The eDNA Society
NEWSLETTER

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Greetings from the President

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

The year 2023 was busy, with two conferences, including an international conference in Otsu in May and the annual meeting in Kyushu in December. We believe that our first international conference was a great success, with approximately 250 participants from 19 countries. For the second conference in Kyushu, we worried about the number of participants, but ended up with 202 participants, making for an even more exciting conference than in previous years. Abroad, societies specializing in environmental DNA (eDNA) studies were formally established in South Korea, China, and Oceania, making 2023 a year in which eDNA studies have flourished both internationally and domestically.

As eDNA studies have progressed, we have observed gradual diversification of the topics presented at eDNA conferences. eDNA analysis of macroorganisms has evolved from the simplest studies, such as detecting DNA of a particular species, to quantitative assessment of DNA concentration and comprehensive detection through metabarcoding, and is now maturing into a tool that can reveal where and how abundantly organisms are distributed. Further, techniques that were previously limited to proof-of-concept status have been developed to a practical level, such as the analysis of complex, difficult-to-identify groups of species and the elucidation of reproductive behavior or physiological status using nuclear/mitochondrial ratios and/or environmental RNA (eRNA). Bottom-up discussion by a wide range of participants is essential to the developments that have occurred in these different areas, and conferences play an important role in this respect.

On the other hand, eDNA technology is now being implemented as a method for elucidating biological distributions. As discussed at the conferences, responses to top-down elements will be important in the future, such as the introduction of eDNA technology into monitoring by governments, requests from the business community in response to the international nature-positive trend, and the development of international standards. The eDNA Society is responding to these issues through revision of its manuals, round tables with ministries and agencies, and participation of board members in related projects and standardization task forces. Despite this progress, we remain behind the curve. We aim to address various issues facing eDNA research in the future with the help of our society members, and we would very much welcome your input.

We face diverse challenges as an organization, including internationalization, but we aim to continue to support the development of this field as an academic discipline and its application to society together with our members. We look forward to your continued support.

Meeting report and greetings from the Organizing Committee

Hiroki YAMANAKA
(Associate Professor, Center for Biodiversity Science, Ryukoku University)

In May 2023, The eDNA Society International Meeting 2023 was held in Shiga Prefecture, Japan. The theme of the conference, "Moving from knowledge into practice," focused on the utilization of rapidly advancing environmental DNA (eDNA) analysis technology and the insights derived from it in societal contexts. Discussion topics ranged from specific monitoring programs implemented in various countries to sampling projects developed for global deployment, as well as strategies to ensure the reliability and value of the generated data to society. This conference provided a valuable platform for envisioning a future in which eDNA analysis is universally applied. We extend our heartfelt gratitude to all participants and supporters who contributed to the success of this event.

The conference was chaired by Dr. Toshifumi Minamoto (Kobe University, Japan), alongside 14 members of the Organizing Committee supported by the society's secretariats. The event took place from May 17th to May 19th, 2023, with excursions to Kyoto and Otsu offered for two days after the core dates. The conference had approximately 250 participants from 19 countries, who made it a highly successful event. The venue, Piazza Omi, which is located on the southwestern shore of Lake Biwa, offered a picturesque view of the lake, where Japanese eDNA research began. Numerous corporate sponsors contributed to the event's success and the foyer overlooking Lake Biwa was adorned with exhibition booths, facilitating lively discussion among exhibitors and attendees. The three plenary symposia, as well as oral presentations and the poster session, stimulated spirited exchanges between presenters and the audience, providing a crucial forum for meaningful discussion at the first face-to-face event since the COVID-19 pandemic.

Snapshots of the meeting

Meeting Summary

On the first day of the conference, an opening ceremony was held. Following this ceremony, Plenary Session 1 focused on evidence-based conservation management utilizing eDNA analysis. An evening banquet was hosted thereafter.

Day two of the conference commenced with Plenary Session 2, which explored the elucidation of biodiversity hotspots using eDNA. Subsequently, corporate workshops, oral presentation sessions, and poster presentations occurred.

The third day of the conference featured Plenary Session 3, which centered on the transition from knowledge to practice in eDNA analysis. Following this session, additional corporate workshops and oral presentation sessions were conducted. The closing ceremony included a keynote address discussing the impacts of eDNA analysis on science and society.

