNA metabarcoding of Amphibia," PP001.

Kaede MIYATA, Hiroshi HONDA, Yasuaki INOUE, Yuto AMANO, Tohru NISHIOKA, Masayuki YAMANE (Kao Corp.), **Takamitsu KAWAGUCHI** (Bioindicator Co., Ltd.), **Osamu MORITA** (Kao Corp.),

"Fish environmental RNA enables precise ecological surveys in rivers with negligible false positives," PP006.

Ryoji SUZUKI, Kunio KAWAMURA, Yuuko MIZUKAMI (Aichi Agric. Res. Cent.),

"Proposal for a simple extraction and detection method for environmental DNA," PP015.

Technical Seminar,
"Environmental DNA Metabarcoding Analysis Q&A"

Report from the lecturer: My experience at the technical seminar at the 4th eDNA society meeting

Masayuki Ushio (Hakubi Center, Kyoto University)

At the 4th Environmental DNA Society, I was invited as a speaker at the technical seminar "Environmental DNA Metabarcoding Analysis Q&A" where the seminar's format was to accept questions from participants in advance, and an unexpectedly large number of questions were sent in. On opening the Q&A file, 36 questions covering various topics, from sampling and experiments to data analysis, were found. Answers were for each of them, but since the opportunity to answer such a wide range of questions in detail is not always available, related literature had to be

reviewed. This was a good opportunity to reaffirm my knowledge of environmental DNA (eDNA).

In the technical seminar, we used a new tool, Pocketalk, for simultaneous interpretation, which I had never tried before. Although the tool had been practiced carefully beforehand, things did not go as planned. The available time was insufficient to check the translation by Pocketalk in real-time during the seminar, but several people found the mistranslation funny. It seems that Pocketalk misinterprets technical terms, especially names of professional software related to data analysis.

As regards the technical seminar, many participants and the very active Q&A session showed a high level of interest in the eDNA technology. The analysis of eDNA requires various techniques, from field sampling, laboratory experiments, to advanced sequence and data analysis, and preparing a detailed manual for all of these tasks is challenging because some tips are difficult to be documented precisely. Additionally, the eDNA technology is developing rapidly, and methods introduced 1–2 years ago may already not be state-of-the-art technologies. Under these circumstances, places where tips and information can be actively exchanged and shared (such as this technical seminar), will play a significant role in disseminating the knowledge to the community. It would be great if the acquisition of "eDNA-based ecological community data" becomes commonplace for everyone. Once we achieve this, the next stage becomes accessible, that is, prediction and management of the dynamics of ecological communities.

Frontier's in Environmental DNA Study (4th)

Introduction of the Research Center for Environmental DNA

Ryohei Nakao

(Graduate School of Science and Technology for Innovation, Yamaguchi University; Associate Professor (Project))

The Research Center of Environmental DNA (CEDNA) was established in July 2018 within the Faculty of Engineering, Yamaguchi University, Ube, Yamaguchi, Japan, as a distinctive research center of Yamaguchi University (Fig. 1). The CEDNA consists of five research divisions (Methods Development, Bioinformatics, Applied Ecology, Fisheries, and Medical and Sanitary), and many researchers, both on- and off-campus, are members of the CEDNA. Additionally, the Environmental DNA Research Consortium has been established for industry-academia collaboration since 2021. In collaboration with these researchers and companies, the CEDNA works on technical developments and advanced studies using environmental DNA (eDNA) analysis.