The toast at the banquet

Meeting Highlights

In the opening ceremony, Dr. Michio Kondoh (Tohoku University, Japan) discussed the use of eDNA analysis in a biodiversity monitoring network (ANEMONE: All Nippon eDNA Monitoring Network), stressing the great potential of eDNA observation for forecasting ecosystem dynamics. He shared his vision on the social applications of eDNA technologies.

The title of Plenary Session 1 was "From innovation to practice: Using eDNA to support evidence-based management," which was organized by Dr. Hiroki Yamanaka (Ryukoku University, Japan). This session provided a platform for discussing the construction of a practical framework for utilizing data obtained through eDNA analysis for biodiversity conservation and fisheries resource management. Four speakers gave presentations on various topics related to optimizing eDNA analysis and streamlining future monitoring programs.

Plenary Session 2, entitled "Understanding biodiversity hotspots using eDNA," was hosted by Dr. Hitoshi Araki (Hokkaido University, Japan) and Dr. Tadashi Kajita (University of The Ryukyus, Japan), along with three additional speakers. The objective of this session was to share foundational information obtained through eDNA analysis about various biodiversity hotspots, including mangrove forests, coral reefs, and marine and freshwater ecosystems.

Plenary Session 3, "Environmental DNA moving from knowledge into practice," organized by Dr. Hideyuki Doi (Kyoto University, Japan), focused on the current state of eDNA research, its potential applications, and the challenges of implementing eDNA surveys for ecological monitoring. Three speakers examined the practical utilization of data obtained from eDNA analysis for biodiversity conservation and resource management, providing participants with valuable insights and opportunities for discussion.

Dr. Mehrdad Hajibabaei (University of Guelph, Canada) delivered the closing keynote address, discussing the significant correlation between biodiversity and human wellbeing. Dr. Hajibabaei emphasized the urgent need for effective tools for biodiversity analysis amidst the ongoing biodiversity crisis. While acknowledging the substantial contributions of eDNA analysis to addressing this crisis, he noted that the utilization of eDNA analysis in environmental assessment and monitoring programs is hindered by the lack of standardized protocols. He recommended advancing the standardization of eDNA protocols, to maximize the technical capacity of this method, to clarify and address the biodiversity crisis. The prospect of advancing eDNA methodologies as standardized tools for biodiversity monitoring aligns with the objectives of this meeting, highlighting the significant value of the meeting as a timely and valuable assembly.

During the conference, 39 oral presentations and 80 poster presentations were given. The Best Poster Presentation Awards were bestowed upon Dr. Narumi Tsugeki and Mr. Fei Xia, who were announced and honored during the closing ceremony.

The Best Poster Presentation Award Winners, with our President in the center.
Left: Dr. Tsugeki. Right: Mr. Xia.

Acknowledgments

The eDNA Society will continue to collaborate with researchers and organizations from various countries, including participants of this conference, to advance eDNA analysis, deepen our understanding of biodiversity, and promote biodiversity conservation. We express our gratitude to all conference participants, sponsoring companies, Shiga Prefecture, Otsu City, and the Richard Lounsbery Foundation for their generous support. A detailed report of the conference is available in Environmental DNA (https://onlinelibrary.wiley.com/doi/full/10.1002/edn3.465).

Group photo of meeting participants

Zooplankton sedimentary DNA as an effective tool for tracking past population dynamics

Narumi TSUGEKI
(Professor, Faculty of Law, Matsuyama University)

Zooplankton play a key trophic role in the transfer of primary production to higher trophic levels, and this production contributes substantially to successful fish recruitment. Despite their importance, knowledge of historical zooplankton dynamics remains limited, due to the lack of long-term data series. To overcome such difficulty, environmental DNA in sediment (sedDNA) has been developed as a tool for reconstructing the past dynamics of organisms. Through the sedDNA approach, great progress has been made in reconstructing the past dynamics of various organisms.

Our study aimed to evaluate the information that can be obtained from zooplankton sedDNA, targeting pelagic copepods (*Calanus sinicus* in the Seto Inland Sea, Japan; *Eodiaptomus japonicus* in Lake Biwa, Japan) and *Daphnia* (*Daphnia galeata* and *D. pulicaria* in Lake Biwa, Japan). We developed a specific primer-probe for each species, applied a quantitative polymerase chain reaction method to sediment cores, and compared the sedDNA concentrations with long-term observation datasets. The sedDNA concentrations of both copepods recovered from sediment layers correlated significantly with in situ biomass, whereas *Daphnia* sedDNA did not correlate with their abundance but, instead, with their resting egg production. These results provide evidence that zooplankton sedDNA is an effective tool for reconstructing past population dynamics while demonstrating that sedDNA recovery differs among species. The application of eDNA analysis to sediments can elucidate historical population dynamics, providing a new monitoring and conservation tool with the potential to clarify from past to present biodiversity.

Listening to the whispers of RNA hidden in the environment

XIA Fei
(Master student, Graduate School of Frontier Sciences, The University of Tokyo)

As an international student, I entered a department heavily focused on the "micro"

aspects of biology, such as molecular biology, but chose a developmental biology lab that works with African clawed frogs due to my desire to interact with "macro" aquatic organisms like fish. There, instead of developmental biology, I encountered environemntal DNA technology and was fascinated by the ease with which it allows for the collection of aquatic biodiversity information from water. I believed that environmental RNA, especially environmental mRNA, whose release into the environment varies with the health of organisms, is key to understanding biodiversity as well as obtaining further information about biota.

Prior to using environmental RNA as a tool, the aim of my study was to acquire foundational information about the types of RNA that organisms release into the environment. For this study, we selected *Xenopus laevis* (Fig. 1) from our department's lab and *Gasterosteus aculeatus* (Fig. 2) from the lab next door, both of which have been well researched in terms of RNA expression patterns. After numerous attempts at the challenging task of environmental RNA sampling, we obtained samples of sufficient quality and quantity and proceeded to conduct analysis using RNA sequencing. The results showed that similar environmental RNA was released by amphibians and fish, including many genes previously reported as stress markers in conventional studies.

Figure 1. *Xenopus laevis*

Figure 2. *Gasterosteus aculeatus*

Based on these findings, we are now exploring the information that can be extracted using environmental RNA from coral reefs (Fig. 3), which are among the most biodiverse areas of the ocean.

Figure 3. Sekiseshoko

6th Annual Meeting of The eDNA Society

Meeting summary

The 6th Annual Meeting of The eDNA Society, chaired by Dr. Satoquo Seino (Kyushu University), was held under the theme "Opening the Way to the Future of Ecology" from December 2nd to 5th, 2023, in Fukuoka, Japan. The event commenced with a water sampling excursion on December 2nd, followed by a public symposium on December 3rd entitled "Environmental DNA-New visions of science and human society provided by innovative technologies".

The public symposium drew a diverse crowd, including young students, citizens, and researchers.

The main academic sessions were conducted at the Ito Campus of Kyushu University on December 4th and 5th, featuring a session on standardization, two plenary sessions, two organized symposiums, and 51 poster presentations. The poster awards were given to the following individuals:

Mika GUNJI (Nippon Koei Co., Ltd.)
"Demonstration test of Passive Sampling tool for use in river fish surveys"

Aoba ITO (Graduate School of Life Sciences, Tohoku University)
"Improving the river fish distribution estimation using eDNA and hierarchical Bayesian modelling"

Tomoharu HINO (Junshin High School)
"Save! *Aphelocheirus nawai*! -Habitat survey with environmental DNA analysis"

Snapshots of the meeting held at the Ito Campus

Reports on sessions and symposiums

Session on standardization: What changes will be made to the next version of the "Environmental DNA Sampling and Experiment Manual" in March 2024?

Toshifumi MINAMOTO (Kobe University), **Teruhiko TAKAHARA** (Shimane University), **Keiko MURAOKA** (Public Works Research Institute)