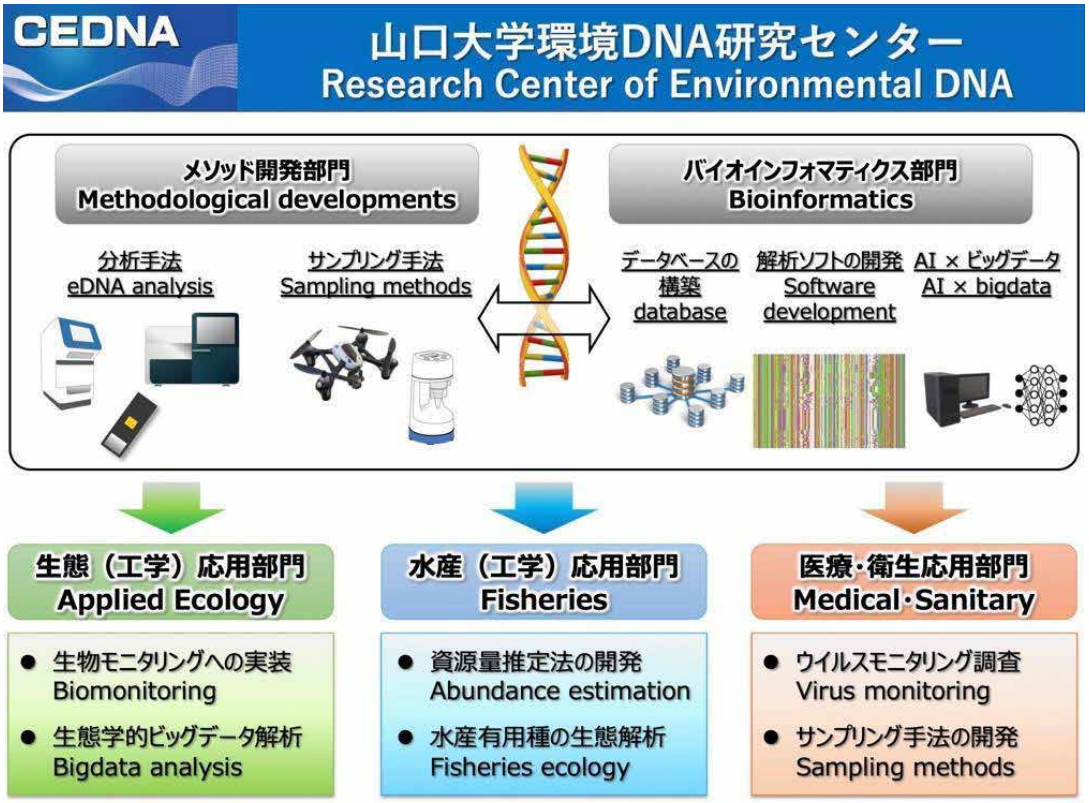


Figure 1　General description of the Research Center for Environmental DNA

As one of our research themes, the CEDNA conducts long-term monitoring of aquatic organisms, such as fishes and insects, using the eDNA analysis, mainly in rivers in the Chugoku district (Fig. 2A). We not only estimate the diversity of aquatic organisms in the river basin using eDNA analysis but also evaluate the response of the organisms to changes in their habitats (e.g., effects of urbanization and natural disasters). Furthermore, taking advantage of the CEDNA located in the Faculty of Engineering, we also conducted the basic study on the ecology of eDNA (e.g., transport and degradation) using hydrographic experiments and hydraulic analysis.

Alternatively, the CEDNA has been developing and introducing advanced monitoring methods for eDNA analysis. These include the development of (1) an eDNA chip for monitoring invasive alien fishes and (2) an airborne eDNA monitoring method (Fig. 2B), and (3) the application of quantitative eDNA metabarcoding (qMiSeq) for fish monitoring. The eDNA chip is based on DNA microarray technology, which enables simultaneous and simple detection of multiple target species. For airborne eDNA monitoring, we are developing the sampling equipment and optimizing survey methods in the field. At the CEDNA, we have introduced the qMiSeq method developed by Ushio et al. (2018) and have begun evaluating the multi-species and quantitative detection of fish eDNA in the above long-term monitoring.

As part of our activities, the CEDNA provides a laboratory service for environmental DNA analysis (Fig. 2C). The CEDNA is equipped with two real-time PCR systems, one thermal cycler and one high-throughput sequencer (Illumina iSeq100), to perform various eDNA analyses ranging from species-specific detection to metabarcoding. We can analyze several taxa, including fish, mammals, birds, reptiles, crustaceans,

aquatic plants, aquatic insects, and algae. We have received offers from researchers, companies, and local governments for environmental DNA analysis. We have also begun introducing the qMiSeq method in our services as an option in eDNA metabarcoding.

Additionally, we have organized the symposium entitled "Frontiers of Environmental DNA Research" (Fig. 2D). Here, companies and researchers are invited as speakers to talk about their work and research on the eDNA study. The eDNA analysis is widely used in various fields and will be a more useful monitoring tool in the future. The CEDNA will continue to contribute to the progress of eDNA study through our research activities and hope to contribute to the spread of eDNA analysis through our analytical services.