The previous version of The eDNA Society's "Environmental DNA Sampling and Experiment Manual" was updated in April 2020, more than three years ago. The Environmental DNA Technology Standardization Committee has scheduled a new version of this manual for completion in March 2024 in response to recent research progress, the development of new technologies, and the originality and ingenuity of researchers. In this session, Toshifumi Minamoto (Kobe University) gave a presentation on "Development of the eDNA experimental manual and international standardization of analytical methods" and Teruhiko Takahara (Shimane University) discussed "Attempt and direction of the eDNA technical standards committee" as topics from the Environmental DNA Technical Standardization Committee of the Environmental DNA Society of Japan, explaining the main points and changes in the revised manual. Teruhiko Takahara explained the background of the standards, points of revision and major changes. To provide further information relevant to the revision of the manual, speakers who have been actively involved in the development and promotion of eDNA technologies explored the topics of "Environmental DNA metabarcoding data analysis" (Wataru Iwasaki, University of Tokyo), "Various filtration methods for environmental DNA" (Shin-ichiro Oka, Okinawa Churashima Foundation), and "Utilization of environmental DNA sampling and experiment manual in ANEMONE observation system" (Hisashi Yamakawa, Kazusa DNA Research Institute). During the Q&A session, participants in the

audience asked questions about the range of potential applications for the new technologies and exchanged opinions about technical issues related to updating the manual.

Plenary session 1: How to Use? Initiatives on eDNA by Japanese ministries

Hideyuki DOI (Kyoto University),**Satoquo SEINO** (Kyushu University),
Keiko MURAOKA (Public Works Research Institute)

Currently, Japanese ministries are undertaking various initiatives toward the implementation of eDNA technologies. This session allowed for the exchange of ideas through lectures and panel discussions regarding the potential for collaboration among ministries and the future expansion of eDNA utilization. Initially, four speakers were present at the event. These speakers included Kosuke Mita from the Ministry of Agriculture, Forestry, and Fisheries (MAFF); Akane Shoji from the Ministry of the Environment; Kazuho Aga from the Ministry of Land, Infrastructure, Transport and Tourism (MLIT); and Naoki Morizumi from MLIT. Each ministry discussed its use of eDNA and initiatives for broader implementation. Following these lectures, a panel discussion took place with the four speakers as well as Noriyuki Koizumi from the National Agriculture and Food Research Organization, Masakazu Hori from the Fisheries Research and Education Agency, Natsuko Kondo from the National Institute for Environmental Studies, Keiko Muraoka from the Public Work Research Institute, and Masaya Hosokawa from the Maritime, Port and Airport Research Institute. The discussion focused on the challenges of and efforts toward eDNA implementation in their respective fields. During this discussion, some concrete plans were proposed, including the establishment of a shared research site for cross-ministerial practical implementation. This session aimed to encourage future collaboration among ministries, researchers, and the government.

Plenary session 2: Contributing to a Nature-Positive World with Environmental DNA

Michio KONDOH (Tohoku University)

An international environmental target of achieving "Nature-Positive" status through halting and reversing the degradation of nature by the year 2030 has been set. eDNA technology is expected to make a significant contribution to the attainment of this goal. In this project session, entitled 'Contributing to a Nature-Positive World with Environmental DNA,' participants, primarily from the business and financial sectors, gathered to share information about recent case studies and to discuss collaboration and future developments aimed at achieving nature-positive status using eDNA and related technologies.

The presentation entitled "Towards a Nature-Positive World - Data Challenges

from the Perspective of TNFD" by Mr. Makoto Haraguchi of the TNFD/MS&AD Insurance Group Holdings provided a clear and insightful explanation of the emerging trend in which the financial sector collaborates with ecologists. This collaboration is giving rise to an economic system that grows while restoring nature. The subsequent panel discussion, facilitated by Dr. Michio Kondo of Tohoku University, featured panelists including Mr. Makoto Haraguchi, Mr. Yuta Hasebe of the Kanagawa Environmental Research Center, Mr. Yuki Tsuda of Fisherman Japan Marketing Co., Ltd., Mr. Yohei Takuso of Nippon Yusen Kabushiki Kaisha, and Mr. Hiroki Unten of NEC Solution Innovators, Ltd.

Following the introduction of initiatives being undertaken by each company, this session delved into the specific mechanisms underlying nature-positive policies, such as the roles of eDNA in fisheries certification and in empowering local communities for ecosystem conservation. The significance of eDNA technology, the potential fusion of information and communication technologies with eDNA technology, and the utilization of eDNA data as a communication tool among stakeholders were explored. The wide array of ideas presented revolved around the effective utilization of eDNA technology toward achieving a Nature-Positive economy.

Symposium 1: Who will build the database? -Challenges and Future Prospects for the DNA Surveys-

Yuta HASEBE (Kanagawa Environmental Research Center)

This symposium explored the current state and challenges of DNA databases, which are an essential component of eDNA research. The first speaker, Utsugu Jimbo (National Museum of Nature and Science), summarized DNA barcoding in Japan and provided a taxonomist's viewpoint. The second presenter, Masaki Takenaka (Shinshu University), gave a lecture on the potential for eDNA from the perspectives of biogeography and phylogenetic research. The third presenter and organizer of this symposium, Yuta Hasebe (Kanagawa Prefectural Environmental Science Center), spoke about the importance of DNA databases from the viewpoint of an eDNA researcher. The fourth speaker, Masaaki Nakamura (Idea Co., Ltd.), gave a presentation from the standpoints of a private company that is entrusted with eDNA analysis and a practitioner who is developing universal primers for shellfish and registering DNA databases. Finally, Yui Ishigami (University of Tartu) introduced "UNITE", a reference database platform that is not yet well known in Japan.

At the end of the symposium, we reviewed the results of a questionnaire on DNA barcoding that we had given to participants at the beginning of the meeting. Among the results, all survey respondents noted that they have had problems in eDNA analysis due to the lack of DNA databases, highlighting the fact that

enhancement of databases is essential to eDNA research. We hope that this meeting served as an opportunity for promoting efforts to enhance DNA databases for various taxonomic groups.

Symposium 2: Bored of sampling water! -Environmental DNA analysis using non-water media-

Masayuki K. SAKATA (Hokkaido University)

At this symposium, unconventional eDNA analyses utilizing substrates such as sediments, soil, and air, in addition to the traditionally employed medium, water, were discussed. The first presenter and organizer, Dr. Sakata, reported eDNA analyses using sediments and soil, providing a brief review of sediment eDNA. The second presenter, Dr. Niko Nakamura (Environmental Research & Solutions Co., Ltd.), presented results from studies utilizing plant rolling and spraying methods targeting terrestrial mammals to detect DNA left by mammals on outdoor plants. The third presenter, Dr. Ryohei Nakao (Yamaguchi University), discussed eDNA analysis of air, comparing collection methods and practical applications in zoos. The fourth presenter, Dr. Ryo Iwamoto (AdvanSentinel Inc.), introduced the COPMAN method (Coagulation and Proteolysis Method using Magnetic beads for detection of nucleic acids in wastewater) for samples containing mixed solids and liquids, such as sewage. This method captures nucleic acids without the need for filtration. This symposium featured presentations and discussions related to eDNA analysis using various media and methods. While the characteristics and optimization of methods in eDNA analysis using non-water substrates is not as well understood as water-based eDNA analysis, this symposium demonstrated the unique utility of other media. For example, detecting terrestrial mammals and birds from environmental water is challenging, but using air or plant-based methods may enhance their detectability. Additionally, in environments with high pollution levels, employing methods such as COPMAN (Coagulation and proteolysis method using magnetic beads for detection of nucleic acids in wastewater) or sediment-based approaches without filtration may overcome existing challenges. I hope that this symposium will serve as a catalyst for development of eDNA analysis using medium other than water.

Environmental DNA database ANEMONE DB and its applications

Minoru KASADA
(JSPS Cross-border Postdoctoral Fellowship (CPD), Graduate School of Life Sciences, Tohoku University)

Extensive data from eDNA-based fish surveys have been released in an open database called ANEMONE DB (https://db.anemone.bio) by Dr. Michio Kondo Laboratory at Tohoku University since 2nd June, 2022. As of January 2024, the database contains 1745 observation data, representing 682 fish species, 202 families and 569 genera. Observation sites are located throughout Japan, from Cape Soya in the north to Iriomote Island in the south, and time-series data have been collected at several sites (Fig. 1). Continuous eDNA observations are being conducted through a biodiversity monitoring network, designated ANEMONE (All Nippon eDNA Monitoring Network, https://anemone.bio), with the participation of private companies and citizens and, thus, the dataset will be enriched in the future.