Figure 2　Introduction of the research activities in CEDNA;
(A) environmental DNA surveys in the river (water sampling) and (B) in the zoo (air sampling), (C) CEDNA website, and (D) CEDNA annual symposium (2019).

Useful tools for eDNA analysis

Takashi Fukuzawa (President, Biryu Planning)

There is no doubt that ecological studies using environmental DNA (eDNA) can be conducted more quickly and easily than conventional methods (visual census or capturing). However, there are many procedures in conducting eDNA analysis by ourselves, and many beginners may suffer from detecting it, even for species-specific detection.

When I was an engineer of PCR instruments, I met Dr. Doi, University of Hyogo, and he let me know the attraction of eDNA and started working in this field. However, since I had never performed DNA extraction, it was hard to conduct filtration and extraction.

After my retirement, I decided to conduct activities for contribution to society under "Biryu Planning," besides enjoying my life. As one of these activities, I chose to cooperate for implementation of eDNA technology to society. Therefore, I created a website called "Introduction to eDNA analysis" based on my experience. This website provides information on procedures including ideas of easier methods for eDNA analysis, especially for beginners.

I feel honored to write this section. So, I would like to introduce some items from the website as "Useful Device" for eDNA analysis. These items were created through trial and error based on my experience of eDNA sampling.

1. Water sampling cup: Fig. 1a
"A-weighted measuring cup being suspended from a fishing line"

Fig. 1a: The modified cup for water sampling

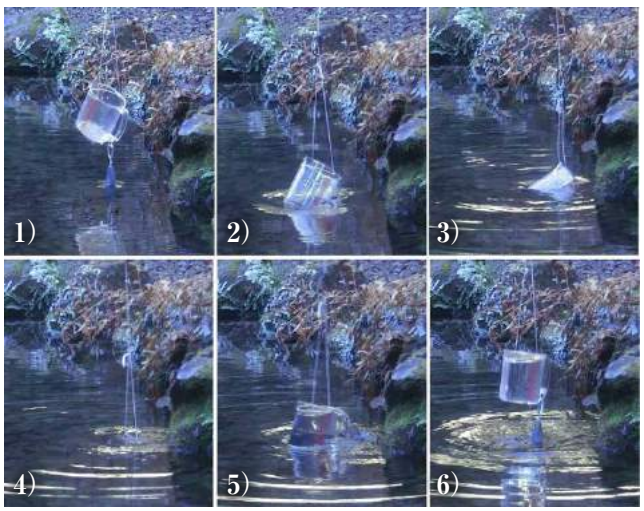


Fig. 1b: The process of water sampling

The first step in eDNA analysis is to collect water. It is unexpectedly difficult to collect water using a bucket with a rope from a high place, such as a bridge. Instead of it, using this modified cup, simply dropping fishing line down and pulling it up, you can easily collect ~500 mL of water from a high place. The key point is to attach a weight of ~150 g to this position **(Fig. 1b.)**

2. Direct water sampling rod: Fig. 2a

"A syringe attached to the tip of a rod, and its piston with a string"

It is useful when a water sampling cup is not available, such as in shallow water or areas with water plants **(Fig. 2b)**. Moreover, you can also collect water at a specific depth, avoiding surface water. When this method is adopted for filtration in fields, this method can prevent contamination by directly collecting water.

Fig. 2a: Water sampling rod

Fig. 2b: Example of using a water sampling rod

3. Biryu filtration supporter: Fig. 3a

"A shoulder belt with a metal plate for setting a syringe"

Fig. 3a: Supporter

Fig. 3b: Previous work

Fig. 3c: Supporter usage

Although using a syringe to filter through Sterivex is simple, it is difficult to filter the large amount of water required for eDNA. At first, I was working as shown in **Fig. 3b**, but it required more strength than I imagined, and my muscles in both arms would soon scream. On the other hand, hanging this supporter on

Fig. 3d: metal plate

your shoulder **(Fig. 3c)**, it becomes easy to filter by hooking the syringe on this jig and pushing it. At this time, the right hand pushing the syringe is almost stretched out and pushes with the force of the shoulder, so it is easy to apply force, while the left hand is not applied force. The structure of the metal plate is a 30 mm square angle cut into 15 cm pieces. The metal plate is made hollow at the center to hook the syringe **(Fig. 3d)**. It is such a great product that I cannot filter without it.