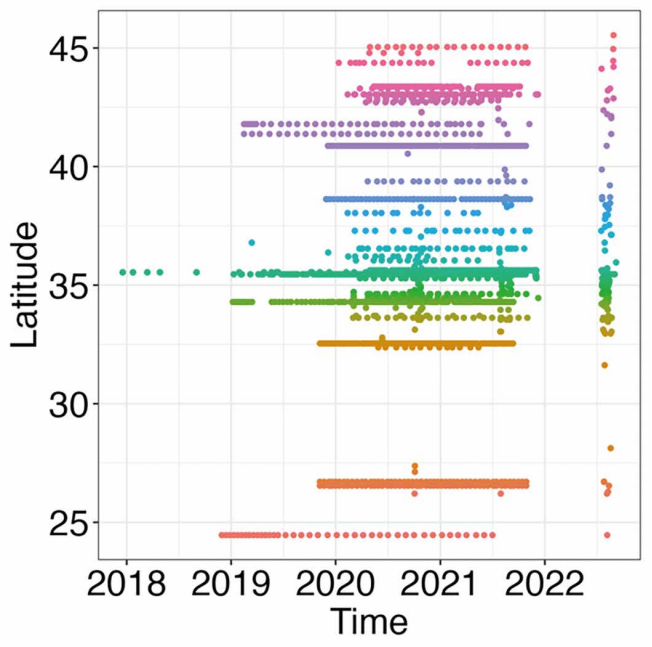

Figure 1. Spatiotemporal distribution of ANEMONE DB samples. X-axis represents time (year) and Y-axis represents the latitude of each observation site.

In this report, I outline the uses and challenges of ANEMONE DB, which is presently in a trial-and-error phase. First, the data can be used for research purposes. The large amount of fish data in ANEMONE DB provides a great

advantage for obtaining ecological information such as species distributions. In addition, the data in ANEMONE DB can be treated as quantitative eDNA data, as the data were collected using the quantification method developed by Ushio et al. (2018). As quantitative eDNA data showing spatiotemporal patterns at the national scale can be obtained from ANEMONE DB, methods such as spatial distribution models and non-linear time-series analysis, which have been rapidly developed in recent years, may be applied. On the other hand, more research is needed to determine the best manner in which to incorporate the relationship between eDNA and biomass into a spatiotemporal model.

Another potential use of ANEMONE DB is as an educational tool. The sampling of eDNA can be conducted without special training. In fact, ANEMONE DB contains abundant data collected by citizen participants. This simplicity of sampling allows for application of this eDNA to teaching about biodiversity in school education (Kitani et al. 2023, Fig. 2). In this context, ANEMONE DB enables to access to data comparable to the data obtained through student sampling. This comparability is expected to deepen biodiversity education. However, ANEMONE DB currently requires user registration and is not designed for easy access by early-career students and teachers and, therefore, improvements are needed to make it easier for beginners to use.

Figure 2. Field work class collecting eDNA at Yaizu Chuo High School (photo by Yuichi Yaoi).

Although ANEMONE DB has these advantages, some issues have arisen since it began operating. The first issue is running costs. Operating a database involves significant human and financial costs. eDNA database management requires knowledge of bioinformatics and database operation, and managers must handle both hardware and software issues. The long-term stable operation of the database requires a team with such knowledge, experience and skills; therefore, we are planning to reinforce our management team and establish a more comprehensive

management system in the future. The second issue is that of data registration. At present, only data obtained from ANEMONE members are available, but if a wider range of users can register their data, the database will become more complete. This report notes the research and educational uses of ANEMONE DB, but a wider range of uses can be considered in the future. We are always open to such ideas. On the other hand, for practical application of ANEMONE DB, the data must be adapted and updated appropriately. We will address these issues through improvement of the ANEMONE DB system, including improvement of its useability.

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Environmental DNA analysis of insects using MtInsects-16S: Comparison with mtDNA COI primers

Masaki TAKENAKA
(Assistant Professor, Department of Biology, Faculty of Science, Shinshu University)

I conduct research in the fields of phylogeography and developmental genetics, using DNA to unravel the evolution of various biological diversity estimates. While engaged in phylogeography research, I had doubts about the use of the mitochondrial DNA (mtDNA) cytochrome oxidase subunit I (COI) region as a DNA barcode for insect species. Therefore, we suggested a novel DNA barcoding region for insects and designed polymerase chain reaction (PCR) primers designated MtInsects-16S, which can be used for insect DNA barcoding and eDNA analysis.

I have been investigating the evolutionary history of various organisms using general DNA barcoding regions, the mtDNA COI and the mtDNA 16S rRNA region. While the mtDNA COI region is suitable for examining intraspecific regional variations, and often exhibits high intraspecific variability, distinguishing between intra- and interspecific variations based on genetic distance is a difficult challenge. This challenge leads to difficulty in identifying a region with high consistency across all insect species. We sought the mtDNA COI region common to all aquatic insects using sequences registered in the database GenBank, but found no such region. For example, we attempted to find a common region at the order level for Ephemeroptera (Fig. 1) and in some cases at the family level, but the search proved challenging. On the other hand, we found multiple common regions of the mtDNA 16S rRNA region, which led to the design of the MtInsects-16S primer set (Takenaka et al., 2023a). We confirmed successful amplification for 14 orders, 43 families, and 68 species with the MtInsects-16S primer set (Takenaka et al., 2023a). Initially, our focus was on aquatic insects, but our results suggest the potential for universal applicability across all insect species. For eDNA analysis using the MtInsects-16S primer set, we constructed a DNA database of Kanagawa Prefecture, particularly of the Sagami River and Sakawa River. In conjunction with GenBank, we registered most species of recorded insects (limited to Ephemeroptera, Plecoptera, and Trichoptera) within Kanagawa Prefecture. Those registrations include 77% of recorded species for the mtDNA 16S region and 83% for the mtDNA COI region.

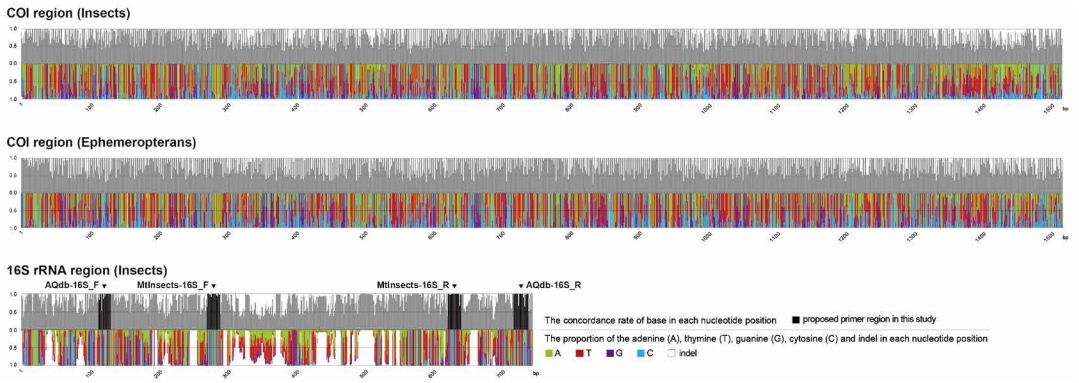

Figure 1. Nucleotide concordance rate (0.0–1.0; upper portion of each panel) and proportion of each nucleotide and indel (0.0–1.0; lower portion of each panel) in the full length of the mtDNA 16S rRNA and COI regions based on mitochondrial genetic sequences (including complete and partial sequences) of all aquatic insects registered in GenBank.

We conducted physical capture surveys and eDNA water sampling at six sites in the Sagami River and Sakawa River. We compared the species lists obtained through eDNA analysis using MtInsects-16S and mtDNA COI region primers (fwhF2-EPTDr2n: Vamos et al. 2017; Leese et al. 2021) with the physical capture survey results. The number of species detected in eDNA using the MtInsects-16S primer set was greater than the number obtained with the fwhF2-EPTDr2n primer set (Fig. 2). eDNA analysis using the MtInsects-16S primer set detected 74.9% of species collected during the physical capture survey. In contrast, eDNA analysis using the fwhF2-EPTDr2n primer set missed nearly half of the species collected during the physical capture survey (40.1%). Even when the mtDNA 16S rRNA region was used, 30% of the collected species remained undetected. Additionally, among the species detected in this analysis, 10.7% were identified exclusively from the mtDNA COI region. Nonetheless, the numbers of both collected insects and detected DNA read counts were low, suggesting that these species are present in rivers with extremely low biomass. Through careful adjustment of the sampling frequency and quantity of water collected, this issue is likely to be resolved.