4. Biryu centrifuge: Fig. 4a

"The tube attached a string to set a 1.5 ml-tube and Sterivex"

According to the manual of the eDNA Society, a large centrifuge should be used to pull down the solution in Sterivex. However, it is expensive and takes time to centrifuge. Usage of Biryu centrifuge is, after setting easily you have only to turn it around for about two seconds using the string, then the residual solution in Sterivex is almost completely collected **(Fig. 4b)**. The structure is as follows: a pipe with an inner diameter of ~18 mm is cut into 60 mm length, and the sides are notched so that the tube can be attached and detached, and squeezed by heating to prevent from fall it off **(Fig 4a)**.

Strictly speaking, contamination could occur using the same pipe for different samples. Therefore, you should use it carefully, and may not use this tool depending on the purpose and situation. But it is so convenient for simplified analysis or on-site extraction for eDNA.

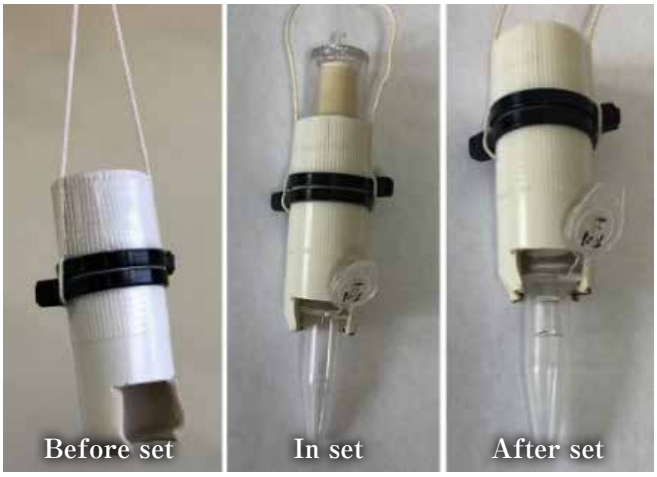


Fig. 4a: Biryu centrifuge

Fig. 4b: How to use it

The above four items are useful for an analysis following the already established protocol such as the eDNA Society Manual. The following is a boldly simplified method for eDNA analysis based on our experience. I would like to introduce this method which the collaboration company "G" is currently developing.

5. Biryu chip(BC) method: Fig. 5a

"The method of using microfluidic chip for filtering and extraction"

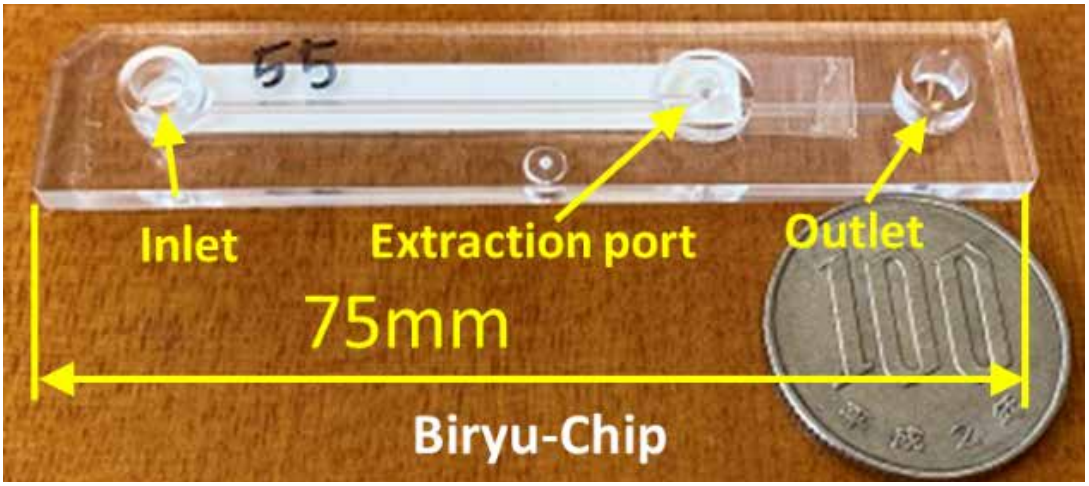


Fig. 5a: Biryu chip

The procedure is simplified from the 12-step of the eDNA Society manual method to 4 steps and can be completed in ~3 minutes **(Fig. 5b)**. Additionally, only the 1/20 volume of the filtration described in the manual is required to perform the same results of quantitative PCR. And it can lead to simplifying water sampling. Furthermore, since all processes are completed in the closed space of the chip, the risk of contamination is greatly reduced. It will be a little while until everyone can use it, but it seems that the work will be much easier once it becomes available.

You can also check the contents in the preprint:
https://www.biorxiv.org/content/10.1101/2021.11.18.468931v1

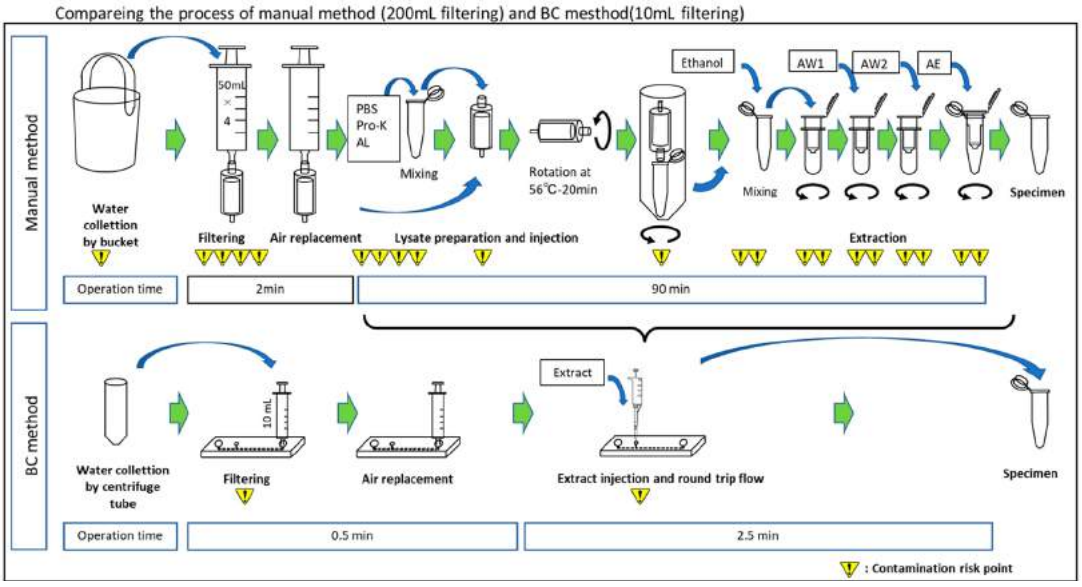


Fig. 5b: Comparison the procedure between BC and the manual of the eDNA society

More information can be found in "Introduction to eDNA analysis (https://biryu-kikaku.com/device)." If you are interested in using our items, please contact us (ryu-edna@biryu-kikaku.com).

Editor's Note

Hiroki Yamanaka (Editor-In-Chief of this newsletter)

The volume of this newsletter is now 4. That is surprising to me, feeling that time pasts so quickly. Thanks to the efforts of four editors, we have managed to publish this volume. As a new challenge, we published this volume in a bilingual setting in Japanese and English, which was a lot of work for both the contributing authors and the editors. However, we have been working on English language support for the internationalization of our annual meetings, and the information contained in this newsletter is too valuable to be published only in Japanese, so these efforts are inevitable. I hope that it will raise the value of this newsletter to all members of society.

In this column in the previous volume, I had written a comment that I wished the spread of the novel coronavirus would end soon. The number of newly infected people had decreased considerably by last autumn, and economic activities became more active. Still, the trend ended soon, and since early in the New Year, it has been reported that a rapid increase in the number of newly infected people again, probably due to a new variant, Omicron. The re-expansion of infection is depressing to us. I wonder when we will have conference meetings and technical seminars in person. However, we must consider this situation as "normal" and think about "how to do essential activities within this situation," People are gradually getting used to the new normal in daily lives and research activities. We will continue to consider flexible management of the activity of the society to proceed smoothly.

Finally, I would like to express my gratitude to all the authors who contributed their manuscripts in a short period despite such a difficult time. We will continue to work on this newsletter to make it more valuable.

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