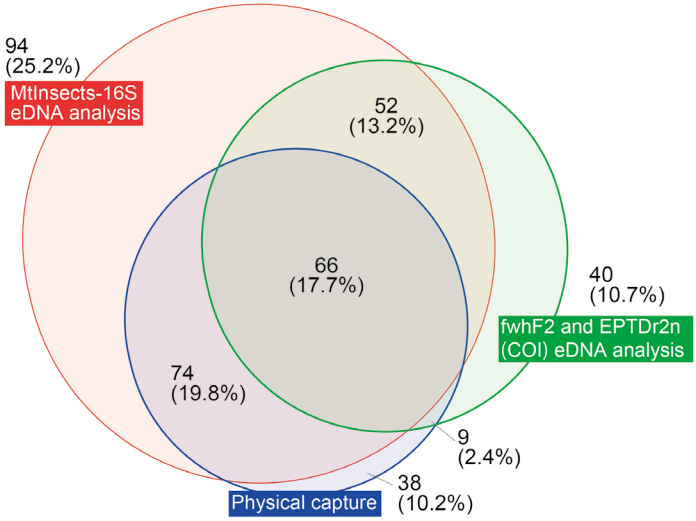

Figure 2. Venn diagrams comparing the species detected through eDNA analysis with two primer sets and a physical capture survey. The number in each section indicates the number of species detected or collected using each method.

Although designing primers capable of improving the efficacy of PCR amplification across all insect species using the mtDNA COI region remains challenging and may be impossible, potential for increasing the number of database records for the mtDNA 16S rRNA region remains. Furthermore, in silico and metabarcoding analyses have reported that the mtDNA 16S rRNA region is suitable for metabarcoding (Marquina et al. 2019). In this study, we have demonstrated the potential for significant improvement if a comprehensive database can be constructed. However, databases that represent numerous geographical regions are urgently needed.

Finally, we express our thanks to Mr. Yuta Hasebe (Kanagawa Environmental Research Center), Prof. Koji Tojo (Shinshu University), Dr. Koki Yano (National Institute for Basic Biology), and Dr. Seiya Okamoto (Aqua Restoration Research Center) for their valuable advice and encouragement. We are grateful to Dr. Noriko Uchida for providing the opportunity to present and introduce this research. Some of the content of this study has been published in Takenaka et al. (2023b) and submitted to an international journal.

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Expert's comment

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

While the field of eDNA research has flourished, the issues facing researchers working with aquatic insects appear not to have changed significantly since I was a student. A critical factor in this shortfall has been the lack of a versatile universal primer set for insects, although studies of aquatic insects generally aim to assess community structure rather than investigate a specific species. Therefore, the development of the MtInsects-16S primer set by Dr. Takenaka is likely to support a breakthrough. In addition, very few reference sequences other than COI regions have been registered to date and, therefore, continuing use of primer sets targeting COI regions has been unavoidable, despite awareness of the many problems associated with those primers. The road to database expansion remains challenging but, as Dr Takenaka has said, "Database expansion is a problem that can be solved." These words suggest that the solution will be simple and broadly agreeable, providing inspiration for future work. With increasing research attention on multi-trophic biota and food webs, new studies of insects will be advantageous. For those researchers with an interest in insect eDNA, why not start by using MtInsects-16S?

Editor's Note / Newsletter Editorial Team

Editor's Note

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

The year 2023 was an important year for The eDNA Society. We held an in-person international meeting in May, which included many people from around the world. Furthermore, the society has accelerated its internationalization efforts, with one of the key milestones being the initiation of efforts to standardize eDNA technologies for biodiversity monitoring. This ongoing initiative is being advanced through collaboration with our international partners.

In this issue, updates on these standardization efforts are provided in various articles, including a message from our president and reports about The eDNA Society International Meeting 2023 and the 6th Annual Meeting. The ANEMONE database, developed by Tohoku University, was featured in the latest Frontiers in Environmental DNA Study series, illustrating its significant contribution to biodiversity monitoring in Japan. Also highlighted in this issue are the novel universal primers for insects, a development that has recently attracted significant attention in the scientific community.

As demonstrated within this issue, steady progress in eDNA science is being made on scientific, technical and social fronts. Let us continue to exchange information and knowledge among our members and the broader community.

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